ผลของกระบวนการผลิตต่อคุณภาพของกาแฟเขียว

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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ปีการศึกษา 2560

EFFECTS OF PROCESSING METHODS ON QUALITY OF GREEN COFFEE

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A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Food Technology
Department of Food Technology
Faculty of Science
Chulalongkorn University
Academic Year 2017

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ฟารียา กุลพิจิตร : ผลของกระบวนการผลิตต่อคุณภาพของกาแฟเขียว (EFFECTS OF PROCESSING METHODS ON QUALITY OF GREEN COFFEE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร.ชาลีดา บรมพิชัยชาติกุล, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ. ดร.อินทาวุธ สรรพวรสถิตย์, ศ.ดีธ ริชาร์ด แคดวอลแลดเดอร์, 269 หน้า.

งานวิจัยนี้มีวัตถุประสงค์ในการศึกษาผลของกระบวนการผลิตต่อคุณภาพของกาแฟเขียวสายพันธุ์อะราบิก้า โดยการแบ่งงานวิจัยเป็น 2 ส่วน ส่วนที่ 1 ศึกษาผลของขั้นตอนกระบวนการจัดการหลังการเก็บเกี่ยวต่อคุณภาพของกาแฟ (postharvest processing) และส่วนที่ 2 ศึกษาผลของกระบวนการอบแห้งด้วยการปั๊มความร้อนต่อสมบัติทางเคมีกายภาพของกาแฟและวิเคราะห์ปริมาณสารระเหยให้กลิ่นที่มีความสำคัญต่อกลิ่นรสของกาแฟ (key odorants) โดยในการศึกษาวิจัยส่วนที่ 1 นี้ ทำการศึกษาถึง 1) ผลของการคัดเลือกระดับความสุกของผลกาแฟต่อคุณภาพของกาแฟเขียวด้วยการวิเคราะห์องค์ประกอบของสารระเหยให้กลิ่น และฤทธิ์การต้านอนุมูลอิสระ 2) การหาอัตลักษณ์ในคัดแยกกาแฟเขียวที่ผลิตกระบวนการต่างๆ คือกาแฟขี้ชะมด (civet coffee) กาแฟที่ผ่านการผลิตแบบเปียก (wet processed coffee) และกึ่งเปียก (semi-dry processed coffee) ด้วยการวิเคราะห์องค์ประกอบของสารระเหยให้กลิ่น, ฤทธิ์การต้านอนุมูลอิสระและการวิเคราะห์ตัวอย่างกาแฟด้วย E-tongue 3) การศึกษาถึงผลของความแตกต่างในกระบวนการผลิต กระบวนการผลิตขั้นตอนย่อย แหล่งผลิต (ละแวกพื้นที่ในการผลิตและประเทศที่ผลิต) และสายพันธ์ต่อองค์ประกอบของสารระเหยให้กลิ่นของกาแฟคั่ว 4) ผลของความแตกต่างในกระบวนการผลิต กระบวนการผลิตขั้นตอนย่อย แหล่งผลิต และสายพันธุ์ต่อคุณลักษณะทางประสาทสัมผัสโดยรวม (over-all difference test) ของกาแฟชงโดยใช้วิธีทดสอบคุณลักษณะทางประสาทสัมผัส pair-comparison test ซึ่งประมวลผลด้วยวิธี multi-dimensional scaling (MDS) ร่วมกับการวิเคราะห์ความแตกต่างของกาแฟชงด้วย E-tongue ผลการทดลองในการวิจัยส่วนที่ 1 พบว่า 1) ผลขององค์ประกอบของสารระเหยและฤทธิ์การต้านอนุมูลอิสระแบบ FRAP (FRAP assay) สามารถนำมาประยุกต์ใช้ในการคัดแยกคุณภาพของกาแฟเขียวตามระดับการสุกของผลกาแฟ โดยมีสารระเหยให้กลิ่นที่มีความจำเพาะในกาแฟเขียวที่ผลิตจากผลกาแฟสุก 17 ชนิด และที่ผลิตจากผลกาแฟที่ยังไม่สุก 5 ชนิด 2) พบสารระเหยให้กลิ่นจำนวน 12 ชนิดที่สามารถนำมาแบ่งแยกอัตลักษณ์ของกาแฟเขียวที่ผลิตจากขี้ชะมดกรรมวิธีแบบเปียกและกึ่งเปียก กาแฟเขียวที่ผลิตจากขี้ชะมดมีฤทธิ์การต้านอนุมูลอิสระแตกต่างจากกาแฟเขียวที่ผลิตด้วยกรรมวิธีแบบอื่นอย่างมีนัยสำคัญ ($p< 0.05$) โดยมีองค์ประกอบของฟีนอลิคทั้งหมด (total phenolic content/TPC) และ FRAP สูงสุดที่ 50.38 mgGAE/g coffee และ 80.67 mgTrolox/g coffee ตามลำดับ ในด้านผลของการวิเคราะห์ด้วย E-tongue พบว่า E-tongue มีประสิทธิภาพที่ดีในการแบ่งกลุ่มกาแฟเขียวด้วยฟังก์ชั่นการวิเคราะห์ PCA ด้วยพล็อตของแกน PC1 (94.5%) และแกน PC3 (1.6%) 3) พบสารระเหยให้กลิ่นจำนวน 20 ชนิดที่บ่งชี้ถึงความแตกต่างในกระบวนการผลิตขั้นตอนย่อย แหล่งผลิต และสายพันธุ์ โดย 2-furfurylthiol เป็น1 ในสารระเหยให้กลิ่นจำนวน 20 ชนิดนี้ที่มีความสำคัญต่อกลิ่นรสของกาแฟสูงที่สุดจากการพิจารณาถึงค่า odor activity value (OAV) ที่สูงสุดเมื่อเปรียบเทียบกับสารระเหยให้กลิ่นชนิดอื่น 4) ผลของการทดสอบความแตกต่างโดยรวมด้านของคุณลักษณะทางประสาทสัมผัสของกาแฟชงร่วมกับการวิเคราะห์ความแตกต่างด้วย E-tongue พบว่าผลจากการทดสอบด้วยการประมวลผลโดยใช้ MDS และ E-tongue สามารถระบุความแตกต่างในขั้นตอนย่อยของกระบวนการผลิตกาแฟ แหล่งที่มาและสายพันธุ์ของกาแฟได้ รวมถึงพบความสัมพันธ์ระหว่างผลการทดสอบทั้ง 2 ที่บ่งชี้ถึงความสัมพันธ์ระหว่าง E-tongue และประสาทการรับรู้ของมนุษย์ สำหรับการศึกษาวิจัยส่วนที่ 2 เป็นการศึกษาถึงผลของการใช้กระบวนการอบแห้งด้วยวิธีปั๊มความร้อนที่อุณหภูมิ 40, 45 และ50 ◦C ต่อคุณภาพของกาแฟที่ผลิตด้วยกรรมวิธีแบบเปียกซึ่งเป็นกระบวนการหลักในการผลิตกาแฟเปรียบเทียบกับกาแฟที่ใช้วิธีการอบแห้งแบบทั่วไป คือ การอบแห้งแบบถาด (tray drying) ที่ 50 ◦C และการตากแดด (sun drying) โดยการวิจัยส่วนที่ 2 นี้ทำการวิเคราะห์สมบัติทางเคมีกายภาพได้แก่ ปริมาณความชื้น วอเตอร์แอคติวิตี้ (a_w) ฤทธิ์การต้านอนุมูลอิสระ การวิเคราะห์ค่าสี การวิเคราะห์องค์ประกอบทางเคมีได้แก่ปริมาณน้ำตาล กรดอะมิโน กรดไขมัน กรดคลอโรจีนิก (chlorogenic acids) ของกาแฟเขียวที่ผ่านการอบแห้งทุกเงื่อนไข รวมถึงการทดสอบความแตกต่างด้านคุณลักษณะทางประสาทสัมผัสของสารระเหยให้กลิ่นในกาแฟชงที่ผลิตจากกาแฟที่ผ่านการอบแห้งทุกเงื่อนไขด้วยวิธี R-index by similar-ranking test และวิเคราะห์ความแตกต่างด้านองค์ประกอบของสารระเหยให้กลิ่นในกาแฟที่มีความสำคัญ จำนวน 40 ชนิด เพื่อการเปรียบเทียบคุณภาพของกาแฟที่ผ่านกระบวนการอบแห้งแบบปั๊มความร้อนกับกาแฟที่ผ่านการอบแห้งด้วยวิธีทั่วไป ผลการทดลองในการวิจัยส่วนที่ 2 นี้พบว่าในเชิงคุณสมบัติทางเคมีกายภาพการอบแห้งกาแฟด้วยวิธีการปั๊มความร้อนทุกเงื่อนไขให้ปริมาณสีที่มีความสว่างกว่าการอบแห้งแบบถาดและการตากแห้ง การอบแห้งด้วยกระบวนการปั๊มความร้อนที่อุณหภูมิ 50 ◦C สามารถอบแห้งกาแฟให้มีระดับความชื้นอยู่ในช่วงปกติ (<12% w.b) และมีค่า a_w ต่ำกว่า 0.6 ด้วยระยะเวลาภายใน 11 ช.ม. นอกจากนี้การอบแห้งกาแฟเงื่อนไขนี้ยังให้ปริมาณฟีนอลิคทั้งหมดและฤทธิ์การต้านอนุมูลอิสระแบบ FRAP สูงที่สุดเมื่อเทียบกับการอบแห้งกาแฟด้วยวิธีปั๊มความร้อนเงื่อนไขอื่น ที่ 53.19 mg GAE/g coffee bean และ 65.27mg Trolox/g coffee bean รวมถึงให้ปริมาณกรดคลอโรจีนิค 3,4-dicaffeoylquinic, 3,5-dicaffeoylquinic และ 4,5-dicaffeoylquinic ที่สัมพันธ์ถึงคุณภาพที่ดีของกาแฟสูงสุด โดยไม่พบความแตกต่างอย่างมีนัยสำคัญจากการวิเคราะห์ปริมาณน้ำตาล กรดอะมิโน และกรดไขมันในกาแฟทุกเงื่อนไขของการอบแห้ง สำหรับผลของการเปรียบเทียบความแตกต่างโดยรวมด้านคุณลักษณะทางประสาทสัมผัสพบว่ากาแฟที่ผ่านการอบแห้งด้วยกระบวนการปั๊มความร้อนที่อุณหภูมิ 50 ◦C ให้ค่า R-index แตกต่างจากตัวอย่างควบคุมน้อยที่สุดที่ 31.67 และกาแฟที่ผ่านกระบวนการอบแห้งแบบถาดให้ค่า R-index แตกต่างจากตัวอย่างควบคุมอย่างมีนัยสำคัญมากที่สุด (73.33) จากผลของค่า R-index ดังกล่าว ในขั้นตอนของการเปรียบเทียบคุณภาพของกาแฟในแง่องค์ประกอบของสารระเหยให้กลิ่นที่มีความสำคัญต่อกลิ่นรสของกาแฟ คณะผู้วิจัยจึงเลือกตัวอย่างกาแฟที่ผ่านการอบแห้งด้วยกระบวนการปั๊มความร้อนที่อุณหภูมิ 50 ◦C มาทำการวิเคราะห์องค์ประกอบของสารระเหยให้กลิ่นเปรียบเทียบกับกาแฟที่ผ่านกระบวนการอบแห้งแบบถาดและการตากแห้งโดยพบว่ากาแฟที่ผ่านกระบวนการอบแห้งแบบปั๊มความร้อนไม่มีความแตกต่างของปริมาณสารระเหยให้กลิ่นจากกาแฟที่ผ่านการตากแดด แต่พบความแตกต่างอย่างมีนัยสำคัญของสารระเหยให้กลิ่นที่มีความสำคัญต่อกลิ่นรสของกาแฟมากที่สุด ได้แก่ 2-furfurylthiol ระหว่างกาแฟที่ผ่านการอบแห้งด้วยการปั๊มความร้อนและการอบแห้งแบบถาด ($p< 0.05$)

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5672906423 : MAJOR FOOD TECHNOLOGY
KEYWORDS: GREEN COFFEE, POSTHARVEST PROCESSING, PHYSICOCHEMICAL PROPERTIES, SENSORY EVALUATION, VOLATILE COMPOUNDS, STABLE ISOTOPE DILUTION ASSAY

FAREEYA KULAPICHITR: EFFECTS OF PROCESSING METHODS ON QUALITY OF GREEN COFFEE. ADVISOR: ASST. PROF.CHALEEDA BOROMPICHAICHARTKUL, Ph.D., CO-ADVISOR: ASST. PROF.INTHAWOOT SUPPAVORASATIT, Ph.D., PROF.KEITH RICHARD CADWALLADER, Ph.D., 269 pp.

To study the impact of processing on quality of Arabica green coffee, the research was divided into two parts: I) the study of the effects of postharvest processing practices on the quality of coffee, and II) the study of the effects of heat pump drying conditions on physicochemical properties of coffee with focus on the determination of the key volatile compounds affected by drying treatments. In part 1, the subtopics of the study were: 1.1) the study of the effects of the selection of coffee cherries differing in maturity (ripe vs unripe) on the quality of green coffee based on volatile analysis and antioxidant activity; 1.2) the study of the effects of different processing methods (digestion by civet, wet and semi-dry processing) on the authenticity of green coffee quality based on volatile analysis, antioxidant activity and the application of an in-house electronic tongue (E-tongue) based on cyclic voltammetry and the principal component analysis (PCA); 1.3) the study of the effects of processing methods (processing with or without civet), subdividing processing (civet feeding conditions; caged vs free-range), the locations of processing (plantation area: Phahee, Doi-Chang, Doi-Tung and Loei), the countries of processing (Thailand, Indonesia and Vietnam), animal species (civet and weasel), and species (Robusta and Arabica) on volatile compounds of roasted coffee; 1.4) the study of the effects of processing methods, of subdividing processing, of the location of processing, of the country of processing, of animal species and of coffee species on overall difference in sensorial properties of coffee brew based on the overall difference method by pair comparison followed by sureness-rating tasks on a four-point scale. This experiment was divided into three sections and the evaluation was based on the difference between samples using E-tongue measurement. The results in the 1st part showed that, in terms of the relationship between the selection of coffee cherries and the quality of green coffee, the volatile compounds and antioxidant activity by FRAP assay were successful in the differentiation of ripe and unripe green coffee. With regard to volatile compounds, 17 vs 5 odorants were identified only in green beans from ripe and unripe berries, respectively. Total phenolic content and DPPH radical scavenging assay could not distinguish between both kinds of beans. The evaluation of the effects of different processing methods on the authenticity of green coffee revealed that there were 12 key volatiles that were successful in the discrimination of civet coffee from others. Total phenolic contents (TPC) and FRAP of civet green coffee (50.38 GAE/g coffee and 80.67 Trolox/g coffee) were significantly different (p<0.05) from the others. The rapid e-tongue also showed its potential to discriminate the profile of multi-processed green coffee into cluster group by the plot of the first principal component 1 (PC1) (94.5%) and the third principal component 3 (PC3) (1.6%). The following set of studies on the effects of processing methods on the quality of 8 roasted coffee samples (civet and without civet) based on volatile analysis indicated that 20 key odorants could be used to separate coffee according to the processing method chosen, subdivided processing, location and species. It was found that 2-furfurylthiol was the most characteristic impact compound with the highest odor activity value (OAV) determined in all coffee samples and it appeared to be successful in indicating the difference between roasted coffee samples from different species and having been subjected to different processing methods. The result of last section in part 1 of the study based on pair comparison/sensory test and the use of E-tongue measurement to evaluate the overall difference in 6 pair of coffee brew showed that there was a relationship between PCA separation via E-tongue and multidimensional scaling (MDS) solutions which revealed a strong correlation between e-tongue and human perception. Both were able to identify differences between civet and non-civet coffees, free-range and caged civet coffees, and civet and weasel coffees. The part 2 of the study, investigated the effects of the use of heat pump drying (40, 45, 50 ºC on quality of wet processed Arabica coffee in comparison with conventional drying process (tray drying at 50 ºC and sun drying), moisture content, water activity (a_w), antioxidant activity measurement, color determination, non-volatile compounds (sugars, amino acids, fatty acids, chlorogenic acid), volatile compounds (40 selected key odorants) and sensory evaluation of the aroma by nose using R-index by similar-ranking test were applied. It was found that all heat pump dried samples had brighter colors than tray dried and sun dried coffee. The application of heat pump drying at 50 ºC could dry coffee to the safe moisture content <12 % w.b. with a_w of less than 0.6 in shortest time (11 h). Heat pump dried coffee at 50 ºC had the highest total phenolic content and reducing antioxidant power of 53.19 mg GAE/g coffee and 65.27 mg Trolox/g coffee, respectively. it also had the highest content of chlorogenic acid isomers (3,4 and 4,5-dicaffeoylquinic acids) which indicated a good quality of coffee. There were no significant differences observed in sugar, free sulfur amino acids and fatty acids contents of all dried coffee. Heat pump dried coffee at 50 ˚C had a closest R-index value to a bench marked coffee (sun dried coffee) at 31.67. Thus, this condition of heat pump dried coffee was selected for further volatile analysis compared with tray dried and sun dried coffee. 2-furfurylthiol was the key odorant that significantly discriminated coffee produced from heat pump drying and the commercial tray drying (p-value <0.05). In term of the calibration in the method used for the determination of high-boiling point volatile, Furaneol® between liquid-liquid extraction and solvent-assisted flavor evaporation, there was no significantly difference observed in the Furaneol® concentration evaluated in these two methods (p> 0.05). Heat pump dried coffee (50 ºC) had the closest similar odor properties to a bench marked coffee (sun dried coffee) with a value at 31.67. Thus, coffee samples subjected to this heat pump drying treatment were selected for further volatile analysis to be compared with tray dried and sun dried coffee. The compound 2-furfurylthiol was the key odorant that significantly differentiated coffee subjected to heat pump drying and the commercial tray drying (p-value <0.05). In terms of the calibration of the method used for the determination of high-boiling point volatile, Furaneol®, between liquid-liquid extraction and solvent-assisted flavor evaporation, there was no significantly difference observed in the Furaneol® concentration evaluated by these two methods (p> 0.05).

Department: Food Technology
Field of Study: Food Technology
Academic Year: 2017

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ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my advisor, Asst. Prof. Dr. Chaleeda Borompichaichartkul, and my co-advisors, Asst. Prof. Dr. Inthawoot Suppavorasatit and Prof. Dr. Keith Cadwallder for their kindness and guidance, encouragement and support through all the years, I have studied both in America and in Thailand. I also would like to thank my examining committees, Assist Prof. Dr. Varapha Kongpensuk, Dr. Sirima Puangpraphant, Dr. Yaowapha Lorjaroenphon and Dr.George Srzednicki for their valuable suggestions which I really appreciate their kind help.

In addition, I would like to give an appreciation to Thailand Research Fund (TRF) Royal Golden Jubilee Ph.D. Program, under Grant No. PHD/0099/255 and the 90th Anniversary of Chulalongkorn University Fund [Ratchadapisek Sompoch Endowment Fund; GCUGR1125594017D] for the supporting funds to do research while I was studying Ph.D as well as a cooperation in coffee research from Dr. Mingchih Fang, Taiwan Food and Drug Administration.

I would like to thank all friends, at the Department of Food Technology, Chulalongkorn University and my friends at University of Illinois at Urbana-Champaign; Bethany Hausch, Sasinee Kanyaboon, Yusuf Chebao, Yun Yin, Fengjia Chen, Chelsea Ickes, Tiendan Wu, Surasit Poonsirikiat, Tisorn Songsermsawas, Pallavi Shukla, Maywee Springfield, Sarochin Santiwarangkul and others for their help and encouragement.

I dedicate this Thesis to my beloved family: Pramint Kulapichitr, Assoc Prof. Dr. Udomluck Kulapichitr and Farin Kulapichitr, I would like to have a special thank to them for their love, encouragement, and emotional support. Without them, I would not have been able to achieve this degree.

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CHAPTER I

INTRODUCTION

1.1 Background

Coffee has been reported as the second most traded global commodity after petroleum. Between 2014-2015, the total coffee production was rise to 143 million bags. Total world coffee consumption was estimated at more than 149 million bags in 2014 with a 2.3% average annual growth rate since 2011 (ICO, 2015). Arabica coffee (*Coffea arabica*) is main coffee species that dominates the world market (70-80% market share), while Robusta coffee (*Coffea canephora*) shares the rest (Bertrand *et al.*, 2003; Farah, 2012). These two species are processed differently, which could make them different in characteristics. Arabica is considered to be more superior than Robusta due to its sensory properties (Bertrand *et al.*, 2003).

Selection of location and coffee cultivars for production, the choice of processing methods and controlling the processing steps activity with separation of ripe/unripe coffee cherries vary depending on the geographic location and climate (Lee *et al.*, 2015; Sanz-Uribe *et al.*, 2017; Sunarharum *et al.*, 2014). The processing specialties are an important factor determining the quality of coffee.

Dry processing and wet (wash) processing are two main techniques to produce green coffee, while semi-dry (semi-wash) process has been reported as an additional combination method to achieve characteristics of both dry and wet processing. The

main difference between these methods is the pulping operation as well as the fermentation and washing process (Clarke & Macrae, 1985; Teixeira *et al.*, 2005).

In addition to above mentioned processes, there is a special method to produce Arabica coffee via animal digestive fermentation (digestive bioprocessing) by Luwak (*Paradoxurus hermaphrodites*) or civet cats. This coffee type is commonly processed using the best ripened red coffee cherries that are selected by special senses of civet cats. After the cherries are eaten, the pulps are digested and the beans are fermented in the digestive system of the cat. The civet cat feces are covering the parchment then sun dry after the completion of digestive process, to reach the same moisture content as coffee processed from method mentioned above and stored in the closed package before the production of green beans. Civet coffee is known by its high price in the green and roasted coffee market because of the claim on its desirable flavour properties, so the authentication and quality determination of civet coffee are seriously considered by consumers and producers (Ali *et al.*, 2012; Jumhawan *et al.*, 2015; Marcone, 2004; Ongo *et al.*, 2012). Additionally, civet coffee has gained the popularity in the production in many countries like Indonesia and Vietnam as well as Thailand. The production of this coffee differs in specific way of each country (for example; feeding methods for civet cat and species of animal used).

As details of green coffee processing have been mentioned above, to date, there are points of research in quality control of coffee that need for further studies. Sorting of green coffee are based on visual parameters and crop yield. Therefore, quality markers of green coffee beans are required in aim to ensure that green beans are from only fully ripe cherries. However, those quality markers are verified mainly in wet processed coffee and less research study in the identification of them in semi-dry

processed coffee. Consider civet coffee processing, a robust protocol for discrimination of civet coffee from regular processed green coffee is needed since the present methods to differentiate civet coffee from regular coffee are only available for the separation of roasted civet coffee (Jumhawan *et al.*, 2015; Marcone, 2004; Ongo *et al.*, 2012). The developed method will reduce potential frauds, such as illegal blending of cheaper coffee into civet coffee. In addition, for character and sensory perception of roasted and brewed coffee, large variation from feeding methods for civet cat, species of animal used, chosen of coffee species, location of processing, origin of processing and relationship between each factor could alter these quality attributes which more studies are essential.

It is very interesting how difference in sub-dividing processing methods of the processing methods themselves affect the quality of coffee since less research study in these effects on quality attribute of coffee.

Aroma, sensory properties and antioxidant activities are qualitative markers of coffee which can be influenced by coffee processing method both green and roasted coffee.

The determination and discrimination of these quality attributes by determination of volatile compounds, antioxidant activity, sensorial properties as well as the use of artificial neural network tools; electronic tongue (E-tongue) have been found successful to be applied as tools to gain a better understanding of difference between coffee characteristics.

Therefore, the application of these methods is very challenging and should be useful in the study of various factors that are likely to affect the quality of coffee.

Between them are cultivars, geographic location of the crop, early postharvest and processing methods. The findings of the study will lead to better quality control and assurance of authenticity of coffee.

Among, the processing operation, drying is a thermal process that is likely to affect the physicochemical properties of green coffee beans and subsequently the crop quality. This applies particularly to mechanical drying at high temperature (Borem *et al.*, 2008; Coradi *et al.*, 2007; Dong *et al.*, 2017). Sun drying is a common practice for coffee drying. There are some drawbacks of sun-drying such as long drying time, cost of labour and requirement of large area. Despite, the drawbacks, sun drying is usually preferred to mechanical drying due to its more desirable sensorial properties enhanced by higher total and reducing sugar contents due to the pre-germination of the embryo of coffee seed during drying

Considering in the role of coffee drying for the enhancement and control of coffee quality, there was still a lack of comprehensive studies on reduction of these drawbacks in coffee post-harvest processing. Due to the benefits consisting of heat-pump drying, being a closed drying system operating at low-temperature, with low energy consumption, and it worthwhile to investigate its potential for coffee drying. To our knowledge, there are no publish studies on the effect of heat pump drying on quality of Arabica coffee. Since, key odorants responsible for the aroma of coffee and the physicochemical properties of coffee can be influenced by the drying methods, it appears worthwhile to study how heat-pump drying can affect these parameters.

Therefore, this study, was divided into two parts aiming at investigating the impact of postharvest processing on quality of coffee as below;

I) Study of the effects of different post-harvest processing methods on quality attribute of coffee.

II) Study of the effect of different heat pump drying treatments on physicochemical properties of Arabica coffee with focus on the key volatile compounds.

1.2 Objectives

To study the impact of overall postharvest processing on quality of green coffee

1.3 Scope of the research

The list of research scope is provided as below and the overall summary in scope of this research is given in Figure 1.

1.3.1 Effects of postharvest processing practices on quality of coffee

1.3.1.1 Maturity of coffee cherries (ripe vs unripe)

1.3.1.2 Processing methods vs the authenticity of green coffee quality

1.3.1.3 Role of processing methods, subdividing processing, locations of processing, countries of processing, animal species involved in coffee fermentation and cultivars in development of volatile compounds of roasted coffee

1.3.1.4 Role of processing methods, subdividing processing, locations of processing, countries of processing, animal species involved in coffee fermentation and cultivars in development of sensorial properties of brew coffee

1.3.2 Effect of heat pump drying on physicochemical properties of Arabica coffee

1.4 Expected outcomes

- To gain information that could separate the differences between ripe and unripe green coffee beans by volatile compounds and antioxidant activity in order to control the selection process of coffee berries.
- To gain information on the effects of processing methods on quality of green and roasted coffee
- To gain information on the application of robust techniques (volatiles analysis antioxidant activity coupled with E-tongue as reliable rapid methods for assessing the authenticity of green civet and regular coffee.
- To gain a better understanding of consumer' s perception of overall differences between coffee that is processed by different methods including civet and non-civet for improvement of postharvest processing of coffee.
- To obtain better knowledge of the relationship between E-tongue and human perception aiming at further development of robust E-tongue
- To determine the optimum condition of heat- pump drying based on physicochemical and sensorial properties including information on the change of key odorants in coffee.

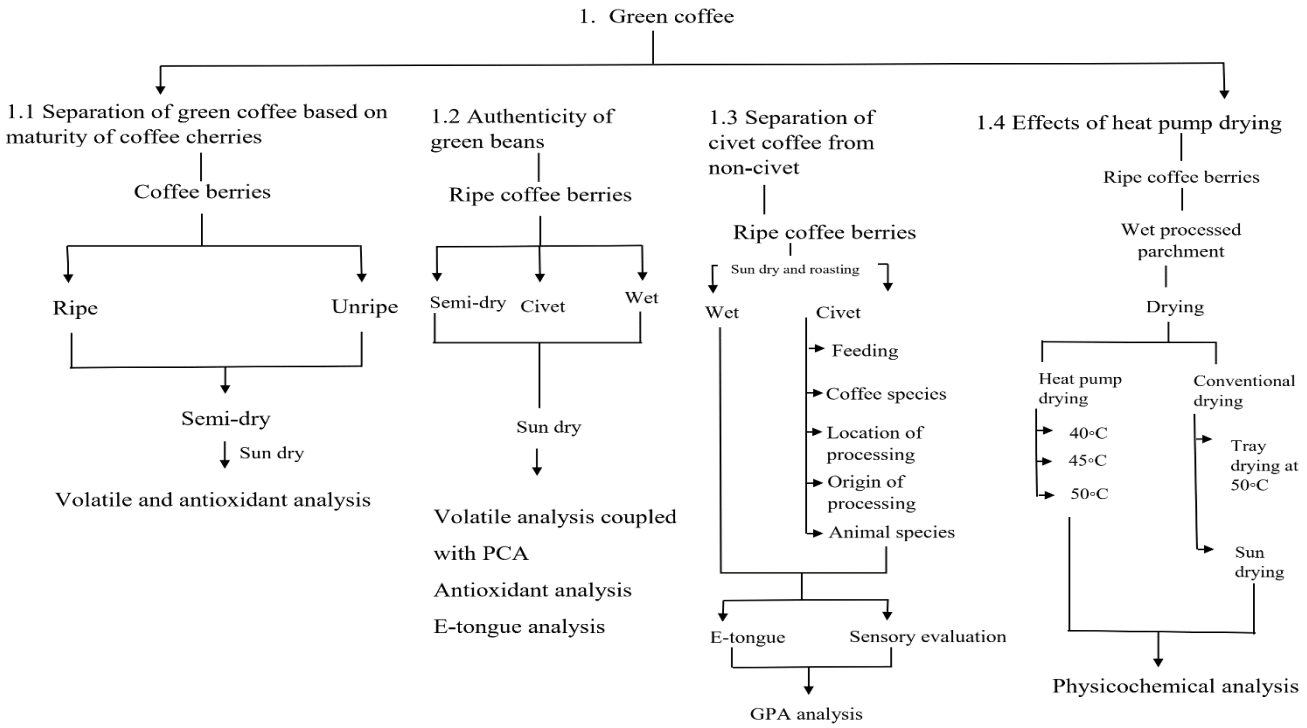


Figure 1 Overall scope of research

CHAPTER II

LITERATURE REVIEW

2.1 Coffee

2.1.1 General information on coffee

Coffee beans are the seeds located in the fruit flesh and are obtained after the removal of pericarp (skin), mesocarp (mucilage), endocarp (parchment) and spermoderm (silver skin), see Figure 2. The seeds may be raw or roasted, whole or ground. Beverages prepared from such seeds are also called “coffee” (Belitz *et al.*, 2009).

Coffee tree is a shrub that can grow from 3-12 m in height depending on the species. The shrubs are generally trimmed to keep their height within 2-2.5 m to be easily harvesting. The evergreen shrubs have leathery short-stemmed leaves and the white flowers commonly release the mild jasmine-like scent. The seed of coffee, cherry-like berries is the product used for produce green coffee beans which usually reach their maturity with a diameter of about 1.5 cm.

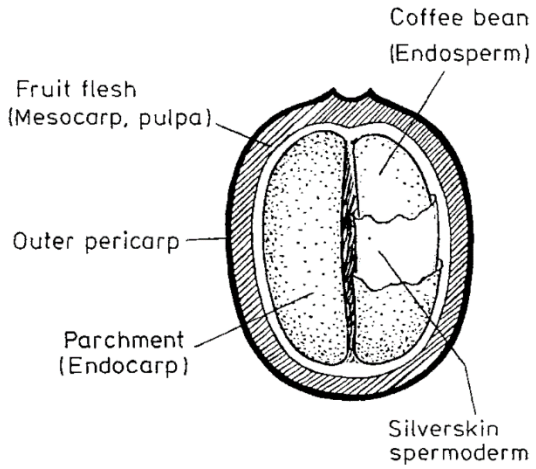


Figure 2 Longitudinal section of a coffee fruit

Source: (Belitz *et al.*, 2009)

Coffee fruit has a green outer skin which will develop to red-violet or deep red color when reaching its maturity within each stage. This outer skin encloses the mesocarp part of coffee which consists of the pulp and the seed called coffee bean (Figure 2).

Two elliptical hemispheres with flattened adjacent sides are surrounded of coffee fruit flesh. A yellowish transparent spermoderm called silverskin, covers each hemisphere. The strong fibrous endocarp called parchment covers the hemispheres and also separates them from each other.

Two species of *Coffea* genus are dominating the coffee cultivation in the world. They are *Coffea arabica* (Arabica coffee) and *Coffea canephora* (Robusta)

respectively. Arabica coffee is generally preferred and produced more in coffee industry than Robusta coffee due to its superior aroma and taste.

Coffee growing areas in Thailand are predominantly producing Robusta in the south and Arabica in the north. Upper Northern Province is a mountainous area with the area of 85,920 km^2 approximately which borders Myanmar to the north and west and Laos to the north and east.

Coffee is grown at altitude between 700 and 1400 meters with the average annual temperature between 18 – 28 ^oC in Thailand, and an annual rainfall of 1,200 - 1,500 mm/year. Because of production at high altitude, they are mostly categorized as watersheds or conservation areas. Many Arabica coffee planting areas are under the coffee promotion program of the Royal Project Foundation (Noppakoonwong *et al.*, 2013). The white coffee flowers with a jasmine like scent commonly bloom 3-4 years after planting and then they are ready to harvest after 6 years of full growth. Coffee fruits can be obtained from the shrub for 40 years of cultivation and provide the maximum yield after growing 10-15 years. The ripening of fruit (called coffee cherries) starts within 8-12 months after the flowers begin to bloom. Considering the cultivation of coffee over the history, only 3 of the 70 species are plant commercially grown.

2.1.2 Green coffee processing

2.1.2.1 Harvesting of coffee

Harvesting of Arabica coffee in northern of Thailand starts from October/November of each year when the coffee fruits develop their full maturity. The berries ripening process begins from the lower level to higher level of coffee plantation above sea level (Angkasith, 2001). Coffee shrub that grows under the shade ripe slower

than the one grows without shading. Cherries in the same branch of coffee tree will not ripe in the same time. It is interesting in this point that lack of equality in the ripening process may influence the diversity of chemical components of green coffee (Sanz-Uribe *et al.*, 2017).

There are two main ways to harvest coffee which are done by hand picking or mechanically picking (Sanz-Uribe *et al.*, 2017). Manual hand picking is generally used in Thai coffee post-harvest processing, laborers will be trained by the coffee company to pick the individual full ripe berry with red color from each shrub and avoid defects like unripe and over-ripe berries. Once the ripe berries have been harvested, it will automatically provoke the unripe to the ripe. To preserve quality of green beans, ripe coffee cherries are usually processed immediately to avoid chemical and/or biological damage which will leading to loss of flavor and flavor defects as shown in the results of previous study in Table 1.

Table 1. Influence of fruit maturity on the brew (State of Sao Paulo): in 1959, the cherries were riper than in 1958, which resulted in a better quality brew

	Harvest 1958		Harvest 1959	
	Average points	Quality of the brew	Average points	Quality of the brew
Mild brew model	7.1	Apparently mild	8.7	Mild
Depulped cherries	8.2	Mild/acid	7.3	Apparently mild
Non-depulped cherries	6.0	Apparently mild	7.3	Apparently mild
Fruit dried on the tree	3.4	Very hard	4.7	Hard
Fruit harvest green	3.0	Very hard, immature	4.4	Very hard
Rioy brew model	1.6	Rioy*	1.1	Rioy
Fruit picked off the ground	0.3	Rioy*	0.5	Rioy

(Source: Modified from Clarke & Macrae, 1985). *Taste being a mixture of iodine, alcohol and acid.

In addition, uncontrolled processing conditions of improper selected of coffee cherries or bad practices could cause medicinal, earthy, musty, moldy, and hidy (tobacco, leather-like) off-flavor (Lingle, 1986) as well as generate defective green coffee beans (Jackels *et al.*, 2014; Smrke *et al.*, 2015). Therefore, after harvested, coffee cherries continue processing within 24 hours to avoid the physicochemical damage when storing in jute-bag before green beans production and a protocol to control the good quality of products to ensure the correctness of maturity of sample should be developed.

2.1.2.2 Postharvest processing methods of coffee and their influence on quality of coffee

The quality and flavor of a coffee brew are mainly affected by genetics, variety, climate, soil, geographic origin, cultivation practices, and especially process methods.

Post-harvesting processing is the needed step to transform coffee cherries to the product, green beans after harvesting. The basic diagram of green coffee processing shown in Figure 3.

