

GC-MS Profiling of Aroma Compounds and Microbial Analysis of Philippine Civet Coffee

Paulina Angela A. Almerido, Denzel Lorenz S. Chanco, and Emmanuel V. Garcia*

Chemistry Department, De La Salle University, Taft Avenue, Manila

Comparison of aroma profiles of civet and non-civet coffee beans of *Coffea canephora* (Robusta variety) and *Coffea excelsa* species were done under three parameters: coffee species/variety, extent of roasting and type (civet vs. non-civet). Different aroma profiles were identified for different coffee species and varieties, as well as for different extents of roasting, and type. Various volatile compounds contribute to the aroma of coffee. For instance, the presence of pyrazines deliver an earthy and burnt odor. Robusta samples contained more pyrazines, phenols and pyridines while Excelsa variety had more furans and pyrans. Aldehydes for all samples increased from light to medium roast but decreased drastically in full roast. For furans and pyrans, the peak intensity increased proportionately with roasting for both Excelsa and Robusta civet varieties. For the microbial analysis, Robusta, Excelsa and commercial civet coffee samples at three levels of roasting were analyzed for fecal contamination. All 7 samples (6 civet and 1 commercial) were within the limits set by FDA Philippines in terms of colony forming units per mL; and gave a negative result for *E. coli*. Although samples were within the acceptable range of CFUs/mL and was negative for *E. coli*, three samples (Robusta civet LR, Robusta civet MR and commercial civet) gave a most probable number (MPN) of fecal coliform per 100 mL count of 150, 4, and 4, respectively, revealing that Robusta civet LR did not pass the MPN per 100 mL count standard set by the FDA Philippines.

Keywords: none

INTRODUCTION

Coffee is one of the most traded commodities in the world. It is the second most consumed beverage after tea. More and more people continue to consume it for its stimulating effects, exquisite taste and flavorful aroma. Each coffee variety produces a different taste, distinct flavor and a diverse aroma perceived by our olfactory senses. Arabica is considered to be superior over other coffee varieties as it

possesses a sweet, caramel like and fuller aroma. Robusta, the cheapest coffee variety, on the other hand contains an earthy and burnt potato like aroma. Different coffee varieties contain different sets of aroma compounds. Higher concentration of maltol, 1-ethenone, 4-ethyl-2-methoxyphenol and furfurylpyrroles in Arabica coffee are the contributors for its smoother and richer aroma (Ryan, et al., 2004). The presence of higher concentration of alkyl pyrazines in

* Author to whom correspondence should be addressed; email: emmanuel.garcia@dlsu.edu.ph

Robusta meanwhile contribute to the earthy and burnt odor. Changing the roasting conditions can also alter the aroma compounds formed and released by the bean. Prolonging the roasting slightly increases the concentration of caffeine. As of present, there are around 1500 chemical constituents of coffee and only 40 of them contribute to the aroma. The market has thus revolved around the search for the most flavorful coffee, in which aroma and taste are heavily scrutinized. From post-harvest processing to brewing methods, from location to variety, the search has expanded to new fronts, some of which have been, and are still being, considered bizarre. One such coffee type, civet coffee, falls into this category. Considered as the most expensive coffee in the world, civet coffee is said to have a more flavorful and richer aroma compared to regular roasted coffee beans. There are certain studies that reveal that the enzymes inside the gastrointestinal tract of the civet cat ferment the raw beans thereby giving it an exotic and exquisite aroma upon roasting. The aroma constituents of the civet coffee are also different from regular ones which are the primary causes of a more flavorful aroma. Moreover, being a defecated product of the animal, it is possible that the civet coffee is contaminated by microorganisms and fecal coliforms. Diseases such as typhoid fever, cholera, dysentery, hepatitis and bacillary could emanate from drinking beverages contaminated with microorganisms.

In this paper, the aroma profile of a Philippine civet coffee obtained from one municipality will be analysed and compare with its non-civet counterpart. A relevant microbial analysis will also be carried out.

METHODOLOGY

Samples. Unroasted (“green”) civet coffee beans and corresponding beans of ripe Robusta variety and Excelsa species cherries were picked and harvested from plantations in the town of Alfonso, Cavite province, Philippines during the harvest season of January 2011 to March 2011. All of the green civet beans were initially in feces defecated by

civet cats (*Paradoxurus hermaphroditus*) when these were picked. The animals are free-roaming in the wild. For non-civet beans, these are coffee cherries that are handpicked from the coffee tree and later on dehulled and processed to obtain the green coffee bean.

