Comparative Physicochemical Analyses of Regular and Civet Coffee

Stephanie Chan[1], Emmanuel Garcia[2]
Chemistry Department, De La Salle University Manila
2401 Taft Avenue, 1004Manila, Philippines
[1]stephanie.c.chan@dlsu.edu.ph
[2]emmanuel.garcia@dlsu.edu.ph

Regular and Civet coffee beans of the *Coffea robusta* variety were analysed for α-tocopherol and caffeine contents by HPLC, surface microstructure by SEM, minerals by EDX. Probably due to absorption, the α-tocopherol content of the civet coffee beans was lower compared to the regular robusta coffee beans. Heating damages the cell membrane and vacuoles, causing an increased release of α-tocopherol and caffeine. Interestingly, calculations showed that roasting produced a more pronounced increase in α-tocopherol content in regular robusta than in the civet counterpart. Meanwhile, the caffeine content increase of the Civet coffee beans may be attributed to the possible formation of amino acids which are precursors of caffeine biosynthesis. SEM revealed that civet coffee beans acquired surface micro-pitting due to the action of digestive enzymes. The roasted regular robusta and civet coffee beans showed a smoother surface due to the fusion of cellulose in the cell wall. The mineral content of the civet coffee beans were lower than that of regular robusta which may have been an effect of absorption by the civet.

Keywords *– Civet coffee, Robusta coffee, caffeine, surface microstructure, α-tocopherol, minerals*

1. INTRODUCTION

Coffee has become a regular drink to everyone - making it one of the most consumed drinks in the whole world. Coffee beans are obtained from the fruit of coffee plants grown in over 80 countries around the world. The Philippines is very fortunate to have the abundance of four varieties of coffee - Arabica, Robusta, Liberica and Excelsa. A few varieties of coffee have made their own mark in the said industry, such as the Jamaican Blue Mountain, Tanzanian peaberry and Civet coffee. Each of these offers its very own distinct flavor making all of them in demand in the coffee market (Juan, P.U. & Francisco, M.R.S., 2007).

Civet coffee, also known as Kopi Luwak in Indonesia and as Kape Alamid in the Philippines, is claimed to be the rarest and most expensive coffee beverage in the world. It was first discovered in the Indonesian islands Java, Sumatra and Sulawesi. For years, it has already existed in some parts of the Philippines, but it is only until recently that people learned and gave much notice about this wonder. Civet coffee boasts of its unique method of production in which the coffee beans are partially processed in the digestive system of the civet (*Paradoxurus hermaphrodites*). This animal is a skilled tree climber which lives in the wild and seeks out to eat only the best and ripest coffee cherries. The consumed coffee cherries are internally fermented by various digestive enzymes and are excreted through their feces. This whole process of internal fermentation is the reason behind the coffee's distinctive flavor. Coffee enthusiasts aspire to get a taste of this drink's smooth, syrupy, and chocolaty flavor (Marcone, 2004).

The rise in popularity of and demand for civet coffee entails a need for additional information concerning the authenticity of the civet coffee available in the Philippines. So far, not much has been scientifically written about it.

2. METHODOLOGY

2.1. Sample Preparation of Robusta beans

The Robusta coffee cherries were dry-processed. Half of the coffee samples were kept in a closed container and the other half were placed in an aluminum foil. The hot plate was pre-heated to 200^{o}C before the beans were roasted. The roasting process lasted for 18 minutes with constant mixing giving a medium roast. After roasting, the coffee beans were all later kept in a polyethylene container prior to analysis. Civet and Robusta beans were obtained from Tagaytay, Philippines.

2.2. α-tocopherol Analysis

The **roasted** coffee beans were ground and approximately 5.0g of coffee were placed in a filter paper making a thimble for the extraction. The simplest oil extraction method was used for the determination of α-tocopherol content. A Soxhlet extraction setup was assembled for the extraction of coffee oil. The coffee in the thimble was extracted using 60ml of n-hexane as the solvent at 69°C for eight hours, siphoning six times per hour. The solvent was evaporated using a vacuum rotary evaporator and the residue was dried at 105^{o}C to obtain the coffee oil. The coffee oils were kept in the refrigerator prior to analysis (Gonzalez, Pablos, Martin, Leon-Camacho &Valdenebro, 2001).