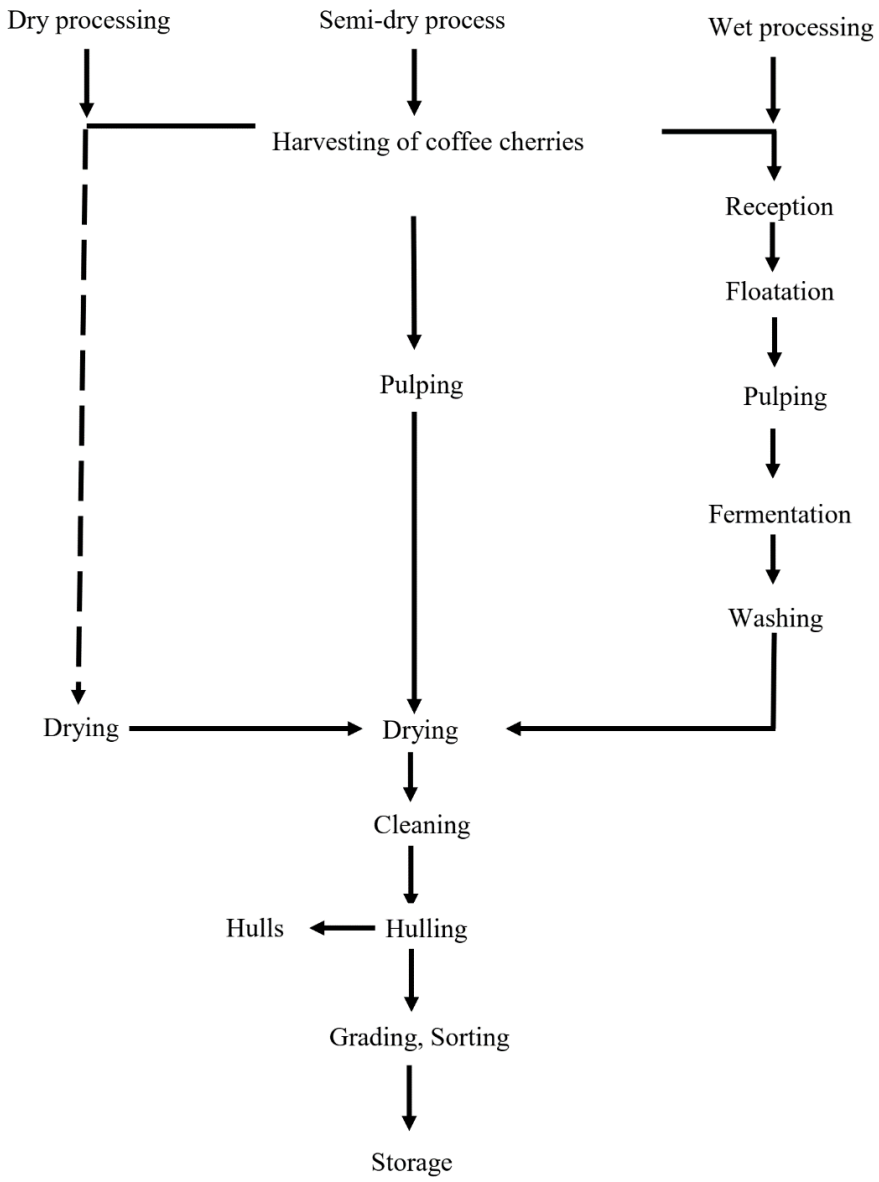


Figure 3 Diagram of overall post-harvest processing of coffee

(Modified from Clarke & Macrae, 1985)

There are few common methods that can be chosen to produce coffee as shown in Figure 3. These processes can modify or increase the final quality of coffee beans and change the organoleptic properties of coffee beverages. (Flament, 2002; Sanz-Uribe *et al.*, 2017). In general, two basic ways (wet and dry methods) are commonly chosen for achieving the stability of coffee products and there are also combinations of general method used as follows;

- **Dry (natural) processing**

Dry processing is employed for drying whole fruits without pulping and elimination of mucilage layer. To begin the process, coffee cherries, sometimes are dried after the harvesting and quality check with/without floatation and with fruit flesh and mucilage layer still intact on the beans until dehulling process takes place followed by polishing after the coffee fruits are dried 15-20 days to obtain the final moisture content of around 10-12% (Clarke & Macrae, 1985; Lee *et al.*, 2015).

Coffee beverages prepared by dry method have characteristics consisting of heavy body are sweet, smooth, and with complex attributes (Lee *et al.*, 2015; Sunarharum *et al.*, 2014).

Robusta coffee is generally produced by this method both in southern Thailand and other countries like Brazil, the world main coffee producer for 90%, Ethiopia and Yemen (Clark & Macrae, 1985). This method is also used when water is not available to process coffee via wet method. In northern Thailand, to produce Arabica coffee, this process, it usually used for small over-ripe cherries at nearly the end of

harvesting periods to maintain coffee taste. In this method, not many factors can possibly influence the bean quality except the control of coffee cherries maturity, and drying practices (Sanz-Uribe *et al.*, 2017). It is highly convenient to use this method where there is not enough available labour to ensure that only ripe fruits are harvested.

- **Wet processing**

Wet processing method is the main process used to produce Arabica coffee due to the superior quality of coffee taste over dry method. It is always used for preparing mild coffee, particularly in Central and south America (Clarke & Macrae, 1985).

Many steps combined in this method can make the difference and change the coffee flavor. To process coffee by washing method, red coffee berries are collected at the reception and passed the quality check by floatation in water tank to select sink coffee berries (higher density than water) that tend to provide best maturities then the fruits are floating. The flesh is removed during pulping process using the machine to obtain coffee parchment which is coated with mucilage. Natural fermentation is performed 12 to 24 hours in order to get rid of coated mucilage layers followed by washing. Wet parchment is dried in the sunlight to obtain the final moisture content at 12% w.b. and stored before the dehulling process to obtain green beans. Pulping and mucilage removal vary between producers and countries which can significantly influence the quality of green beans and coffee quality as a

result of the fermentation step (Clarke & Macrae, 1985; Sanz-Uribe *et al.*, 2017).

- **Semi-dry method (Pulped natural)**

Semi-dry method or pulped natural is an additional combined process of the wet and dry methods (Sanz-Uribe *et al.*, 2017; Sunarharum *et al.*, 2014). In this type of process, the removal of mucilage is not or only partially included.

To obtain the coffee parchments, coffee cherries are pulped and the seeds are dried while surrounded by the mucilage, with the limitation of mucilage removal (Teixeira *et al.*, 1995). The beans are then dried under sun light with the same final moisture content with wet and dry process before further steps to produce the green beans. This process provides coffee with special sweet flavor, closer to wet process via the combination of 2 methods.

This method results in a significant reduction of the quantity of water used in the process. So, the choice of post-harvesting process chosen is a factor needed to be considered in order to achieve a suitable quality of coffee. The driving parameters that influence the distinct flavor of coffee in each process are coffee mass homogeneity, water quantity, oxygen (aerobic or anaerobic) and microorganisms involved (Penuela-Martinez *et al.*, 2013; Schwan & Graham, 2014). By controlling these parameters, the aromatic diversity can be enhanced the price of quality coffee increased.

Fermentation is a naturally occurring spontaneous process caused by microorganisms growing in the environment. Variety, initial condition of the beans, climate and fruit maturity play the major role to fermentation due to their influence on the timing of microorganism activity and substrate formation. Unique to coffee flavor the changes in fermentation happen via biochemical reaction of microbes while degrading different substrates mainly carbohydrates and lead to increase in temperature and decrease in pH (from ca. 6.5 to 4.1) (Avallone *et al.*, 2001; Penuela-Martınez *et al.*, 2010; Velmourougane, 2013)

The change of microbial population while fermentation process occurs can also affect the flavor production. Growth of bacteria is predominant at the beginning following with multiple species of yeast during the middle of fermentation and eventually, the development of filamentous fungi that grow under aerobic environment (Avallone *et al.*, 2001; Evangelista *et al.*, 2015; Silva *et al.*, 2008). Considering the wet method, the degradation of the mucilage adhered to the coffee parchment naturally occurs through the enzymatic reaction of natural microorganisms which usually produce pectinases during fermentation (Avallone *et al.*, 2002; Masoud & Kaltoft, 2006).

Fermentation also occurs in semidry and dry processes via the decrease of coffee moisture (Evangelista et al., 2013) but somehow contrasts with wet method due to the use of water, time and availability of O2 in the wet method (Velmourougane, 2013).

Every step in fermentation, their interactions and changes need to be considered since they contribute to the diversity of coffee composition and the

formation of important precursors involved in aroma and taste formation which affect coffee sensorial properties.

Nowadays, the industrial perspective, the trend in process variation is to deliberately change the flavor of coffee. Today's consumers are interested to discover new flavors and aromas. In terms of coffee drinks, they should be no longer a beverage produced from exotic origins, or the traditional processes.

In addition to above mentioned processes, there is a special method to produce coffee via animal digestive fermentation (digestive bioprocessing) from Luwak (*Paradoxurus hermaphrodites*) or civet cats. The coffee from civet's fermentation process, called civet coffee, has been reported as the world's most expensive coffee (U.S. $150-227/lb) due to its exotic and unexpected production process (Ongo *et al.*, 2012).

Civet coffee grew in popularity just before the 1950s, when coffee plantations expanded heavily during the reign of the Dutch in the East Indies.But at that time it only happened to be known among gourmet coffee enthusiasts in the 1980s. In 2010, it made the headlines in Australia after the President of Indonesia, Susilo Bambang Yudhoyono, gave Luwak coffee to the Australian Prime Minister, Kevin Rudd, during his visit to Australia (Sanz-Uribe *et al.*, 2017).

This coffee type is generally processed using the best ripened red coffee cherries that are selected by special senses of civet cats. After the cherries are eaten, the pulps begin to digest and fermentation occurs within the digestive system of the cat. Civet cat feces are washed as soon as the digestive process is

completed. The civet cat feces covering parchment are usually then dried to reach the final moisture content 12% w.b. and are stored in the closed package before the production of green beans. Desirable flavors of civet coffee that are hypothetically produced during transient fermentation inside the civet's gut make the coffee beans are commonly aromatic, less acidic and less bitterness (Yusianto *et al.*, 2012). Because of its high price in the green coffee market, authentication and quality determination of civet coffee are seriously considered by consumers and producers.

The present effective and reliable methods to differentiate civet coffee from regular coffee are based upon the determination of sensory perception, visual examination, and metabolomics approach in roasted civet coffee (Jumhawan *et al.*, 2013; Marcone, 2004; Ongo *et al.*, 2012). These methods are available for the roasted coffee only. In contrast, the discrimination of green civet coffee from the regular ones is also important in coffee trading. However, there is no report about the methods for 'green' coffee beans discrimination of civet from non-civet ones.

Malic and citric acid content are used as chemical fingerprint to trace and identify real Luwak coffee from mixed or fake beans (Jumhawan *et al.*, 2013). Civet coffee well processed are considered as kosher following Indonesian Ulema Council, Majelis Ulama Indonesia, through fatwa No. 4, dated July 20, 2010 (Sanz-Uripe *et al.*, 2017).

Hence, a robust protocol for discrimination of civet coffee from coffee produced by different methods is needed. The developed method can reduce

potential frauds, such as illegal blending of cheaper coffee into civet coffee. In addition, due to the distribution of roasted civet coffee to many countries that usually have their own specific processing, the authentication method of civet coffee will be useful.

It would be interesting to know how civet coffee could be distinguished the conventionally processed coffees within the variability of the processing method and locations.

To date, only study of Cheong *et al.* (2013) compared the difference in aroma profile, antioxidant activities and sensory profile of one roasted civet coffee with regular coffee processed from different area. There were no new studies exploring the relationship between cultivar, processing location and methods used that affect the quality of civet and non-civet coffee. Hence, this topic is very challenging to observe for the new elucidation of information.

2.1.2.3 Coffee drying methods

Drying is one of the important steps in post-harvest processing that affects the quality of Arabica coffee. This step needs to be monitored carefully as lack of good drying practice can be detrimented to the overall quality of coffee in the further steps of green bean processing. Because of the high moisture content of washed coffee, 52.7-53.5% (Puerta Quintero, 2005) its chemical composition, and humid weather conditions in coffee plantation are favourable for the growth of microorganisms, mainly molds and yeasts. The contamination by microbes usually comes from the field, trees (total coliforms), contact with the picker hands, the facilities of the coffee mill and from the water used for washing (Archila, 1985).

Hence, an appropriate moisture content at 10-12% is required for food safety proposes. The common water activity is 0.65-0.68, at which microorganisms growth and metabolic activity are reduced. The minimum water activity for the growth of Aspergillus ochraceus varies from 0.77 to 0.83 and for producing ochratoxin A (OTA) is from 0.83 to 0.87 (Urbano *et al.*, 2001).

There are two coffee drying techniques that are commonly used for coffee drying including (1) hot air drying (40-60 ^{o}C) (2) natural sun drying (5-7 days of drying). The final moisture content of coffee bean is usually reduced down to 11-12% (w.b.) to prevent microbial contamination during storage of parchment coffee before exporting, roasting and brewing.

In general, the production of Ocharatoxin A (OTA) cannot be eliminated by high temperature during roasting.

Sun drying is a common practice for coffee drying. It is usually carried out by spreading the coffee on clean dry ground, trays, a solid concrete surface and on bamboo mesh (Clarke & Macrae, 1985). The berries must be spread out on a drying surface in a thin layer only 30-40 mm thick, especially at the start of the drying period to prevent growth of microorganisms. Frequent raking is allowed to help the distribution of heat and uniformity of drying in order to prevent the discoloration of coffee parchment. The length of sun drying mainly relies on the weather conditions including the psychrometric characteristics of the ambient air. It also depends upon the size of the berries, their degree of ripeness and initial moisture content. This method consumes less energy and is less expensive. However, it consists of many drawbacks including

long drying, cost of labour, requirement of large area and the lack of moisture content uniformity due to the unpredictable weather.

In contrast to that, mechanical drying, requires small machines with a static capacity or multiple small compartments which can complete the drying in shorter time. However, it is important to ensure that the product be not exposed to excessive temperature, and that the final moisture content be homogeneous to avoid the re-drying of coffee. The speed of drying is undeniable controlled by the limited range of drying temperature. Fluctuation of high temperature during drying can generate defects to green coffee beans as fragile brittle crystallized beans are easily found when the initial moisture content of the beans high (Wintgens, 2004). Discolored, pale beans are the common defective beans, caused by inappropriate drying. Over-dried coffee will become brittle and produces too many broken beans during hulling. They are considered to be defective beans. Coffee that is not dried sufficiently will be too moist and prone to rapid deterioration caused by the attack of fungi and bacteria (Hicks, 2002).

To dry Arabica coffee, the range of temperature usually recommends not to exceed 45 ˚C, since higher temperatures cause the deterioration to the beans, mainly through enzymatic and chemical reactions. Robusta, or coffee processed by dry method, on the contrary, requires a high initial air temperature (85-95 ˚C) and a final temperature of 50-60 ˚C because it does not require pulping and the initial moisture content is high. In the terms of the effect of drying on the physicochemical properties, there were studies claiming about the negative effects of high temperature to the final cup quality of coffee (Borem *et al.*, 2008; Coradi *et al.*, 2007; Saath *et al.*, 2010).

The study of Coradi *et al.* (2007) reported on the influence of drying on physicochemical characteristics and cup quality of coffee by using two-fixed bed techniques of mechanical dryer under hot air at 40 ˚C and 60 ˚C, compared with the natural sun-drying. It was found that high temperature to 60 ˚C had effect on the deterioration of coffee quality by the degeneration of cellular membranes that leaded to the significant increase of potassium leaching value, the electrical conductivity, total titrable acidity and the reduction of reducing sugar.

The possible effect on the flavor precursors also relates to the lower sensory scores in the sensory analysis in this study as the sensory score of wet processed coffee was reduced to 68.1 when compare with the control (74.3).

The degeneration of the cellular membranes from the high temperature causes the leak of the lipid compounds from the beans. The damage to coffee beans cell membrane system correlates to extravasations of fatty acids present in the cell interior (Taveira *et al.*, 2015). This leads to the occurrence of oxidative or catalytic reactions and the forming of undesirable by-products which are harmful to the sensorial properties of the beverage as well as lead to the increasing of electrical conductivity from the bean exudates.

Strange flavors of wood and oil were frequently found in the coffee dried at 60 ◦C (Taveira *et al.*, 2015). Study of Taveira *et al.* (2015) found that modification of drying techniques by combination of natural drying with heated air at 60/40 ˚C was more effective. It gave the better sensorial score (79.05) when compare with the basic hot air-drying at 60 ˚C (77.64). Comparison of flavor observed in two drying techniques found that long period of sun drying leads to pre-germination of coffee that results in a

more favorable taste from the embryo within the seed than the use of mechanical drying (Borem *et al.*, 2014). From the abovementioned coffee drying techniques, it is a challenge to develop the new drying technique that overcome the drawback of common methods and maintain the quality of coffee. At present, this point still need more research to apply the knowhow in order to enhance the coffee drying practice.

2.1.2.4 Heat pump drying

Heat pumps (HPs) has been reported as energy-efficient process for drying operations because of their potential in heat recovery and relatively high energy utilization efficiencies (Tong *et al.*, 2010). In these drying systems, the sensible and latent heat of the evaporated moisture in the dryer can be recovered and recycled back to the dryer by re-heating the dehumidified air. This system also has benefit in ability to control the drying gas temperature and humidity (Colak & Hepbasli, 2009a, 2009b), see Figure 4 and 5. The composition of the heat pump dryer with conventional hot-air dryer is shown in Table 2.

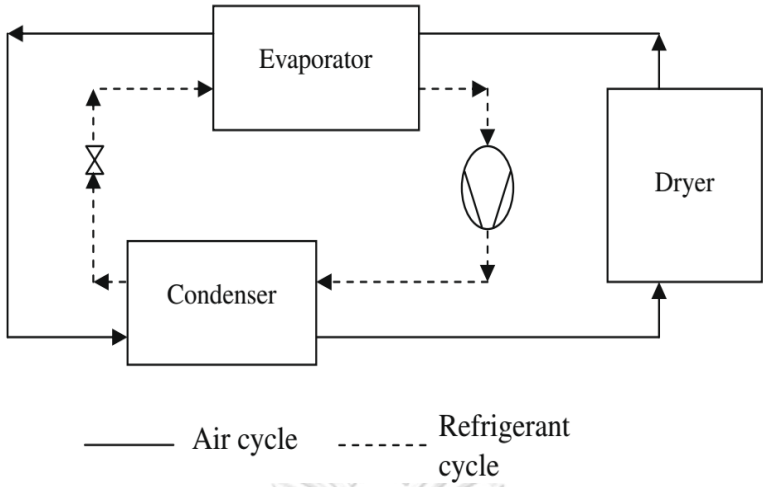


Figure 4 A schematic illustration of a heat pump drying system
Source: (Colak & Hepbasli, 2009a)

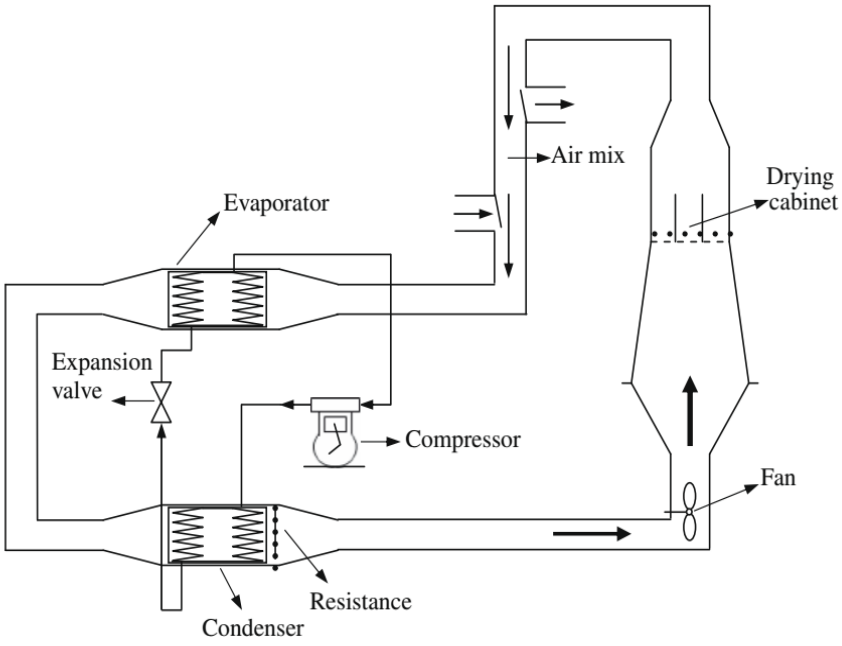


Figure 5 A Closed cycle heat-pump dryer

Source: (Colak & Hepbasli, 2009a)

During the past few decades, heat pump drying systems and their food applications have been very popular due to their low operating cost (Zielinska *et al.*, 2013).

Table 2 General comparison of heat pump dryers with conventional hot-air

Parameters	Hot-air drying	Heat pump drying
SMER (kg H_2O/kwh)	0.12-1.28	0.05-4.0
Drying efficiency (%)	35-40	>100
Operating temperature range (°C)	40-90	-20-65
Operating % RH range	Variable	10/65
Capital cost	Low	Moderate
Running cost	High	Low

(Reproduced from Soylemez (2006))

Many studies on this drying technique of fruits and vegetables have shown that heat pump drying is an effective option to preserve quality of many food products resulting to the retaining of antioxidant compounds, color and taste like ginger, macadamia nut, apple, pear and mango due to low-temperature used and the design of closed-system (Borompichaichartkul, 2013; Hawlader *et al.*, 2006; Hwa Chong *et al.*, 2013). Heat pump drying was found to significantly retain the polyphenol content from 44% to 73% of dried cocoa beans compared to other drying methods ($p < 0.05$) in the study of Hii *et al.* (2012). This technique also helps to maintain the stability of color in apple, pear and mango. It was found that change of total color (ΔE) in sample dried

with continuous heat pump drying was lower than of samples dried by other combined methods (Hwa Chong *et al.*, 2013).

Borompichaichartkul *et al.* (2013) reported that the undesirable lipid oxidation and the energy consumption were successfully reduced in macadamia nut dried by modified atmosphere heat pump drying used two-stage drying which the first stage was the drying under nitrogen 40 ˚C followed by 60 ºC under air in the second stage.

Due to the benefits deriving from heat-pump drying namely a closed drying system with low-temperature, a less energy consumption, this method appears to be useful for coffee drying and overcome the problems of common coffee drying practices. Dong *et al.* (2017) had recently studied the effects of heat pump drying at 50 ˚C coupled with other drying techniques on the caffeine, trigonelline, fat, protein, amino acid, fatty acid composition and the volatile profile of green coffee beans. The authors identified the success in producing a higher number of aroma components and a lighter color (L^*, a^* and b^*) of green coffee. However, to-date, research on the optimization of heat pump drying temperature to maximize the quality attributes of green coffee and also further study on the sensory properties of the heat pump dried coffee still needed to be done.

2.1.2.5 Quality management of green coffee before distribution

After drying, coffee beans are usually stored as parchment coffee with the endocarp remaining around the beans for up to two years with control of safe moisture content (no more than 12% w.b) to prevent microbial growth (Selmar *et al.*, 2008). This period can depend on customer demand, while the storage conditions depend on where the coffee beans are stored. The dry husk of parchment are acting as a good barrier

protect coffee against moisture transfer (Clarke & Macrae, 1985). In the amount of appropriate periods of storage before further roasting. Green beans usually pass the dehulling process to remove husks and grading coffee by separating the defective beans from the non-defective then the visual assessment is performed to separate color of coffee bean with these defects (Wintgens, 2004) as follows:

- Mouldy beans. These beans show the development of mould with white, grey or greenish colors. This will affect respectively in coffee flavor and aroma.
- Infested beans. Coffee which has been badly damaged by insects during storage. Depending on the level of the damage this may be considered to be a serious defect.
- Bleached beans. High temperatures and high levels of moisture will impact bean color, particularly when the beans are stored for a lengthy period.

After evaluating the beans by the visual assessment to separate the faulty of the good and finding the acceptable, the beans will be roasted in order to characterised by expected flavour.

2.2 Roasting process

Roasting is the key process and undoubtedly among the most important factor in the coffee value chain. It is a dry heat treatment to transform the green coffee precursors to roasted coffee with desirable color and flavour characteristics (Belitz *et al.*, 2009; Flament *et al.*, 2002; Lee *et al.*, 2015; Sunarharum *et al.*, 2014)

The process usually occurs in the temperature range between 100 ˚C to the final temperature of around 200 ˚C to cause the profound changes within the beans. After roasting, the beans increase their volume (50–80%) and the degree of weight loss is 15-20% by weight. The specific gravity of green to roasted beans falls from 1.126–1.272 to 0.570–0.694 (Belitz *et al.*, 2009). During roasting, beans become dark and brittle with porous texture and are ready for grinding and beverage extraction (Clarke & Macrae, 1985). Many changes in the green coffee composition depend on coffee species, degree of ripeness, post-harvest processing methods and can determine the flavor quality during roasting (Farah & Donangelo, 2006; Farah *et al.*, 2006; Sunarharum *et al.*, 2014; Variyar *et al.*, 2003; Viani & Petracco, 2007).

The intrinsic quality of green beans definitely depends on the precursors composition. During roasting only heat treatment in this process can make the evolution and unlock the full potential of those precursors in flavour generation by applying appropriate roasting conditions. Table 3 provides the information of green beans composition and precursors.

Table 3 Chemical Composition of Raw Arabica and Robusta Coffee Beans

Chemical Composition of Raw Arabica and Robusta coffee Beans			
Constituents	Content (% Based on Dry Weight)		Components
	Arabica	Robusta	
Soluble carbohydrates	9-12.5	6-11.5	
Monosaccharides	0.2-0.5	0.2-0.5	Fructose, glucose, galactose, arabinose (traces)
Oligosaccharides	6-9	3-7	Sucrose (90%), raffinose (0-0.9%), stachyose (0-0.1%)
Polysaccharides	3-4	3-4	Heteropolymers from galactose (55-60%), mannose (10-20%), arabinose (20-35%), glucose (0-2%)
Insoluble carbohydrates	46-53	34-44	
Hemicellulose	5-10	3-4	Heteropolymers from galactose (65-70%), Arabinose (25-30%), mannose (0-10%)
Cellulose, □(1-4) mannan	41-43	32-40	

Table 3 Chemical Composition of Raw Arabica and Robusta Coffee Beans (Cont.)

Chemical Composition of Raw Arabica and Robusta coffee Beans			
Lignin	1-3	1-3	
Acids and phenols			
Organic acids	2-2.9	1.3-2.2	Citric acid, malic acid, quinic acid
Chlorogenic acids	6.7-9.2	7.1-12.1	Feruoylquinic acid, mono- and di-caffeoyl quinic acid
Constituents	Content (% Based on Dry Weight)		Components
Lipids	15-18	8-12	
Coffee oil	15-17.7	8-11.7	Major fatty acids: linoleic acid (C18:2 and palmitic acid (C16:0)
Wax	0.2-0.3	0.2-0.3	
N-compounds	11-15	11-15	
Free amino acids	0.2-0.8	0.2-0.8	Major amino acids: glutamic acid, aspartic acid, asparagine
Proteins	8.5-12	8.5-12	
Caffeine	0.8-1.4	1.7-4.0	Traces of theobromine and theophylline
Trigonelline	0.6-1.2	0.3-0.9	
Minerals	3-5.4	3-5.4	

Source: modified from (Belitz *et al.*, 2009)

Components of green coffee: carbohydrates, nitrogen (N)-containing compounds (mainly proteins, trigonelline, and caffeine), lipids, organic acids, and water are important drives of flavor and color development.

However, the main principal flavor precursors that involving to coffee quality are known to be sugars, proteins, free amino acids, trigonelline, and chlorogenic acids (CGA). Those precursors and their constituents that can be affected by many factors and to the creation of aroma and taste are discussed in the following sections.

2.2.1 Main flavour precursors in green coffee

2.2.1.1 Carbohydrates

Carbohydrates fraction comprises between 40-65% of the dry matter of green coffee which can be separated to water-soluble and water-insoluble carbohydrates according to Table 3, polymers of arabinose, galactose, glucose, and mannose consist of soluble polysaccharides and insoluble fraction. These components also belong to the structure of cell walls along with proteins and CGA (Bradbury & Halliday, 1990). The complex structures of cellulose, galactomannan, and arabinogalactan account for the major fraction of coffee beans (up to 45% of dry matter) while the soluble disaccharide accounts for the rest (Trugo, 1985).

The soluble fraction of green coffee is widely known to be responsible as the most important precursor pool to the formation of key odorants, taste and color of coffee (Nunes & Coimbra, 2001). Miscellaneous degradation of carbohydrate take place at the early stage of roasting. The water-soluble constituents of carbohydrate are divided into two fractions; high molecular weight (HMW) and low molecular weight (LMW) fraction (De Maria *et al.*, 1994). Water-soluble HMW comprise galactomannans and arabinogalactans, with the latter accounting for 14-17% dry matter (Bradbury & Halliday, 1990; De Maria *et al.,* 1994). Arabinogalactans, generally are represented in form of highly branched molecules that are covalently bound to proteins. This part is called arabinogalactan proteins (AGPs). Heat treatment during roasting

causes to the structural change of the AGPs including depolymerization of main and side chains, which then release free arabinose, which acts as an important sugar precursor (Wei *et al.*, 2012).

The arabinose residues of arabinogalactan side chains play a role in acid formation, i.e., formic and acetic acid (Ginz *et al.*, 2000). As free galactose, the other constituent of arabinogalactans, it could take part in aroma generation, however it is only detected in significant amounts in green beans and is rapidly degraded (Redgwell *et al.*, 2002). The water-soluble LMW fraction comprising free sugars, trigonelline, and CGA. mono- and disaccharides plays major role as important flavor precursor. Mono- and disaccharides are the minor constituents of carbohydrate fraction, however, they have potential to form key odorants of coffee by caramelization and Maillard-type reactions. The major constituents, sucrose (disaccharide composed of glucose and fructose) is the most important disaccharide responsible for the complex aroma and overall flavor of Arabica coffee (Flament, 2002; Belitz *et al.*, 2009) this point was also been studies by (Farah & Donangelo, 2006).

Oligosaccharides (stachyose, raffinose) and monosaccharides (fructose, glucose, galactose, arabinose) are found in trace amounts in green coffee. In the early period of roasting, concentration of glucose and fructose rises steadily due to the constant degradation of sucrose. While roasting is occurring, most of all free sugars are lost due to Maillard reaction and caramelization, giving rise to water, carbon dioxide, color, aroma, and taste.

About 12-24% of the polysaccharides are degraded in light roasted coffee, 35-40% upon dark roasting which result from the degradation of the arabinogalactan side chains to arabinose, whereas cellulose and mannans remain almost intact in the roasted

coffee (Bradbury, 2001). It was previously studied that during roasting, polysaccharides do not take part in aroma formation, it is somehow impart to the organoleptic properties of coffee brew which are viscosity and mouth feel (Redgwell *et al.*, 2002). From the studies mentioned above, monosaccharides and the disaccharide sucrose are very important in their sensitivity to degradation by heat within few minutes of roasting.

In addition, to follow the quality of green coffee to aroma formation of coffee, the determination of mono and disaccharides in green coffee should be included.

2.2.1.2 Acids

The acidic fraction in green coffee amounts to about 8% of raw coffee. It comprises volatile aliphatic, non-volatile aliphatic and phenolic acids. Major non-volatile acids are chlorogenic acids (CGA), citric, malic and quinic acid (Maier, 1993). The major volatile acids of green coffee are formic and acetic acids (Viani and Petracco, 2007). They are accumulated via fermentation process in postharvest treatment and can also be formed through Maillard reactions upon roasting (Davidek *et al.*, 2006). CGA and their derivatives have been found in higher concentration in coffee plants than the other acids, their concentration in green coffee was between 3.4-14% of dry matter (Farah & Donangelo, 2006; Ky *et al.*, 2001). Other phenolic compounds, such as tannins, lignans and anthocyanins are also present in coffee seeds but in minor amounts. CGA are generally a group of phenolic compounds derived from the esterification of hydroxycinnamic acids (caffeic, ferulic, and *p*-coumaric acids) and quinic acid. About 80% of the total CGA is caffeoylquinic acid (CQA) which is the most abundant isomer of chlorogenic acid (5-CQA) (56-62%) (Farah, 2012). The main groups of CGA found in green coffee beans are presented in Figure 6, according to their chemical identity, number and position of acyl residues.

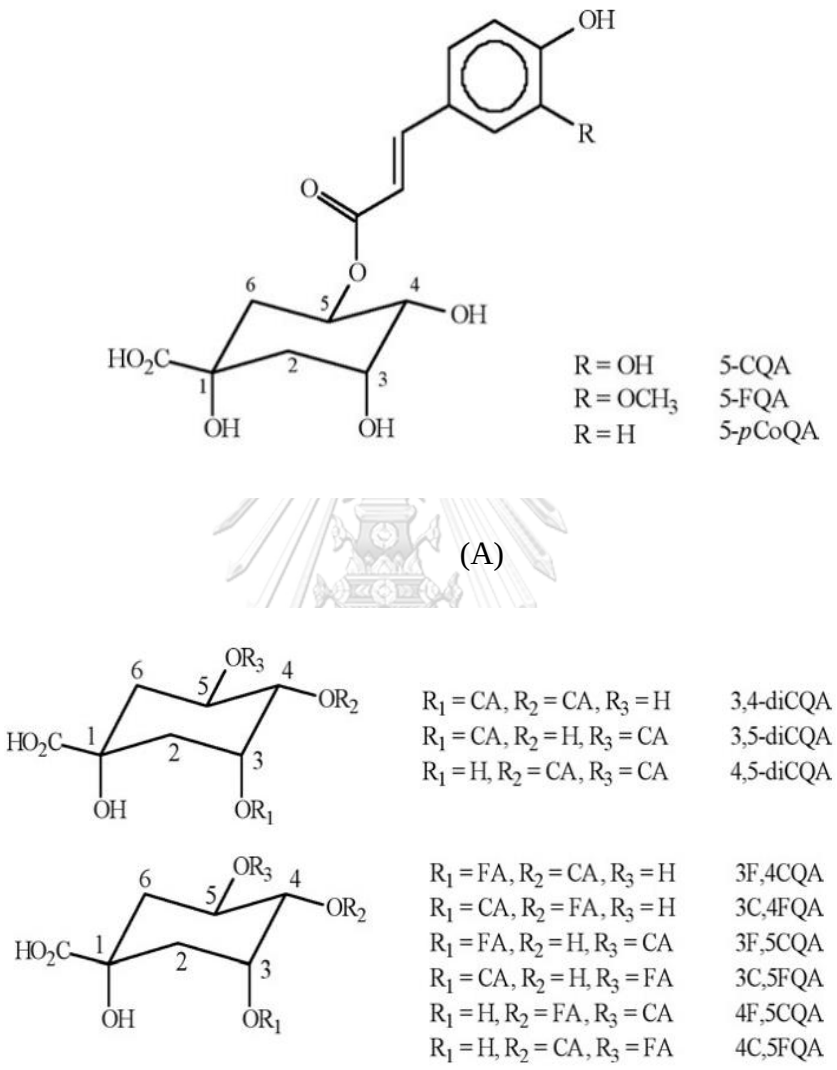


Figure 6 Chlorogenic acids and related compounds according to chemical characteristics. (A) Basic compounds; (B) monoesters of quinic acid with hydroxy-cinnamic acids (example of 5-isomers)

Source: (Farah & Donangelo, 2006)

These groups of compounds include: caffeoylquinic acids (CQA), with 3 isomers (3-, 4- and 5-CQA); dicaffeoylquinic acids (diCQA), with 3 isomers (3,4-diCQA; 3,5-diCQA; 4,5-diCQA); feruloylquinic acids (FQA), with 3 isomers (3-,4- and 5-FQA); *p*-coumaroylquinic acids (*p*CoQA), with 3 isomers (3-, 4- and 5- pCoQA), and six mixed diesters of caffeoylferuloyl-quinic acids (CFAQ) which were named according to the preferred IUPAC numbering system (Clifford, 1985, 2000, 2003).

CGA, which are present in high concentrations in green coffee seeds have a remarkably influence in determining coffee quality and play an important role in the formation of coffee flavor and tastes (Farah, 2012; Farah *et al.*, 2006; Variyar *et al.*, 2003). The diversity of total CGA content of green coffee beans are vary according to genetics – species and cultivar, degree of maturation and, less importantly, agricultural practices, climate and soil (Clifford, 1985; Farah *et al.*, 2005a). The diversity of methodology used to determine CGA is another important factor in establishing levels, since there may be a certain discrepancy between results obtained by high resolution chromatographic methods and those obtained by less sophisticated methods. CGA are the major products of the mechanism in phenylpropanoid pathway, one branch of the phenolic metabolism in higher plants that is induced in response to environmental stress conditions such as infection by microbial pathogens, mechanical wounding, and excessive UV or high visible light levels (Haard & Chism, 1996; Hermann, 1995).