Processing of Coffee Beans Prior to Roasting. *Raw Civet Coffee Beans.* Raw Civet beans were washed several times in running water. The washed samples were then sun-dried for 24 hours. Identification based on shape of Robusta and Excelsa bean varieties were done and the civet beans were dehulled and were washed again in running water. The Civet beans were then oven-dried afterwards for 4 hours at 40 °C. Civet coffee samples were classified by their respective coffee variety either Excelsa or Robusta according to the beans’ size, shape and appearance. Oval or elliptical beans with obtuse base were classified as Robusta civet beans while ovoid and flat form beans with acuminate apex tip were classified as Excelsa civet beans.

Ripe Coffee Cherries. The coffee cherries’ pericarp were removed, leaving only the hull or endocarp of the coffee cherries. Samples were sun-dried for 24 hours until the hull was brittle. The dried coffee cherries were then dehulled, washed in running water and were oven-dried for 4 hours at 40 °C.

Coffee Bean Roasting. *Sand Preparation.* Sand was used to facilitate the roasting of coffee beans. Two (2) cups of sand were washed several times in running water and then boiled for 15 minutes. The cleaned sand was then soaked in 6 M Hydrochloric acid solution for 48 hours. The sand was then washed several times with running water while maintaining the pH of the sand at 7.0.

Degree of Roasting. Four (4) grams of Non-Civet and Civet coffee beans were roasted in the sand roaster set-up which consisted of the following: beaker, three (3) tablespoons of sand, magnetic stirrer and thermocouple thermometer. Roasting of beans were done at a constant temperature of 240 °C. Three varying roasting conditions were achieved by varying the length of operation: light roast, LR

(2 min), medium roast, MR (4 min), and full roast, FR (9 min). Percent moisture or weight lost was the roasting indicator used. Samples were stored in a tightly capped vial (2 cm × 10 cm) for 2 hours prior to aroma analysis.

Aroma Analysis. *Headspace Solid-Phase Microextraction (SPME) Sampling.* Four (4) grams of coffee bean samples were ground in a coffee grinder and were placed in a 50 mL Erlenmeyer flask capped with a septum. The flask was first heated for 10 min at 60 °C before SPME syringe was exposed to the headspace above the sample to achieve condensation of volatile compounds on the fibers. A 57324-U SPME fiber was used to adsorb the gas in the vial for 10 minutes. The collected gas was injected to the GC-MS.

GC Analysis. Analyses were carried out on a Perkin Elmer Clarus 500 Gas Chromatograph-Mass Spectrometer equipped with split injector and a PerkinElmer Elite-5MS capillary column (30m × 0.25mm × 0.25µm; -60 – 325/350 °C). The GC oven temperature program was: 40 °C for 10 min followed by an increase of 5 °C/min to 80 °C, held for 12 min, to a maximum temperature of 300 °C. The pressure rate of carrier gas flow (He) was 20.0 psi.

Microbial Analysis. *Heterotrophic Plate Count (Goldman and Green, 2009).* Ten-fold serial dilution of the coffee samples were performed using 9 mL diluent of increasing number of dilution of sterile lactose broth. One mL of the sample was aseptically transferred into the first tube. The same procedure was done for the succeeding tubes. One mL of the suspension was aseptically transferred to a sterile petri dish. Pour plate technique was employed by mixing 20 mL sterile melted agar to the inoculated petri dishes. The same procedure was done for each dilution. Three trials were done in the experiment. The plates were incubated at 37 °C for 18-24 hours.

Multiple Tube Fermentation Technique (EPA SW-846, 1980). Presumptive Test. Three double strength and six single strength lactose broth with Durham tubes were prepared and

sterilized for the presumptive test. Three 10 mL, 1 mL and 0.1 mL of sample were transferred aseptically in the three double strength lactose broth and six single strength lactose broth, respectively. The tubes were incubated at 37 °C for 48 hours. All tubes with gas formation inside the fermentation tube were reported as positive for the presumptive test and were carried over to the confirmed test.

Confirmed Test. One to two loopfuls of samples from each of the positive presumptive test tubes were aseptically inoculated in each Eosin Methylene Blue agar plates using clock-streak method. The plates were incubated at 37 °C for 18-24 hours. The appearance of dark-colored colonies especially with metallic green sheen were reported as positive for the confirmed test. Samples, which tested negative for the confirmed test, were declared safe for consumption. All samples which tested positive for the confirmed test was carried over to the completed test.

Completed Test. Colonies from each of the plates which tested positive for the confirmed test were aseptically inoculated into each of sterile nutrient agar slants and single strength lactose broth tubes with Durham fermentation tubes. Tubes were incubated at 37 °C for 24-48 hours.