Stock standard solutions of α-tocopherol were prepared using a 400IU commercially available sample (MYRA-E) in n-hexane. Working standard solutions with concentrations of 0.1, 1, 5, 10 and 20ppm were prepared from the stock solution by dilution in n-hexane. Ten mg of the coffee oil extracted was dissolved in 10ml n-hexane. The solution was then filtered through a disposable filter unit. A 20μl aliquot sample from this solution was injected into the chromatograph. The amount of the α-tocopherol in the coffee samples was obtained from the calibration graphs. Five trials were performed.

2.3. Caffeine Analysis

The coffee beans were ground and approximately 0.6g was transferred to a clean beaker. The beaker was then added with 50mL of boiling distilled H_2O and the mixture was stirred. Using a pipet, 5mL was transferred into a 25mL volumetric flask and diluted to the mark with 50:50 MeOH-H_2O.

A series of caffeine standards with concentrations of 15, 25, 50, 75ppm were prepared in 100mL volumetric flasks using 50:50 MeOH-H_2O.

The standards were filtered through a syringe filter. A 20μl aliquot of the sample starting with the least concentrated was injected to the instrument for analysis. From the calibration graphs, the caffeine contents were obtained. Three trials were performed. Quantitative analysis of α-tocopherol and caffeine were performed using an Agilent Series 1200 model HPLC system equipped with Supelco PLC-18 column. In the isocratic mode, the mobile phase for α-tocopherol was n-hexane/propan-2-ol (99:1, v/v) and 50% aqueous methanol for caffeine. The flow rate was 1.0ml/min. The detector was set at 295nm for α-tocopherol and 280nm for caffeine.

2.4. Microstructure Examination and Elemental Analysis

The coffee beans were attached directly to the carbon tape for the microstructure examination while for the elemental analysis, the coffee beans were ground. A small amount of each ground sample was attached to the carbon tape settled in an aluminum stub. The samples were subjected to gold coating for 15 seconds. The samples were then analyzed using the SEM/EDX JEOL JSM-5310 scanning microscope combined with Oxford Link Isis.

3. RESULTS AND DISCUSSION

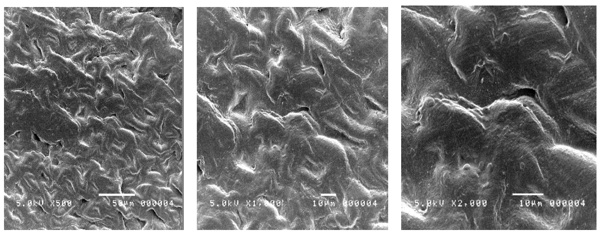
The content of α-tocopherol in coffee oils was found to increase when the coffee beans were subjected to roasting. The average quantity of α-tocopherol calculated for unroasted and roasted regular robusta beans were 0.419mg/kg and 1.320mg/kg coffee oil, respectively; while the unroasted and roasted civet coffee contained 0.328mg/kg and 0.349mg/kg coffee oil, respectively. This suggests that heating increases the α-tocopherol content of both the regular robusta and civet coffee beans. One of the many effects of heating on the bean cellular structure is damage to the cell membrane where the α-tocopherols are located, triggering the release of α-tocopherols. Increases in the α-tocopherol content of coffee beans are very essential as it enhances its antioxidant activity. When comparing the effect of how the robusta beans were processed inside the digestive system of the civet, it suggests a 27.8% decrease in the α-tocopherol content. The unroasted regular robusta beans with 0.419mg/kg coffee oil were found to have a higher α-tocopherol content than the unroasted civet coffee with 0.328mg/kg coffee oil. This may be due to the absorption with the aid of bile salts by the civet.