Plant phenolic acids are regularly synthesized from phenylalanine and tyrosine via the shikimic acid pathway, which converts simple carbohydrate precursors, derived from glycolysis and the pentose phosphate shunt (phospho-enol-pyruvate and

D-erythrose-4-phosphate), into aromatic amino acids. The most active pathway is showed in Figure 8. The parent trans-cinnamic acid is formed from L-phenylalanine by the action of phenylalanine ammonia-lyase, a key enzyme in the biosynthesis of phenolic compounds that is activated in response to different stress conditions. Quinic acid is synthesized from 3-dehydroquinate, an intermediate metabolite of the shikimic acid pathway. Hydroxy-cinnamicacids (*p*-coumaric, caffeic, ferulic and sinapic) could be formed from cinnamic acid by hydroxylation or alternatively from tyrosine by the action of tyrosine aminolyase.

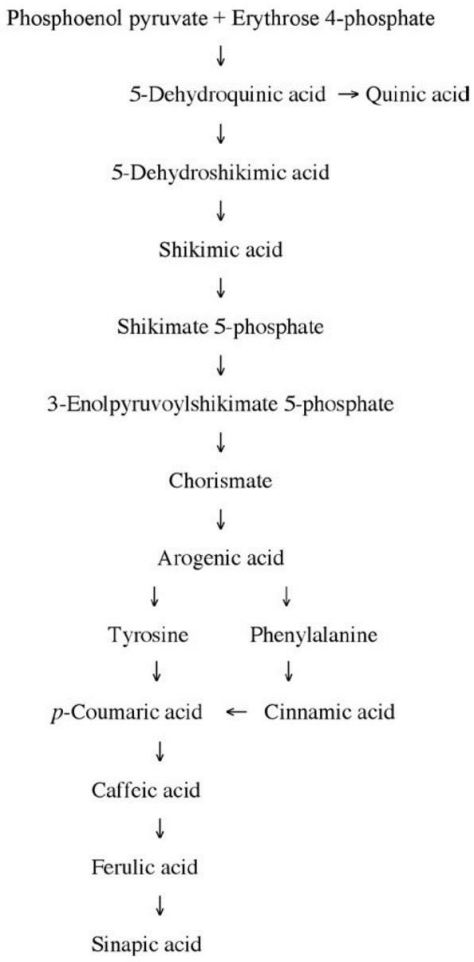


Figure 7 Biosynthesis of cinnamic/hydroxycinnamic acids and quinic acid via the shikimic acid pathway

Source: (Farah and Donangelo, 2006)

The synthesis of 5-CQA, 5-FQA and 5-*p*-CoQA has been investigated in several studies. They were formed via the binding of trans-cinnamic acid to coenzyme A (CoA) by a CoA lyase, followed by transfer to quinic acid by a cinnamoyl transferase (Gross, 1981). The origin of the CGA with acyl groups in positions 3- and 4- is unclear, although the possibility of acyl migration has been considered (Gross, 1981) after the pathway of formation those CGA could be spontaneously be substrate of enzymes such as polyphenol oxidase (Mazzafera & Robinson, 2000) leading to polymerization products such as insoluble brown pigments and lignin that contribute to the plant defense mechanisms and to the synthesis of plant cell wall constituents.

CGA are normally distributed on the surface of coffee seeds, in association with the cuticular wax, and in the cytoplasm adjacent to the cell walls of the endosperm parenchyma (Clifford, 1987), part of the cell wall CGA may be associated with caffeine, as a 1:1 or 2:1 molar complex (Clifford, 1985). During roasting CGA may be almost completely degraded into phenol derivatives. While roasting occurs, part of CGA is isomerized, part is transformed into quinolactones due to dehydration and formation of an intramolecular bond and part is hydrolysed and degraded into low molecular weight compounds (Farah & Donangelo, 2006; Trugo & Macrae, 1984). CGA also participate in the formation of polymeric material like melanoidins (Menezes, 1994).

The degradation of CGA is responsible for the formation of important volatile phenols from the class of guaiacols like 2-methoxyphenol (guaiacol), 4-ethyl-2-methoxyphenol (4-ethylguaiacol), and 4-vinyl-2-methoxyphenol (4-vinylguaiacol) (Poisson *et al.*, 2017). They are involved to the typical smoky, woody, and ashy

characteristics of roasted coffees. In term of the correlation of CGA with coffee quality, high concentration of 5-CQA, 4-CQA, 4-FQA and 5-FQA was previously claimed on their strongly correlated with Rio off-flavor of coffee cup (r^2 = 0.93, 0.94, 0.82 and 0.90, respectively), 3-CQA was not mentioned as definiting a specific pattern (r^2 =0.73) but the high content of this isomer in coffee tend to correlate to fraud of coffee. In contrast to those aforementioned group, 3,4-diCQA, 3,5-diCQA and 4,5-diCQA were claimed as the phenolic group indicating a good quality of coffee (Farah *et al.*, 2006).

2.2.1.3 Nitrogen (N) Containing Compounds

N-compounds of green coffee are mainly proteins, they account to 11-15% of green coffee dry matter. The total protein content account for 10% of both Arabica and Robusta green coffee. Part of the proteins is linked to the water-soluble polysaccharide arabinogalactan to form the accumulated AGPs. Even free amino acids represent less than 1% of all components in green coffee, their high contribution to the final flavor of roasted coffee is undeniable. Besides mono- and disaccharides, they are considered to be key precursors in the Maillard reaction as well as in the Strecker degradation, resulting in yielding of many potent odorants.

Three main free amino acids of green beans are glutamic acid, aspartic acid, and asparagine. Many studies claim the importance of the single amino acids that influence the development of aromatic profile via Maillard reaction (Wong *et al.*, 2008). The degradation of proteins and peptides can lead to the generation of reactive molecules which take part as aroma precursors in Maillard reaction (De Maria *et al.*, 1996). Over entire period of roasting, free amino acids are almost completely degraded. Other nitrogenous part of green coffee; caffeine, the alkaloid compound and trigonelline can also contribute to development of flavour and taste during Maillard

reaction. During roasting, trigonelline can partially degraded and converted into nicotinic acid and volatile compounds such as pyridines and pyrroles (Viani & Horman, 1974).

2.2.1.4 Lipids

The lipids fraction in green coffee constitutes to 15-18% of dry matter in Arabica coffee (Viani & Petracco, 2007). This part is composed of coffee wax coating the bean and triglycerides.

Linoleic acid (40-45%) and palmitic acid (25-35%) are the main fatty acids that play a role in coffee sensorial properties. The lower amounts of various fatty acids (arachidic, behenic and lignoceric), they are naturally present in the raw coffee waxes in the esterified form, with hydroxytryptamide. Diterpenes (cafestol, kahweol) and sterols in free and esterified form are also being part of the total lipid fraction. Role of lipid in aroma development upon roasting is the formation of aldehydes through thermal degradation. They further react with other coffee constituents (Belitz *et al.*, 2009).

2.2.2 Changes in green bean during roasting

Under the appropriate condition of roasting process, it leads to physical and chemical transformations besides the development of color from blue-greenish to brown. The intact bean generally acts as an essential “mini-reactor” for the chemical reactions. Major four phases can be distinguished in roasting process. They are dehydration, development, decomposition and full roasting. Green bean begins to change at or above 50 ˚C as the denaturation of protein in cell tissue and evaporation of free water are occurring (Belitz *et al.*, 2009). Considering to the dehydration step, a

considerable amount of water represent within the green bean is an important factor on the generation of further chemical reactions (Poisson *et al.*, 2017).

The actual total water content and water activity at different stages of the roasting process play a key role for the kinetics of chemical and physical changes in the bean because not only the temperature that determines the reaction but also the speed of important flavor-generating chemical reactions also rely on the availability of water. If the moisture content of the beans falls below a certain critical value or behave differently, it can slow down some chemical reactions (Poisson *et al.*, 2017).

Next, the development of brown compounds occurs when temperature rises above 100 ˚C due to pyrolysis of organic compounds as well as the swelling and the beginning of the dry distillation when volatile products include water vapour, carbon dioxide and carbon monoxide start to be released at about 150 ˚C. These result in an increase of the bean volume**.** The decomposition phase, when the exothermic reactions start to occur when roasting temperature rises to180–200 ˚C, then a size expansion of the beans due to the drying of the beans can be seen. This happens under the increasing pressure, so that the beans are forced to pop and burst. The sound of beans cracking or first crack can be noticed at this stage as well as the formation of bluish smoke and the release of coffee aroma. Eventually, under optimum caramelization, the full roasting phase is achieved, the moisture content of the beans then drops down to 1.5–3.5%.

Roasted product is then discharged rapidly to cooling sifters or is sprinkled with water (water quenching) in order to avoid over-roasting or burning and aroma loss. While roasting, conduction process is occurring when the distribution of the heat is transferred by contact between the beans and the walls of the roasting apparatus. Convection process is obtained from the contact of moving hot air or combusted gases.

The roasting process can be selected to be controlled electronically or manually by sampling roasted beans. During roasting, vapors formed and cell fragments (silverskin particles) are removed by suction of an exhauster and, in larger plants, incinerated.

The grades of roasting vary between countries or company styles. In the USA and Central Europe, beans are roasted to a light color (200–220 ˚C, 3–10 min, weight loss 14–17%), and in France, Italy and the Balkan states, to a dark color (espresso, 230 ˚C, weight loss 20%) (Belitz *et al.*, 2009).

2.2.3 The main chemical reactions influence to aroma development of coffee

During roasting, multiple changes are happening such as the formation of low molecular weight compounds, the development of brown color, releasing of carbon dioxide and the formation of flavour molecule responsible for roasted, caramel, earthy and toasted aroma of the roasted beans (Belitz *et al.*, 2009; Poisson *et al.*, 2017).

The key reactions affecting the coffee aroma characteristics are shown in Table 4. The non-enzymatic browning, Maillard reaction is the key reaction driving these phenomena. Change of the bean color comes from the generation of high molecular weight colored melanoidins during the reaction. Additionally, antioxidative effect and chemoprevention result from Maillard-type reactions.

The Maillard reaction consists of complex series of numerous reactions starting with an amino-carbonyl coupling of reducing sugars and amino acids (or peptides and proteins) leading to N-substituted glycosylamines (Schiff base), which are rearranged to the first stable intermediates like Amadori and Heyns products. The schematic of the chemistry of flavour formation during Maillard reaction is presented in Figure 8.

These conjugations of the activated reactive sugars are easy to degrade and generate further small fragments which are spontaneously forming a multitude of volatile and non-volatile products (Ledl & Schleicher, 1990). Sucrose, the main sugar components of green coffee and the main flavour precursor is activated to degrade into glucose and fructose when receiving heat treatment. They will then react with other precursors.

The intensity and diversity of the Maillard products and consequently the composition of the roasted coffee can be influenced by the composition of the precursors in the green bean. Besides, to develop coffee aroma via Maillard reaction involves many factors as the type of the reactive species (sugars and amino acids), reaction temperature, time, pressure, pH, and moisture content (Ho *et al.*, 1993; Ledl & Schleicher, 1990).

Acid compounds contribute to the total sensory perception of acidity as acetic acid and formic acid are commonly formed via the degradation of carbohydrate precursors during the early stage of Maillard reaction and caramelization (Davidek *et al.*, 2006). However, those acids are degraded or evaporated due to the higher temperatures during the final stages of roasting. Even though the concentrations of short-chain volatile acids are slightly increasing during roasting, in contrast, the sensory perceivable total acidity is decreasing during the course of roasting which is indicating their limited role in coffee acidity.

The Strecker degradation is one of the subside reaction taking part during the main Maillard reaction. It is involved in aroma formation and contributes to the spectrum in the formation of volatile aldehydes with the characteristics of malty (2/3-methylbutanal) and potato (methional) notes.

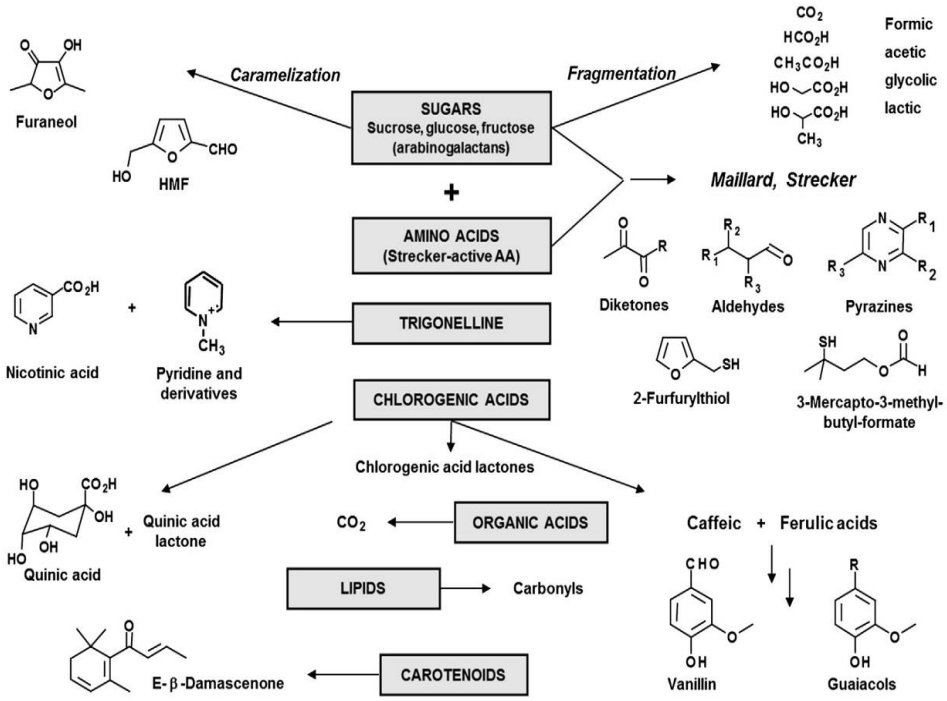


Figure 8 Schematic presentation of the most important flavor precursors in green coffee and the transformation into key aroma compound

Source: (Yeretzian *et al*., 2002)

The Strecker degradation is produced through a deamination and oxidative decarboxylation of the amino acids which result in the yielding of Strecker aldehyde (R-CHO). This reaction also contributes to the formation with earthy and roasty note like pyrazines (Poisson *et al.*, 2017).

During Maillard reactions, the reaction of sulfur-containing amino acids as cysteine and methionine yield volatile thiols and sulfides. Those odorants like 3-mercapto-3-methylbutylformate, 3-methyl-2-butene-1-thiol, methanethiol and especially the major impact compound, 2-furfurylthiol, odorant have very low odor thresholds that contribute to the characteristic aroma of coffee (Holscher & Steinhart,

1992). With rising temperature, the caramelization of sugars leads to caramel and seasoning notes. However, it was found that the development of aroma components is favoured by the Maillard route due to lower activation energy in the presence of reactive nitrogen species (e.g. amino acids). During roasting, CGA continue to be vigorously degraded. This results in hydrolysis of products like quinic acid and ferulic acid which continue to be further degraded and form the important volatiles phenolics like guaiacol and 4-vinylguaiacol. It was previously reported that after 9 min of roasting, 90% of total CGA have been decomposed (Farah *et al.*, 2005b). Highly potent odorants; hexanal, enals and dienals groups are formed from the lipid oxidation of unsaturated fatty acids, even these aldehydes do not belong to the key volatiles of coffee but they can further react with other coffee components through the cyclization through coffee roasting (Belitz *et al.*, 2009). Hexanal is commonly a volatile indicator of lipid oxidation in various foods and as a volatile indicates to coffee staling among other compounds (Sanches-Silva *et al.*, 2004; Spadone & Liardon, 1989).

Most of the polymeric carbohydrates, lipids, caffeine and inorganic salts are stable during entire process. In alkaloid groups, caffeine contents are relatively constant and only trigonelline is degraded and form the volatile compounds (Viani & Horman, 1974).

Table 4 Key reactions of coffee roasting that impact aroma characteristics

Reactions	Precursors Involved	Compounds Formed (aroma descriptor)
Maillard reaction	Reducing sugars N-compounds	Diketones (buttery) Pyrazines (earthy, roasty,nutty) Thiazoles (roasty, popcorn-like) Enolones (caramel-like, savory) Thiols (sulfury, coffee-like) Aliphatic acids (acidic)
Strecker degradation	Amino acids Diketones deriving from Maillard reaction	Strecker aldehydes (malty, green, honey-like)
Caramelization	Free sugars (sucrose after inversion)	Enolones (caramel-like,savory)
Degradation of chlorogenic acids	Chlorogenic acids	Phenols (smoky, ashy, woody, phenolic, medicinal) Lactones (bitter)
Lipid oxidation	Unsaturated fatty acids	Aldehydes (fatty, soapy, green)

Source: (Poisson *et al.*, 2017)

2.3 Brewing of coffee

This process unlocks the transformation of aroma molecules within roasted beans and produced coffee beverage with good cup quality by the balance of aroma, taste and mouthfeel. Brewing steps are commonly performed to extract the odorants and taste compounds from prepared roasted coffee matrix. Various methods of brewing

are used depending on origin, culture, and ultimately consumer preference. Each brewing methods involves a number of factors to play a role in order to extract flavor into the beverage and balance the ingredients.

With the propose to prepare the coffee beverage, the beans are well protected from contact with oxygen until brewing. In general, before brewing, roasted coffee needs to maintain its equilibration of flavor at least 12 h for a steady release of volatile compounds. During this period, the aroma starts to develop as the beans release carbon dioxide with some volatiles outside.

The development of flavor goes on during the stabilization of roasting chemistry and the equally distribution of water within the beans. Extraction condition is a key factor very influencing flavor balancing in a cup of coffee. Factors like grinding size and temperature needs to be controlled to prepare coffee beverage, otherwise a balance between the compounds might change. Table 5 shows the information about these factors.

Table 5 Parameters influencing final cup quality of coffee brews

Water	Coffee	Resulting Variables
Quality	Weight	Water/coffee ratio
Quantity	Particle size and shape	Pressure
Temperature	Particle size distribution	Flow time
	Compaction	Flow rate
	Shape of coffee bed	

Source: (Poisson *et al.*, 2017)

The temperature of water has to be considered as a factor playing a role in the kinetic energy of the water molecules. This is because high temperature will activate the leaching of extracted compounds from the coffee bed into the brew.

During brewing, volatiles from coffee particles will move into liquid phase. The solubility of gases is also temperature dependent following Henry's Law, so that the increase of the water temperatures will definitely transfer the volatile aroma compounds into gas phase (Sanchez-Lopez *et al.*, 2016). Then, odorants will be released in the air during brewing, and this will be stimulated by higher water temperatures. The aroma release during extraction and release before and during serving contributes to the overall aroma perception and experience by the consumer, but decreases the in-cup concentrations of these highly volatile compounds.

Considering the driving forces of volatile extraction in coffee brewing, the main role is pressure. It is the consequence of the balance between the applied force on top of the bed (through the water) and the resistance of the coffee bed against the filtration of water.

Each extraction method has its own driving force and its typical coffee bed properties. The variation of coffee bed preparation is very important for the character of coffee as characteristics of the coffee bed determine the water permeability and how quickly it will pass through the coffee bed (Corrochano *et al.*, 2015). A too low bed permeability can give rise to (too) high extraction pressures, low overall flow rates, and too long extraction times possibly leading to over-extraction (Corrochano *et al.*, 2015). There are a number of brewing methods applied for coffee beverage preparation which can generally be classified under decoction (boiled, turkish, percolator, vacuum

coffees), infusion or steeping (filter, neapolitan) and pressure methods (plunger, moka, espresso) (Belitz *et al.*, 2009).

The brewing method will influence to bring the best cup quality of coffee and interfere to the determination of coffee sensory quality while cupping. Hence, the Specialty Coffee Association of America (SCAA) has published the official protocol, Steep cup methodology for cupping coffee with the preserve of balance in quality (SCAA, 2015).

This method provides an advice on the optimum ratio of coffee per water (about 8 g and 150 mL of boiled water), standard grinding size (slightly coarser powder), optimum temperature on how to prepare coffee cupping as this conforms to the mid-point of the optimum balance recipes for the standard golden cup. Previous studies of Lyman *et al.* (2003) and Marcone (2004) have been successfully applied in Steep up protocol on coffee beverages preparation in the propose to evaluate the difference in sensory properties of civet coffee and non-civet coffee as well as the cupper sensory perception of coffee roasted to different degree. Therefore, this method is robust in the consistency of coffee beverage preparation by control the brewing condition and avoiding the interferences between volatile release and sensory analysis.

2.4 Determination of volatile compounds in coffee

Coffee good flavor characteristic have been claimed on the basis of a balance of pleasant sensation of flavor, body and aroma (Mori *et al.*, 2003). Hence, flavor is the most important criterion for consumer buying choice and warrants thorough investigation from a sensory and compositional perspective (Mori *et al.*, 2003).

Volatile compounds produced during coffee bean roasting are the most important quality-determinant of coffee. Only a small group of key odorants are responsible to the flavor and aroma characteristics of coffee (Nijssen, 1996). More than 1000 volatiles and non-volatiles were identified in coffee. A sophisticated analytical approach has been required combining sensory and instrumental evaluations to narrow down the number of relevant compounds to carefully focus on the important odorant groups. The complexity of coffee flavor requires a high level of analytical expertise to choose the most appropriate methodology for a given task to accomplish, in combination with data treatment and molecular interpretation (Kerler & Poisson, 2011).

Much knowledge of character impact odorant in coffee is needed to be elucidate in order to unfold the connecting between the processing conditions in the coffee value chain and the green bean composition. Details of research focusing on the isolation and identification of coffee flavour have been obtained using an innovative approach relating information between sensory and chemical analysis. This approach consisted in the application of sensory methods (e.g., sniffing and tasting) in the identification of aroma- and taste-active compounds that really matter for the perception of overall flavour (Blank, 2002; Blank *et al.*, 1992; Ottinger *et al.*, 2001).

The results from the use of gas chromatography olfactometry (GC-O) combined with gas chromatography mass spectrometry (GC-MS) and the odor activity value (OAV) calculation has been unfold a limited number (20-30) of aroma-relevant volatiles that describe coffee sensorial characteristics (Blank, 2002; Grosch, 2001). The increasing performance of analytical instruments has been considered as an important breakthrough allowing enhanced separation of components in coffee matrix on the chromatographic side due to higher sensitivity and selectivity of detection

devices, mainly mass spectrometry (MS). The general steps of aroma separation and identification are shown in Figure 9.

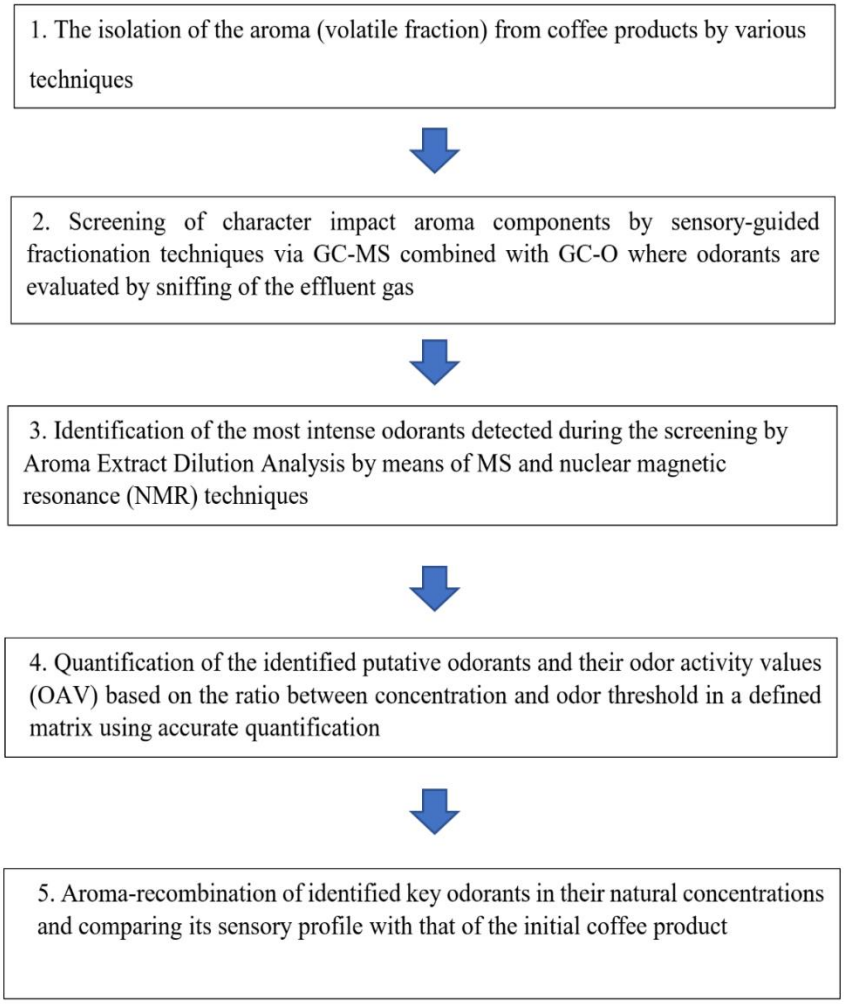


Figure 9 Sequence of steps to identify and quantify the important odorants of coffee

Source: (Poisson *et al.*, 2017)

First of all, the analytical procedure usually begins with the sample preparation step with the isolation of the volatile from the non-volatile fraction in coffee matrix using techniques with the aim of obtaining a representative extract of the original aroma (Gloess *et al.*, 2013). The complexity of flavor isolation in coffee matrix has been successfully overcome by a mild and exhaustive distillation technique called solvent-assisted flavor evaporation (SAFE) (Engel *et al.*, 1999; Fang & Cadwallader, 2013) coupled with acid/neutral/base fractionation to avoid the interference of matrix and separate the class of odorants in order to reduce the complexity of chromatographic results. Such technique was previously successfully used in the previous studies (Czerny & Grosch, 2000; Semmelroch & Grosch, 1996; Semmelroch *et al.*, 1995).

Other sample preparation methods can be used without the inclusion of distillation clean up steps of SAFE in the determination of coffee volatiles. The liquid-liquid extraction (LLE) using multiple solvents without the fractionation has been described as a successful technique in coffee volatiles isolation (Cheong *et al.*, 2013; Pickard *et al.*, 2013; Pickard *et al.*, 2014). They are less time consuming than SAFE but, the interference of matrix could cause an error in the concentration of target odorants. Therefore, this technique sometimes needs a variation of calibration. There are also solvent free techniques such head space solid phase micro-extraction (HS-SPME) that were successful in the determination of coffee volatile analysis in order to determine the important target key odorants of coffee that are not category of high-boiling point volatile compounds (Fang & Cadwallader, 2013; Mestdagh *et al.*, 2014; Sun *et al.*, 2018) and avoid multiple steps of sample preparation that may cause artefact and consume time like SAFE method did.

Next step, after the first pre-isolation of coffee volatiles, the compounds isolated are screened for character impact aroma or taste components by sensory-guided fractionation techniques. Screening of odorants is usually done by GC separation of the aroma extract combined with olfactometry where odorants are evaluated by sniffing of the effluent gas (Blank, 2002; Poisson *et al.*,2017). According to this technique, the human nose is used as sensitive detector to differentiate potent odorants from the overpopulated odorless volatile components. Fractionation techniques are used for the characterization of those components resulting in multiple fractions which are determined using GC-O. After the completion of these steps, the most intense odorants perceived by Aroma Extract Dilution Analysis (AEDA) from GC-O are identified by means of GC-MS or nuclear magnetic resonance (NMR) techniques to confirm the structure of volatile compounds. The identified recognized potent odorants are quantified using stable isotope dilution assay technique and their odor activity values (OAV) are calculated (ratio between concentration and odor threshold in a defined matrix) using various type of accurate quantification methodology.

After executing the above mentioned steps, in order to confirm the importance of those potent odorants calculated by OAV concept, the aroma model of coffee can be created by mixing the preidentified key odorants in their present concentration in coffee and comparing its sensory profile with that of the initial coffee product. The relative importance of the key volatiles for the overall coffee flavor model can be evaluated by omitting single compounds or a group of compounds in the test model. Volatile compounds leading to a significant change of the overall sensory profile can be referred to as impact compounds of coffee.

2.4.1 Stable Isotope Dilution assay

Stable isotope dilution assay (SIDA) is a quantitative method characterized by both, high precision and accuracy, applied to detect and quantify food flavors (Fang, 2013). Using this technique, the isotopically labeled internal standard, is spiked into the sample matrices prior to sample preparation, has great similarity in both chemical and physical properties with its unlabeled counterpart, which represents the target compound. The problem of food matrix complexity like coffee can be overcome by the application of SIDA for the quantification of key odorants.

2.4.1.1 Principle of SIDA

SIDA is a state-of-the-art method that has been claimed to have high precision and accuracy in the quantitative analysis since the first publication in 1996. The authors of the study described the use of the deuterated D-glucose as an isotope tracer for glucose determination (Sweeley *et al.*, 1966). A stable isotope used in SIDA is typically chosen to be deuterium or carbon-13 for labelling purpose. The generation of the isotopically labelled compounds make them be an analogue of an unlabelled analyte which can be applied as the internal standard during quantitative analysis. The properties of the labelled isotope could be considered as the ultimate internal standard due to its great similarity in both physical and chemical properties to its unlabelled counterpart, the target compound (Fang, 2013; Fang and Cadwallader, 2013).

After spiking of the labelled compounds and the equilibration of a known amount of the stable isotope in the sample, the ratio of labeled and unlabeled compounds is maintained throughout the multiple steps of volatile analysis including extraction, workup and analysis as shown in Figure 10 .

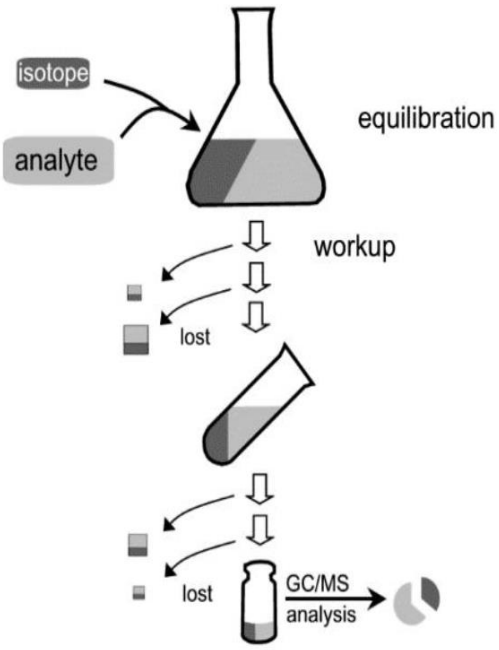


Figure 10 Concept of SIDA, where the isotopologue ratio remains stable throughout extraction and analysis
(Source: Fang, 2013)

Consequently, the two isotopologues, which differ in mass, are detected and differentiated by a mass spectrometer (MS). The mass ion ratio is used to determine the abundance of the target analyte in the initial sample as shown in the equation 2.1 (Fang, 2013).

$$\text{Mass of analyte} = \frac{(\text{Extracted ion chromatogram area of analyte})\times}{(\text{area of labelled internal standard})} \text{ mass of internal standard x response factor} \quad (2.1)$$

This technique has been widely used in order to accurately quantify the amount of the key odorants in coffee both of the low-boiling point and high boiling point groups combined with the sample extraction technique like SAFE and SPME.

2.4.2 Injection techniques for high boiling point key volatiles

A conventional Programmable temperature vaporizer (PTV) with cold-splitless technique is normally used for the injection of sample with extracts containing heat labile compounds. The sample could be injected to cold liner with the control of heating holding below or near the solvent boiling point. In PTV injectors the vaporization chamber can be heated or cooled rapidly. This injection unit contains a sophisticated control unit which is designed to perform the heating at a pre-selected rate. In addition, in order to cool the liner, cold air/ expanding CO_2 or liquid nitrogen can be applied to facilitate rapid heating and cooling while transferring the heat sensitive analytes. Then the system would reduce ring temperature and transfer analyte to chromatographic system. This technique also avoids the discrimination of low boiling point volatiles and prevents degradation of compounds. It also offers the handling of sample large volume with remaining traces of solvent vapour from the liner (Giardina & McCurry, 2016; Stajnbaher & Zupancic-Kralj, 2008; Zrostlikova *et al.*, 2001). Hence, from the mentioned benefits, the PTV injection in cold-splitless type is suitable for the quantitation of heat sensitive extracts containing trace level of high boiling point volatiles. However, although this technique provides benefit of the cold injection and of having an inlet that could be cleaned and changed, it still causes a problem due to matrix effect and give an over estimation of analyte concentration that needs to be eliminated using many calibration methods (Stajnbaher & Zupancic-Kralj, 2008).

In contrast to PTV cold-splitless injection, cool-on-column capillary injection has been claimed to offer a high precision of results after injection (Giardina & McCurry, 2016). It was described as having an easy sample insert to apply

for capillary gas chromatography analysis with a control rate of volume injection especially when samples contain thermally labile analites or mixtures with components that have numerous labile odorant and have complex matrix with very different volatilization properties of target compounds. The system was also described as providing analites discrimination for the high boiling substances.

Comparing with other conventional injection techniques, on-column injection delivers the extract directly into the column via a special thin injection needle with the slow injection to oven temperature of less than 40°C that also prevent the re-condensation of solvent during injection without evaporation of heat sensitive volatiles in a heated space by using an initial temperature below boiling point of the solvent and, hence, the discrimination being precluded (Zrostlikova *et al.*, 2001; Giardina & Mccurry, 2016). In addition, on-column injection commonly provides the accurate standard deviations that are naturally much lower than the conventional injection (Galli & Trestianu, 1981). However, high amount of analyte entering the column is an additional source of active sites in the GC system in samples containing matrix than in pure solvent (Stajnbaher & Zupancic-Kralj, 2008), so the dirty samples like extract from plant materials should be avoided.

Both on-column and cold-splitless injection techniques that are used for accurate test are suitable for analyzing trace analytes containing high boiling point volatiles (analytes in the ppm level or less in the final injected sample) but the matrix effect that usually come from extract should minimized. Besides, each technique offers advantages over another considering from their injector design is frequently the choice of technique that are a compromise between varieties of samples.

2.5 Odorants in coffee

2.5.1 Odorants in green coffee

Most of the aroma research has been performed on roasted coffee or the corresponding beverage. The volatile composition of green coffee was previously studied as well (Cantergiani *et al.*, 2001; Spadone *et al.*, 1990). The aroma of green coffee is reported to be green, hay- and pea-like (Gretsch *et al.*, 1999), and the taste as sweet, astringent (Viani & Petracco, 2007). Odorants in green coffee have been studied for their identified off flavor before roasting and focus on the potent odorants of unstored green coffee as well as their change indicate and might predict the quality of the roasted coffee produced from coffee stored under different conditions (Cantergiani *et al.*, 2001; Scheidig *et al.*, 2007; Spadone *et al.*, 1990).

Volatile compounds of green coffee beans are around 300 which is much lower and less complex than roasted coffee. Some of them are not affected by roasting and can be found unchanged in the roasted product (e.g., 2-isobutyl-3-methoxypyrazine formed enzymatically in green beans), whereas the others decrease during roasting by evaporation (e.g., ethyl-3 - methylbutyrate) or degradation. The other aroma-active components of coffee which are linalool, lipid degradation products, biologically derived alcohols, aldehydes, and organic acids are present in green coffee before the generation of the complex chemical reactions during roasting (Cantergiani *et al.*, 2001; Holscher & Steinhart, 1994).

However, as mentioned above in the discussion of reviewed literature very few studies on the authenticity of green coffee produced in each process and the separation of raw coffee produced from ripe and non-ripe cherries process from Thai

Arabica via the application of volatile analysis. Most of the published research on green coffee volatiles reports only the identification results via mass spectrometry and calculation of RI and made the comparison of volatile using peak areas. Only study of Scheidig *et al.* (2007) made the comparison of important volatile classes as concentration determined using internal standard. Therefore, to gain the better understanding of green coffee volatiles, more studies on quantitation of those key odorants are required.