IMViC Biochemical Test (American Society for Microbiology Microbe Library). Indole Production Test. A loopful of sample, which tested positive for the Confirmed Test, was inoculated in sterile Tryptic soy broth. Tubes were incubated at 37 °C for 48 hours. Ten drops of Kovac's reagent was added to the tubes after incubation. The same procedure was done for each positive Completed test tubes.

Methyl Red Test. A loopful of sample, which tested positive for the Confirmed Test, was inoculated in sterile MR-VP medium. Tubes were incubated at 37 °C for 48 hours. Four to five drops of methyl red indicator was added after incubation. The same procedure was done for each positive Completed test tubes.

Vogues Proskauer Test. A loopful of sample, which tested positive for the Confirmed Test, was inoculated in sterile MR-VP medium. Tubes were incubated at 37 °C for 48 hours. Five drops of 40% KOH and 10 drops of alpha-naphthol were added after incubation. The same procedure was done for each positive Completed test tubes.

Citrate Utilization Test. A loopful of sample, which tested positive for the Confirmed Test, was inoculated in sterile Simmons citrate agar slant. Tubes were incubated at 37 °C for 48 hours. The same procedure was done for each positive completed test tubes.

RESULTS AND DISCUSSION

Roasting. Table 1 summarizes the roasting conditions used both on the non- civet beans and their civet coffee counterparts.

Table 1. Amount of Moisture Lost at Varying Roasting Conditions.

Degree Roast of Temperature	Length of Exposure at 240 °C (min)	Average % Moisture Lost (± 0.5 %)
Light Roast (LR)	2	7.1
Medium Roast (MR)	4	9.9
Full Roast (FR)	9	10.8

Temperature was kept constant at 240 °C while varying the length of roasting time in order to observe the changes in chemical reactions occurring during roasting. As the roasting temperature increases, the beans lose approximately more than 5% of their dry weight due to the volatilization of substances, in addition to loss of moisture (Arya and Rao, 2007).

Analysis of Volatiles. Identification of Volatile Compounds. Volatile compounds were detected using the GC-MS with the aid of the NIST MS 2.0 library. The fragmented ions of the mass spectrum of the compounds were also analyzed to confirm the data shown in the library. Retention time of the compounds detected was compared to the reported retention time of the compounds published in literature. A total of 96 volatiles were

determined. Almost all of the volatile compounds detected had been previously identified in previous studies.

Figures 1 and 2 show samples of full scan GC-MS chromatograms of civet and non-civet coffee samples for Robusta and Excelsa at Light Roast (LR).

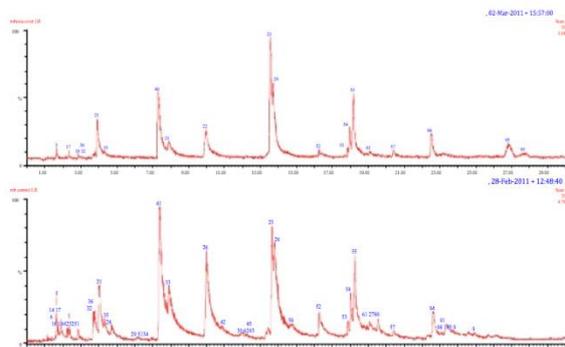


Figure 1. Chromatogram of Robusta LR: Civet (top), Non-civet (bottom).

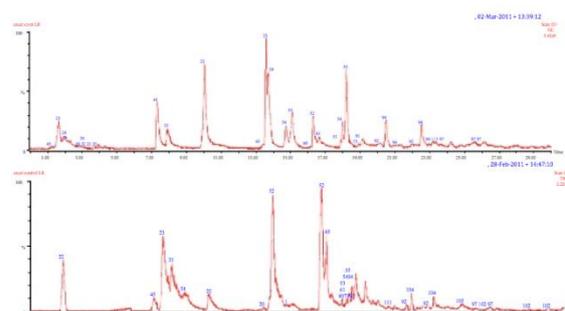


Figure 2. Chromatogram of Excelsa LR: Civet (top), Non-civet (bottom).

The compounds belong to different functional groups all of which have been grouped by chemical class to simplify the comparison of different samples. A representative aroma was common from each class. Caramelic notes are attributed to furans and pyrans group, burnt potato and woody odor for the pyrazine group, smoky aroma for phenols, fruity scent for aldehydes and ketones. The major groups that existed were hydrocarbons, alcohols, aldehydes and ketones, acid anhydrides, furans and pyrans, esters, phenols, pyrroles, thiazoles, pyridines, pyrazines, amines and other nitrogen heterocyclic compounds. Table 2 summarizes these compounds.

Table 2. Peak Area (from chromatogram) per Chemical Class of Aroma Compounds.