Similar trends were observed concerning the effect of roasting on the caffeine content of the coffee beans. The unroasted regular robusta coffee with a caffeine content of 39.978mg/kg coffee increased by 13.4% to 44.922mg/kg coffee when it was roasted. Akin to regular robusta beans, the content of caffeine in the unroasted civet coffee amounting to 41.772mg/kg coffee increased by 14.0% after undergoing the roasting process to 47.599mg/kg coffee of caffeine. These findings show that heating increases the caffeine content of the regular robusta and civet coffee. The increase of the caffeine content may be due to damage of the cell membrane as well as the vacuoles where caffeine is usually located, caused by the heating process. This damage is an effective way of increasing the release of caffeine. In evaluating the consequence of processing the coffee cherries inside the civet's body system, it can be asserted that the caffeine content of the civet coffee beans is higher than the regular robusta beans (Ashihara, Monteiro, Gillies & Crozier, 1996). The unroasted regular robusta and civet coffee beans contained 40.0mg/kg and 41.8mg/kg coffee of caffeine, respectively. It shows a slight increase (4.49%) in the caffeine content of the control beans after having passed through the civet's digestive system. Caffeine is not only accumulated in the diet but is recognized to be biosynthesized from purine nucleotides.

Table 1.
Caffeine and α-tocopherol content in the unroasted and roasted regular robusta and civet coffee

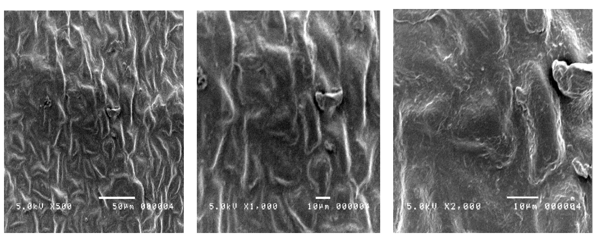
	Unroasted Robusta (mg/kg)	Roasted Robusta (mg/kg)	Unroasted Civet (mg/kg)	Roasted Civet (mg/kg)
α-tocopherol	0.419	1.320	0.328	0.349
Caffeine	39.978	44.922	41.772	47.599

The SEM images at x2000 magnification showed that roasted regular robusta beans had smoother surfaces than the unroasted regular robusta beans (Figures 1 and 2). Heating causes the melting of cellulose which results to a bound and smoother surface (Pittia, Rosa, & Lerici, 2001).. At x500 magnification (Figure 4), the examination of the unroasted regular robusta beans showed a smoother surface than the unroasted civet coffee beans. The structure of the unroasted regular robusta beans at x500 magnification illustrates a raw bean that is compact with a few small pores. At x1000 magnification, the unroasted civet coffee beans exhibits surface micro-pitting (see Figures 1 and 3). This was illustrated by the several dark regions that can be seen representing cavities or pores. This suggests that different enzymes and acidic substances were reacting with the beans' surface.



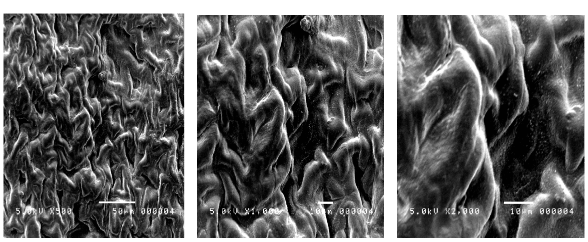
(a) (b) (c)

Figure 1. **Scanning electron micrographs of unroasted regular robusta coffee beans at (a) x500 magnification (b) x1000 magnification (c) x2000 magnification**



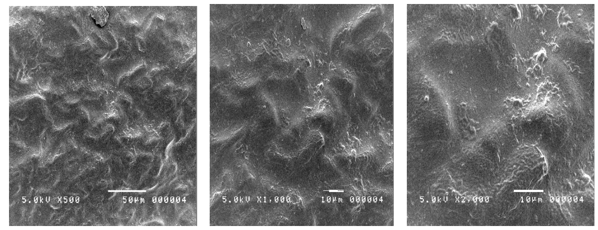
(d) (e) (f)