2.5.2 Odorants in roasted coffee

According to the previous discussion on the main chemical reactions happening during roasting, the development of coffee aroma characteristic comes from change in the complex network of physical and chemical structures of green beans during roasting. Previous research has explored on the number of roasted coffee odorants which have been found to be more than 1000 compounds nowadays (Nijssen, 1996). Because of the complexity of coffee matrix the overall balance of those volatile fractions accounts for around 0.1% of total roasted coffee weight. Hence the concentration of these key volatiles ranges from parts per trillion (ppt) to higher part per million (ppm) levels (Hertz-Schunemann *et al.*, 2013). Only 20-40 of key odorants are responsible to the sensorial properties of coffee as previously mentioned (Blank *et al.*, 1992; Grosch, 1998; Kerler & Poisson, 2011).

One group of these impact odorants, around 40 compounds, were normally found in most of investigated foods, e.g. roasted beef, red wine and chocolate (Poisson *et al.*, 2017). Those identified key odorants are in the classes of thiols, sulfides, aldehydes, pyrazines, dicarbonyls, phenols, and furanones. The overview of key

odorants identified in roasted coffee from various studies is shown in Table 6 (Blank *et al.*, 1992; Kerler & Poisson, 2011; Semmelroch & Grosch, 1996).

It was elucidated in the separated results from omission experiments that among all of these key odorants, 2-furfurylthiol is the major impact compound involved in characterizing coffee (Belitz *et al.*, 2009; Semmeroch *et al.*, 1995; Semmeroch and Grosch, 1996; Flament, 2002; Holscher and Steinheart, 1992, Poisson *et al.*, 2017). Its aroma is referred to coffee-like note. The precursors of this compound are claimed to be polysaccharides containing arabinose like arabinogalactans as well as cysteine in the free and bound form. 2-Furfurylthiol and the other thiols shown in the Table 6 are bound form of disulfide and group of cysteine, SH-peptides and proteins in roasted coffee. The formation of 2-furfurylthiol in roasted coffee is provoked by the amount of water and the slightly acidic pH value of the beans which activates the partial hydrolysis of polysaccharides (Belitz *et al.*, 2009).

Arabica coffees are usually composed of more sweet/caramel-like odorants than Robusta. On the hand, the latter consists of alkylpyrazines and phenols in significantly higher concentrations. 3-isobutyl-2-methoxypyrazine and 3-alkyl-2-methoxypyrazine are the main compounds that account for the perceivable pea-like and potato-like note according from their highest aroma value. These alkylpyrazines are stable through the entire roasting process but somehow, generally are being suppressed from the odor threshold of other odorants. The recognize of the off-note, potato like is perceived in case that the concentrations of the alkylmethoxypyrazines rise significantly.

Table 6 Profile of the potent odorants of roasted Arabica and Robusta coffee and their relative abundance (more abundant in Arabica Coffee [A], more abundant in Robusta Coffee [R], similar in Arabica and Robusta [A/R] as reported by different authors

Odorants	Aroma descriptor	Relative Abundance
Methanethiol	Sulfur, garlic	R
Dimethyl sulfide	Sulfur, cabbage	A/R
Dimethyl disulfide	Sulfur, cabbage	A/R
Dimethyl trisulfide	Sulfur, cabbage	A/R
2-Furfurylthiol	Sulfury, roasty	R
3-Mercapto-3-methylbutyl formate	Catty, blackcurrant-like	A/R
3-(Methylthio)propionaldehyde (methional)	Potato	A
2-Methylbutanal	Green, solvent, malty	R
3-Methylbutanal	Malty, cocoa	A/R
2,3-Butanedione	Buttery	A/R

Table 6 Profile of the potent odorants of roasted Arabica and Robusta coffee and their relative abundance (more abundant in Arabica Coffee [A], more abundant in Robusta coffee [R], similar in Arabica and Robusta [A/R] as reported by different authors (Cont.)

Odorants	Aroma descriptor	Relative Abundance
2,3-Pentanedione	Buttery	A
2-Ethyl-3,5-dimethylpyrazine	Earthy, roasty	R
2-Ethenyl-3,5-dimethylpyrazine	Earthy, roasty	R
2,3-Diethyl-5-methylpyrazine	Earthy, roasty	R
2-Methoxy-3-isobutylpyrazine	Pea, earthy	A
2-Methoxyphenol	Smoky	R
4-Ethyl-2-methoxyphenol	Spicy, clove-like	R
4-Vinyl-2-methoxyphenol	Spicy, clove-like	R
3-Hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon)	Fenugreek, curry	A/R

Source: (Blank *et al.*, 1992; Kerler & Poisson, 2011; Schenker *et al.*, 2002; Semmelroch & Grosch, 1996).

2.5.3 Odorants in coffee brew

Comparing the difference of those potent odorant group between roasted coffee and coffee beverage as shown in Table 7, it was found that the sensorial note of ground coffee gave more intensive phenolic, buttery, caramel like note and a lower roasty note. The major influences are from the change of potent odorants concentration

during the performance of water extraction in order to prepare coffee beverages (Belitz *et al.*, 2009). Considering the difference of aroma group which are detected in Arabica and Robusta coffee, it was found that 2,3-diethyl-5-methylpyrazine and 4-ethylguaiacol were predominant in the Robusta coffee and 3-mercapto-3-methylbutylformate, sotolon and abhexon in the Arabica coffee. Further significant differences were found in the concentration of 2-methyl-3-furanthiol, phenylacetaldehyde, 3,4-dimethyl-2-cyclopentenol-l-one, 2-/3-methylbutanoic acid and linalool, which are predominant in the Arabica coffee, and for 3-methyl-2-buten-l-thio1, which prevail in the Robusta coffee.

The comparison in term of coffee sensory attributes showed a higher note of caramel-like, sweet-roasty in Arabica coffee, while the spicy and earthy-roasty were more pronounced in the Robusta species. The results of Semmelroch and Grosch (1996) indicated that the amounts of diacetyl and 2,3-pentandione were higher in Arabica than in Robusta coffee brew which correlated with the presence of the buttery top-notes in the mild aroma of Arabica coffee. This becomes more clear from the comparison of OAV, The higher OAV of diacetyl indicated that this dione contributed more significantly to the aroma of the brews than 2,3-pentandione.

Table 7 Concentrations and Odor Activity Values of potent odorants of brews prepared from Arabica and Robusta Coffees

Potent odorant in coffee brew	Concentration		Odor Activity Value	
	Arabica	Robusta	Arabica	Robusta
2-furfurylthiol	19.1	39.0	1910	3900
2-ethyl-3,5-dimethylpyrazine	13.1	35.2	82	220
2,3-diethyl-5-methylpyrazine	3.2	9.3	36	103
(*E*)-□-damascenone	1.3	1.5	1730	2000
methional	5.7	2.8	29	14
3-mercapto-3-methylbutylformate	5.5	4.3	1570	1230
guaiacol	170	1230	68	490
4-vinylguaiacol	1640	5380	82	270
4-ethylguaiacol	51	635	1	13
vanillin	220	740	9	30
4-hydroxy-2,5-dimethyl-3(2H)-furanone	4510	2480	450	250
3-hydroxy-4,5-dimethyl-2(5H)-furanone	77	31	257	103
5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone	8.7	4.4	1	<1
2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone	840	670	42	29
2,3-butanedione	2750	2400	183	160
2,3-pentanedione	1570	750	52	25
2-isobutyl-3-methoxypyrazine	1	0.17	200	34
propanal	435	435	44	44
methylpropanal	800	1380	1140	1970
2-methylbutanal	650	1300	500	1000
3-methylbutanal	550	925	1570	2640
methanethiol	210	600	1050	3000

Source: (Semmelroch and Grosch, 1996)

Furaneol® (4-hydroxy-2,5-dimethyl-3(2H)-furanone) is one of the most odorant contributors to the caramel-like characteristic of coffee that differ from Robusta and can be one of the key compounds distinguish between two coffee species (Blank *et al.*, 1992). The concentration of Furaneol® was 4.5 time higher in the brew of Arabica coffee than in the corresponding sample of the Robusta species. The OAV of Furaneol® in Table 7 confirmed its strong influence on the flavour of Arabica coffee.

The concentrations of sotolon (3-hydroxy-4,5-dimethyl-2(5H)-furanone) and abhexon (5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone) were reported to be lower than those of Furaneol®, however these compounds prevail in the Arabica coffee. Their predominance in Arabica coffee may explain the preference of the consumers for this coffee species. The presence of the prementioned key volatiles responsible for the desirable characteristics of coffee is undeniable and therefore they need to be investigated in order to compare and improve the post-harvest processing of coffee and that preserves these compounds.

2.6 Rapid methods for evaluation of overall difference in product characteristics

2.6.1 Electronic-tongue (E-tongue)

Sensory characteristics of food may be assessed through the senses of smell, taste, sight, hearing, and touch. Odor and taste are undeniably the two most crucial factors in determining the deliciousness. To date, with the advances of science and sensor technology, electronic noses (Gardner & Barlett, 1994) and electronic tongues (Di Natale *et al.*, 1996; Otto & Thomas, 1985) offer promising analytical instrument as a taste and an odor sensor, respectively. The concept of both devices does not rely on discrimination of chemical constituents, but focuses on the recognition of the odor or taste itself via some quantitative expression (Lopetcharat & McDaniel, 2005). Both instruments consist of a sensing array, a measuring system and a multivariate analysis. Electronic noses have long been applied to examine food quality (Gardner & Barlett, 1994; Loutfi *et al.*, 2015). Applications of electronic tongues for food quality assessment are rather more recent and emerging as a promising tool, which have been demonstrated on many food stuffs and beverages, such as coffee (Buratti *et al.*, 2015; Domínguez *et al.*, 2014). Despite several studies on artificial sensory

systems, a question remains on their ability to match human perception. Real food dishes, which comprise many natural substances, mixing and suaveness of the tastes and odors, pose an interesting challenge for such systems.

Hence, to compare artificial sensory system, such as electronic tongues, with human perception, two approaches have been widely applied. One is to relate quantitatively specific chemicals for a particular food attribute to measurements by an electronic tongue (Legin *et al.*, 2003; Toko, 1996; Várvölgyi *et al.*, 2015). Owing to either the complexity of chemical constituents in food and their possible interactions or the lack of information on the specific chemicals, this approach is not often applicable. The other approach is based on a sensory test with either an expert panel or a group of consumers, which relies on a questionnaire with number scoring tasks on particular or overall attributes of food samples (Cetó *et al.*, 2015; Várvölgyi *et al.*, 2015).

The study of the relationship between the electronic tongue outputs and certain food attributes, may be achieved by a combination of various statistical analyses, such as principal component analysis (PCA), partial least squares (PLS), supported vector machine (SVM) and artificial neural network (ANN).

2.6.2 Pair-comparison test with multi-dimensional scaling (MDS)

Upon judging fine qualities of food stuffs, like coffee in most cases, consumers are aware when products are different, but they usually have a hard time to describe what make those differences (as either the differences are intricate or there are no suitable words to describe their experiences). In these situations, when any specific characteristics that differentiate samples from each other are not known, or obtaining reliable descriptors is not possible, asking consumers to judge the overall difference

between the samples become an effective and appropriate task to gauge the perception of consumers. To gain information regarding how different products are, many tasks may be used, e.g. sorting, napping, direct rating of overall difference, pair-comparison (Lawless & Heymann, 1999). Pair-comparison tasks are more suitable for a few products with a carry-over effect than evaluation tasks involving many independent samples, due to possible memory loss (Lau *et al.*, 2004). In the latter case, the Multi-Dimensional Scaling (MDS) analysis are usually performed (Lawless & Heymann, 1999) to unfold the perceptual space of a product set, whereby small distances indicate more similarity and large distances indicates less similarity (Tang & Heymann, 2002). A proximity/distance matrix, or a square matrix indicating the level of similarity/dissimilarity between a series of samples, is the input for the MDS analysis.

MDS algorithms are designed to minimize the difference between the disparity matrix created from models (written specifically for specific algorithms) and the distance matrix obtained from the actual data set. The absolute model, a rule to create the disparity matrix, is aimed to equate the disparity to the dissimilarity of the starting matrix. The difference between the disparity matrix and the distance matrix is called Stress. The lower the Stress, the greater the similarity between the disparity and the distance matrices. Normally, the amount of Stress less than 0.1 is acceptable for considering that the disparity matrix is able to represent the original distance matrix (Kruskal & Wish, 1978).

2.6.3 Generalized Procrustes Analysis (GPA)

Generalized Procrustes Analysis (GPA) is a mathematical technique commonly used in consumer studies to investigate the similarities and differences of

multiple data sets that maximizes the correlations between structures by creating new dimensions that capture correlations between the structures (Lopetcharat & McDaniel, 2005). The principle of GPA mathematically handles variations caused by subjects due to different understanding of attributes (in this case, different meanings and scale usages) (Arnold & Williams, 1986). Hence, this method allows one to study the relationship between very different measurements such as E-tongue and consumer's evaluation of overall differences without a complete set of pairings for all the samples.

2.6.4 R-index similar ranking test

To discriminate two confusable food stimuli or other products with sensory attributes with propose to control quality, reformulate food products, develop new formulas, specify the ingredients, identify shelf-life, sensory difference tests are frequently used. The exact evaluation of sensory difference/similarity between foods, as well as consumer acceptance/preference and concepts, is undoubtedly needed to optimize and maintain food quality (Lee *et al.*, 2009).

The R-Index is one of the sensory test methods to measure the magnitude of difference/similarity which was previously tested for the development of sensory difference tests to use for food quality control, product development and others. This measurement method is based on signal detection theory which is free of the response bias that will cause to invalidate difference testing methods including categorization and same/different and A-Not A tests. It is a nonparametric test that making no assumption about sensory distribution resulting into the easy interpretation to compute. The R-Index is also flexible in its application. Methods based on R-Index analysis have been used as detection and sensory difference tests, as simple alternatives

to hedonic scaling, and for the measurement of consumer concepts. The benefit on using of R-Index analysis is that it provides a size of different/similar among test products which is unlikely to obtain using the traditional sensory analysis due to the fact that those results only provide significance checks (Green & Swets, 1988; Lee *et al.*, 2004; Macmillan & Creelman, 2005). In addition, the R-Index analysis also can be applied to test products with more than 2 stimuli (a signal compared with a noise) at the same time (several signals compared with a noise) (Brown, 1974; O'Mahony, 1983, 1988). In terms of the application of R-index to the sensory discriminating tests, its values also can be compared over many experiments, if the same testing protocols are constantly used over the experiments as mentioned previously (Lee *et al.*, 2007).

R-Index analysis has also potential to be applied for multiple comparisons sensory test where there is a reference product and many other test products. During the test, the reference product (control) is treated as the noise and the test products are treated as the signals. The power of the discrimination of such this test is that the panelist are familiarized with the reference product before being advised to compare each test set to the reference one (Argaiz *et al.*, 2005; Lee *et al.*, 2007). This method can be applied in term of ranking test to rank the index value from ranking data when ranking between products is practical. This procedure was confirmed by Brown's (1974) prediction that a higher value would be calculated with ranking than with a rating procedure because of its forced choice nature (Chang & Carr, 2007). The protocol of R-index by similarity ranking is to rank the multi-test samples that include one product that has same similarity to a reference. The reference product will act as noise while the values provided from samples will be the signal. In this analysis, the frequencies for each rank are counted and the response matrix can be derived as in

signal detection rating. The number of categories in the response matrix equals to the number of products.

The procedure of R-index provides a more reliable result due to its natural forces panelists to re-taste the stimuli, whose taste/aroma they have forgotten. This design avoids the 'reversal errors', stimuli that should be assigned higher scores (e.g. more intense stimuli for intensity scaling) not being given those higher scores, because judges had forgotten the sensations elicited by stimuli tasted earlier in the experiment (Kim & O'Mahony, 1998; Lee *et al.*, 2001). After the interpretation of results, the R-index value is ranging from 100% through 50% to indicate the degree of similarity of test products compared with the control. Higher R-index refers to less similarity of sample to the control reference than the noise reference. In addition, degree of the difference can also be obtained in this test due to the R-index ranging from 50% through 0% indicating the degree of difference of products. The higher the R-index, the more different is the test product to the reference. The value 50% of R-index indicates the parity of test products with the control reference.

Considering the benefits of R-index similar by ranking, the studies on the importance of key volatiles to the character of targeted food had success on the application of this method combined with aroma recombination and omission test to find the important contributor odorants by omitting groups of odorant or single odorants from the aroma model (Buttara, 2014; Dharmawan *et al.*, 2009; Kiatbenjakul *et al.*, 2015; Lorjaroenphon *et al.*, 2008). When aroma model mixtures were served in three-digit coded sniffing bottles, panelists were asked to rank the degree of similarity of aroma attributes between complete recombination models and omitted models using R-

index method. In this case, R-index was present as the percentage of times of the sample which was ranked less similar to the control sample. After the comparison and determination of R-index value which usually could be calculated by John Brown computation (R-index by JB), the results could be used to compare with critical value from R-index statistical table two tailed test and confirmed the contribution of odorants prepared in model to the observed food matrix (Bi & O'Mahony, 1995).

From the principle of R-index by similar ranking and the previous applications, it is appropriate to use it in order to distinguish and applied to the complex test samples like coffee which comes from various origins in order to choose/adjust process and correlate the magnitude of coffee beverage differences to the volatile analysis and chemical determination.

2.6.5 Rapid-HS-SPME analysis

To control the quality of products that could be influenced by postharvest processing, robust techniques have been widely used not only to determine the amount of target defectives where the profile of results after analysis could be used as further information to control food processing. Volatile compounds have been claimed to be one of quality determinants of coffee. There are many studies indicating that those components could be used as quality indicators of both green and roasted coffee beans such as the change of key odorants of raw coffees during storage in various conditions and the choice of postharvest processing methods as shown in the studies of Scheidig *et al.* (2007) and Gonzalez-Rios *et al.* (2007). Toci&Farah (2008); Toci&Farah (2014) also found that volatile compounds can be used as potential marker to differentiate defective and non-defective beans in both raw and roasted coffee beans.

Three dominant volatiles; which are tridecane, dodecane, and tetradecane, were identified in potato taste notes of green coffee associated with Antestia bug (Jackels *et al.*, 2014).

Thus, the use of volatile profile could be an alternative way to separate the frauds/defects and indicate the difference between coffee during process in order to control its quality.

2.6.6 Antioxidant activities determination

Not only was volatile component verified as quality marker, but also antioxidant activity, which is contributed by phenolic compounds in green coffee beans (Cheong *et al.*, 2013; Somporn *et al.*, 2012). Phenolic compounds are distributed in various parts of coffee, such as coffee pulp, skin, and bean. In green coffee beans, chlorogenic acids, group of esters formed between quinic and hydroxycinnamic acids, are the predominant compounds ((Farah & Donangelo, 2006; Somporn *et al.*, 2012). Beside phenolic composition, total phenolic content, diphenyl-1-picrylhydrazyl (DPPH) assay, and ferric reducing antioxidant potential (FRAP) assay are common in vitro methods used for antioxidant activity determination (Cheong *et al.*, 2013; Ramalakshmi *et al.*, 2008; Somporn *et al.*, 2011, 2012). They were successful in the discrimination of green coffee produced under shade and unshaded method (Somporn *et al.*, 2012) as well as in green and roasted civet coffee produced by one species as described in Cheong *et al.* (2013).

Hence the analysis of antioxidant activity could provide more data for the discrimination of various coffee sample within the location, processing, and species.

CHAPTER III

MATERIAL AND METHODS

3.1 Raw Materials and Equipment

3.1.1 Raw materials preparation for the study of effects of postharvest processing practice on quality of coffee

3.1.1.1 Maturity of coffee cherries (ripe *vs* unripe)

Ripe and unripe coffee berries (*Coffea Arabica* L. cv. '*Catimor*'; harvested in February, 2015) were provided by Doi Tung Wildlife breeding center, Mae Fa Luang District, Chiang Rai, Thailand. The color of ripe berries was completely red (whole fruit), while the unripe ones were mix in colors from green to yellow, and pale red (Ortiz *et al.*, 2004; Smrke *et al.*, 2015). Coffee parchments of both ripe and unripe berries were obtained by passing through semi-dry process. The beans were dried under sun light in order to get dried parchment with not over 12% (w.b). Dried coffee parchments were packed separately in laminated sealed pouch and kept in desiccator containing silica gel at ambient temperature (about 25 °C) prior to analysis. To obtain green coffee bean samples, silverskins and hulls were gentle removed from parchments with porcelain mortar and pestle. Ground green coffee beans were prepared based on the methods described by Gonzalez-Rios *et al.* (2007) and Jackels *et al.* (2014) with some modifications. The green coffee beans were cooled down with liquid N_2 for 10 min, then ground at Hi-speed in blender with stainless steel jar (Waring Commercial, model 7011HS, New Hartford, CT, USA) for 1 min. Green coffee powders from every

treatment were kept in laminated sealed pouch and stored at –40 °C prior further analysis.

3.1.1.2 Effects of processing methods and the authenticity of green coffee quality

Coffee berries (*Coffea arabica* L. cv. '*Catimor*'; harvested in February, 2015) were provided by Doi Tung Wildlife Breeding Center, Mae Fa Luang District, Chiang Rai Province, Thailand (same plantation as in the section 3.1.1.1). To process civet coffee, the color of ripe berries were completely selected to be red (whole fruit) before feeding to civet cat to begin the digestive bio-processing for 4 h. After the completion of digestive process, the civet cat feces covering of the parchment were dried by sun drying for 12 h to reach the final moisture content of 12% (w.b.) and stored in laminated sealed pouch. The samples were kept in a desiccator containing silica gel at ambient temperature (about 25 °C) prior to analysis. Semi-dry processed coffee samples were prepared using red coffee berries from the same plantation as civet coffee. After the beans passed the process of pulping and drying, they were packed separately in the closed pouches before the production of green beans using the same method as civet coffee. Coffee parchment received from Slow Coffee^{TM}, Co. processed by wet processing at the plantation near Doi Tung Wildlife Breeding Center were used as control in this study. The fruit flesh was removed from selected ripe coffee berries by pulping process. Then the wet parchments underwented the fermentation process for 18-24 h. Wet parchment coffee was then dried under sunlight on bamboo trays to reach same the final moisture content and then stored prior to analysis as the previous

samples. All green coffees were prepared according to Gonzalez-Rios *et al.* (2007) and Jackels *et al.* (2014) as described in section 3.1.1.1.

3.1.1.3 Processing methods, subdividing processing, locations of processing, countries of processing, animal species, and coffee species. Effects on volatile compounds of roasted coffee

Parameters of this study included processing methods (civet and non-civet processing), subdivided processing (civet feeding condition: caged and free-range), locations of processing (plantation area: Doi-Chang, Doi-Tung and Loei), countries of processing (Thailand, Indonesia and Vietnam), animal species (civet and weasel) and coffee species (Robusta and Arabica). The samples were obtained as follows:

Thai civet digested coffee beans with different feeding methods (caged (Cg) and free-range(Fr) were collected from different harvesting areas: 1) Doi Tung Wildlife breeding center, Mae FaLuang District, Chiang Rai (Arabica, 2014-2015 harvest season) from caged and free-range animals) 2) Doi Chaang, Maesai District, Chaing Rai (Arabica; 2014-2015 harvest season from free-range animals) (Doi Chaang Coffee™, Chiang Rai) and 3) Loei Province (Robusta 2014-2015 harvest season from free-range animals). Wet-processed samples of Thai Arabica coffee beans (from Doitung, Chiang Rai) and (Slow Coffee™, Chiang Rai) and the commercial samples of Arabica civet processed coffee from Indonesia and Vietnam were obtained in order to compare the effects of treatments in this study. All the beans collected from harvesting

area, except the commercial ones supplied from Indonesia and Vietnam, were dried to 11% (w.b) and were professionally roasted to medium-roast level according to each estate's roasting technique. The roasted samples were vacuum packed and stored at 4 ˚C in sealed aluminum bags fitted with a CO_2 degassing valve before determined physicochemical properties. Ground roasted coffee beans were prepared using the same method as green coffee powder as mentioned in section 3.1.1.1. Details of locally purchased roasted beans of different species, purity and location are showed in Table 8, which sample number 1 and number 4, they were harvested in same plantation in section 3.1.1.1 but different lot and time.

Table 8 Codes of roasted coffee beans sample

Sample	**Estate and origin**	**Coffee type**	**Processing method**	**Feeding condition**	**Note/Brand**
1	Doi Tung, Chiang Rai, Thailand	Arabica	Civet	Caged	
2	Doi Tung, Chiang Rai, Thailand	Arabica	Civet	Free range	
3	Doi Chang, Chiang Rai, Thailand	Arabica	Civet	Free range	Doi Chaang Coffee™
4	Doi Tung, Chiang	Arabica	Normal wet	N/A	Premium Slow Coffee™

	Rai, Thailand				
5	Doi Chang, Chiang Rai, Thailand	Arabica	Normal wet	N/A	Premium Doi Chaang Coffee™
6	Loei, Thailand	Robusta	Civet	Free range	
7	Vietnam	Arabica	Weasel	Caged	Lien Saigon Weasel Coffee
8	Indonesia	Arabica	Civet	Caged	Commercial Mandailing Estate Coffee™

3.1.1.4 Processing methods, subdivided processing, locations of processing, countries of processing, animal species and coffee species. Effects on sensorial properties of coffee brew

Roasted coffee was used in this section of study. The majority of samples were the same as described in section 3.1.1.3 except the addition of Indonesian civet coffee of high purity grade. The list of roasted coffee is showed in Table 9.

Table 9 Codes of coffee bean samples

Sample	Estate and origin	Coffee type	Processing method	Feeding condition	Note/Brand
C-T-A-Cg-Tg	Doi Tung, Chiang Rai, Thailand	Arabica	Civet	Caged	
C-T-A-Fr-Ch	Doi Chang, Chiang Rai, Thailand	Arabica	Civet	Free range	Doi Chaang Coffee™
C-T-A-Fr-Tg	Doi Tung, Chiang Rai, Thailand	Arabica	Civet	Free range	
N-T-A	Doi Tung, Chiang Rai, Thailand	Arabica	Normal wet	N/A	Premium Slow Coffee™
C-I-A-Cg-Com	Indonesia	Arabica	Civet	Caged	Commercial Mandailing Estate Coffee™
C-I-A-Cg-Pure	Indonesia	Arabica	Civet	Caged	High purity Burgayo Coffee™
C-T-R-Fr	Loei, Thailand	Robusta	Civet	Free range	
W-V-A	Vietnam	Robusta	Weasel	Caged	Lien Saigon Weasel Coffee

3.1.2 Raw materials preparation for studies of the effect of heat pump drying on physicochemical properties of Arabica coffee

3.1.2.1 Raw material preparation

Arabica coffee cherries (*Coffea arabica* L. cv. *Catimor*) were collected from Doi Chang, northern Thailand, Chiang Rai province at the harvesting period of November, 2016. To prepare heat pump dried coffee, the sample of wet parchment Arabica coffee were dried in a heat pump dryer at Faculty of Engineering, Mahasarakham University. The drying air temperature was 40, 45 and 50 ˚C at 23, 16 and 11 h, respectively with an air velocity of 0.6 m/s. The two other drying treatments were tray drying and sun drying. For the tray drying, parchments coffee was uniformly distributed over stainless steels trays (53 × 72 × 3cm) in a layer of 0.5 cm thickness and dried at 50 ˚C for 18 h in an electric convection hot-air dryer (Kluay Nam Thai, Bangkok, Thailand). The sun dried coffee was prepared by uniformly distributing the wet parchment coffee on a fabric with a cover of polymer mesh to prevent contamination. The drying was performed in a ventilated open area, which allowed natural air flow to dry the samples at an ambient room temperature of 20 to 30 ˚C and 70% relative humidity for 3 days.

The samples for heat pump drying treatments were compared with the tray dried ones (50 ˚C) and the sun dried coffee (control) produced from the same lot and plantation.

The samples subjected to any of the drying treatments needed to have a moisture content below 12% (w.b.) and a water activity less than 0.6. The accuracy of heat pump dryer was $\pm$ 1°C. After drying, all samples of coffee parchment were

vacuumed packed and stored in laminated sealed pouches prior to roasting. To obtain green coffee bean samples for the determination of antioxidant activities, all green coffee were prepared in the way described in section 3.1.1.1

The green coffee samples (100 grams) from each drying treatment were roasted with a single batch coffee sample roaster (Sample Pro-G2, Nexu International, LTD., Hong Kong) at 200 °C, 15 min in order to prepare the medium roasted coffee. Once the roasting process was terminated, cool air was immediately blown into the chamber for 4 min to cool down the roasted beans. The roasted coffee was then removed from the roaster and placed in a metal container to cool until it reached room temperature. The roasted samples were then vacuum packed and stored 4 ˚C in sealed aluminum bags with a CO_2 de-gassing valve. The roasted beans were brought out to reach equilibrium with the room temperature prior to every analysis.

3.1.3 Chemicals and reagents

3.1.3.1 Chemicals and reagents for antioxidant activity determination

Name	Company	Country
Folin-Ciocalteu's phenol reagent	Merck	Germany
97% Gallic acid	Sigma	USA
Diphenyl-1-picrylhydrazyl (DPPH)	Sigma	USA
2,4,6-tris-2-pyridyl-1,2,5-triazine (TPTZ)	Sigma	USA
Ferric chloride hexahydrate	Merck	Germany
(±)-6-Hydroxy-2,5,7,8-tetramethylchromane -2-carboxylic acid (Trolox)	Sigma	USA
Methanol (HPLC grade)	Merck	USA
Acetone (HPLC grade)	Merck	USA

3.1.3.2 Chemicals and reagents for chlorogenic acids determination

Name	Company	Country
Carrez's reagents Solution I &II	Sigma	USA
Methanol (HPLC grade)	Sigma	USA
Chlorogenic acid (5-Caffeoylquinic acid (5-CQA)	Sigma	USA

Neochlorogenic acid (3-Caffeoylquinic acid (3-CQA))	Sigma	USA
Cryptochlorogenic acid (4-CQA)	Sigma	USA

3.1.3.3 Chemicals and reagents for caffeine determination

Name	**Company**	**Country**
Carrez's reagents Solution I &II	Sigma	USA
Methanol (HPLC grade)	Sigma	USA
Acetic acid	Merck	Germany

3.1.3.4 Chemicals and reagents for sugar determination

Name	**Company**	**Country**
Acetonitrile	Merck	Germany
Sucrose (analytical standard)	Sigma	USA
Glucose (analytical standard)	Sigma	USA
Fructose (analytical standard)	Sigma	USA

3.1.3.5 Chemicals and reagents for volatile determination

3.1.3.5.1 General chemical

Name	Company	Country
Anhydrous diethyl ether	Fisher Scientific	USA
Anhydrous sodium sulfate	Fisher Scientific	USA
Dichloromethane	Fisher Scientific	USA
Hydrochloric acid	Fisher Scientific	USA
Methanol (HPLC grade)	Fisher Scientific	USA
Sodium bicarbonate	Fisher Scientific	USA
Sodium chloride	Fisher Scientific	USA
Sodium hydroxide	Fisher Scientific	USA
95% L-cysteine	Sigma	USA

3.1.3.5.2 Unlabeled reference standards

All authentic reference standards listed in Appendix A, including n-alkane standard (C5-C30), 2-methyl-3-heptanone and 1-heptanethiol were purchased from Sigma-Aldrich Chemical Co. (USA), except for 4-vinylguaiacol that was purchased from Lancaster Co. (USA).

3.1.3.5.3 Isotopically labeled standards

The following labeled compounds were obtained from commercial sources; [$^2H_3$]-Acetic acid, [$^2H_2$]-3-Methylbutanal, [$^2H_3$]-Guaiacol and [$^2H_3$]-*p*-Cresol (CDN, Canada); [$^2H_6$]-Dimethyl trisulfide (Sigma-Aldrich, USA). The following compounds were synthesized according to published procedures:

Name	**Published procedures**
[$^2H_2$]-2-Methylpropanal	(Wu, 2017)
[$^2H_2$]-2-Methylbutanal	(Wu, 2017)
[$^2H_3$]-3-(Methylthio)propanal	(Sen & Grosch, 1991)
2-[$^2H_2$]-Furfurylthiol	(Sen & Grosch, 1991)
[$^{13}C_2$]-Furaneol®	(Sen *et al.*, 1991)
[$^2H_2$]-Linalool	(Steinhaus *et al.*, 2003)
[$^2H_2$]-3-Methylbutanoic acid	(Steinhaus & Schieberle, 2005)
[$^2H_4$]-β-Damascenone	(Kotseridis *et al.*, 1998)
[$^{13}C_2$]-2-Phenylethanol	(Schuh & Schieberle, 2006)
[$^{13}C_2$]-2,3-Pentandione	(Hausch *et al.*, 2015)

Name	**Published procedures**
[$^2H_4$]-Hexanal	(Steinhaus *et al.*, 2009)
[$^2H_3$]-1-Octen-3-ol	(Lin *et al.*, 1999)
[$^2H_3$]-2-Methyl-pyrazine	(Fang & Cadwallader, 2013)
[$^2H_5$]-2-Ethylpyrazine	(Fang & Cadwallader, 2013)
[$^2H_3$]-2,3-Dimethylpyrazine	(Fang & Cadwallader, 2013)
[$^2H_3$]-2,6-Dimethylpyrazine	(Fang & Cadwallader, 2013)
[$^2H_{10}$]-2,6-Diethylpyrazine	(Fang & Cadwallader, 2013)
2-[$^2H_3$]-3,5-Trimethylpyrazine	(Fang & Cadwallader, 2013)
[$^2H_3$]-2,3-Diethyl-5-methylpyrazine	(Fang & Cadwallader, 2013)
[$^2H_5$]-3-Ethyl-2,5-dimethylpyrazine	(Fang & Cadwallader, 2013)
[$^2H_3$]-Methanethiol	(Guth & Grosch, 1994)
[$^2H_3$]- 4-Vinylguaiacol	(Scheidig *et. al.*, 2007)
[$^2H_5$]-4-Ethylguaiacol	(Lahne, 2010)

3.1.4 Apparatus

Instruments	Model	Company
Tray dryer	-	Kluay Nam Thai, Co. Ltd., Thailand
Sample roaster	Sample Pro-G2	Nexu International, Ltd., Hong Kong
Chromameter	CR-300	Minolta, Japan
Water activity meter	-	AquaLab, USA
High performance Liquid Chromatography (HPLC)	1100 series	Agilent Technology, USA
High performance Liquid Chromatography (HPLC)	3000 series	Fisher Scientific, USA
Gas Chromatography Mass Spectrometry (GC/TOF-MS)	7890 N	Agilent Technology, USA
Gas Chromatography Mass Spectrometry (GC-MS)	6890N/5973N	Agilent Technology, USA
1,2 cm SPME fiber	DVB/CAR/PDMS	Supelco, USA
Coffee Grinder	80335R	Hamilton Beach®, USA

Instruments	Model	Company
pH meter	CyberScan pH 1000	Eutech, Singapore
Spectrophotometer	Genesys^{TM} 20	Fisher Scientific, USA

3.2 Effects of postharvest processing practice on quality of coffee

3.2.1 Maturity of coffee cherries (ripe *vs* unripe)

3.2.1.1 Determination of antioxidant activity

3.2.1.1.1 Extraction of samples

To extract the phenolic compounds, coffee bean powders were extracted according to protocol of Somporn *et al.* (2012) with some modification. One gram of coffee powder was placed into a test tube and mixed with 40 mL of methanol/water mixture (50:50). To adjust pH 2.0, HCl was added. The tube was then shaken at room temperature for 1 h. After centrifugation at 2500 g for 10 min, the supernatant was collected. Forty mL of acetone/water mixture (70:30) were added to the coffee bean residue, then shaken and centrifuged. The supernatant was collected and mixed with the one extracted by methanol/water mixture. The phenolic extracts were used to determine the total phenolic content and antioxidant activity of green coffee beans. All analyses were conducted in duplicate.