Chemical Class	Robusta						Excelsa					
	Civet			Non-Civet			Civet			Non-Civet		
	LR	MR	FR	LR	MR	FR	LR	MR	FR	LR	MR	FR
Hydrocarbons				0.93								
Alcohols	0.23			0.08								
Aldehydes	0.96	0.24	0.06	2.84	0.42	0.99	1.04	0.19	0.07	0.19 0.9		
Ketones	0.33	0.13	0.44	0.37	0.7	1.8	0.31	0.08	0.4	1.43 3.76		
Acids	0.16			2.36	0.98		0.18			1.12 0.71		
Ester	0.06			1.22	0.27		0.32		0.37	0.16 0.8		
Phenols	5.85	2.39	1.41	2.73		6.1	1.08		4.45	1.13	1.17	2.23
Furans and Pyrans	12.12	41.14	44.04	37.76	41.51	20.31	22.33	48.16	42.65	46.9	68.14	37.55
Pyrroles	1.28	0.48	1.44	1.77	1.04	2.03	1.33	0.4	0.48	0.73	1.2	3.62
Thiazoles	11.97			0.04			1.33		0.4			
Pyridines	5.89	0.13	5.51	3.86	0.83	3.38	1.79	1.51	5.96	0.44	1.34	10.7
Pyrazines	40.08	51.78	35.83	41.46	39.3	37.77	62.14	41.44	42.73	32.34	20.06	29.41
Amines and miscellaneous nitrogen compounds	0.25	0.06	0.38	0.36	0.47	1.4	0.31		21.1	0.41	0.06	
Other N heterocyclics				1.22	1.19	1.28	0.08			0.15		
Unidentified	21.7	2.77	5.99	18.37	5.83	24.8	9.65	5.37	2.63	16.75	2.97	10.03

Notable Aroma Constituents. From the above, previously-established notable compounds were picked for more focused differentiation of Civet and Non-Civet Coffee. Table 3 lists the notable aroma compounds common in both civet and non-civet coffee samples.

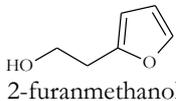
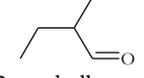
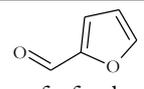
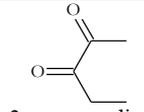
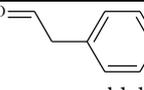
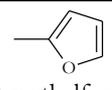
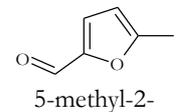
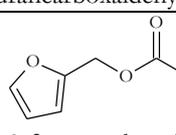
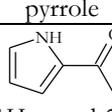
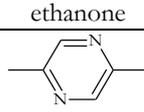
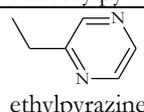
2-Furanmethanol. Having been identified as products of the thermal degradation of cysteine and xylose in tributyrin (Ledl and Severin, 1973), 2-furanmethanol was detected from the studies of Shelddon et al. (1986) in a heated cysteine glucose model system (Flament, 2002). This aroma compound has a known burnt and slightly caramellic, oily odor. For civet Robusta and Excelsa beans, the peak area of 2-furanmethanol increases with increasing roasting conditions. The non-civet counterpart on the other hand increases from light to medium roast but plummets down at full roast. Civet beans also exhibited a higher concentration of 2-furanmethanol as opposed to ordinary beans.

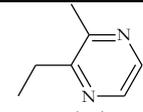
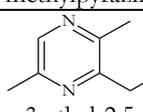
2-Methylbutanal. 2-methylbutanal is formed in the pyrolysis of isoleucine (Merritt, et al., 1963) by the enzyme polyphenol oxidase (Sheldon, et al., 1986) and is also generated

through Maillard reaction (Silwar and Lullmann, 1993). Having a buttery chocolate-like aroma, 2-methyl butanal decreases in concentration with an increase in roasting. In all of the samples, the said aroma compound displayed consistent behavior.

Furfural. Usually formed from the oxidation of furfuryl alcohol, furfural can also be formed by the decomposition of pentosans through the dehydration of the furanose form of arabinose (Smith, 1963). This is a feature of lightly roasted coffee to which it imparts a flavor like that of roasted cereals. Studies of Mottram (1994) have shown that furfural can also be formed from the Amadori compound of a pentose and an intermediated 3-deoxyosone (Motoda, 1979). This aroma constituent has been identified in the products of thermal degradation of cysteine and xylose in tributyrin (Ledl and Severin, 1973) and in a heated cysteine/glucose model system (Sheldon, et al., 1986). Based from the data generated from the analysis, the percent peak area of furfural increases from light roast to medium roast and suddenly plummets down at full roast. This trend has been explained by Hughes and Smith (1998) that the furfural

Table 3. Notable Aroma Compounds with Retention Times (mins.) and Identifying Fragment Ions (m/z).