Figure 2. **Scanning electron micrographs of roasted regular robusta coffee beans at (d) x500 magnification (e) x1000 magnification (f) x2000 magnification**



(A) (B) (C)

Figure 3. **Scanning electron micrographs of unroasted civet coffee beans at (A) x500 magnification (B) x1000 magnification (C) x2000 magnification**



(D) (E) (F)

Figure 4. **Scanning electron micrographs of roasted civet coffee beans at (D) x500 magnification (E) x1000 magnification (F) x2000 magnification**

With the exception of bromine and copper, the mineral content of coffee generally decreased most probably as a result of absorption while in the digestive tract of the civet. A reason for the increased amount of copper and bromine is currently unknown.

Table 2.
Elemental analysis of different coffee samples

	Unroasted regular robusta	Roasted regular robusta	Unroasted civet	Roasted civet
C	43.24%	58.90%	64.02%	64.86%
O	44.77%	30.72%	31.59%	25.89%
Na	-	0.74%	-	0.66%
Mg	1.02%	0.72%	-	0.44%
K	5.76%	1.87%	0.75%	2.67%
Ca	0.85%	0.34%	0.18%	0.44%
Fe	-	0.69%	-	0.14%
Zn	1.24%	1.40%	-	0.18%
Br	1.73%	2.52%	3.37%	3.10%
Cu	1.39%	2.11%	0.09%	1.62%

4. CONCLUSIONS

This study successfully illustrated the major differences between regular robusta and civet coffee beans in terms of their α-tocopherol, caffeine and mineral contents, and structural properties. The α-tocopherol content of the civet coffee is less than the amount present in regular robusta beans because it has been absorbed by the civet's digestive system. However, after the regular robusta and civet coffee beans were roasted, the level of α-tocopherol increased because heating causes increased release of α-tocopherol. For the caffeine content, the results revealed that civet coffee has higher caffeine content than regular robusta beans. A similar trend for both roasted and unroasted regular robusta, and civet coffee were reported. Scanning electron microscopy revealed that at x1000 magnification, the civet coffee beans exhibit surface micro-pitting caused by the digestive enzymes, whereas, the entire roasted beans' surface was found to be smoother than their unroasted counterpart. Except for a notable increase in bromine and copper, elemental analysis showed that the minerals in civet coffee beans were lower than the regular robusta beans.

ACKNOWLEDGEMENTS

We are thankful to Ms. Ailine Dimapilis for her assistance during the sampling process. We would also like to express our deepest appreciation to Mr. Michael Ajero, Mr. Irving Chiong, and Mr. Rey Coralde for their technical assistance in the instrumental laboratory.

REFERENCES

Marcone, M.F. (2004). Composition and properties of Indonesian palm civet coffee (Kopi Luwak) and Ethiopian civet coffee. *Food Research International*, 37(9), 901-912.

Kemsley, E.K., Ruault, S., & Wilson, R.H. (1995). Discrimination between coffea arabica and coffea canephora variant robusta beans using infrared spectroscopy. *Food Chemistry*, *54*(3), 321-326.

Juan, P.U. & Francisco, M.R.S. (2007). *An introduction to coffee.* Pasig, Philippines: Anvil Publishing Inc.

Gonzalez, A.G., Pablos, F., Martin, M.J., Leon-Camacho, M., Valdenebro, M.S. (2001). HPLC analysis of tocopherols and triglycerides in coffee and their use as authentication parameters. *Food Chemistry*, 73, 93-101.

Pittia, P., Rosa, M.D., Lerici, C.R. (2001). Textural Changes of Coffee Beans as Affected by Roasting Conditions. *Lebensm-Wiss Food, Science and Technology*, 34, 168-175.