3.2.1.1.2 Determination of total phenolic content

The determination of total phenolic content (TPC) was carried out using the Folin-Ciocalteu method according to Cheong *et al.* (2013). Gallic acid was used as a standard. In order to measure the TPC, 1 mL of the phenolic extract was diluted with deionized water. One mL of the diluted extract was then mixed with 5 mL of 10% diluted Folin-Ciocalteu's reagent (Merck Chemicals, Darmstadt, Germany) in deionized water. The mixture was allowed to stand for at least 3 min before 4 mL of 7.5% (w/v) sodium carbonate were added. The reaction mixture was then mixed and allowed to stand at room temperature for 60 min. The absorbance of the solution was measured at 765 nm, using a spectrophotometer (Genesys^{TM} 20, Themo Fisher Scientific, Waltham, MA, USA). A control sample was prepared by replacing the diluted extracts with deionized water. A standard curve of gallic acid with an exact amount of concentration ranging from 5-80 mg/L (correlation coefficient: $r^2 = 0.9806$) was prepared for TPC calculation using the same method of determination. The calibration is shown in Appendix A.8

3.2.1.2.3 Determination of the free radical scavenging activity by DPPH assay

The free radical scavenging activity of the phenolic extract was determined according to the method described by Cheong *et al.* (2013). An aliquot of 3.9 mL of diphenyl-1-picrylhydrazyl (DPPH) solution (7.5 mg DPPH in 100 mL of methanol) was mixed with 0.1 mL of each phenolic extract. The mixture was well mixed and placed in the dark at room temperature for 10 min. The absorbance at 517 nm was then measured. A control was prepared by mixing 3.9 mL of DPPH

solution with 0.1 mL of methanol. The free radical scavenging activity for each solution was calculated using the following formula showed in equation 3.1:

$$\text{Radical Scavenging activity } (\%) = \frac{(A_{control} - A_{sample})}{(A_{control})} \times 100 \qquad (3.1)$$

3.2.1.1.4 Determination of the antioxidant activity by ferric reducing antioxidant potential (FRAP) assay

The FRAP assay was performed following the method described by Cheong *et al.* (2013). FRAP reagent was prepared by mixing of 2.5 mL of 10 mM 2,4,6-tris-2-pyridyl-1,2,5-triazine (TPTZ) solution in 40 mM HCl, 2.5 mL of 20 mM ferric chloride hexahydrate in deionized water, and 25 mL of 0.3 mM acetate buffer (pH 3.6). The mixed solution was then incubated at 37 ˚C for 30 min. Fifty microliter of the phenolic extract were diluted to 1 mL with deionized water. To evaluate the reducing power, 900 µL of freshly prepared FRAP reagent were mixed with 90 µL of deionized water and 10 µL of the phenolic extract. After incubating at 37 °C for 30 min, the absorbance was measured at 595 nm. The reducing power of coffee beans was expressed in mg Trolox/g coffee beans. A standard curve of Trolox in ethanol with an exact concentration ranging from 100-800 mg/L (correlation coefficient: $r^2 = 0.9775$) was prepared following this procedure for reducing power of green coffee beans calculation as shown in Appendix A.9.

3.2.1.2 Determination of volatile compounds

Two types of green coffee beans processed from ripe and unripe coffee berries were characterized for their volatile components by Head Space Solid Phase Micro Extraction-Gas Chromatography/Mass Spectrometry (HS-SPME-

GC/MS) technique using 2-methyl-3-heptanone as an internal standard as per procedure described in Appendix A.1.

3.2.1.3 Statistical analysis

The means of scores from antioxidant and volatile analysis were analyzed for significant difference at $p \leq 0.05$ by using an independent sample t-test. All statistical analyses were performed using SPSS Statistics software version 13.0 (SPSS Inc., Chicago, IL, USA).

3.2.2 Effects of processing methods on the authenticity of green coffee qualities

3.2.2.1 Determination of antioxidant activity

3.2.2.1.1 Sample extraction

Extraction of antioxidant in all green beans used the same method as described by Somporn *et al.*, (2012).

3.2.2.1.2 Determination of total phenolic content

Determination of total phenolic content (TPC) was conducted using the method of Cheong *et al.* (2013) as previous described in section 3.2.1.1.2.

3.2.2.1.3 Determination of the free radical scavenging activity by DPPH assay

The free radical scavenging activity of the phenolic extract was determined according to the method described by Cheong *et al.* (2013).

3.2.2.1.4 Determination of the antioxidant activity by ferric reducing antioxidant potential (FRAP) assay

The FRAP assay was performed following the method described by Cheong *et al.* (2013).

3.2.2.2 Determination of volatile compounds

Volatile compounds of all green coffees were extracted by HS-SPME/GC-MS as described in 3.2.1.2.

3.2.2.3 Determination of Odor Activity Value (OAV)

This method was used to determine the potential of odorant identified in this study to characterize coffee sample. An approximate odor activity value (OAV) of a compound was calculated as the ratio of its concentration in coffee to its odor threshold in water per equation 3.2. The odor detection thresholds in water of the selected target volatiles were obtained from the literature.

$$\text{Odor Activity Value (OAV)} = \frac{\text{Concentration of volatile compounds}}{\text{Odor thresholds in water reported in literature}} \quad (3.2)$$

3.2.2.4 Evaluation of coffee brew by electronic tongue (E-tongue)

To determine the differences between coffee samples, electronic tongue was build in-house and equipped by a low-noise data acquisition circuit with the NI-6009 interface and controlled by the Labview software package. The E-tongue's electrode comprised gold wire (1-mm diameter), carbon (Staedtler^{TM} 2H, 2-mm diameter), and platinum wire (0.3-mm diameter of 0.5 cm in length), embedded in a Teflon rod. The Pt wire was a reference electrode. To obtain green coffee powder, hulls

and the silver skin of coffee parchments of samples were removed. Green coffees were then ground with a coffee grinder. The steep cup methodology described by Lyman *et al.* (2003) with a slight modification were used to prepare coffee brew sample. A fixed volume (21.4 mL) of water were boiled (98-100 °C) for 4 minutes. Then, 1.4 g of each green coffee powder was steeped through cotton strainer and cooled down to 50 ± 2 °C. Deionised water was used as control to calibrate the ability of e-tongue to discriminate green coffees. The electrode was submerged approximately one centimeter under the coffee samples surface. The electrode was manually cleaned with a detergent solution, rinsed and calibrated with deionized water before measurement in every analysis.

3.2.2.5 Statistical Analysis

The significant difference of means calculated from the antioxidant analysis of green coffees as well as concentration of volatiles were analyzed by One-way ANOVA, the multiple comparison test, least significant difference (LSD). LSD of the analyzed samples were performed using SPSS Statistics software version 13.0 (SPSS Inc., Chicago, IL, USA). Cyclic voltammertic (CV) characteristic data of food samples on E-tongue were preprocessed by the first derivative of signals for row preprocessing (Oliveri *et al.*, 2010). CV measurements were conducted with the scan range of -1.5 to +1.5 V, the rate of 0.2 V/s with three scan loops. The CV current data for each loop were preprocessed by extracting averaged current values at a 0.25 V interval. All E-tongue data were arranged in a matrix and preprocessed by column centering technique as shown in Oliveri *et al.* (2010). The data acquisition and the PCA of this instrument were obtained using the LabVIEW software package. The mean value and the standard deviation of each parameter were determined for the data set. A zero-centered co-variance matrix was constructed as an input for the PCA calculations.

3.2.3 Processing methods, subdivided processing, locations of processing, countries of processing, animal species and coffee species. Effects on volatile compounds of roasted coffee

3.2.3.1 Determination of volatile compounds

Roasted sample number 1-8 were characterized by the volatile profiles determined by head space solid phase microextraction-gas chromatography/mass spectrometry (HS-SPME/GC-MS) with slight modification of the conditions. The same chemicals and equipment as described in 3.2.1.2 were used. The detail of this determination is described in Appendix A.2.

3.2.3.2 Determination of Odor Activity Value (OAV)

To find the potential of odorant identified in this study to importance to the characterize the coffee. An approximate odor activity value (OAV) of a compound was calculated using equation 3.2.

3.2.3.3 Statistical Analysis

The significant difference in the concentration of volatiles were analyzed by using the multiple comparison test, least significant difference (LSD). LSD of the analyzed samples were performed using SPSS Statistics software version 13.0 (SPSS Inc., Chicago, IL, USA). Multivariate analysis of the significant key volatiles data sets of 8 coffees was carried out using principal component analysis (PCA) by XLStat v.2018.2.10 (Addinsoft, Paris, France). A score plot of PCA was used to study the distribution of the significant key volatiles determined by univariate analysis (One-way ANOVA) relative to the overall variability of the data sets obtained from three brew coffees. A biplot was then plotted on a single scaling plot where objects (8 coffees)

were pointed and the variables were represented by eigenvectors relating each variable to each component.

3.2.4 Processing methods, subdivided processing, locations of processing, countries of processing, animal species, and coffee species. Effects on sensorial properties of coffee brew

3.2.4.1 Coffee brew preparation

All coffee bean samples were prepared on the day of the testing by grinding using a coffee grinder set at the grinding level of 6 to obtain the fine ground particle size. The ground coffee bean samples were packed separately in laminated resealable pouches. The steep cup methodology was used to prepare coffee beverages. About 80 grams of a coffee bean sample was steeped through a cotton strainer using 100 mL hot water 98-100 ˚C) for 4 min (Lyman *et al.*, 2003). Then, the ground coffee particles were discarded. The coffee samples were served in a 60 mL plastic cup (EPP, Thailand) with 20 mL of freshly brewed coffee in the cup at 70 ± 2 ˚C. Samples were presented in pair, with a holding time in the cup of no more than 2 min to reach the final temperature of approximately 40 ± 2 ˚C.

3.2.4.2 E-tongue measurement

In section 3.2.2.4, the E-tongue has already been provided. This quasi Pt reference electrode was described as suitable for electrochemical applications (Kasem & Jones, 2008). It is use of the Pt quasi-reference electrode for electronic tongue applications was demonstrated (Chodjarusawad *et al.*, 2016). The performance of this quasi-reference electrode in comparison to the standard Ag/AgCl reference has also been investigated and will be published elsewhere. This robust design allowed the

coffee samples to be measured by E-tongue at the serving temperature, alongside the panel test. From each sample of the eight coffee beans, 30 mL of brewed coffee were collected, stored at 70 ± 2 °C and allowed to cool down to approximately 40-45 °C before each measurement. After each measurement, the electrodes were applied as section 3.2.2.4.

3.2.4.3 Overall difference testing by consumers

The overall difference test by consumers was divided into three experiments as follows:

1) Comparing civet coffee from different feeding conditions (caged vs. free range)

2) Comparing Thai civet coffees from the same area (Doitung, Chiang Rai, Thailand) and near-by area (Doichang, Chiang Rai, Thailand) with different feeding conditions (caged vs. free-range)

3) Comparing civet coffees from different countries (Thailand vs. Vietnam vs. Indonesia), processing method (civet vs. weasel) and bean types (Robusta vs. Arabica)

For each panel test experiment, 12-14 consumers (different consumers for each experiment) were asked to rate the overall difference of six pairs of coffee samples on a four-point overall-difference scale using two-step questioning procedure: 1st) asking the consumers to declare their perception of differences (Yes/No) and 2nd) asking them to declare the level of sureness of their differences decision (Sure/Not sure). This resulted in four categories of overall differences: 1 = Not different-sure, 2 = Not-

different-not sure, 3=Different-not sure and 4=Different-sure. For the sensory test, consumers who were all coffee drinkers were provided with consent forms consistent with human subjects' approval and a ballot as shown in Appendix B.2. All responses were collected using paper ballots.

The order of presentation was randomized with the Williams design to account for presentation order effects. Panelists were prompted to taste the sample by sipping the sample presented on the left first followed by sample on the right. Sipping water between the two samples was mandatory to rinse their palettes. In addition, to prevent carry-over effects from one pair test to another, panelists were obliged to follow a rinse procedure of sipping, then, spitting the water out.

3.2.4.4 Statistical analysis

3.2.4.4.1 E-tongue

The statistical analysis of E-tongue was done by PCA analysis as described in section 3.2.2.5.

3.2.4.4.2 Overall Difference Test

The overall difference test was divided into three groups, with four coffee samples in each group. Each experiment was designed to address a certain set of hypotheses by making a comparison between a specific set of coffee samples. For each experiment, a proximity matrix was created directly from the proportion of consumers who rated that "the pair of coffee samples were surely different". The estimate of difference-sureness rating means for each coffee samples were done using Analysis of Variance (ANOVA). Then the matrices were subjected to separate Multi-Dimensional Scaling (MDS) analyses. Scaling by Majorizing a Convex Function (SMACOF), a

matrix MDS algorithm with Absolute MDS option, was used with the criterion of Kruskal's Stress1 less than 0.1. All statistical analyses were performed at 95% confidence level using XLStat v.2014.1.10 (Addinsoft, Paris, France).

3.2.4.4.3 Finding relationship between E-tongue and MDS solutions using Generalized Procrustes Analysis (GPA)

Generalized Procrustes Analysis (GPA) was performed on the PCA loading scores (3 dimensions extracted) of all eight samples from E-tongue evaluation and three sets of MDS-coordinates (using 3-dimension solutions) of the four samples from each experiment. There were a total of four data matrices: E-tongue, Experiment 1, Experiment 2 and Experiment 3 in this analysis. Gower procedure with an option of incomplete data set was used to study the correlation between perceptual construct similarities and differences between E-tongue and consumers' overall difference spaces (represented by MDS spaces from 3 studies). GPA dimensions that were highly correlated were further investigated for attributes that may have caused the significant correlations (PCA factor loading). All statistical analyses were performed at 95% confidence level using XLStat v.2014.1.10 (Addinsoft, Paris, France).

3.3 Effect of heat pump drying on physicochemical properties of Arabica coffee

The dried green coffee samples from all heat pump dried coffee, tray drying and control (sun drying) was measured for the following properties (see 3.3.1.).

3.3.1 Physical properties

3.3.1.1 Color evaluation

Green coffee powder color sampling from different drying treatments were directly measured with a Minolta chroma meter (CR-300, Minolta, Japan). To obtain the values of lightness (L*), redness (a*), yellowness (b*), chroma (C*), hue (h^0) and color difference (ΔE*) were compared with control treatment (sun drying).

3.3.1.2 Determination of moisture contents and water activity in green coffee

Moisture contents of all green coffee were determined in triplicate according to ISO 6673 (Reh *et al*., 2006). Approximately 10 g of whole green coffee beans are dried for 16 ± 0.5 h. via an electrically heated drying oven with forced air ventilation at 105 ˚C. Water activity (a_w) was measured by an instrument using the chilled mirror dew point technique (Series 3 TE, Aqualab, Pullman, WA, USA).

3.3.2 Chemical properties

3.3.2.1 Extraction of chlorogenic acids from green coffee

All coffee beans were milled with MM 400 homogenizer at 30 Hz for 1 min (Retsch, Haan, Germany). Powdered sample (0.5 g) each was ultrasonicated with 40% aqueous methanol 10 mL in a 15-mL centrifuge tube for 20 minutes at room temperature. The tube was centrifuged (5000 × g for 5 min) and 1 mL supernatant was taken. The followed clarification was done by adding of Carrez's reagents (solution I, hexacyanoferrate(II) trihydrate 15 mg/mL; and solution II, zinc sulfate heptahydrate 30 mg/mL) 100 μL, respectively (Duarte *et al.*, 2010). The mixture was centrifuged and then filtered through 0.22 μm PVDF filter. The sample solution was diluted with 40%

aqueous methanol before analysis. Standards of chlorogenic acids were prepared in 40% aqueous methanol.

3.3.2.2 HPLC analysis of chlorogenic acids from green coffee

The separation and identification of all chlorogenic acids were performed on high performance liquid chromatography coupled with photodioarray detector (DAD) and masss selective detector (MS) of which the specific details and the calculated response factors (R_f) are given in Appendix A.3.

3.3.2.3 Antioxidant activities determination

3.3.2.3.1 The extraction of phenolic compounds from green coffee matrix

To obtain green coffee bean samples for antioxidant extraction, sample were prepared using the same method as described in 3.2.1.1.1 as well as the phenolic extraction from ground green coffee.

3.3.2.3.2 Determination of total phenolic content

Determination of total phenolic content (TPC) was conducted using the method described by Cheong *et al.* (2013).

3.3.2.3.3 Determination of the free radical scavenging activity by DPPH assay

The radical scavenging activity of the phenolic extract was tested according to the method described by Cheong *et al.* (2013).

3.3.2.3.4 Determination of the antioxidant activity by ferric reducing antioxidant potential (FRAP) assay

The FRAP assay was performed following the method described by Cheong *et al.* (2013).

3.3.2.4 Caffeine determination

To determine the caffeine content, caffeine was extracted from green coffee powder samples using hot water according to AOAC (1995). Carrez I and II solution (Sigma, Germany) were added for the clarification after the extraction was done, it was filtrated through filter paper no.1, 0.45 µm pore size. The aliquot of 20 µL of extract was injected to HPLC, Agilent 1100 series, with diode-array detector (Agilent Technologies Inc., Palo Alto, CA, USA) to separate on Zorbax-Eclipse-XDB-C18 column (4.6 mm × 250 mm, 5 µm) with isocratic gradient using methanol: water: acetic (80:19:1) with the monitored wavelength 276 nm. Caffeine concentration was determined based on peak area/peak height of standard caffeine. The analysis was performed in duplicate.

3.3.2.5 Sugar determination

The determination of total sugars in green coffees was performed according to AOAC method 982.14 (AOAC, 2000). A sample (2 g) of fine green coffee powder was extracted twice with 50 ml of petroleum ether to remove fat prior to analysis. After the extraction was done, the residual petroleum ether was removed with gentle stream of N_2. A volume (100 ml) of methanol/deionized water (1:1) was added and weighed. The sample was then incubated to 80 ˚C for 25 min, stirred, then cooled to room temperature and added alcohol to original weight. The extract was filtered

through 0.45 µm nylon syringe filter. Sugar extracts (10 µL) were injected into Zorbax NH_2 column (4.6 x 250 mm, id., 5µm) being part of HPLC, Agilent 1100 series apparatus with diode-array detector (Agilent Technologies Inc., Palo Alto, CA, USA). The flow rate of mobile phase consisted of Acetonitrile: deionized water (70:30) was 1.5 ml/min. The analysis was performed in duplicate. Amounts of sugars were quantified based on peak area and height of each sugars compared with sugar standard solutions (fructose, glucose, sucrose) (Sigma-Aldrich Chemical Co., St. Louis, MO).

3.3.2.6 Determination of amino acids

3.3.2.6.1 Total amino acid profile determination

Total amino acids of green coffee were determined by HPLC using Hypersil Gold C-18 column connected with fluorescence detector. All green coffee beans were cooled down with liquid N_2 for 10 min, then ground at hi-speed in blender with stainless steel jar (Waring Commercial, model 7011HS, New Hartford, CT, USA) for 1 min to prevent the loss of bioactive components. Green coffee powder was hydrolysed and derivatized by 6 N HCl and heated at 110 ˚C for 22 h. The samples were then mixed with AccQ-fluor derivatization reagent and heated to 50 ˚C for 10 min. Sodium acetate buffer (pH 4.9) with 60% acetonitrile was used as eluent.

3.3.2.6.2 Free amino acid determination

The content of free amino acids of green coffee and standards were determined by (HPLC). About 100 mg of green coffee powder was extracted with deionized water and derivatized with AccQ-Fluor reagent before determination with Hypersil Gold C-18 column connected to fluorescence detector. Binary gradient consisting of 9.1% (v/v) AccQ-Tag Eluent A concentrate (mobile phase A) and 60%

(v/v) aqueous acetonitrile (mobile phase B) were used to elute the extract. The analyse were performed in triplicate.

3.3.2.7 Total Fatty acid determination

Total oil of green coffee powders were extracted with soxhlet extraction according to EEE (Firestone, 1989). The oil of sample was derivatized to fatty acid methyl esters (FAMEs) and measured by gas chromatography-mass spectrometry (GC-MS) (Firestone, 1989).

3.3.3 Sensory properties of coffee

3.3.3.1 Determination of sensory analysis: R-index by ranking test

The purpose of this test is to study the effects of heat pump drying on sensorial properties of coffee as well as select coffee samples to further determination of the selected key volatiles and to make a comparison between treatments. Coffee brew from every drying treatment were evaluated their overall difference in aroma characteristics in comparison with benchmark product (control), which was the sun dried coffee. R-index by similar ranking test was applied for this comparison. Roasted coffee samples were brought out to room temperature (~25 ˚C) to reach equilibrium before further processed. Samples were frozen at -70 ˚C, 1 h prior to grinding with Hamilton Beach Fresh-Grind Coffee Grinder to obtain a fine particle size with a particle size between 1.77-2.36 mm. Ground roasted coffee and coffee brew were prepared on the date of sensory analysis no later than 12 h before the conduct of the test to preserve freshness. To prepare coffee brew, a modification of steep cup methodology (Lyman *et al.*, 2003) was used in this study in order to prepare coffee

beverages following gold standard cup of SCAA. About 8.5 gram of coffee powder were brewed with 150 mL of hot odorless water (98-100 ˚C) for 5 min. The human subject protocol number 17658 was approved by The University of Illinois at Urbana-Champaign Institutional Review Board (IRB) to conduct this sensory analysis as described on Appendix B.1. Coffee brews were then cooled immediately in an ice bath to stop the chemical reaction during brewing. All brew samples (50 mL) were then transferred to 125-mL Teflon sniff bottles (Nalgene PTFE wash bottle without siphon tube; Nalge Nunc International, Rochester, NY) covered with aluminum foil to avoid assessor bias. A set of 5 test samples, including 1 reference bottle; sun dried coffee and another sun dried sample as blind control sample were labeled with random 3-digit codes, and presented to the panelists with control sample. The serving order was random and balance. Thirty students and staffs (21 females and 9 males; 18-54 years old) participated in this test. They were asked to sniff the expressed air by gently squeezing the bottle, and ranked the test samples on how similar they were to the control sample. The other details are showed on Appendix B.1. John Brown computation as described by O'Mahony (1992) was used to calculate the R-index which is the percentage of times the sample is ranked less similar to the control sample. The percentage of R-index was compared to the critical value (n = 30) for two-tailed test at $\alpha = 0.05$ to detect the significant differences from sun dried coffee.

3.3.4 Volatile profile of coffee

3.3.4.1 Determination of target key volatile compounds in coffee brew

Three tests were selected from the results of previous analysis; antioxidant activity determination, chlorogenic acid content and R-index similar by

ranking result. Headspace-solid phase microextraction (HS-SPME) coupled with SIDA and liquid-liquid extraction were used to determine and calculate the target 40 volatiles of coffee brew which belong to low boiling point groups, sulfur volatile group and high boiling point volatile groups. Further details are given in Appendix A.4.

3.3.4.2 Determination of odor activity values (OAV)

The odor activity value (OAV) of each target key odorants was calculated as per equation 3.2.

3.3.4.3 Calibration of Furaneol® determination

In order to calibrate the accuracy of Furaneol® content in coffee which was determined by the LLE method, the use of stable isotope dilution analysis (SIDA) and direct solvent extraction-solvent-assisted flavor evaporation (DSE-SAFE) were applied to determine Furaneol® concentration to make the comparison and ensure the correctness of rapid method. The detail of this calibration is given in Appendix A.5.

3.3.5 Statistical Analysis

The means of scores from volatile determination and chemical analysis were analyzed for significant difference at $p \leq 0.05$ by using One-way ANOVA, Least Significant Difference test (LSD). All statistical analyses were performed using SPSS Statistics software version 13.0 (SPSS Inc., Chicago, IL, USA). Multivariate analysis of the significant key volatiles data sets of three coffees was carried out using principal component analysis (PCA) by XLStat v.2018.2.10 (Addinsoft, Paris, France). A score plot of PCA was used to study the distribution of the significant key volatiles determined from univariate analysis (One-way ANOVA) relative to the overall variability of the data sets obtained from three coffee brews. PCA computed those

volatile variables into new variables, PC dimensions/factors which indicated the overall variance of data sets. A biplot was then plotted on a single scaling plot where objects (three coffee brews) were pointed and the variables were represented by eigenvectors relating each variable to each component.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Effects of post-harvest processing practice on quality of coffee

4.1.1 Maturity of coffee cherries (ripe *vs* unripe)

4.1.1.1 Antioxidant activity of green coffee beans

The determination of antioxidant activity of green coffee beans in this section was expressed in terms of total phenolic content (TPC), DPPH assay, and FRAP assay based on the earlier reports about the rapid-feasibility, reliable and low cost of these methods when applied to evaluate antioxidant activities of coffee (Liang & Kitts, 2014). The antioxidant activity values in both ripe and unripe green coffee beans are shown in Table 10.

Table 10 Antioxidant activity of ripe and unripe green coffee beans

Antioxidant determination	**Green coffee beans**	
	Ripe	**Unripe**
Total phenolic content^{ns} (mg GAE/g coffee)	59.94±4.14	54.56±0.73
DPPH radical scavenging activity ^{ns} (%)	71.74±5.26	70.68±4.78
FRAP assay (mg Trolox/g coffee)	66.98^{a}±4.70	52.72^{b} ±3.20

^{a,b}values with different letter in a row are significantly different ($p \leq 0.05$); ^{ns}not significant GAE: gallic acid equivalent; DPPH: diphenyl-1-picrylhydrazyl; FRAP: ferric reducing antioxidant power

The TPC determination was based on a redox reaction. According to the results (Table 10), it was found that the TPC of ripe (59.94 mg GAE/g coffee) and unripe (54.56 mg GAE/g coffee) beans were not significantly different (p>0.05). However, they were higher than those reported by Somporn *et al.* (2011) and Cheong *et al.* (2013) that TPCs of ripe green coffee beans (*Coffea arabica* L. '*Catimor*') which were 34.32 and 43.07 mg GAE/g coffee. This might be because of the differences in fermentation process since beans used in this research was passed through semi-dry process, while the others were wet process.

The DPPH radical scavenging activity assay was used to investigate antioxidant potential of green coffee bean extracts. Table 10, shows that there was no significantly difference between ripe and unripe green coffee bean extracts. Both ripe and unripe green coffee extracts showed DPPH radical scavenging activity of about 70% inhibition. The results reported by Somporn *et al.* (2011; 2012) show that the % inhibition was higher (~92%) in fully ripe green coffee extract. In addition, the ferric reducing antioxidant power (FRAP) of each extract was determined and expressed in mg Trolox/g coffee. FRAP of ripe bean extract (66.98 mg Trolox/g coffee) showed higher ferric reducing capacity than unripe extract (52.72 mg Trolox/g coffee) (p< 0.05). The increasing value of FRAP of green coffee beans would be occurred during coffee cherry ripening since the chlorogenic acid content and composition vary during coffee fruit maturation (Farah & Donangelo, 2006).

Among these three modes of assessment of antioxidant activity, only FRAP assay could differentiate ripe from unripe green coffee beans. It comparing DPPH and FRAP assay, their reaction mechanisms are different. DPPH is used to determine antioxidants based on 2 mechanisms, the transfer of a single electron (SET) and hydrogen atom (HAT), while FRAP is a non-specific, redox-linked, colorimetric assay that is related to the molar concentration of the antioxidants present in coffee matrix that quantifies molecules that transfer only a single electron (Cheong *et al.*, 2013; Liang *et al.*, 2014). According to Jeszka-Skowrona *et al.* (2016), Liang *et al.* (2014, 2016), several classes of water soluble components; caffeine, trigonelline, nicotinic acid and chlorogenic acids and fat-soluble compounds; kahweol and cafestol, tocopherol as well as the trace metal contents (like selenium, calcium, copper, etc.) and their interactions between each fractions of antioxidant extracted compounds are responsible for the different mechanisms of antioxidant activities in green coffee and could be influenced by the antioxidant analysis methods chosen, physical state of analyzed samples and concentration of antioxidant extracts. The composition responsible for FRAP mechanism in green coffee was usually reported as mostly water-soluble components that react in the aqueous solution with the reducing capacity being determined in the absence of molecules that may have protective capacities to detoxify reactive oxygen species (Liang *et al.*, 2014). This difference indicates that the development of water soluble antioxidant compounds; caffeine, trigonelline, nicotinic acid and chlorogenic acids was enhanced by the ripening process and may result in higher FRAP value in ripe green coffee than in unripe one. The majority of water soluble antioxidants in coffee are chlorogenic acids, distributed on the surface of berries, in association with the cuticular wax, and in the cytoplasm adjacent to the cell

walls of the endosperm parenchyma (Clifford, 1987), these compounds are formed through biosynthesis of cinnamic/hydroxycinnamic acids and quinic acid during the maturation of coffee berries (Clifford, 1985, Farah *et al.*, 2005a). Hence, to gain more understanding about the effect of ripening process, the individual determination of chlorogenic isomers and the correlation with antioxidant activity will be helpful to confirm to the result of rapid FRAP assay.

The aim of study in this section was to separate green coffee processed from ripe and unripe cherries by using volatile analysis and antioxidant activity as the rapid tools for the discrimination by maturity of the coffee cherries. The volatile compounds profile and antioxidant activity by FRAP could be used as quality markers in order to evaluate the selection process of coffee berries during the semi-dry process of coffee post-harvest treatment.

4.1.1.2 Volatile compounds in green coffee beans

As mentioned earlier, volatile profile, one of quality markers of coffee, had been previously showed as successful in distinguishing the degree of ripeness of green coffee and separate the defective and non-defective green coffee processed by wet processing method (Smrke *et al.*, 2015; Toci & Farah, 2008; Toci & Farah, 2014). Besides the wet processed coffee, semi-dry processed coffee has gained its popularity in the production of coffee due to its favorable characteristic of the brew which is claimed to be the combination between dry processed and wet processed coffee. The difference in coffee berries maturity could influence the final cup quality of semi-dry coffee beverages which need to be controlled by maintaining the optimum quality of semi-dry green coffee. Head Space Solid phase micro extraction-gas

chromatography/mass spectrometry (HS-SPME-GC/MS) had been applied in this study to separate the ripe and unripe semi-dry green coffee. From volatile composition analyses, more than 200 peaks of volatile compounds were found in both unripe and ripe green coffee beans (chromatograms are not shown). From among those hundreds of compounds, selected volatile compounds of both ripe and unripe green coffee beans and their approximate concentrations categorized following the calculated retention index (RI) are shown in Table 11. A total of 45 volatile compounds were identified in ripe and unripe green coffee beans.

Table 11 Concentrations of selected volatile compounds in ripe and unripe green coffee beans

Compounds	Retention index[a] (RTX-5)	Concentration (ng/kg)[b]		Identification method[d]
		Semi-dry process		
		ripe	unripe	
Ethanol	477	8.4	21.5	MS, RI
Dimethyl sulfide	506	1.32	0.06	MS, RI
Acetic acid methyl ester	538	3.63	0.44	MS, RI
Dihydro-3,5-dimethyl-2(3H)-furanone	553	n.d.[c]	2.50	MS
2-Methyl-3-pentanol	558	2	0.60	MS
Acetic acid	584	4.1	4.50	MS, RI
2-Butanone	602	n.d.[c]	0.50	MS, RI
2-Methylfuran	611	1	n.d.[c]	MS, RI
3-Methylbutanal	655	0.03	n.d.[c]	MS, RI
2-Methylbutanal	666	0.02	n.d.[c]	MS, RI
Pentanal	691	0.4	n.d.[c]	MS, RI
2-Pentanol	709	0.84	n.d.[c]	MS, RI
3-Methyl-1-butanol	738	11.6	13.9	MS, RI
(*S*)-2-Methyl-1-butanol	744	5.25	n.d.[c]	MS, RI
Methyl 3-methylbutanoate	780	20.4	0.7	MS, RI
Methyl-2-methylbutanoate	782	11.7	0.3	MS, RI
2,3-Butanediol	793	n.d.[c]	32.1	MS, RI
Hexanal	803	13.5	2.6	MS, RI
Methyl (2*E*)-2-butenoate	852	1.1	n.d.[c]	MS, RI
3-methylbutanoic acid	857	42.1	1.6	MS, RI

Table 11 Concentrations of selected volatile compounds in ripe and unripe green coffee beans (Cont.)

Compounds	Retention index[a]	Concentration (ng/kg)[b]		Identification method[d]
		Semi-dry process		
		ripe	unripe	
Ethyl 3-methylbutanoate	861	1.3	0.4	MS, RI
2-Methylbutanoic acid	867	16	0.6	MS, RI
1-Hexanol	877	15.4	n.d.[c]	MS, RI
3-Methyl-1-butanol acetate	884	0.04	n.d.[c]	MS, RI
2-Heptanol	905	2.9	n.d.[c]	MS, RI
2,6-Dimethylpyridine	909	0.4	0.36	MS, RI
Butyrolactone	925	5.13	3.41	MS, RI
Ethyl 3-methyl-2-butenoate	929	0.2	0.17	MS, RI
Methyl hexanoate	930	0.9	n.d.[c]	MS, RI
2,7-Dimethyloxepine	940	0.6	n.d.[c]	MS, RI
□-Pinene	942	0.44	n.d.[c]	MS, RI
2,2-Dimethyl-3,4-pentadienal	947	1.1	n.d.[c]	MS
Benzaldehyde	969	1.4	0.7	MS, RI
1-Octen-3-ol	984	3.51	0.7	MS, RI
2-Ethyl-1-hexanol	1032	1.7	n.d.[c]	MS, RI
Limonene	1036	1.5	1.47	MS, RI
Benzeneacetaldehyde	1052	5.2	1.5	MS, RI
3-Ethyl-2,5-dimethylpyrazine	1087	n.d.[c]	0.4	MS, RI
2-Ethylhexyl hexyl oxalate	1090	n.d.[c]	7.8	MS, RI
2-Phenylethanol	1121	7.9	1.8	MS, RI
(*E*)-2-Nonenal	1163	2.21	n.d.[c]	MS, RI

Table 11 Concentrations of selected volatile compounds in ripe and unripe green coffee beans (Cont.)

Compounds	Retention index[a] (RTX-5)	Concentration (ng/kg)[b]		Identification method[d]
		Semi-dry process		
		ripe	unripe	
Methyl phenylacetate	1181	1.7	n.d.[c]	MS, RI
2-Isobutyl-3-methoxypyrazine	1185	7.36	2.15	MS, RI
Methyl salicylate	1204	0.4	n.d.[c]	MS, RI
4-Vinylguaiacol	1324	2	1.3	MS, RI

[a]Retention index determined on RTX-5 column. [b]Approximate concentration, ng/g coffee bean from triplicate analysis, mean ±S.D <10%. [c]Not detected. [d]Identification criteria: mass spectra (MS); retention index (RI).

Acids, alcohols, esters, and aldehydes were found to be predominant groups of compounds in ripe beans, while the majority volatiles in unripe one were alcohols and ketones.

These results are in agreement with the study by Gonzalez-Rios *et al.* (2007), which mentioned that the Mexican *Coffea arabica* bean presented larger numbers and quantities of acids, alcohols, aldehydes, and esters due to the fermentation process. In term of compound quantity, overall concentration of ripe coffee bean (206.48 ng/g coffee bean) was higher than unripe bean (71.95 ng/g coffee bean). In terms of changes during ripening process, there were more clearly developed in the group of alcohol, aldehydes, acids, and esters. This phenomenon generally occurs upon ripening of coffee berry because of the forming of degradation products (Ortiz *et al.*, 2004), especially in coffee parchment obtained from dry and semi-dry processes.

The aroma-active groups of coffee; dimethyl sulfide, 2/3-methylbutanal, 4-vinylguaiacol, 1-octen-3-ol, benzeneacetaldehyde, 2-isobutyl-3-methoxypyrazine were identified in green coffee in the present study which agrees with the results of Cantergiani *et al.* (2001) and Holscher & Steinhart (1994) that those odorants could be detected in green coffee before the increase in some of their concentration due to Maillard reaction during coffee roasting.

The ripening process is confirmed to enhance the production of those aromas since some of them were in trace amounts and increased in ripe green coffee. During ripening process, aroma precursors like sugar components are increased (Sanz-Uripe *et al.*, 2017), leading to increase of the content of aroma-active components in green coffee processed from ripe coffee cherries. In addition, to the other potent odorant group of coffee, methoxyalkylpyrazines are not formed by Maillard reaction but are from the biogenetic origin. They are usually formed from the condensation of amides of α-amino acids and α-dicarbonyl derivatives (Flament, 2002). 2-isobutyl-3-methoxypyrazine was identified in both ripe and unripe green coffee but in higher concentration in ripe berries which is in accordance with the findings that the ripening process leads to the formation of aroma precursors and may enhance the biogenetic reaction resulting in the forming of methoxypyrazines products.

From Table 11, shows 17 compounds found only in ripe beans, including 2-methylfuran, 3-methylbutanal, 2-methylbutanal, pentanal, 2-pentanol, (*S*)-2-methyl-1-butanol, methyl (2*E*)-2-butenoate, 1-hexanol, 3-methyl-1-butanol acetate, methyl hexanoate, 2,7-dimethyloxepine, □-pinene, 2,2-dimethyl-3,4-pentadienal, 2-ethyl-1-hexanol, (*E*)-2-nonenal, methyl phenylacetate, and methyl salicylate. In

addition, 5 compounds were only found in unripe green beans: dihydro-3,5-dimethyl-2(3H)-furanone, 2,3-butanediol, 2-butanone, 3-ethyl-2,5-dimethyl pyrazine, and 2-ethylhexyl hexyl oxalate.