Peak No.	t _R	Compound	Fragment Ions
22	10.21	 2-furanmethanol	98(B), 81, 97
17	2.35	 2-methylbutanal	57(B), 58, 86
33	7.92	 furfural	96(B), 95, 67
18	2.84	 2,3-pentanedione	57(B), 100
57	20.41	 benzeneacetaldehyde	91(B), 92, 65
5	1.69	 2-methylfuran	82(B), 53, 81
52	16.33	 5-methyl-2-furancarboxaldehyde	110(B), 109, 53
53	17.91	 2-furanmethanol acetate	81(B), 52, 98
35	4.30	 pyrrole	67(B)
92	20.16	 1-(1H-pyrrol-2-yl)- ethanone	94(B), 109, 66
23	13.60	 2,5-dimethylpyrazine	108(B), 81
24	13.75	 ethylpyrazine	107(B), 108
32	3.66	 pyrazine	80(B), 53

Peak No.	t _R	Compound	Fragment Ions
55	18.20	 2-ethyl-3-methylpyrazine	121(B), 122, 67
94	22.56	 3-ethyl-2,5-dimethylpyrazine	135(B), 136, 56
21	3.92	 pyridine	79(B), 52, 51

content in coffee was high in the early stages of roasting, and then fell rapidly as the extent of roasting increased. Furfural decomposes at higher temp based from study of Silwar and Lullmann (1993). Moreover, furfural has a honey, sweet and almond like aroma.

2,3-Pentanedione. Studies of Heyns, et al. (1966) identified 2,3-pentanedione as a primary volatile product formed by thermal degradation of furaneol. It can also be produced when heating glucose. This aroma compound possesses a sweet caramel like odor. It gradually decreases in concentration as the roasting process lengthens.

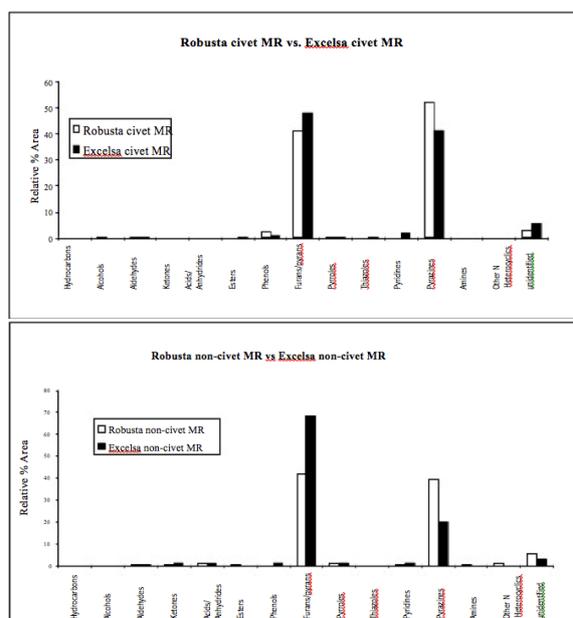


Figure 3. Comparison between Robusta and Excelsa at Medium Roast for Civet and Non-civet Coffee.

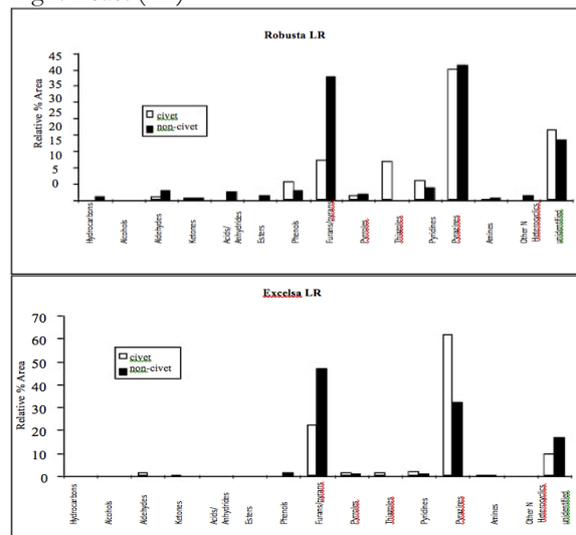
Benzeneacetaldehyde. Benzeneacetaldehyde only existed in the Robusta beans having a floral, pungent green odor. It is formed from phenylalanine by polyphenol oxidase (Michigan Department of Environmental Quality Water Division, 2004).

In the comparison of Robusta against Excelsa samples under similar roasting conditions, the chromatograms showed a consistent trend that pyrazines, phenols and pyridines are higher for Robusta beans for both civet and non-civet at all roasts. Figure 3 shows, as an example, the comparison at Medium Roast.

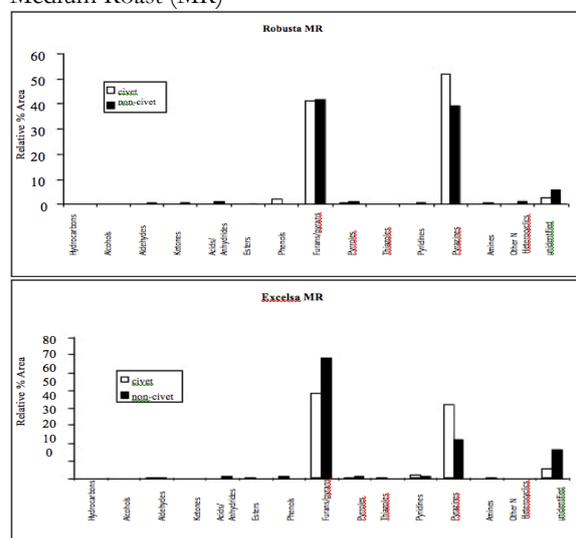
Excelsa variety on the other hand contains more furans and pyrans constituent while the presence of aldehydes and ketones are relatively similar for both Robusta and Excelsa beans. Moreover, Robusta in general have more aroma constituents than Excelsa beans. However instances like the presence of additional constituents like methoxypyrazines are known suppressants of odorous compounds found in Robusta beans. This being the case, more aroma constituents detected in the sample does not necessarily guarantee a better and fuller aroma.

In the analysis regarding varying roasting conditions (Figure 4) a consistent trend for aldehyde behavior was revealed. Its peak area increases from light to medium roast but drastically decreases in full roast. The aldehydes are suspected to break down and decompose under intense heat resulting to its drastic decline in its peak area value. Moving on with the pyrrole group, the trend goes as medium roast being the lowest peak followed by the light and full roast for Robusta civet and non-civet and Excelsa civet varieties. For furans and pyrans, the peak intensity increases proportionately with roasting for both Excelsa and Robusta civet varieties. The non-civet Robusta showed a reverse trend as furans and pyrans decrease in concentration as we increase roasting magnitude. The ketone group showed consistency for both civet samples as it is least available for medium roast followed by light and full roast. The non-civet kind has an increasing peak area for ketones as we increase roasting. However,

Light Roast (LR)



Medium Roast (MR)



Full Roast (FR)

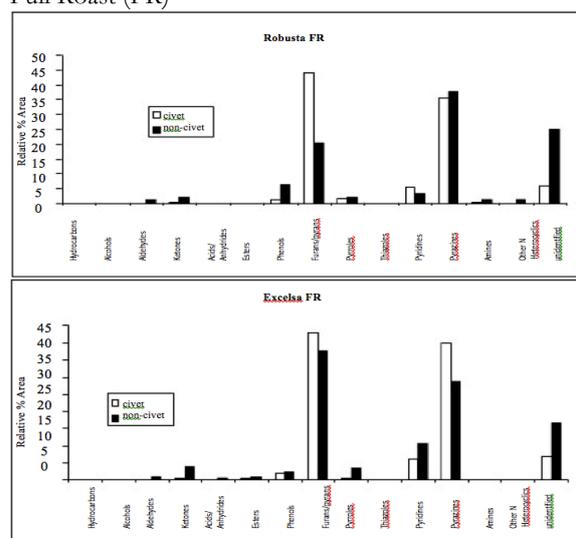


Figure 4. Relative Percentage Peak Areas of Aroma Compounds in from Robusta and Excelsa at Varying Extents of Roasting.

major aroma constituent family pyrazines showed no trending behavior throughout the twelve samples.

Microbial Results. Heterotrophic Plate Count. Of the 7 samples analyzed, 4 samples (Excelsa civet LR, Excelsa civet MR, Excelsa civet FR and Robusta civet LR) gave a numerical value of average colony forming units per mL while the remaining three samples (Robusta civet MR, Robusta civet FR and Commercial sample) gave very few colonies in a span of 24 hours incubation at 37 °C. Colonies formed beyond 24 hours were not counted as this study is specific only on heterotrophic bacteria which are viable and naturally occurring bacteria within 24 hours. TFTC are reported whenever countable colonies are less than 30. Based from the results in Table 4, a 61% and an 80% decrease of average CFUs/mL was observed as the time of exposure to high temperature of coffee beans through roasting was increased from 2 to 4 minutes then to 9 minutes. A decreasing trend in heterotrophic microorganisms was observed as the degree of roast was increased. Prolonged exposure of beans to 240 °C roasting temperature was effective enough to kill significant number of microorganisms present in the sample. This trend was also observed in Robusta civet samples since Robusta civet MR and Robusta civet FR yielded less than 30 countable colonies (TFTC).