With the help of rapid analysis by HS-SPME/GC-MS, the present study elucidates that these odorants could be used as quality markers to distinguish between ripe and unripe green coffee beans.

4.1.2 Processing methods and the authenticity of green coffee quality

4.1.2.1 Antioxidant activity of green coffee beans

Continuing from previous section 4.1.1, the rapid determination in antioxidant activities of green beans had again applied in this section as another quality determinant to assess the quality of green beans indicated to different processes. It was proposed to find the suitable antioxidants activity tests that could be used as rapid tools for separation of green beans in each processes. The antioxidant activity of green coffees was determined and expressed in terms of total phenolic content (TPC), DPPH assay, and FRAP assay. The results of the antioxidant activity and phenolic contents of green coffee beans processed by different methods are shown in Table 12.

Table 12 Antioxidant activity of green coffee beans processed by different methods.

Antioxidant determination	Green coffee beans[c]		
	Wet process	Semi-dry process	Civet process
Total phenolic contents (mg GAE/g coffee)	47.29±2.94[b]	46.68±5.09[b]	50.38±6.12[a]
DPPH radical scavenging activity (%)	79.96±6.55	81.97±2.47	89.96±6.18
FRAP assay (mg Trolox/g coffee)	42.63±2.70[b]	66.98±4.26[b]	80.67±7.11[a]

[c]Mean values based on the analysis of triplicates. S.D. <15 %. [a,b]values with different letter in a row are significantly different ($p \leq 0.05$). [ns]not significant. GAE: gallic acid equivalent; DPPH: diphenyl-1-picrylhydrazyl; FRAP: ferric reducing antioxidant power.

The Total phenolic contents determination (TPC) was based on a redox reaction. It is indicated that the TPC contents of green coffee processed via the civet process (50.38 mgGAE/g coffee) was significantly different ($p<0.05$) from wet process and semi-dry process coffee (47.29 and 46.68 mgGAE/g coffee). In contrast, there was no difference observed between green coffee processed from semi-dry and wet methods ($p>0.05$). This result confirms the results of Duarte *et al.* (2010) which reported that the content of CGA, the main phenolic compounds were not significantly different between semi-dried and wet-processed coffee when comparing various cultivars of *Coffea arabica* (Red Cataui, Rubi, Yellow Burbon and Topázio).

The high TPC content of civet coffee could be explained that the fermentation process in the animal digestive tract with the use of less water than wet process method. This increases some derivative contents of phenolic compounds, chlorogenic acids, according to the study of Cheong *et al.* (2013) that the total phenolic contents and caffeic acids of green civet coffee/Kopi luwak in their study was higher

(30.87 and 0.57 mg/g green coffee) than Doichang green coffee (29.55 and 0.18 mg/g green coffee). The lower contents of TPC in green coffee processed from wet-method was in accordance to the interpretation of Komes&Vojvodić (2014) that water soluble components could be easier lost by the leaching and fermentation steps. However, when comparing the TPC contents of coffee samples in the present study to the study of Somporn *et al.* (2012) (34.32 mg GAE/g coffee) and Cheong *et al.* (2013) (43.07 mg GAE/g coffee), the values in their study were lower due to the difference in cultivars, plantation area, variation of phenolic compounds content within whole green bean before grinding, packing and processing method (Clifford, 1985; Farah *et al.*, 2005a).

When considering to the determination of antioxidant activity, by two test, DPPH radical scavenging activity and FRAP assay, assays are naturally differing from each other based on the reaction mechanisms.

Civet green coffee extracts showed the highest DPPH radical scavenging activity at about 90% inhibition while for semi-dried processed coffee and wet processed coffee, their % inhibition were ~80%. Since DPPH is a lipophilic radical which reacts as an antioxidant in alcoholic solvent, ethanol (Liang *et al.*, 2014), not only water soluble phenolic compounds exhibit antioxidant activity but also fat soluble compounds like tocopherol, kahweol and cafestol. Therefore, the higher DPPH of civet green coffee than other two processes type may relate higher extracting rate of those compounds via bio-digestive fermentation by civet. Although the highest % inhibition could be detected in civet green coffee, nonetheless there was no significant difference observed in the % inhibition of DPPH activity among the three processed coffees. For FRAP assay civet coffee confirmed its significant difference in the higher value

(p>0.05) of the ferric reducing antioxidant power (80.67±3.31 mg Trolox/g coffee) vs wet processed and semi-dry processed coffee (42.63±4.66 and 66.98±4.70 mg Trolox/g coffee) respectively as well as in agreement with the highest content of TPC in civet green coffee. In spite of that, even semi-dry processed coffee showed the higher potential of FRAP value than wet processed coffee, however, they were not significance difference from each other and in agreement with the previous discussion about the total phenolic contents comparing between these two processes (p>0.05).

Determination of the rapid low cost antioxidant activities provided the information that green civet coffee could be separated from regular process based on TPC and FRAP value. In addition, the results confirmed the ability of postharvest processing methods to produce differences in antioxidant activity in green coffee. Due to the fact that civet in this study had been fed with the selected full ripe coffee berries with the same grade of ripe berries as used in wet and semi-dry process, the fermentation that takes place within civet digestive tract do not involve water used and reactivity of different microbes. This is the case that in opposite to wet process and semi-dry process, and could be well correlated to the significant value of FRAP in civet green coffee. For a better understanding about the different activities of antioxidant molecules in green coffee, other antioxidant activities should be investigated as well as the phenolic composition of green beans.

4.1.2.2 Volatile compounds of green coffee beans

In the previous section, volatile component analysis by gas chromatography (GC) showed that it was successful in differentiating the processed beans from coffee cherries of different ripeness. In this section aims to discriminate green coffee processed by different post-harvest methods and find the authenticity of

green coffee, especially civet coffee, based on volatile profile. Hence, HS-SPME-GC/MS had been applied to find the key volatile that have potential to discriminate green beans having undergone different processes and identify their authenticity based on volatile profile. Total 300 peaks of volatile constituents were identified in green coffee subjected to semi-dry process, wet process or civet process. Hydrocarbons, acids, alcohols, esters, and aldehydes were found to be predominant in all green coffee beans samples.

Among of these volatiles, 17 key odorants previously identified in roasted and brew coffee and have been reported on their importance to coffee flavor characteristic were detected in green coffees of this study (Belitz *et al.*, 2009; Czerny & Grosch, 2000; Flament, 2002; Poisson *et al.*, 2017), their identification criteria were based on retention indices and mass spectra. After the identification, the important of these odorant was then categorized for further PCA analysis based on the calculation of their odor activity value (OAV) which in the final selection, the group of odorants were selected by the volatile compounds provided the OAV value >1. The approximate concentration and OAV of these compounds were given in Table 13.

Considering from the results of the approximately identified volatiles, their concentration and OAV, there are total 8 volatiles that represent the uniqueness of civet coffee are 2-methylpropanal, 2 and 3-methylbutanal, methional, 1-octen-3-ol, hexanal, indole and (*E*)-β-damascenone. While for semi-dry processed and wet processed coffee, most of their identified volatiles were similar but different in concentration.

Ethyl 2-methylbutanoate and ethyl 3-methylbutanoate which were previously described as potent odorants of green coffee were not detected in civet coffee (Czerny & Grosch, 2000; Scheidig *et al.*, 2007) but somehow detected both in wet and semi-dried processed green coffee with the high OAV of 293 and 183 respectively. Their concentration in this study (1.76 and 1.89 µg/kg) agreed with the range of previous detection (0.15-2.5 and 2.3-13.3 µg/kg). They were lesser them in the study of Scheidig *et al.* (2007) that reported their highly concentration rise to 32 and 8 µg/kg. This is due to the difference in cultivars, location, the condition of HS-SPME/GC-MS and the difference in the sample preparation.

Table 13 Concentrations and odor activity value (OAV) of selected volatile compounds in green coffee subjected to semi-dry process, wet process and civet process

Selected compounds	Retention Index (RI) (RTX-5)[a]	Odor Threshold (ng/kg)	Concentration of selected compounds (ng/g)[b]			Odor Activity Value		
			Processing			Processing		
			Civet process	Wet process	Semi-dry process	Civet process	Wet process	Semi-dry process
Dimethyl sulfide	506	300[I]	n.d.[c]	0.57	1.32	n.d.[c]	2	4.4
2-Methylpropanal	567	700[II]	6.1	n.d.[c]	n.d.[c]	9	n.d.[c]	n.d.[c]
3-Methylbutanal	655	350[II]	1.04a	0.04b	0.03b	3	0.1	0.1
2-Methylbutanal	665	1300[II]	1.21[a]	0.05[b]	0.03[b]	0.93	0.04	0.02
Hexanal	804	4500[III]	3.72[a]	1.1[b]	1.41[b]	0.83	0.24	0.31
Ethyl 2-methylbutanoate	859	6[IV]	n.d.[c]	1.76[a]	0.37[b]	n.d.[c]	293	62
Ethyl 3-methylbutanoate	862	10[IV]	n.d.[c]	1.89[a]	0.71[b]	n.d.[c]	189	71
Methional	913	200[V]	0.51	n.d.[c]	n.d.[c]	3	n.d.[c]	n.d.[c]
1-Octen-3-ol	983	1000[II]	22.7[a]	n.d.[c]	3.7[b]	23	n.d.[c]	4
2,3,5-Trimethylpyrazine	1014	9100[VI]	1.17	n.d.[c]	n.d.[c]	0.01	n.d.[c]	n.d.[c]
Benzeneacetaldehyde	1053	4000[VII]	2.21[a]	4.1[b]	0.7[c]	0.6	1	0.2
3-Ethyl-2,5-dimethylpyrazine	1087	8600[III]	0.81	n.d.[c]	n.d.[c]	0.1	n.d.[c]	n.d.[c]
Nonanal	1106	1000[VI]	0.05	n.d.[c]	n.d.[c]	0.05	n.d.[c]	n.d.[c]
2-Phenylethanol	1122	1000000[k]	10.3[a]	12.3[b]	7.9[a]	0.01	0.01	0.01

Table 13 Concentrations and odor activity value (OAV) of selected volatile compounds in green coffee subjected to semi-dry process, wet process and civet process (Cont.)

Selected compounds	Retention Index (RI) (RTX-5)[a]	Odor Threshold (ng/kg)	Concentration of selected compounds (ng/g)[b]			Odor Activity Value		
			Processing			Processing		
			Civet process	Wet process	Semi-dry process	Civet process	Wet process	Semi-dry process
(*E*)-2-Nonenal	1163	150[VI]	1.2[a]	n.d.[c]	2.3[b]	8	n.d.[c]	15
2-Isobutyl-3 methoxypyrazine	1185	5[II]	10.1[a]	11.6[a]	7.45[b]	2020	2320	1490

[a,b,c,d,e]values with different letter in a row are significantly different (p<0.05) with mean ± S.D < 15%. [I](Persson & Von Sydow, 1973). [II]Semmelroch&Grosch (1996). [III]Guth&Grosch (1993). [IV]Takeoka *et al.* (1995). [V]Guadagni *et al.* (1972). [VI]Heiler (1995).[VII]Buttery *et al.* (1969). [a]Retention index determined on RTX-5 column. [b]Approximate concentration, ng/g coffee bean from triplicate analysis, mean ±S.D <10%. [c]Not detected.

The consists of depulping process in semi-dry and wet procedures are previously mentioned as increasing the quantities of esters and ketones which were correlated to the high development of those contents in the finding of this study and may explain the lesser development of important aldehyde odorants compared with civet process. As reported by Duboc&Milo (2003), the strain of microbes during water fermentation (yeast/lactic acid bacteria) contribute to the formation of fruity and floral attributes of coffee as they take part in the conversion of 2- and 3-methylbutanal to their corresponding alcohols their corresponding alcohols, 2-and 3-methylbutanol during fermentation. This prior finding could relate to the significantly difference in the higher contents of 2 and 3-methylbutanal in civet green coffee (1.21 and 1.04 µg/kg) in this study since the strains of microbes in bio-digestive fermentation method of civet processing are likely to differ from the strains that active during fermentation in wet and semi-dry processing.

Methional, a key odorant responsible for potato note of coffee usually generated from Strecker degradation of methionine during Maillard reaction, and normally increases with degree of roasting. It was found only in civet green coffee at 0.51 µg/kg. This compound had been claimed as one of potent odorant of green and roasted coffee by Czerny and Grosch (2000).

2-methylpropanal, a malty note odorant was detected only in green civet coffee (6.10 µg/kg), this compound was identified in green coffee for the first time in this study. It usually forms from the pyrolysis of alanine, valine and leucine and increases significantly in roasted coffee (Flament, 2002). According to prior studies, the protein hydrolysis through civet's gastrointestinal tract and the permeation of

digestive enzymes and gastric juices through the endocarp of coffee cherries and bean surface causes a change in the amino acids composition of green coffee (Marcone, 2004). The release of amino acids from proteolytic enzymatic reaction may explain the generation of volatile compounds that form during the thermal degradation of free amino acids like methional and 2-methylpropanal in green civet coffee.

1-Octen-3-ol and hexanal are the lipids derived odorants that are mainly produced from the autoxidation of linoleic acid, main unsaturated fatty acid in green coffee. They are odorants indicating off-flavor (mushroom, cardboard and green-notes) and staling of coffee during storage and improper practice. In this study, they were developed in highest amounts in civet coffee namely 22.67 and 3.72 µg/kg. These volatiles were normally preidentified in wet-processed green coffee in previous studies (Cantergiani *et al.*, 2001; Czerny & Grosch, 2000; Spadone *et al.*, 1990) but in different concentration to that in this study due to the identification method used and variation of sample as discussed above. It is challenging to study further the activities of lipolytic enzymes from lactic acid bacteria, the main culture group within civet digestive tract and the combination step of green beans washing after the excretion of the beans from the digestive tract. The waiting period while drying of civet green coffee under sun light will provoke the degradation of stored lipid within cell wall of coffee, producing free short chain fatty acids and enhancing the development of the lipid derived odorants through the enzymatic reaction of lipid oxidation. Since, the living species that have been reported with regard to their metabolic rate which responsible to induce the generation of unique flavor in civet coffee are lactic acid bacteria (Muzaifa *et al.*, 2018), the tracking of the lipolytic enzyme activity that occurs within the bio-digestive

fermentation of civet coffee should need to be studied further to find a clear explanation to the relationship with hexanal formation.

(*E*)-β-damascenone, key odorant of coffee that contribute to cooked apple note and in formed via the degradation of carotenoid by heating and oxidation, oxidation and isomerization (Czerny & Grosch, 2000; Flament, 2002) was found in civet coffee with the highest OAV calculated from the approximate concentration in green coffee (0.08 µg/kg) at 107. The OAV calculated in this study was contrasting with that in the study of Czerny & Grosch (2000) which indicated that this compound is not important as an odorant of green coffee. However, this is the first time that it was found in green civet coffee and confirmed the accomplishment of robust GC-MS analysis in order to discriminate green civet coffee from the conventional commercial process.

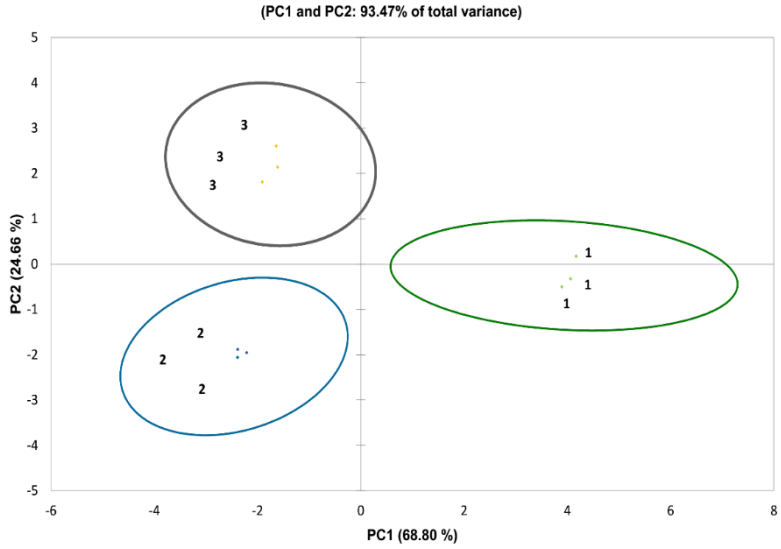
After the completion of green coffee volatile profile discriminations, 12 key odorants were selected based on their contribution to the significant differences of volatile profiles within green coffee samples. Two dimensional PCA visualization plot was performed to distinguish coffee subjected to different processing methods based on volatile profiles. The score plot and bi-plot of PCA analysis are shown in Figure 11, the first two principle components; PC1 (68.80%) and PC2 (24.66%) demonstrate that the main extracted information disclosed the volatile profiles and the total cumulative variance among samples at 93.47%. The plot of PC1 and PC2 could discriminate and segregate coffee samples with a distinct volatile characteristic to a clear cluster with a boundary of 95 % confidence eclipse. The distances of each cluster correlate to the proximity in the expression of significant volatile constituents.

The dominant characteristic of civet coffee was identified on the positive side of PC1 while the negative side along this axis indicated the contrast character of this animal processed coffee from wet processed coffee. The relationship of wet processed and semi-dried coffee could be seen via the close distance between two confidence boundaries clustered on positive and negative side of PC2 axis which confirmed the results of non-significant difference between these samples in most volatile groups.

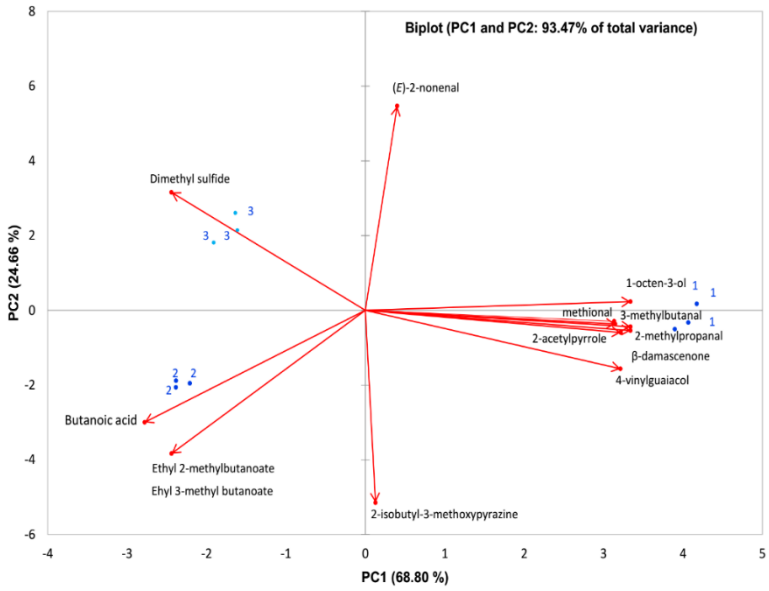
To gain more better understanding in the discrimination among coffee via different processes, a bi-plot and its eigen vector (Figure 11) were plotted to identify the importance of volatile variables contribution in each sample cluster. Eigen vectors direction and the high positive correlation (>0.7) of 2-methylpropanal, 3-methylbutanal, 1-octen-3-ol, 4-vinylguaiacol, β-damascenone, methional, butanoic acid and 2-acetylpyrrole confirmed that three coffees could be separated mainly on the basis of volatile profiles which belong to the characteristics of green civet coffee. The direction of eigen vectors and negative coefficients of butanoic acid (-0.825), ethyl 2-methylbutanoate (-0.725) and ethyl 3-methylbutanoate (-0.825) to the PC1 refer to the main characteristics of wet processed coffee volatile profile. Semi-dried coffee also shared those characteristics with wet-processed coffee, however the presence of dimethyl sulfide, made it different from wet-processed type.

Therefore, from the PCA analysis, the separation of coffee based on volatile profile has been confirmed and correlates well with the results of one-way ANOVA analysis of volatile factor loading in total variances of the data sets. From the result of volatile analysis in this study, the goal of the study can be reached namely to

identify the key compounds for coffee discrimination and find the rapid tool for the determination of authenticity of green coffee for the propose of quality control of green beans subjected to the different processing methods to prevent the contamination of civet coffee by the fake beans.



A



B

Figure 11 (A) PCA score plot (PC2 against PC1) from the concentration of 12 key volatile compounds of three processed coffees (B) PCA biplot (PC2 against PC1) of three processed coffees

(1) Civet coffee; (2); Wet-processed coffee (3) Semi-dried coffee.

4.1.2.3 E-tongue evaluation

An electronic tongue (E-tongue) has been claimed as a rapid and low cost technique used for quality control of food and beverages based on the ability to classify substances. Similar by human taste receptors, E-tongue uses electrochemical sensors (Ciosek & Wroblewski, 2011; Lopetcharat & McDaniel, 2005; Ongo *et al.*, 2012; Toko, 1996). The sensor can classify the differences among test samples by the electrochemical cyclic voltammetric measurements using inert electrodes combined with the Principal Component Analysis (PCA). In this section of the study, this technique has been applied for the rapid determination of the quality markers (volatile analysis and antioxidant activity) of green coffee in order to determine the clear discrimination pattern of green coffee within each process. Cluster groups were separated by E-tongue and the results combined with those of other quality markers analysis for the determination of the green beans authenticity. Samples of green coffee subjected to three different processing methods were tested by E-tongue. The results were analyzed by PCA and are shown in Figure 12.

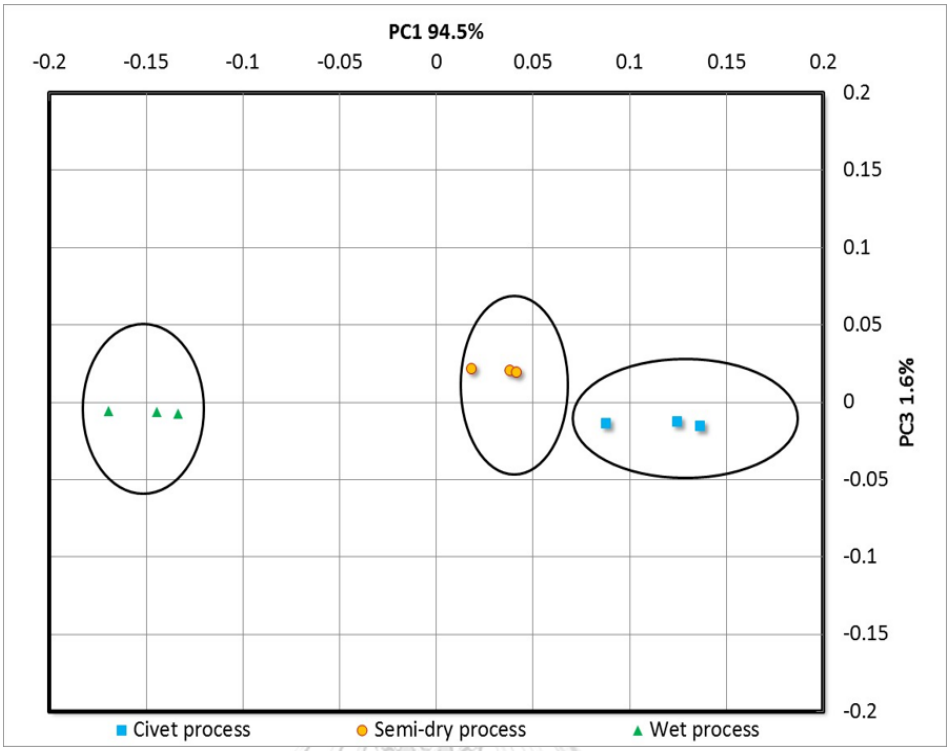


Figure 12 PCA plot of green coffee processed by different methods and tested by E-tongue

The use of E-tongue could successfully discriminate the profile of civet processed, wet-processed and semi-dried green coffee into cluster groups via the plot of PC1 (94.5%) and PC3 (1.6%) with the validation set of n=3. Coffee samples were clustered according to occurrence of perturbation (both animal digestion and regular coffee processing; wet and semi-dry process) along PC1 axis. The score plots are clearly clustered into three groups as civet, wet and semi-dry to a much greater extent than the spread of each data due to experimental errors. Regardless of the closer antioxidant activities between semi-dry and wet process coffee that was observed in the extracts of green coffee, the result of E-tongue indicates the difference between two coffees via the distance of the semi-dry group from wet process group in the first

principal component (PC1) due to the conductivity of samples. This result also correlates with the previous clear separation of the clusters in PCA analysis based on volatile profiles. Moreover, the civet, semi dry and wet groups are also clearly separated in the third principal component (PC3).

Results of this section demonstrate the ability of E-tongue to determine the difference between green coffee sample subjected to different processing methods. This shows that E-tongue is a suitable tool for rapid analysis of the sample applicable to determine their authenticity. Combining the results of volatile analysis and antioxidant activity from the previous sections, the discrimination of civet coffee from the other processing methods could also be continued by the results of E-tongue measurement. Similar pattern of antioxidant activity of wet-processed and semi-dry processed coffee was observed but the analysis by E-tongue showed that results were diverging from each other which could be explained by the differences due to brewing of coffee. The contact between water and coffee beans and the brewing time could affect the extraction of compounds, both volatile and non-volatile due to the difference in polarity (Mestdagh *et al.*, 2014). Hence the difference between those two brew samples of green coffee should reflect the overall difference between green coffee compositions including volatiles and non-volatiles through differences in conductivity measured by E-tongue. According to analytical result presented in this section, it could be established that the application of rapid analysis methods; volatile analysis by head space solid phase micro extraction-gas chromatography/mass spectrometry (HS-SPME/GC-MS), antioxidant activity determination and the use of electronic tongue (E-tongue) appear to be appropriate techniques determine the authenticity of green coffee.

4.1.3 Processing methods, subdivided processing, locations of processing, countries of processing, animal species, and cultivars. Effects on volatile compounds of roasted coffee

4.1.3.1 Volatiles of roasted coffee

Following on the studies of the chemical compounds and properties of green coffee presented in section 4.1.2, it could be concluded that the key volatiles could be used as qualities markers to discriminate green coffee subjected to different processing methods. The authenticity of civet green coffee could also be provided using HS-SPME/GC-MS. However, volatiles formed during roasting of coffee beans contribute the perception of coffee flavor when roasted coffee has been brewed and served to consumer. Several factors implicated in post-harvest processing could affect volatile profile of roasted coffee and influence the overall sensory properties of coffee brew. Among published reports, there is no evidence of research on effects of processing methods (civet and regular process), sub-divided processing methods (feeding condition), cultivars (Robusta and Arabica), animal species (civet and weasel), locations (planting area) and countries of processing (e.g. Thailand, Vietnam and Indonesia) on the difference of volatile compounds occurring both in civet and non-civet coffee. Therefore, in this section of the study, impact of these factors on volatile profile of roasted coffee were examined. This included the odorants of roasted coffees number 1-8, following the determination list given in Table 8. Samples number 1 and 4 were roasted coffee prepared from caged civet and wet processed green coffee samples in section 4.1.2 harvested from the same plantation area and processes, there were evaluated in this section to determine the more complicate in effects of processing methods from green beans to volatile compounds of roasted coffees.

A total of approximately 400 peaks of volatile compounds were found in the roasted coffee samples, their identification criteria were based on retention indices and mass spectra. Out of them, 77 volatiles were identified and their approximately quantitated concentrations are showed in Table 14. Most of volatiles indicated the difference between roasted coffees samples characterized by different processing methods, origin and location of processing, coffee species and animal species. The calculation of odor activity value (OAV) could indicate how the investigated odorants influence the characteristics of coffee. Hence, it is necessary to make clear comparison of volatile profiles observed in all roasted samples. A group of 40 interesting compounds had been chosen. They had been previously reported their odor threshold in literature. Hence, it was possible to calculate the Odor Activity Value (OAV) based on the approximate concentration showed on Table 15. These compounds include 1 hydrocarbon, 7 aldehydes, 10 pyrazines, 2 furans, 3 ketones, 4 acids, 4 pyrroles, 1 pyran, 3 phenols and 5 sulfurs. Their OAV values are provided in Table 4.6.

The results provide the information that among 40 odorants, there were around 20 volatiles corresponding to the characteristics of roasted coffee samples as seen from their high OAV (>1). Considering the results, there were variations in the concentration and OAV of the major important odorants previously reported in literatures. Among these compounds, the main dominant compounds were chosen in order to determine the role of the experimental factors in the aroma development.

Considering the aldehydes fraction of all roasted coffee. 2-methylpropanal (malty note), 2/3-methylbutanal (malty/fruity/chocolate note) were the volatiles already identified in the previous section of the volatile analysis in green coffee. They were also described to be responsible for malty/fruity/chocolate note of

roasted and brew coffee (Belitz *et al.*, 2009; Semmeroch & Grosch, 1996). This aldehyde group was successful in discriminating civet processed coffee from wet processed coffee ($p<0.05$) with the highest concentration and OAV of 2-methylbutanal observed in sample number 4 at 10.33 ppm and 7945.6 respectively. The intensity of the 2-methyl isomer grows higher than 3-methyl isomer in every roasted coffees which were in accordance with the claim of previous reports about the importance of this aldehyde isomer in coffee aroma and sensorial properties than any other odorant (Flament, 2002).

Among the civet processing method, considering to the same country of processing (Thailand) but with the different locations and feeding condition, the OAV of 2-methylbutanal were ranked from 2>1>3>6 which mean that among the near-by process area (1,2 and 3), civet coffee from Doi-tung had a significant higher content of 2-methylbutanal than the one processed from Doi-Chang area even they were claimed to be processed from the same grade of ripe coffee cherries. To compare the contents among feeding condition; the one which was the sample from caged civet process was significantly higher than the free-range (3 and 6) and for the factor of cultivars; Arabica samples number 1-3 also had a significantly higher content of 2-methylbutanal than 6, the civet Robusta coffee. This confirms the impact of feeding condition and coffee species on the malty note of civet coffee processed in the same country. Within the factor of different countries of processing, the content of this compounds were developed highest in civet coffee came from Vietnam which 8>2>1>3>6 and confirmed the variation of volatile from countries of processing.

2-methylpropanal is the compounds contributing to the fruity/pungent note of roasted coffee. It was found that the OAV of 2-methylpropanal in all roasted

coffee ranged in the order from 16.13 $x10^3$ (3) to 38.88 $x10^3$ (5) which confirmed the importance of this volatile for the character of roasted coffee. Considering, the same country of processing, 2-methylpropanal was present in the concentration highest in wet processed group, 4 and 5. In contrast, 1, 2, 3 and 6 had the lower OAV and this again confirmed the significant difference between regular coffee and civet coffee within the same country of production.

Considering the results, of the civet coffee processed in Thailand, these were clearly significant different both within the different locations (south and north of Thailand) and species (Robusta and Arabica). Comparing between different countries of production, this effect is also present in significant variation of 2-methylpropanal content between Thailand > Indonesia > Vietnam if include the count of wet-process results. In comparison in the influence of origin within civet coffee, civet coffee from Indonesia showed the highest concentration of this fruity note. According to the discussion in the previous section of green coffee volatile analysis, the protein hydrolysis through civet's gastrointestinal tract and the permeation of digestive enzymes and gastric juices through the endocarp of coffee cherries and bean surface cause a change in the amino acids compositions of green coffee. The release of amino acids from proteolytic enzymatic reaction may explain the generation of 2-methylpropanal in civet coffee which differed between the process location and species.

Among all coffee volatiles, sulfur compounds play an important role in the formation of coffee flavor (Budryn *et al.*, 2011; Nebesny & Budryn, 2006; Schutte & Teranishi, 1974) because of their extremely low thresholds and strong odor which influence coffee sensory characteristic (Dulsat-Serra *et al.*, 2016.). This study has

discovered and identified 5 sulfur compounds: dimethyl sulfide, dimethyl-disulfide, methional, 2-furfurylthiol, 3-Mercapto-3-methylbutanol. Among all of them, considering to the effect of processing methods within the near-by plantation, this effect caused no significant difference to volatile profile since there were no detection of dimethyl sulfide in roasted coffee number 3 and 5. The disappearance under the influence of processing methods also applied to dimethyl-dimethyl sulfide as it could not be detected in samples coded 3,4,5.

3-mercapto-3-methylbutanol was identified only in wet process coffee from Doitung, (sample number 4) with the concentration of 0.07 ppm and OAV>1 (at 37).

Between these sulfur volatile compounds, 2-furfurylthiol is the well known potent odorant, as it provides the coffee-like note to the final products. This compound has been claimed to be the most influence character impact odorant responsible to the describe of coffee character (Semmelroch *et al.*, 1995; Semmelroch and Grosch, 1996; Belitz *et al.*, 2009; Flament, 2002; Mestdagh *et al.*, 2014). Hence, the importance of this odorant would correspond to the exhibit character of roasted coffee within processes. The result indicates that between civet coffee and wet processed coffee produced in Thailand, this odorant was successful to discriminate civet and non civet coffee with the highest concentration found in civet coffee processed from Robusta cultivar (6), and between the nearby location and same wet process method, sample number 4 and 5 did not significantly differ from each other (p>0.05). Considering the factor, coffee species within the civet process, civet coffee processed from Robusta tend to have stronger 2-furfurylthiol while those civet coffee processed

from Arabica provided the lower coffee note. This same trend appears when comparing roasted civet coffee processed from Robusta species and wet process coffee processed from Arabica. The difference of this character impact compound in civet coffee processed in Indonesia and Vietnam confirmed the lower variation in the concentration of this 2-furfurylthiol compared with Thai coffee which mean location of origin influences this impact compound as well. Within civet processed coffee not only the origin results in the lower content of 2-furfutylthiol in Indonesian and Vietnamese coffee but also the animal species that determines the release of this compound. Since the reactive amino acids influence the development of 2-furfurylthiol (Flament, 2002; Weerawatanakorn *et al.*, 2015), it may be related to different bio-digestive fermentations in civet cat and weasel which might yield differ amino acids resulting in significantly different 2-furfurylthiol content.

As for pyrazines groups, alkylpyrazines are generally the second largest volatile fraction detected from roasted coffee with some of them providing benefit of antioxidative activities and mostly exhibit nutty/roasty/hazelnut-like and coffee-like odor (Flament, 2002; Pickard *et al.*, 2013). They are generally originating from the pyrolysis of serine and threonine (Pickard *et al.*, 2013). As indicated from the results in Table 4.6, there is a large diversity in the development of total pyrazine contents in civet coffee, and the highest abundance was found within the wet processed group (4 and 5).

In this study, 13 pyrazines were detected in our study and showed the significantly difference in their detected concentration but only 2,3,5-trimethylpyrazine, 3-ethyl-2,5-dimethylpyrazine, 2,6-diethylpyrazine, 2,3-diethyl-5-methylpyrazines were the group of nutty odorants that provided the high contribution

to roasted coffee flavor considering from the OAVs in all sample (OAV >1). Within this pyrazine group, 2,6-diethylpyrazine showed the highest contribution to the roasted coffee flavour with the highest OAVs at 1664.56 in wet processed sample coded 5. Despite the same process, samples 4 and 5 showed the significantly difference in pyrazine content between each other as 5 was drastically lower than 4 or vice-versa. Considering civet process, the different area of processing affected to the diversity of 2,6-diethylpyrazine, the concentration observed in 3 rise to 7.01 ppm while 6 and 8 were lower (p<0.05). In addition, 2,3-diethyl-5-methylpyrazine, despite of the unclear separation between Indonesian civet coffee and weasel coffee as well as wet processed coffee group but the different in concentration of this compound show the clearly separation of civet coffee among the feeding condition (1,2,3,6). The concentration of 2,3,5-trimethylpyrazine were significantly different among roasted coffee sample (p< 0.05) which confirmed the influence of factors observed in this study. The OAVs ranged from 29.35 (3) to 167.96 (7). In spite, the observed significant difference, the concentration detected in the pairs of 4 and 5 and 3 and 6 showed the close relationship among the same processing method and processing country of roasted coffee when compared the concentrations among roasted coffee processed in Thailand.