Table 4. Heterotrophic Plate Count Results.

Sample	Roasting Condition	Average CFUs/mL
Excelsa Civet	LR	LR3.43E+05
Excelsa Civet	MR	MR1.32E+05
Excelsa Civet	FR	FR2.62E+04
Robusta Civet	LR	LR4.00E+05
Robusta Civet	MR	MRTFTC
Robusta Civet	FR	FRTFTC
Commercial Sample	not applicable	TFTC*

*TFTC: Too Few To Count (<30 colonies)

Reported values of CFUs/mL in Table 2 were all below the maximum microbial limits set by the Food and Drug Administration (FDA) of the Philippines, which is 1.0×10^6 (Bureau of Food and Drugs, 2007).

Multiple Tube Fermentation Technique. Reported MPN per 100 mL values of Robusta civet MR and Commercial sample in table 5 were all within the microbial limits set by FDA, which is 10 MPN per 100 mL,¹⁸ while Robusta civet LR did not pass the microbial limit for fecal coliform. This is due to the fact that light roasted coffee samples received the least exposure to heat. However, IMViC biochemical test of the three samples, which tested positive for fecal coliforms gave a negative result for the presence of *Escherichia coli* giving a result of + - + + instead of + + - - . The identity/ies of the microorganism cannot be plainly determined by using the results yielded by IMViC Biochemical Test which is specific only for the identification of *Escherichia coli*, *Enterobacter aerogenes* and *Klebsiella pneumoniae*.

Table 5. Most Probable Number of Fecal Coliform.

Sample	MPN per 100 mL	Test for Coliform	IMViC Result for the presence of <i>E. coli</i>
Excelsa Civet LR	0	-	-
Excelsa Civet MR	0	-	-
Excelsa Civet FR	0	-	-
Robusta Civet LR	150	+	-
Robusta Civet MR	4	+	-
Robusta Civet FR	0	-	-
Commercial Civet	4	+	-

Although CFUs/mL of Robusta civet LR are within the acceptable value, Robusta civet LR cannot be regarded as free from microbial contamination. Only the samples which passed the CFUs/mL and MPN/100 mL standards set by FDA can be regarded as safe for consumption. The high MPN value per 100 mL and CFUs/mL of Robusta civet LR may indicate the presence of other enteropathogenic microorganism, other than *Escherichia coli*. It must be also noted that the presence of coffee constituents such as

phenols, volatile, non-volatile compounds, etc. possess antimicrobial properties, adding more reason to the low to non-existent microbial presence in roasted coffee.

SUMMARY AND CONCLUSION

By comparing the GC-MS profiles of coffee under three different parameters namely 1) extent of roasting, 2) bean variety/species, and 3) coffee type, a behavior of aroma constituents was established.

In summary (Figure 5), for the Robusta varieties, civet beans have lower aldehyde, furan and pyran content, although it drastically increases as the extent of roasting is intensified; while to opposite trend occurs for phenols. It is therefore conclusive that civet Robusta beans become more aromatic and odorous in full roast while non-civet beans are more superior at low roasting conditions. As for the Excelsa species, aldehyde and ketone content in civet beans are lower than the civet counterpart. However, similar to Robusta, furan and pyran content increases for civet, accompanied by a decrease for non-civet, as the extent of roasting intensifies. This is however exceptional for the non-civet medium roasts.

For the microbial analysis, among 7 samples tested for fecal coliform, none tested positive for microbial contamination.

REFERENCES

American Society for Microbiology Microbe Library. Laboratory Protocols [Internet]. [Place unknown]: American Society of Microbiology; [cited 18 Jul 2011]. Available from: <http://www.microbelibrary.org>.

Arya M, Rao LJ. An Impression of Coffee Carbohydrates. *Crit Rev Food Sci Nutr*. 2007; 47:51-67.

Bureau of Food and Drugs. Philippine National Standard [Internet]. [Place unknown]: [Publisher unknown]; 2007 [cited 10 Jul 2011]. Available from:

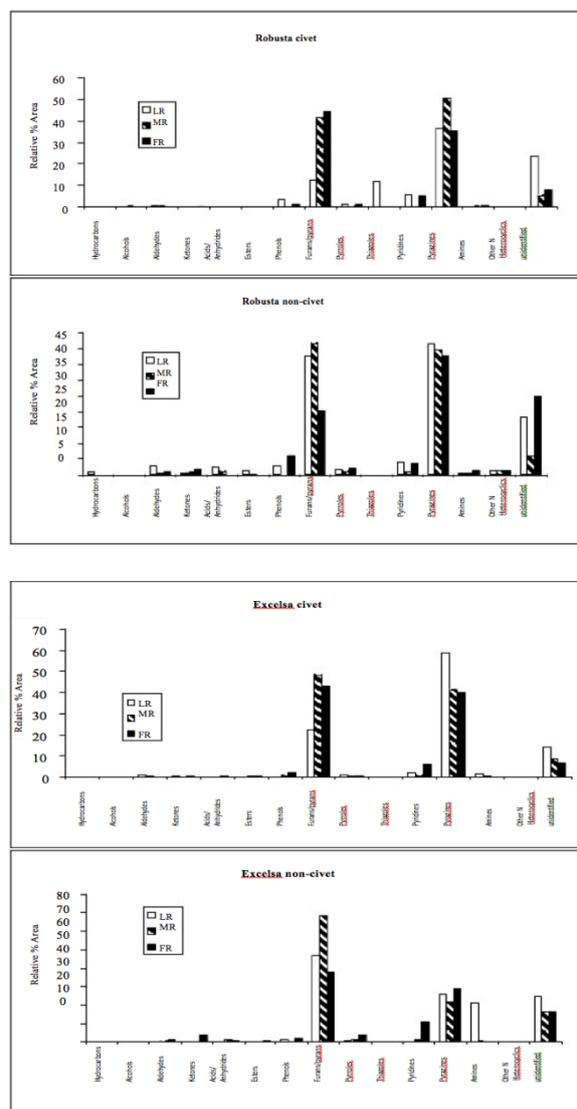


Figure 5. Summary of Comparisons between Civet and Non-civet Robusta and Excelsa Aroma Profiles at Varying Extents of Roasting.

http://www.bfad.gov.ph/cms/pdf/PNS/Re_PNS-BFAD-17-2007.pdf.

Environmental Protection Agency Test Methods for Solid Waste Hazards. SW-846 [Internet]. [Place unknown]: US Environmental Protection Agency; 1980 [cited 18 Jul 2011]. Available from: <http://www.epa.gov/solidwaste/hazard/testmethods/sw846/pdfs/9131.pdf>

Flament I. Coffee Flavor Chemistry. New York, USA: John Wiley and Sons; 2002.

Goldman E, Green, LH. Practical Handbook of Microbiology. 2nd ed. CRC Press; 2009.

- Heyns K, Stute R, Scharmann H. Massenspektrometrische Untersuchungen. XII. Die Massenspektre von Furanen. *Tetrahedron*. 1966; 22:2223-2235.
- Ledl F, Severin T. Thermische Zersetzung von Cystein und Xylose in Tributyrin. *Chem Mikrobiol Technol Lebensm*. 1973; 2:155-160.
- Merritt C, Bazinet M, Sullivan J, Robertson D. Mass spectrometric determination of the volatile components from ground coffee. *J Agric Food Chem*. 1963; 11:152-155.
- Michigan Department of Environmental Quality Water Division. Environmental Health Fact Sheet: Coliform Bacteria and Drinking Water [Internet]. [Place unknown]: [Publisher unknown]; 2004 Oct [cited 20 Jul 2011]. Available from: http://www.ewashtenaw.org/government/departments/environmental_health/wells_septic/well_septic_pdf/eh_coliformfactsheet.pdf.
- Motoda D. Formation of aldehydes from amino acids by polyphenol oxidase. *J Ferment Technol*. 1979; 57:395-399.
- Ryan D, Shellie R, Tranchida P, Casilli A, Mondello L, Marriott P. Analysis of roasted coffee bean volatiles by using comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry. *J Chromatogr A*. 2004; 1054:57-65
- Sheldon SA, Russel GF, Shibamoto T. Photochemical and thermal activation of model Maillard reaction systems. In: Fujimaki M, Namiki M, Kato H, editors. *Amino-Carbonyl Reactions in Food and Biological Systems. Proceedings of the 3rd International Symposium on the Maillard Reactions*. New York: Elsevier; 1986. p.145-154.
- Silwar R, Lullmann C. Investigation of aroma formation in robusta coffee during roasting. *Café, Cacao, The*. 1993; 37:145-151.
- Smith R. The determination of caffeine in coffee and in coffee mixtures. 1st Int Coll Chem Coffee (Paris, 20-22.5.1963). *Café, Cacao, The*, 1963; 3:223-230.