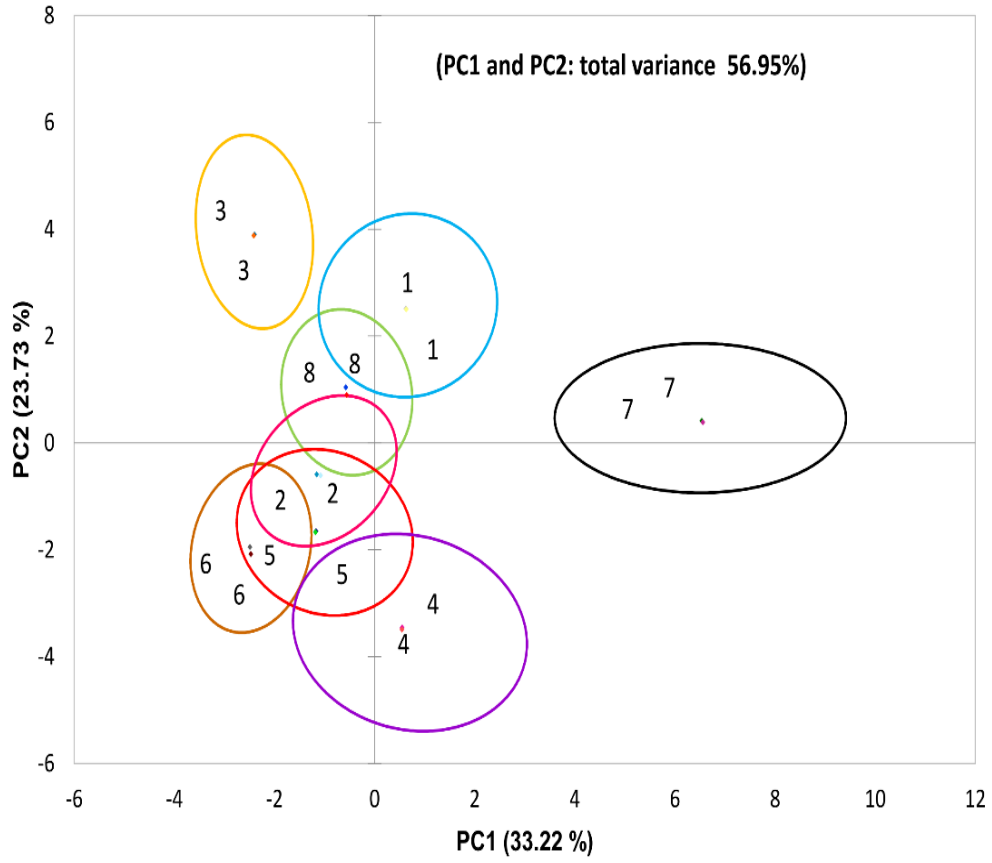
The results in this part of the study showed that HS-SPME/GC-MS could be used as a tool to provide the information on 20 key odorants showing difference between the volatile profiles in roasted coffee influenced by the processing method chosen (civet and non-civet process), different feeding conditions, location area of processing, coffee species and animal species. Among all of these 20 key odorants, 2-furfurylthiol was found to be the predominant character impact compound with the highest OAV determined in all coffee which indicates the difference of roasted coffee

under the impact of post-harvesting practice. Hence, after the determination of OAVs, 20 key odorants were selected based on their contribution to the significant differences between volatile profiles among three samples. Those concentrations were further used to conduct PCA analysis to relate the relationship and separate coffees based on volatile profiles. According to the visualization of PCA score plot showed in Figure 13, the first two principal components; PC1 (33.22%) and PC2 (23.73%) represented the major information underlying the volatile profiles and the total cumulative variance between coffee samples at 56.95%. The score plot of PC2 against PC1 also separated and segregated coffee samples with distinct volatile characteristic via different factors of post-harvest processing to a clear cluster with a boundary of 95% confidence eclipse. The distances between clusters correlate with the proximity in the expression of significant volatile constituents. The axis of PC1 showed the clearly significantly different characteristic of weasel coffee number 7 from both other civet coffees and regular coffees which confirmed the effects of animal species used in civet coffee processing. The separation of coffee clusters via axis of PC2 also indicates the discrimination of civet coffee (2,3,6,7,8) from regular coffee (4,5) as well as points out to the distinguished coffee groups based on location of processing (1,3 and 6), countries of processing (1-6 and 7) and feeding condition (1 and 2,3,6) from the information of volatile profile.

The biplot between PC1 and PC2 show that volatile variables loaded on PC1 axis; 2,3-butanedione, 4-ethylguaiacol, skatole, dimethyl sulfide, trimethylpyrazine, 2/3-methylbutanal, 2,6-dimethylpyrazine, 2-vinylpyrazine were responsible for the discrimination among samples since they were positively correlated to dimension of PC1 >0.7 and they also showed distinct characteristics of weasel coffee

(7) through the representative of these volatile components. This means that the factors such as animal species and origin of processing were confirmed as causing alteration to the flavor characteristics of roasted coffee. It is worthwhile mentioning that the direction of eigenvector of guaiacol indicates the dominant characteristic of caged-civet coffee from Doi-Tung (1) that is clearly separated from free range civet coffee from the same area (2) and different location (6) as well as segregation from the regular processed coffee (4) and (5). The volatile factor loading on PC2 with the positive correlation of more than 0.7 were responsible for the discrimination of coffee based on coffee species effect as observed in the eigen vector direction via hexanal and 2,6-diethylpyrazine. These two volatiles could provide a separation between coffee Thai free range civet (3) and Indonesian caged civet (8) from Thai Robusta free range civet coffee (6).

It is indicated that the result obtained from the multivariate analysis, showed PCA as successful tool in demonstrating variations between coffee processing based on volatiles profile and confirmed the effectiveness of these selected key volatiles through the results of univariate analysis. One-way ANOVA is used to indicate the variances in the total data sets.


(PC1 and PC2: total variance 56.95%)
PC2 (23.73 %)
PC1 (33.22 %)
8
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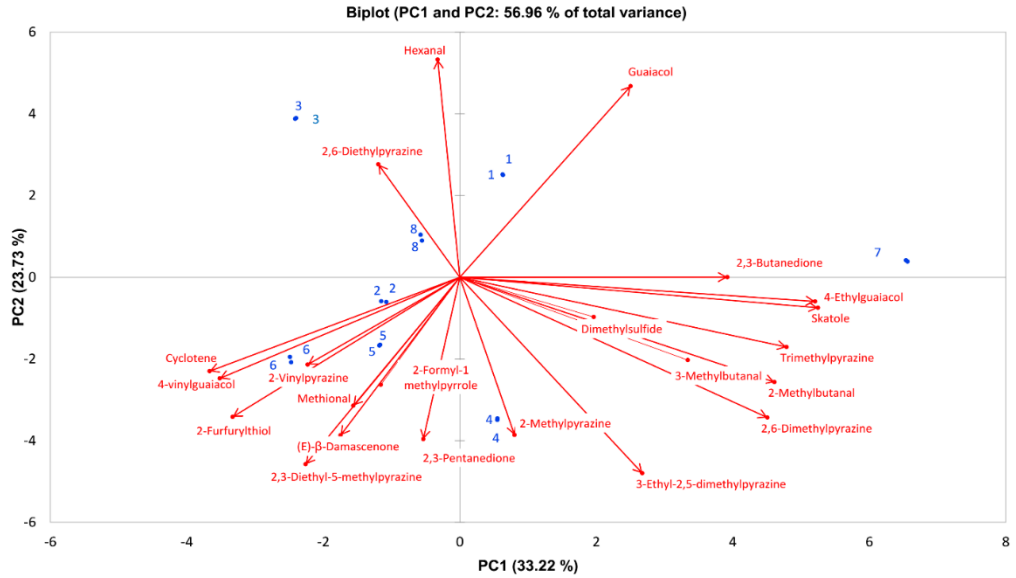
(A)



(B)

Figure 13 (A) PCA score plot (PC2 against PC1) from the concentration of 20 significant key volatile compounds of 8 roasted coffees (B) PCA biplot (PC2 against PC1) of 8 roasted coffees

1 = Caged-civet coffee from Doi-Tung, 2 = Free-range civet coffee from Doi-Tung, 3 = Free-range civet coffee from Doi-Chang, 4 = Wet-processed coffee from Doi-Tung, 5 = Wet-processed coffee from Doi-Chang, 6= Free-range civet coffee from Loei, 7= Weasel coffee from Vietnam and 8= Caged civet coffee from Indonesia, commercial sample.

Table 14 Retention index and approximate concentration of volatile compounds in 8 roasted coffee samples

Volatile compounds	Retention Index (RI) (RTX-5)[a]	Approximated concentration of selected compounds (mg/kg)[b]							
		Roasted bean treatment							
		1	2	3	4	5	6	7	8
Dimethyl sulfide	531	0.05[a]	0.04[a]	n.d.[c]	0.01[d]	n.d.[c]	0.15[b]	0.13[c]	0.05[a]
Methyl acetate	538	0.98[c]	n.d.[c]	0.04[a]	0.52[b]	0.13[d]	0.04[a]	0.35[b]	0.03[a]
2-Methylpropanal	560	7.87[d]	10.01[e]	11.29[c]	22.7[h]	27.22[g]	13.72[f]	17.89[b]	19.81[a]
2,3-Butanedione	601	7.81[b]	5.18[b]	1.36[a]	4.25[c]	6.09[b]	1.23[a]	7.98[b]	2.55[a]
2-Methylfuran	610	0.55[b]	0.1[a]	0.08[a]	0.97[c]	0.19[a]	0.1[a]	0.45[b]	0.07[a]
Acetic acid	626	17.62[d]	14.21[e]	7.61[c]	26.63[h]	22.43[g]	12.07[f]	21.44[b]	11.45[a]
3-Methylbutanal	655	0.66[d]	0.44[ab]	0.16[c]	0.92[e]	0.15[c]	0.45[b]	0.72[b]	0.41[a]
2-Methylbutanal	660	5[d]	6.79[e]	1.74[c]	10.33[f]	3.54[a]	3.68[a]	12.66[b]	3.28[a]
2,3-Pentanedione	702	9.81[d]	7.09[e]	1.43[c]	30.29[h]	35.68[g]	3.41[f]	2.08[b]	3.04[a]
Propanoic acid	709	4.1[d]	3.71[e]	2.74[b]	19.18[h]	7.18[g]	0.96[f]	1.58[c]	0.86[a]
2,5-dimethylfuran	711	0.85[e]	0.91[e]	0.13[d]	0.11[d]	0.24[f]	0.26[f]	0.57[c]	0.01[a]
Acetoin	716	1.07[c]	0.02[a]	0.1[ab]	1.63[d]	0.96[b]	0.12[ab]	0.18[ab]	0.4[a]
3-Methyl-3-buten-1-ol	736	0.76[c]	0.74[c]	0.02[a]	3.43[e]	0.65[c]	0.19[d]	0.06[b]	0.03[a]
Pyrazine	738	3.68[c]	3.21[d]	0.75[b]	5.29[g]	4.12[f]	1.49[e]	0.89[a]	0.94[a]
Methyl 2-hydroxypropanoate	743	n.d.[c]	n.d.[c]	0.01[a]	n.d.[c]	0.02[a]	0.01[a]	n.d.[c]	0.01[a]
1-Methylpyrrole	745	0.28[b]	0.17[c]	0.06[a]	0.79[d]	0.3[b]	0.25[b]	0.24[b]	0.08[a]
Dimethyl disulfide	748	n.d.[c]	n.d.[c]	0.01[a]	n.d.[c]	n.d.[c]	0.06[b]	0.03[a]	0.02[a]
Pyridine	755	11.65[d]	9.37[e]	0.71[c]	18.2[h]	19.2[g]	5.78[f]	14.43[b]	1.76[a]
Pyrrole	762	0.71[d]	0.59[e]	0.1[c]	n.d.[c]	n.d.[c]	1[f]	1.65[b]	0.05[a]
Isobutyric acid	764	0.01[c]	0.04[a]	0.02[c]	0.04[a]	0.05[a]	0.05[a]	0.09[b]	0.04[a]
Methyl trans-2-butenoate	771	0.04	0.04	n.d.[c]	0.04	n.d.[c]	n.d.[c]	n.d.[c]	n.d.[c]
1-Hydroxy-2-butanone	775	0.54[b]	0.59[a]	0.13[c]	1.34[e]	0.7[d]	0.47[b]	0.51[b]	0.58[a]

Table 14 Retention index and approximate concentration of volatile compounds in 8 roasted coffee samples (Cont.)

Volatile compounds	Retention Index (RI) (RTX-5)[a]	Approximated concentration of selected compounds (mg/kg)[b]							
		Roasted bean treatment							
		1	2	3	4	5	6	7	8
Butanoic acid	804	0.28[d]	0.2[e]	0.23[c]	1.12[g]	0.55[f]	0.17[c]	4.43[b]	0.38[a]
Hexanal	807	0.11[d]	0.07[e]	0.78[c]	0.16[a]	0.06[e]	0.1[f]	0.22[b]	0.15[a]
Dihydro-2-methyl-3(2H)-furanone	813	2.07[d]	2.45[e]	2.37[c]	18.17[g]	9.07[f]	2.21[d]	1.16[b]	2.98[a]
1-Ethylpyrrole	821	0.07[d]	0.05[a]	0.02[c]	0.16[b]	0.1[e]	0.1[e]	0.15[b]	0.04[a]
4-Methylthiazole	827	0.11[e]	0.06[b]	0.04[d]	0.2[c]	0.13[e]	0.06[b]	0.19[c]	0.07[a]
2-Methylpyrazine	831	10[e]	8.11[f]	3.23[d]	21.19[i]	13.75[h]	12.1[g]	11.3[c]	6.59[a]
Furfuryl methyl ether	838	0.58[f]	0.58[f]	0.11[e]	0.67[gi]	0.43[h]	0.39[g]	0.45[b]	0.19[a]
Furfural	842	5.85[f]	6.15[f]	3.36[e]	14.08[h]	10.1[g]	5.98[f]	3.61[b]	12.28[a]
2-Allylfuran	861	0.11[b]	n.d.[c]	0.03[c]	n.d.[c]	n.d.[c]	n.d.[c]	0.11[b]	0.05[a]
2-Methylbutanoic acid	881	0.23[d]	2.51[e]	2.38[c]	n.d.[c]	1.54[g]	3.55[f]	4.12[b]	0.9[a]
Methional	895	0.05[c]	0.04[a]	0.04[a]	0.09[g]	0.07[f]	0.18[d]	0.34[b]	0.04[a]
2-Furfurylthiol	914	1.76[c]	2.37[d]	1.52[b]	2.02[g]	1.88[f]	2.23[e]	0.98[a]	1.08[a]
2-Acetylfuran	917	13.38[d]	1.99[e]	4[c]	16.95[h]	8.86[g]	6.87[f]	9.31[b]	8.2[a]
2,6-Dimethylpyrazine	918	7.92[d]	10.5[e]	4.81[c]	12.11[f]	9.83[a]	8.19[d]	14.67[b]	9.76[a]
2-Ethylpyrazine	922	6.26[d]	9.22[e]	1.06[c]	8.45[f]	6[d]	2.43[b]	3.88[b]	2.62[a]
γ-Butyrolactone	923	3.9[d]	3.87[d]	1.03[c]	7.98[g]	6.36[f]	1.67[e]	1.53[b]	1.79[a]
2,3-Dimethylpyrazine	928	4.06[d]	5.8[e]	0.71[c]	5.86[e]	3.83[g]	1.89[f]	2.56[b]	1.67[a]
2-Vinylpyrazine	938	6.26[d]	5.56[e]	1.06[c]	8.45[g]	6[d]	2.46[f]	3.88[b]	2.62[a]
5-Methyl-2(5H)-furanone	950	0.7[c]	n.d.[c]	0.03[b]	0.3[e]	0.14[d]	n.d.[c]	n.d.[c]	0.04[a]
Benzaldehyde	969	0.34[d]	0.19[c]	0.18[c]	0.37[f]	0.62[e]	0.18[c]	0.46[b]	0.31[a]
5-Methyl-2-furancarboxaldehyde	971	7.08[d]	5.36[e]	4.21[c]	14.92[h]	8.28[g]	5.27[f]	5.46[b]	11.71[a]
3-Mercapto-3-methylbutanol	980	n.d.[c]	n.d.[c]	n.d.[c]	0.07	n.d.[c]	n.d.[c]	n.d.[c]	n.d.[c]

Table 14 Retention index and approximate concentration of volatile compounds in 8 roasted coffee samples (Cont.)

Volatile compounds	Retention Index (RI) (RTX-5)[a]	Approximated concentration of selected compounds (mg/kg)[b]							
		Roasted bean treatment							
		1	2	3	4	5	6	7	8
Phenol	988	14.79^{d}	14.95^{e}	10.87^{c}	3.07^{h}	3.2^{g}	7.89^{f}	5.64^{b}	11.61^{a}
2-Pentylfuran	994	n.d.^{c}	0.04^{c}	0.03^{b}	n.d.^{c}	n.d.^{c}	0.02^{d}	n.d.^{c}	0.05^{a}
2-Furanmethanol, acetate	998	66.27^{d}	43.44^{e}	37.63^{c}	14.49^{h}	14.6^{g}	15.87^{f}	9.4^{b}	46.17^{a}
2-Ethyl-6-methylpyrazine	1004	5.61^{d}	6.37^{e}	4.32^{c}	11.14^{h}	13.09^{g}	8.91^{f}	10.17^{b}	8.5^{a}
2-Ethyl-3-methylpyrazine	1006	0.06^{d}	0.04^{e}	0.04^{c}	0.09^{h}	0.19^{g}	0.2^{f}	0.11^{b}	0.06^{a}
2,3,5-Trimethylpyrazine	1008	3.98^{d}	1.55^{e}	2.67^{c}	9.28^{h}	7.64^{g}	2.98^{f}	15.28^{b}	5.15^{a}
2-Formyl-1-methylpyrrole	1013	4.84^{d}	3.49^{e}	10.65^{c}	27.71^{h}	24.02^{g}	18.1^{f}	5.55^{b}	22.37^{a}
Furyl ethyl ketone	1014	5.04^{d}	4.72^{e}	2.01^{c}	5.8^{h}	4.89^{g}	2.67^{f}	2.16^{b}	2.28^{a}
2-Formylpyrrole	1015	n.d.^{c}	0.84^{b}	n.d.^{c}	n.d.^{c}	n.d.^{c}	1.07^{c}	n.d.^{c}	3.28^{a}
Limonene	1033	n.d.^{c}	n.d.^{c}	n.d.^{c}	n.d.^{c}	n.d.^{c}	n.d.^{c}	0.05	n.d.^{c}
Cyclotene	1035	4.21^{c}	4.84^{d}	7.36^{b}	9.02^{g}	18.15^{f}	15.21^{e}	n.d.^{c}	18.95^{a}
2-Acetyl-5-methylfuran	1037	7.31^{d}	6.13^{e}	3.24^{c}	8.64^{h}	9.55^{g}	3.11^{f}	3.45^{b}	5.94^{a}
2-Acetylpyrrole	1070	31.56^{d}	31.39^{e}	8.16^{c}	44.12^{h}	35.18^{g}	32.64^{f}	4.94^{b}	15.79^{a}
2-Acetyl-1-methylpyrrole	1082	1.87^{d}	1.66^{b}	4.13^{c}	6.46^{g}	4.61^{f}	2.96^{e}	1.6^{b}	1.41^{a}
3-Ethyl-2,5-dimethylpyrazine	1085	0.25^{b}	0.37^{c}	0.14^{e}	0.34^{c}	0.29^{bd}	0.3^{cd}	0.36^{c}	0.24^{a}
Furan, 2,2'-methylenebis-	1086	2.55^{d}	2.31^{e}	1.53^{c}	2.83^{h}	3.23^{g}	1.04^{f}	4.26^{b}	1.47^{a}
2,6-Diethylpyrazine	1092	5.83^{d}	3.41^{e}	7.01^{c}	1.45^{h}	9.99^{g}	4.53^{f}	3.05^{b}	4.72^{a}

Table 14 Retention index and approximate concentration of volatile compounds in 8 roasted coffee samples (Cont.)

Volatile compounds	Retention Index (RI) (RTX-5)[a]	Approximated concentration of selected compounds (mg/kg)[b]							
		Roasted bean treatment							
		1	2	3	4	5	6	7	8
Guaiacol	1097	7.1[d]	7.5[e]	6.26[c]	2.55[g]	2.65[g]	5.39[f]	7.91[b]	5.71[a]
Maltol	1121	2.46[d]	2.24[e]	3.44[c]	2.27[g]	2.26[g]	4.98[f]	4.12[b]	6.73[a]
5H-5-Methyl-6,7-dihydrocyclopentapyrazine	1151	0.7[d]	0.63[e]	0.06[c]	0.79[h]	0.72[g]	0.5[f]	1.2[b]	0.1[a]
2,3-Diethyl-5-methylpyrazine	1160	0.04[c]	0.02[a]	0.01[b]	0.03[d]	0.03[d]	0.03[d]	0.02[a]	0.02[a]
3,5-diethyl-2-methylpyrazine	1162	0.6[d]	0.56[d]	0.01[c]	0.87[g]	0.62[f]	0.42[e]	0.14[b]	0.02[a]
2-Furfuryl-5-methylfuran	1183	1.18[d]	1.09[e]	0.79[c]	0.65[h]	0.95[g]	0.04[f]	0.88[b]	0.35[a]
(*E*)-Cinnamaldehyde	1284	0.03[c]	n.d.[c]	0.01[b]	0.12[d]	0.11[d]	n.d.[c]	0.09[a]	n.d.[c]
4-Ethylguaiacol	1289	1.26[d]	1.21[e]	0.36[c]	2.15[f]	2.1[f]	0.38[c]	8.18[b]	0.65[a]
Difurfuryl ether	1307	2.88[b]	2.7[d]	0.98[c]	3.21$_g$	3.58[f]	0.9[e]	2.88[b]	1.07[a]
Indole	1309	0.15[d]	0.09[a]	0.04[c]	0.39[f]	0.25[e]	0.09[a]	0.64[b]	0.08[a]
4-Vinylguaiacol	1325	11.72[d]	12.14[e]	27.81[c]	29.98[h]	22.55[g]	27.89[f]	6.19[b]	7.56[a]
(*E*)-β-Damascenone	1398	0.01[c]	0.02[d]	0.01[c]	0.09[f]	n.d.[c]	0.21[e]	0.04[b]	0.03[a]
Skatole	1406	0.09[b]	0.04[a]	0.02[a]	0.1[b]	0.09[b]	0.04[a]	0.28[c]	0.03[a]
n-Hexadecanoic acid	1962	0.11[b]	0.09[b]	0.07[b]	0.25[c]	0.12[b]	0.07[b]	0.13[b]	0.01[a]

[a,b,c,d,e]values with different letter in a row are significantly different (p<0.05) with mean ± S.D < 15%. [a]Retention index de termined on RTX-5 column. [b]Approximate concentration, ng/g coffee bean from triplicate analysis, mean ±S.D <10%. [c] Not detected

Table 15 Odor thresholds and approximate Odor Activity Value (OAV) of 20 selected compounds in 8 roasted coffee

Volatile compounds	Retention Index (RI) (RTX-5)[a]	Approximated concentration of selected compounds (mg/kg)[b]							
		Roasted bean treatment							
		1	2	3	4	5	6	7	8
Phenol	988	14.79[d]	14.95[e]	10.87[c]	3.07[h]	3.2[g]	7.89[f]	5.64[b]	11.61[a]
2-Pentylfuran	994	n.d.[c]	0.04[c]	0.03[b]	n.d.[c]	n.d.[c]	0.02[d]	n.d.[c]	0.05[a]
2-Furanmethanol, acetate	998	66.27[d]	43.44[e]	37.63[c]	14.49[h]	14.6[g]	15.87[f]	9.4[b]	46.17[a]
2-Ethyl-6-methylpyrazine	1004	5.61[d]	6.37[e]	4.32[c]	11.14[h]	13.09[g]	8.91[f]	10.17[b]	8.5[a]
2-Ethyl-3-methylpyrazine	1006	0.06[d]	0.04[e]	0.04[c]	0.09[h]	0.19[g]	0.2[f]	0.11[b]	0.06[a]
2,3,5-Trimethylpyrazine	1008	3.98[d]	1.55[e]	2.67[c]	9.28[h]	7.64[g]	2.98[f]	15.28[b]	5.15[a]
2-Formyl-1-methylpyrrole	1013	4.84[d]	3.49[e]	10.65[c]	27.71[h]	24.02[g]	18.1[f]	5.55[b]	22.37[a]
Furyl ethyl ketone	1014	5.04[d]	4.72[e]	2.01[c]	5.8[h]	4.89[g]	2.67[f]	2.16[b]	2.28[a]
2-Formylpyrrole	1015	n.d.[c]	0.84[b]	n.d.[c]	n.d.[c]	n.d.[c]	1.07[c]	n.d.[c]	3.28[a]
Limonene	1033	n.d.[c]	n.d.[c]	n.d.[c]	n.d.[c]	n.d.[c]	n.d.[c]	0.05	n.d.[c]
Cyclotene	1035	4.21[c]	4.84[d]	7.36[b]	9.02[g]	18.15[f]	15.21[e]	n.d.[c]	18.95[a]
2-Acetyl-5-methylfuran	1037	7.31[d]	6.13[e]	3.24[c]	8.64[h]	9.55[g]	3.11[f]	3.45[b]	5.94[a]
2-Acetylpyrrole	1070	31.56[d]	31.39[e]	8.16[c]	44.12[h]	35.18[g]	32.64[f]	4.94[b]	15.79[a]
2-Acetyl-1-methylpyrrole	1082	1.87[d]	1.66[b]	4.13[c]	6.46[g]	4.61[f]	2.96[e]	1.6[b]	1.41[a]
3-Ethyl-2,5-dimethylpyrazine	1085	0.25[b]	0.37[c]	0.14[e]	0.34[c]	0.29[bd]	0.3[cd]	0.36[c]	0.24[a]
Furan, 2,2'-methylenebis-	1086	2.55[d]	2.31[e]	1.53[c]	2.83[h]	3.23[g]	1.04[f]	4.26[b]	1.47[a]
2,6-Diethylpyrazine	1092	5.83[d]	3.41[e]	7.01[c]	1.45[h]	9.99[g]	4.53[f]	3.05[b]	4.72[a]

Table15 Odor thresholds and approximate Odor Activity Value (OAV) of 20 selected compounds in 8 roasted coffee (Cont.)

Selected compounds	Odor Threshold in water (μg/L)	Odor Activity Value							
		Roasted bean treatments							
		1	2	3	4	5	6	7	8
2-Methylbutanoic acid	540[d]	0.42	4.66	4.41	n.d.[a]	2.86	6.57	1.67	7.62
Methional	0.2[k]	259.58	207.50	176.15	447.54	373.27	900	187	1719.65
Dimethyl disulfide	3[h]	n.d.[a]	n.d.[a]	4.66	n.d.[a]	n.d.[a]	20	6.18	11.44
2-Acetylfuran	80000[l]	0.17	0.13	0.5	0.21	0.11	0.11	0	0.12
2-Furfurylthiol	0.01[e]	176000	199300	152000	202000	188000	223000	108000	98000
2,6-Dimethylpyrazine	1500[k]	5.28	6.15	3.2	8.07	6.55	5.46	7	9.78
2-Ethylpyrazine	21000[j]	0.3	0.28	0.05	0.4	0.29	0.12	0	0.18
2,3-Dimethylpyrazine	2500[k]	1.62	1.55	0.28	2.34	1.53	0.76	1	1.03
2-Vinylpyrazine	2000[m]	3.13	2.78	0.53	4.23	3	1.23	2619	1.94
Benzaldehyde	350[a]	0.98	0.56	0.52	1.05	1.77	0.51	0.9	1.31
3-Mercapto-3-methylbutanol	2[n]	n.d.[a]	n.d.[a]	n.d.[a]	37	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]

Table 15 Odor thresholds and approximate Odor Activity Value (OAV) of 20 selected compounds in 8 roasted coffee (Cont.)

Selected compounds	Odor Threshold in water (μg/L)	Odor Activity Value							
		Roasted bean treatments							
		1	2	3	4	5	6	7	8
2-Ethyl-3-methylpyrazine	130[o]	0.44	0.31	0.28	0.66	1.49	1.54	0.4	0.86
2,3,5-Trimethylpyrazine	91p	43.69	17.03	29.35	102.01	83.9	32.75	56.57	167.96
2-Formyl-1-methylpyrrole	37[q]	130.7	144.86	287.78	748.94	649.32	489.19	604.61	150.05
Limonene	60[r]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]	1.55
Cyclotene	300[l]	14.03	16.13	24.55	30.07	60.49	50.7	63.17	n.d.[a]
2-Acetylpyrrole	170000[q]	0.19	0.08	0.05	0.26	0.21	0.19	0.09	0.03
3-Ethyl-2,5-dimethylpyrazine	8.6[b]	29	41.86	16	40	33.55	35	27.8	42.37
2,6-Diethylpyrazine	6[e]	971	569.17	1168	242	1664.56	755	786.8	507.79
Guaiacol	2.5[s]	2841	3000	2503	1021	1058.77	2156	2284.1	3164.38
Maltol	9000[d]	0.27	0.2	0.25	0.25	0.25	1	0.7	0.46
2,3-Diethyl-5-methylpyrazine	0.09[b]	416	41.86	158	337	348.55	333	236.1	176.81
4-Ethylguaiacol	50[e]	25	24.2	7	43	41.92	8	13	163.51

Table 15 Odor thresholds and approximate Odor Activity Value (OAV) of 20 selected compounds in 8 roasted coffee (Cont.)

Selected compounds	Odor Threshold in water (µg/L)	Odor Activity Value							
		Roasted bean treatments							
		1	2	3	4	5	6	7	8
Indole	90[t]	1.71	1.01	0.4	4.34	2.82	1	0.84	7.07
4-Vinylguaiacol	20[e]	586.15	606.85	1390.71	1499.09	1127.33	1394.5	7556	309.3
(*E*)-β-damascenone	7.5 x 10^{-7}[c]	9095.6	31.33x10^3	15.72 x10^3	116.09 x10^3	n.d.[a]	28 x10^4	3.90 x10^3	49.85 x10^3
Skatole	3[t]	30.42	13.7	6.49	34.29	29.93	13.33	9.77	93.59

[a]Perrson and von Sydow (1973). [b]Semmelroch & Grosch (1996). [c]Semmelroch *et al.* (1995). [d]Rychlik *et al.* (1998). [e]Guth&Grosch (1993). [f]Salo (1970). [g]Calabretta (1975). [h]Shankaranarayana *et al.* (1982). [i]Teranishi (1971). [j]Koehler *et al.* (1971).[k]Guadagni *et al.* (1972). [l]Brule (1971). [m]Wagner *et al.* (1999). [n]Flament (2002). [o]Buttery *et al.* (1971). [p]Czerny&Wagner (1995). [q]Buttery (1999). [r]Ahmed *et al.* (1978). [s]Semmelroch & Grosch (1996). [t]Moss *et al.* (1993). [a]Not detected.

4.1.4 Processing methods, subdivided processing, locations of processing, countries of processing, animal species, and coffee species. Effects on sensorial properties of coffee brew.

4.1.4.1 E-tongue evaluation

In this section after the separation of coffee in according to the processi ng method based on volatile profile discrimination by E-tongue was applied. The aim was to discriminate coffee brew produced from the roasted samples subjects to various processing treatments. The list of sample codes in this part of study is provided in Table 9. The results in Figure 14a) shows the clear separation between Robusta coffee (C-T-R-Fr) and Arabica coffees.

In addition, the distance of regularly wet processed coffee (N-T-A) indicates a well separation between the samples from the others (Figure 14). The first and the second principal components (PCs), PC1 (50.5%) and PC2 (21.4%), drive the separation. PC1 separates Robusta civet coffee (C-T-R-Fr) from Arabica civet/weasel coffees and PC2 separates civet coffees from the normal-process coffee (N-T-A) with additional separation between Robusta and Arabica coffees in the bottom region (Figure 14a).

Meanwhile, the third PC (PC3 (8.6%)) appears to a certain extent to separate coffee origins within the country of processing as Indonesian civet coffees were on the negative side, Thai civet and normal wet-processed coffees were in the middle range (0 to 0.6) and Vietnamese weasel coffee was well-separated to the top right (Figure 14b).

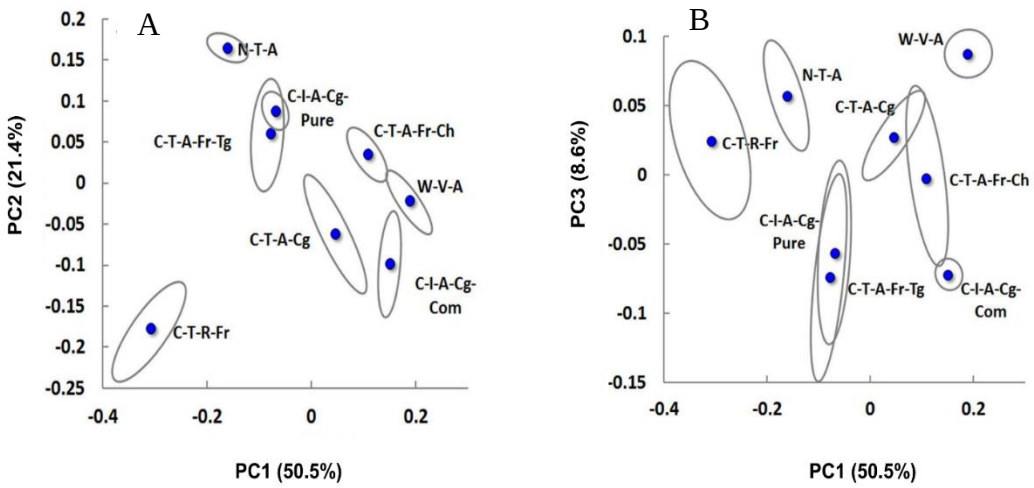


Figure 14 PCA score plots of E-tongue measurements for all coffee samples: A) PC1 and PC2, B) PC1 and PC3. Each point represents the centroid with a data boundary as shown.

This separation is less definite in the case of high purity Indonesian civet coffee (C-I-A-Cg-Pure) and Thai free-range civet coffee from Doitung (C-T-A-Fr-Tg), particularly if the 95% confidence boundaries from three repetitions displayed for each data point are taken into consideration. The distinction between the origins of coffees samples by PC3 most likely occurs as a result of the processing method.

In addition, this different processing methods effects are obvious in the separation via PC2 and PC3 when the same area of Doitung, Chiang Rai are considere d (i.e. N-T-A, C-T-A-Cg, C-T-A-Fr-Tg). Not only the civet and the non-civet coffees are separated, but also the different feeding conditions (free-range¬ (Fr) vs caged (Cg)) may be distinguished. On the whole, E-tongue measurements are able to distinguish the civet and the non-civet coffees, the type of coffee beans (Arabica vs. Robusta), the processing methods and the coffee origins (Indonesia, Thailand and Vietnam).

4.1.4.2 Overall difference tests

To determine the effects of processing methods on the sensorial properties of civet and regular coffee brews with the inclusion of the same factors as in the previous volatile analysis section and to calibrate the results of E-tongue, sensory analysis of overall difference test was used. This part of the study was divided into 3 experiments as follows;

Experiment 1: Comparing civet coffee from different feeding conditions (caged vs. free range)

In this experiment, civet coffees with different feeding conditions: caged (Cg) (C-T-A-Cg and C-I-A-Cg-Pure); and free-range (Fr) (C-T-A-Fr-Tg) were studied (with an addition of normal wet processed coffee (N-T-A) as a reference).

Table 16 shows the percent of the consumers who declared that coffee-pairs were different with "sure" conviction (Yes-sure) and averaged overall-difference rating for corresponding pairs. The differences between the coffee samples are more clearly illustrated in Figure 15. All Thai coffees were from the same Doitung area. Civet and non-civet (normal wet processed samples) were significantly distinguished by consumers (Yes-sure of 92.3 %). Civet coffees with different feeding conditions (free-range and caged) were found significantly different (78.6%). The high purity civet coffee from Indonesia produced from caged civet cats was not significantly different from the others, regardless of free-range and caged feeding conditions.

Table 16 Percent of consumers who declared "Yes, sure" and the ANOVA-averaged overall-difference ratings (1-4 points) for all coffee pairs from the three experiments

Exp.	Pair		Different - sure (%)	Overall-difference rating
1	C-T-A-Cg	N-T-A	92.3	3.8
	C-T-A-Fr-Tg	C-T-A-Cg	78.6	3.6
	C-T-A-Fr-Tg	N-T-A	64.3	3.4
	C-I-A-Cg-Pure	C-T-A-Cg	64.3	3.2
	C-I-A-Cg-Pure	C-T-A-Fr-Tg	57.1	3.4
	C-I-A-Cg-Pure	N-T-A	50	3.1
2	C-T-A-Cg	N-T-A	92.3	4
	C-T-A-Fr-Tg	N-T-A	85.7	3.8
	C-T-A-Fr-Tg	C-T-A-Cg	78.6	3.5
	C-T-A-Fr-Ch	C-T-A-Cg	71.4	3.5
	C-T-A-Fr-Ch	N-T-A	57.1	3.2
	C-T-A-Fr-Ch	C-T-A-Fr-Tg	42.9	2.8
3	W-V-A	C-T-A-Cg	92.3	4
	W-V-A	C-I-A-Cg-Com	76.9	3.7
	W-V-A	C-T-R-Fr	76.9	3.8
	C-T-A-Cg	C-I-A-Cg-Com	69.2	3.4
	C-T-R-Fr	C-I-A-Cg-Com	69.2	3.4
	C-T-R-Fr	C-T-A-Cg	53.8	3.3

The distinction between civet and non-civet coffees as perceived by the consumers also disappears (Yes-sure of 50%) in the case of the Thai non-civet and the Indonesian civet coffees. Comparing the sizes of the percent "Yes-sure" (Table 16) to the distances between the coffee data points in E-tongue space (Figure 14a) yields a very convincing result.

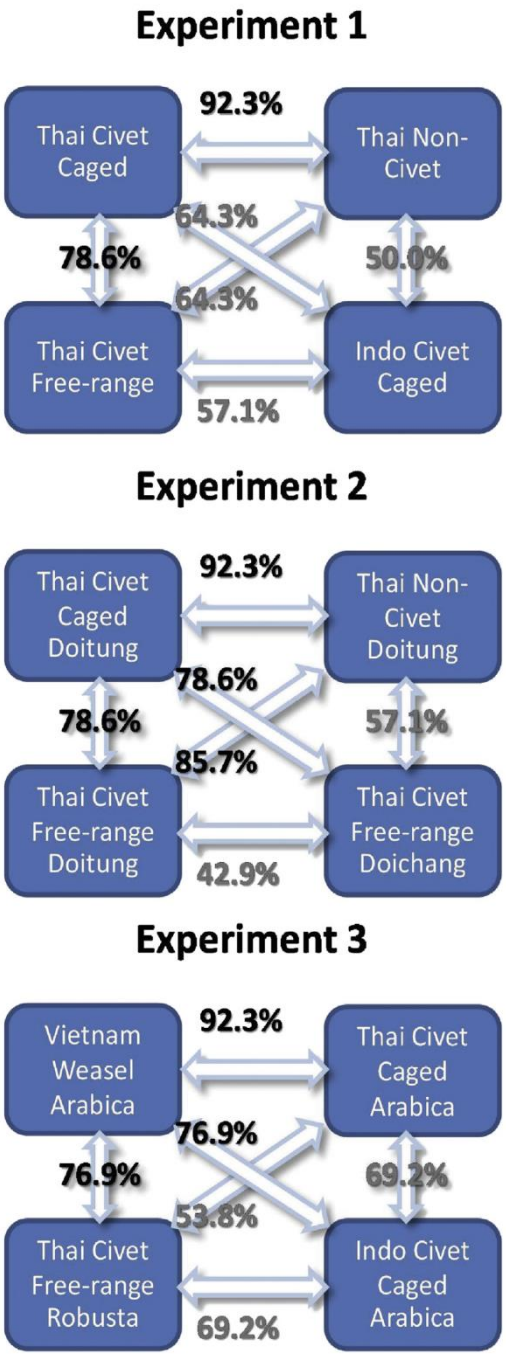


Figure 15 Summary of responses of consumers who declare "Yes, sure" for all coffee pairs from all three experiments

The closer the coffees in the PCA space, the lower the percentages of "Yes-sure". This indicates that consumer's overall-difference perception was captured with PC1 and PC2 of E-tongue with some influences of PC3. Figure 16 shows consumer's overall-difference perceptual space unfolded by the MDS analysis.

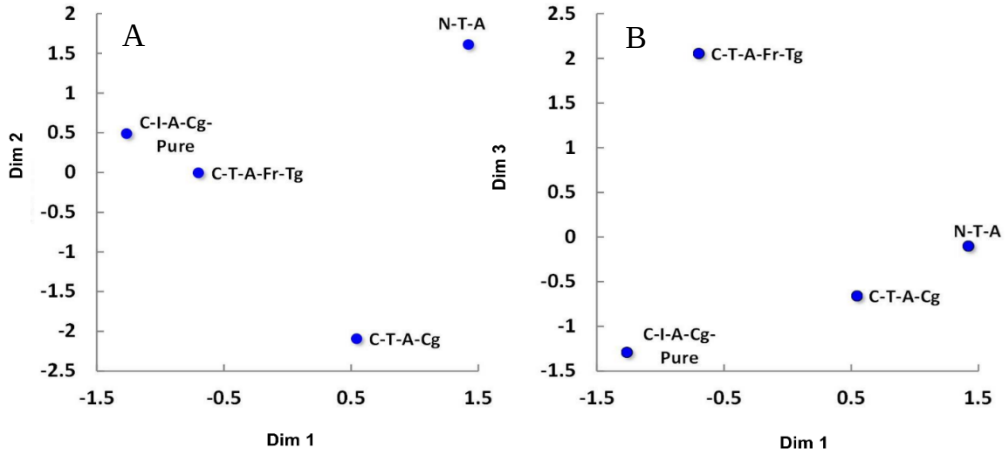


Figure 16 MDS Overall difference perceptual space from Experiment 1, Kruskal's stress (1) of 3-dimensional solution was 0.00003. (A) Dimensions 1 and 2, (B) Dimensions 1 and 3.

To capture the differences among the coffee samples, three dimensions were required. Comparing Figure 16a to Figure 14a shows some similarity between the two spaces, especially the closeness of C-I-A-Cg-Pure and C-T-A-Fr-Tg and the large separation of C-T-A-Cg and N-T-A. Interestingly, the dimension 1 and 3 (Dim1 and Dim3) of MDS structure (Figure 16b) and PC1 and PC3 of E-tongue (Figure 14b) exhibits quite a similar arrangement of C-T-A-Cg, N-T-A and C-T-A-Fr-Tg coffees. These results indicate that E-tongue space from PCA analysis may have some correlation with consumers' overall-difference perceptual space obtained in MDS analysis.

Experiment 2: Comparing Thai civet coffees from the same area (Doitung, Chiang Rai, Thailand) and near-by area (Doichang, Chiang Rai, Thailand) with different feeding conditions (caged vs. free-range).

In this experiment, only Thai civet coffees from Chiang Rai province were evaluated against one another. N-T-A as a reference was an Arabica (A) coffee from the same Doitung area as C-T-A-Cg and C-T-A-Fr-Tg. C-T-A-Fr-Ch was harvested from nearby area, Doichang. The results are shown in Table 16. The percentages of "Yes-sure" amongst these coffees are better depicted in Figure 15. The civet and the normal-process coffees from the same Doitung area show the most significant difference. Besides the differences between processing conditions (normal wet vs. civet processed), different feeding conditions (caged vs. free-range) within the same area (Doitung) was the third most significant difference.

As shown in Figure 16, the effect of feeding condition were disappear when civet coffee samples from different areas are compared (Indonesia vs. Thailand in Experiment 1 and Doichang vs. Doitung in Experiment 2). In Experiment 2, the difference (78.6%) between coffees from different area with different feeding conditions (free-range Doichang vs. caged Doitung) is still significantly larger than that (57.1%) of the coffees from different areas and different processing conditions (civet vs. normal wet process). Based on this trend, the civet coffees from different area with the same feeding conditions (free-range) were not significantly different from each other (42.9%).

Interestingly, the impact of different areas appears to reduce the impact of feeding and processing conditions. The distances between coffee samples in the E-

tongue PCA space in Figure 14 are roughly in agreement with the “Yes-sure” percentages from the consumers (Table 16). The MDS analysis in Figure 17 again confirms a similar pattern of the MDS space to the E-tongue space (Figure 14), with the Doichang (Ch) civet coffee well-separated from the other three. In addition, the Dim3 axis of MDS experiment 2 differentiates the different feeding (caged vs. free-range) and processing (civet vs. normal wet processes) conditions.

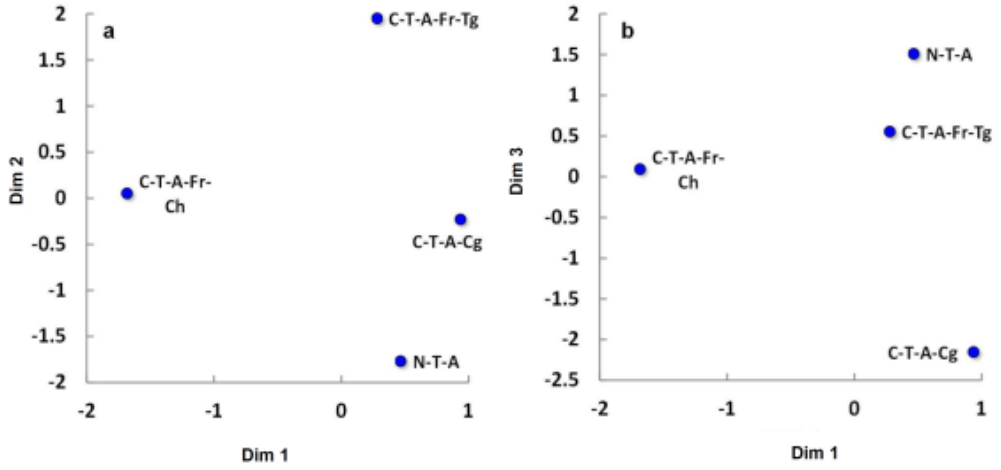


Figure 17 MDS Overall difference perceptual space from Experiment 2, Kruskal's stress (1) of 3-dimensional solution was 0.00003. (A) Dimensions 1 and 2, (B) Dimensions 1 and 3

Experiment 3: Comparing civet coffees from different countries (Thailand vs. Vietnam vs. Indonesia), processing method (civet vs. weasel) and coffee species (Robusta (*Coffea canephora*) vs. Arabica (*Coffea arabica*))

The goal of this experiment was to compare civet coffees from different countries namely from Indonesia, Thailand and Vietnam. However, as mentioned earlier, Vietnamese coffee samples commercially available as "civet coffee" were, in fact, processed by weasels (W). The results are presented in Table 16 and depicted in Figure 15. The "weasel" coffee exhibits the largest differences from the other civet coffees from Thailand and the commercial grade caged civet coffee from Indonesia, regardless of the type of coffee beans used (Robusta or Arabica). This is in agreement with the cluster separation of PCA analysis based on volatile profile in previous section 4.1.3 which confirms the distinct characteristic of weasel coffee. The result of MDS analysis with three-dimensional solutions elaborates this difference in Figure 18.

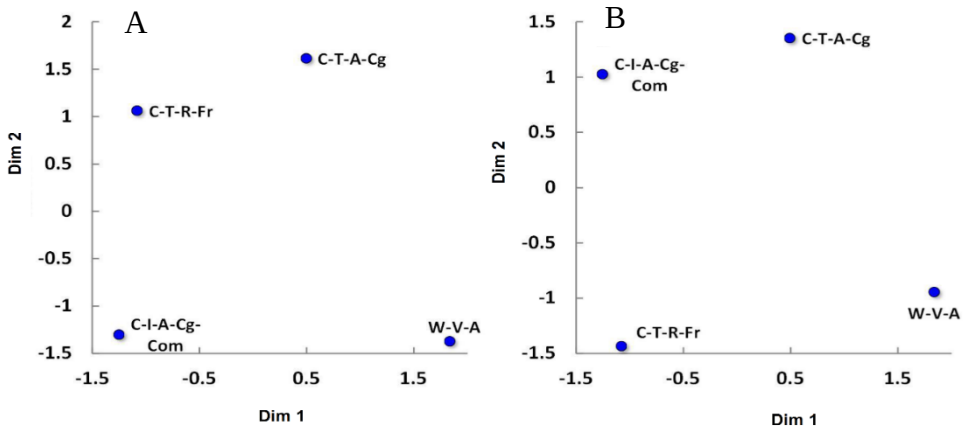


Figure 18 MDS Overall difference perceptual space from Experiment 3, Kruskal's stress (1) of 3-dimensional solution was 0.00002. (A) Dimensions 1 and 2, (B) Dimensions 1 and 3

In addition, the civet coffees from Thailand and Indonesia are rather different (Figure 15), but the differences are lower than when compared to the Vietnamese weasel coffee. Comparing coffees of different species (Robusta vs. Arabica) and different feeding conditions (free-range vs. caged) from the same country (Thailand) does not show a different result. This contrasts with the PCA analysis in previous section based on volatile profile. However, the distribution of the data points in the MDS space for Experiment 3 (Figure 18) again resembles that in the E-tongue space (Figure 14), with a clear separation of the weasel coffee.

4.1.4.3 Relationship between E-tongue measurement and consumers' perception of overall differences of civet coffees

Figure 19 shows the locations of all coffees samples from the three experiments in a combined space from E-tongue and the MDS analysis from the three experiments. The space is analogous to the PCA score plot. With three dimensions, all coffees are distinguishable from each other. The GPA spaces in Figure 19a and Figure 19b clearly resemble the E-tongue spaces in Figure 14a and Figure 14b, respectively, since the GPA results are essentially the E-tongue space refined by the MDS results from the overall difference tests. GPA1 (48.3% variance) seems to differentiate coffee species (Robusta vs. Arabica). GPA1 and GPA2 (33.2%) together (Figure 19a) could differentiate processing and feeding conditions (normal wet-process, civet caged and civet free-range).

The correlations between E-tongue principal component axes (PC1-3) and the MDS dimensions from the three experiments are obtained from GPA, as shown in Table 17. Dimensions with strong correlations (with the magnitude above 0.7) to the GPA axes GPA are highlighted in bold. This allows matching of multi-dimensional structures/spaces, in analogy to the PCA loading plot. In this study, matching the E-tongue space with the three perceptual spaces from the three experiments provides the meaning to E-tongue measurements based on human perception of overall differences between coffees.

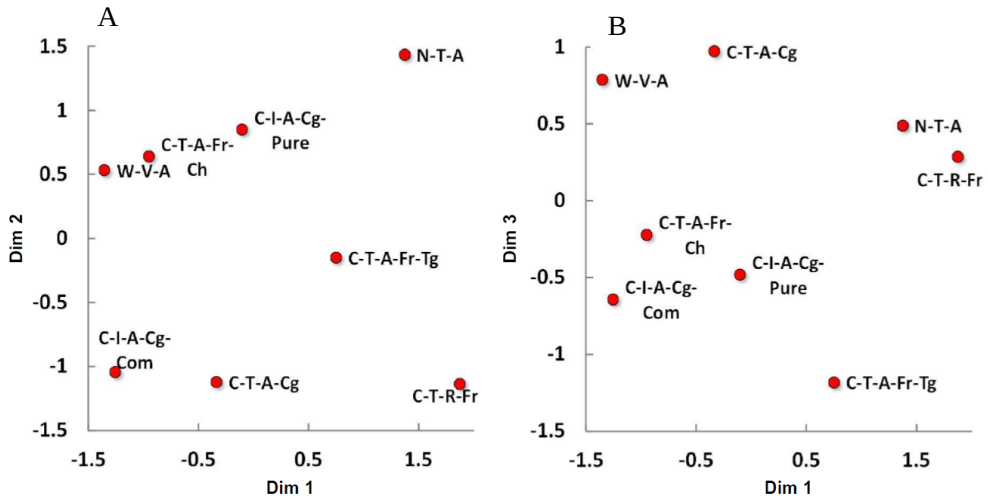


Figure 19 GPA space of all coffee samples representing combined space from E-tongue and MDS-spaces from the three experiments (A) Dimensions 1 and 2, (B) Dimensions 1 and 3.

Table 17 Correlation between E-tongue PC-scores and MDS dimensions from the three experiments. The numbers indicate the degree of correlation to GPA dimensions.

Original Dimension	GPA1 (48.3%)	GPA2 (33.2%)	GPA3 (18.5%)
E-tongue1	**-0.98**[a]	0.03	0.03
E-tongue2	0.08	**0.88**[a]	-0.18
E-tongue3	0.07	0.25	**0.90**[a]
Exp1-1	0.48	0.11	**0.76**[a]
Exp1-2	**0.78**[a]	**0.97**[a]	-0.33
Exp1-3	0.51	-0.17	-0.59
Exp2-1	0.59	-0.40	0.36
Exp2-2	-0.20	-0.51	**-0.79**[a]
Exp2-3	0.64	**0.89**[a]	-0.47
Exp3-1	-0.47	**0.83**[a]	**0.77**[a]
Exp3-2	**0.70**[a]	-0.62	0.48
Exp3-3	-0.50	-0.43	-0.16

[a]Bold font indicates >0.7 correlation to GPA dimensions

E-tongue space (PC1-3) is well correlated to the three GPA dimensions, GPA1, GPA2, and GPA3, respectively. This confirms the E-tongue space as the main contribution to the GPA space. PC1 shows strong negative correlation with Dim2 of experiment 1 and experiment 3 of MDS analysis, whereas PC2 exhibits strong positive correlation with Dim 2 of experiment 1, Dim 3 of experiment 2 and Dim1 of experiment 3. The large negative values also signify that the data are highly correlated but in the opposite sense, which only depends on the order of the data in the matrices. PC3 shows strong positive correlation with Dim1 of experiment 1 and 3 and strong negative correlation with Dim2 of experiment 2.

The meanings of each E-tongue axes are best extracted using the summary of overall different characteristics from the MDS analysis of the three experiments in Table 18. The characteristics listed for each dimension start from the

most significant one. The characteristics that are well distinguished by the overall difference tests include processing methods (normal wet vs. civet), feeding conditions (free-range vs. caged), animals used in the process (civet vs. weasel), countries of origin. The others that are less distinct including coffee species (Robusta vs. Arabica) and local areas (Doitung vs. Doichang). When these characteristics are related to Table 17 the physical meanings may be assigned to the E-tongue space.

Table 18 Qualitative summary of overall different characteristics extracted from the MDS analysis of the three experiments.

Original dimension	MDS overall different characteristics
Dimension 1 of MDS analysis from experiment 1	Processing (normal wet vs. civet) Countries (Thailand vs. Indonesia)
Dimension 1 of MDS analysis from experiment 2	Processing (normal wet vs. civet)
Dimension 3 of MDS analysis from experiment 1	Feeding (Free-range vs. caged) Processing (normal wet vs. civet) Countries (Thailand vs. Indonesia)
Dimension 1 of MDS analysis from experiment 2	Nearby area (Doitung vs. Doichang)
Dimension 2 of MDS analysis from experiment 2	Processing (normal wet vs. civet) Feeding (free-range vs. caged)
Dimension 3 of MDS analysis from experiment 2	Feeding (free-range vs. caged) Processing (normal wet vs. civet)
Dimension 1 of MDS analysis from experiment 3	Processing (civet vs. weasel) Countries (Thailand, Indonesia, Vietnam)
Dimension 2 of MDS analysis from experiment 3	Countries (Thailand vs. Indonesia & Vietnam) Processing (civet vs. weasel)
Dimension 3 of MDS analysis from experiment 3	Coffee species (Robusta vs. Arabica)

PC1 of E-tongue (correlated to dim2 of experiment 1 and 3) is able to distinguish mainly the processing methods, including normal wet, civet and weasel processes. The countries of origins can also possibly separated by PC1 but more Indonesian and Vietnamese coffees are needed for a more conclusive study. PC2 (correlated to dim2 of experiment 2 and 3, dim3 of experiment 2) is able to separate the feeding and the processing conditions (free-range, caged, normal wet, civet). PC3 (correlated to dim 1 of experiment 1 and 3, and dim2 of experiment2) shows a similar characteristic to PC2, but with the addition of ability to distinguish civet and weasel coffees. The remaining two characteristics that are not correlated to the E-tongue space are the coffees from nearby areas (Doitung vs. Doichang) and the coffee species (Robusta vs. Arabica), even though the two coffee samples responsible for these characteristics (C-T-A-Fr-Ch and C-T-R-Fr) are well separated from the rest on the E-tongue space (Figure 14). The reason is that these two characteristics are not conclusively distinguished by the consumers with low percentages of "Yes-sure" in the overall difference tests.

The result in this part of study showed a more clear discrimination of roasted coffee produced within different processes, subdivided processing (feeding condition; caged and free-range civet), country of processing and cultivar following from the previous section in term of sensory quality when brewed coffee, final product delivered to consumer was test. The application of E-tongue, overall-difference sensory test and the determination of the relationship between the results provided by E-tongue and overall difference test by GPA help in better understanding of the impact of processing methods on the complex composition and large variation with 8 coffee sample in this study.

4.2 Effects of heat pump drying on physicochemical properties of Arabica coffee

4.2.1 Physical properties

4.2.1.1 Color evaluation

Visual assessment consists in the observation of external coffee quality attributes by coffee processor. Hence, the determination of color value can be used to assess the effects of drying conditions on the color of dried product. The color values according to CIE $L^*a^*b^*$ system, color space of dried coffee beans on hue and chroma in Munsell color subjected to the different drying methods are shown in Table 4.10. For ΔE evaluation between dried green coffee samples, sun dried coffee (control) was used as reference sample to evaluate the total difference of color value within drying treatments. Sun dried coffee was also used to calibrate the difference of ΔE of sun dried sample itself.

The use of Mechanical drying (heat pump and tray drying) caused an increase in the L* compared to sun dried sample. In contrast to L*, the value of a* and b* decreased when all drying methods were applied to coffee beans.

Table 19 Color values (L*, a* and b* of green coffee processed by heat pump drying (40, 45 and 50˚C), tray drying (50˚C) and sun drying

Color values	Green coffee drying treatments				
	Heat pump drying			Sun drying	Tray drying (50 ˚C)
	40 ˚C	45 ˚C	50 ˚C		
L*	61.18±1.12[a]	60.51±0.55[a]	60.74±0.41[a]	55.07±0.46[b]	59.20±0.76[b]
a*	2.74±0.10[a]	3.12±0.10[c]	3.10±0.05[bc]	4.32±0.24[d]	3.44±0.10[e]
b*	10.94±0.62[a]	11.74±0.41[ab]	12.53±0.49[b]	15.50±0.34[c]	13.67±0.83[d]
Hue angle	75.1±0.09[a]	75.3±0.13[a]	74.9±0.43[a]	76.03±0.88[bc]	76.12±0.13[bc]
Chroma	11.99±0.15[a]	12.34±0.04[a]	12.81±0.27[b]	15.2±0.07[b]	15.60±0.24[b]
ΔE	7.78	6.721	6.51	2.21	4.59

[a,b,c,d,e]values with different letter in a row are significantly different (p<0.05).

; ΔE > 2.3 corresponds to just noticeable difference (Sharma, 2003).

Maximum color lightness was found for heat pump drying at 40 ˚C (61.18±1.13), followed by 50 ˚C (60.74±0.61) and tray drying 50 ˚C (59.20±0.76). All heat pump dried coffee showed a significant difference with tray dried and sun dried sample (p<0.05). Increasing of L* values indicated to the brightening color of coffee passed heat pump drying. The lesser of a* showed the trend towards increasing of greenish shade in coffee sample. Heat pump dried coffee at 40 ˚C has the lowest a* color at 2.74±0.10 followed the heat pump dried coffee at 50 ˚C (3.10±0.05). The value of the blue-yellow (b*) coordinate parameter ranged from 10.94+0.62 (Heat pump

drying at 40 ˚C) to 15.50±0.34 (Sun drying). These variations in the value of L*, a*, and b* may explain to the degradation of pigments or non-enzymatic Maillard browning during drying (Dadali *et al.*, 2007). The significant difference of b* showed that between heat pump dried sample, heat pump dried at 40˚C was significant difference from the heat pump dried sample at 50 ˚C. The mechanical dried sample (heat pump dried and tray dried coffee) had the significantly lesser value (p<0.05) of b* than sun dried sample. The total color change of coffee beans (ΔE) indicates that the highest ΔE was observed in heat pump dried coffee at 40 ˚C. A higher ΔE value of heat pump dried coffee at 40˚C might be due to the changes in the L*, a* and b*values of the sample that were treatments higher than in the two other heat pump dryings. The higher a* and b* values of sun dried and tray dried coffee indicated the slightly darker coffee beans with more shades of red and yellow that were obtained through the natural sun drying and mechanical drying.

The result of color assessment showed that coffee dried by heat pump drying tended to give a brightener shade of green coffee.

4.2.1.2 Moisture contents and water activity in green coffee

Moisture content and water activity are the important factors that influence to growth of microbes and the activation of enzymatic and non-enzymatic chemical reactions which lead to the deterioration of coffee beans during storage, trading and export. The normal acceptable range of moisture content after drying of coffee beans is 10-12 % (w.b.) After drying, the water activity and moisture content values showed that dried coffee samples were acceptable to be stored at room temperature as shown in Table 20.

The water activity of the dried coffee was considered safe from microbial spoilage because the value was lower than the minimum level of water activity required for microbial growth which is 0.6 (Kerley *et al.*, 1985).

Table 20 Drying time, Moisture content, water activity of green coffee dried by heat pump dryer (40, 45 and 50 ˚C), tray dryer (50 ˚C) and sun drying

Properties	Green coffee drying treatments				
	Heat pump drying			Sun drying	Tray drying (50 ˚C)
	40 ˚C	45 ˚C	50 ˚C		
Drying time (h)	23	16	11	72	18
Moisture content (% w.b.)	9.66±0.49	10.48±0.32	10.00±0.07	10.71±0.27	9.10±0.67
Water activity (a_w)	0.59±0.048	0.59±0.04	0.59± 0.005	0.6±0.01	0.56±0.03

Mean±S.D < 10% from triplicate analysis

In this study, it was found that final moisture contents of green coffee in all drying treatment below 11% (w.b.) as shown in Table 20. The drying time in the heat pump dryer at 40, 45, 50˚C was 23, 16 and 11 h respectively. Heat pump drying was effective in coffee drying with the lowest drying time of 11 h at 50 ˚C compare with the conventional tray drying 50 ˚C (18 h) and natural sun drying (72 h).

4.2.2 Chemical properties

4.2.2.1 Chlorogenic acids contents

Phenolic compounds of coffee are responsible for the astringency, aroma development, flavor and antioxidative activity (Farah & Donangelo, 2006; Cheong *et al.*, 2013). The mains phenolic acids in coffee belong to chlorogenic acid (CGA) group. The variation of CGA isomer classes is responsible for

good quality and fraud indicative in coffee and was successful in distinguishing between defect and non-defect green and roasted coffee (Farah *et al.*, 2006; Farah *et al.*, 2005a; Toci & Farah, 2008; Toci & Farah, 2014). These phenolic groups were determined in this study in order to investigate the effects of thermal treatment applied during heat pump drying to green coffee on the quality of coffee via the changed CGA content. Eight CGA which were 5-caffeoylquinic acid (Chlorogenic acid/5-CQA), 3-caffeoylquinic acid (Neochlorogenic acid/3-CQA), 4-caffeoylquinic acid (4-CQA), 4-feruloylquinic acid (4-FQA), 5-feruloylquinic acid (5-FQA), 3,4-dicaffeoylquinic acid (3,4-diCQA), 3,5-dicaffeoylquinic acid (3,5-diCQA) and 4,5-dicaffeoylquinic acid (4,5-diCQA) were previously mentioned as indicators of quality of green coffee. They were identified and confirmed their structures in this study. Their contents are shown in Table 21.

Table 21 Content of chlorogenic acids and their derivative in all dried green coffee samples

Green coffee No.[a]	Concentration (mg/100g dry weight)[b]								
	Chlorogenic acids derivatives								
	5-CQA	3-CQA	4-CQA	5-FQA	4-FQA	3,4-diCQA	3,5-diCQA	4,5-diCQA	Total CGA
1	2797.41	264.67^{a}	401.11^{a}	350.37^{a}	65.67^{a}	91^{a}	236.3^{a}	63.33^{a}	3885^{ab}
2	2715.73	287^{ab}	420.22^{b}	334.83^{b}	70.67^{b}	101.67^{ab}	217.03^{b}	52.05a	3757^{ab}
3	2687.77	249.67^{a}	375.19^{c}	344.44^{c}	64.67^{a}	100.33^{a}	282.59^{c}	65.92^{ab}	3795^{ab}
4	2909.63	254.67^{b}	431.48^{d}	361.48^{d}	81.67^{d}	115^{b}	224.08^{d}	82.58^{b}	4159.67^{b}
5	2590.76	276.67^{ab}	357.17^{e}	345.51^{c}	74.67^{e}	95.33^{a}	228.08^{e}	57.56^{a}	3707.67^{a}

[a]1= Heat pump drying 40 ◦C, 2 = Heat pump drying 45 ◦C, 3= Heat pump drying 50◦C, 4= Sun drying, 5 = Tray drying 50◦C.[b]Mean ± S.D ≤ 20% with triplicate analysis (n=3)

Despite the significant difference between CGA contents, the results showed in Table 21 indicate that sun drying had the total highest CGA (4159.67) followed by heat pump drying at 40 ˚C (3885) and 50 ˚C (3795) and the lowest amount was observed in tray dried coffee (3707.67). In terms of the correlation between CQA isomers and quality of coffee, high concentration of 5-CQA, 4-CQA, 4-FQA and 5-FQA were previously claimed as being strongly correlated with Rio-off flavor of coffee (r^2 = 0.93, 0.94, 0.82 and 0.90, respectively) and claimed to be in accordance with high astringency flavor of coffee (Farah, 2012; Farah *et al.*, 2006), 3-CQA was not mentioned in the definite specific pattern (r^2 =0.73), however, according to Farah *et al.* (2006), its high content in coffee tends to correlate with fraud of coffee. As shown in Table 21, among all heat pump drying conditions, the concentration of those qualitative indicators (3CQA, 5-CQA, 4-CQA and 4-FQA) were lowest in heat pump dried coffee at 50 ˚C. The concentration were proportionally at 2687.77, 249.67 375.19 and 64.67 mg/100g dry weight coffee, respectively. In contrast to other dried samples, sun dried coffee exhibits the highest contents of these chlorogenic acids isomers. Despite of the non significant difference (p>0.05) between sun dried coffee and heat pump dried coffee at 50˚C in the content of 5-CQA, however, tray dryer at 50 ˚C tend to provide coffee with lower 5-CQA.

In contrast to the aforementioned group, 3,4-diCQA, 3,5-diCQA and 4,5-diCQA were claimed as the phenolic group indicating to good quality of coffee (Farah *et al.*, 2006). Sun drying had the highest content of 3,4-diCQA and 4,5-diCQA at 115 and 82.58 mg/100 g dry weight. In spite of that, considering to influence in the concentration of total CGA, 5-CQA, 4-CQA, 4-FQA and 5-FQA,

sun dried coffee seems to be of lesser quality than heat pump dried coffee as heat pump dried coffee had a lower content of these undesirable phenolic compound. Considering among all heat pump drying condition, heat pump dried coffee at 50 ◦C had the highest contents of 3,4-diCQA, 3,5-diCQA and 4,5-diCQA at 100.33, 282.59 and 65.92, respectively.

A higher contents of CGA in sun dried green coffee was correlated to the previous discussion on color determination about the darker shade of sun dried coffee in the section of effect of heat pump drying on physical properties of coffee. According to Farah *et al.* (2006) as sample quality decreased from the high content of major CGA (5-CQA), the color intensity of green arabica beans increased significantly ($r^2 = 0.96$). A high correlation between intensity of color and the content of 5-CQA has been claimed in their study since the more 5-CQA increased, the darker shade of green coffee was observed.

CGA isomers provide an important contribution to color intensity of coffee, a portion of them are likely to be the major substrate for activation of the enzyme polyphenol oxidase (Mazzafera & Robinson, 2000). During sun drying, there has been a period of long time drying under the presence of oxygen, frequent stirring of coffee parchment and uncontrol atmosphere, hence, the possibility to explain this circumstance is the generation of ortho-quinones, formed by the action of polyphenol oxidase on CGA that results to the darker shade of the beans. Moreover, Amorim *et al.* (1977) related the action of polyphenol oxidase, triggered by structural changes of bean cell membranes, as a possible cause of the Rio-off-flavor which relate to the high content of 5-CQA. Therefore, this explanation agrees with the results

produced from sun dried coffee and explains the correlation between natural sun drying practice and the activation of polyphenol oxidase, the high content of 5-CQA and the shade in green dried coffee.

4.2.2.2 Antioxidant activity of dried green coffee

TPC, DPPH assay, and FRAP assay have already been described in this study as rapid in vitro methods in the previous studies section for the determination of antioxidant activity. In this section, the antioxidant activity of all green coffee beans was determined and expressed in terms of TPC, DPPH assay, and FRAP assay in order to determine the effects of different drying treatments. The results of the antioxidant activity and phenolic contents determination in all dried green coffee beans are shown in Table 22.

Table 22 Antioxidant activities of dried green coffee

Antioxidant Activities	Condition of drying				
	Heat pump drying			Tray dry 50 ˚C	Sun drying
	40 ˚C	45 ˚C	50 ˚C		
Total phenolic content^{ns} (mg GAE/g coffee)	44.09±2.94^{a}	48.02±1.64^{ab}	53.19±4.67^{b}	41.30±2.49^{a}	48.97±1.45^{ab}
DPPH radical scavenging activity (%)	80.46±0.09	89.99±2.11	90.99±0.95	87.16±3.44	90.99±0.61
FRAP assay (mg Trolox/g coffee)	39.78±20.47^{a}	38.42±17.47^{a}	65.27±22.93^{b}	56.47±49.38^{b}	64.69±30.22^{b}

^{a,b}values with different letter in a row are significantly different (p<0.05), mean+S.D < 15%.

The results in Table 22 show the highest TPC contents in green coffee dried in a heat pump dryer at 50 ˚C (53.19±4.67). Considering that the major phenolic compounds determined in the section 4.2.2.1 namely the CQA isomers were found to have highest concentration in the sun dried and the heat pump dried (40 ◦C) samples, there are also minor phenolic compounds like sinapic or *p*-coumaric acid that may contribute to the antioxidant activity (Cheong *et al.*, 2013; Farah, 2012; Liang *et al.*, 2014). When considering results, two antioxidant activity assays, DPPH and FRAP assay between green coffee drying treatments ($p>0.05$). Heat pump dried coffee, tray dried coffee and sun dried coffee showed the % inhibition (≈92%) of sun dried green coffee extract reported by Somporn *et al.* (2012). In addition, the FRAP of each extract was determined and expressed in mg Trolox/g coffee. FRAP of coffee extract from heat pump dried coffee at 50˚C (65.27±22.93) had higher values than those of coffee dried at 40 and 45˚C (39.78±20.47, 38.42±17.47 mg Trolox/g coffee) as well as and tray dried coffee (56.47±49.38 mg Trolox/g coffee) ($p<0.05$). This value is comparable with FRAP from sun drying (64.69±30.22) since no significant difference was observed in both treatments ($p>0.05$). In addition, the highest TPC contents and FRAP of heat pump 50 ˚C could be explained that the mild condition of drying using shorter time (11 h) combined with the closed system with no exposure to light and uniform drying air distribution. These could overcome to the problem of bioactive compounds loss in conventional mechanical hot air drying due to the long time exposure of heat, the photodegradation and the degeneration of the cellular membranes from the high temperature used in mechanical dryer that causes the leak of the oils, reducing sugars and acids in the beans (Borem *et al.*, 2008; Coradi *et al.*, 2007; Taveira *et al.*, 2015). The extravasations of fatty acids present in the cell interior due to long drying time at

high temperature by mechanical dryer has been claimed the activate to oxidative or catalytic reactions between coffee beans metabolites and forming undesirable by-products (Borem *et al.*, 2008; Coradi *et al.*, 2007;). This could contribute to the lower CGA content in tray dried coffee compared with heat pump dried coffee. Despite the lower of total CGA contents of heat pump dried coffee at 50 ˚C than at 40 ˚C and sun dried coffee, the high FRAP value may be due to the action from different class of phenolic acids within matrix of coffee like sinapic acid or *p*-coumaric acid (Cheong *et al.*, 2013; Farah, 2012; Liang *et al.*, 2014;).

It may also be related to the extraction method of antioxidant compounds because CGA comprises different isomers. The interaction between these CGA isomers in the extract of green coffee could affect the variation in antioxidant activities according to Liang *et al.* (2016). The TPC found in this study were in agreement with values in Arabica coffee reported by Somporn *et al.* (2012) and Cheong *et al.* (2013) (34.32 - 53.76 mg GAE/g coffee) with slightly higher. FRAP values in green coffee detected in this study experiment also as compared with Cheong *et al.* (2013) (123.40 –147.46 mg Trolox/g coffee). This might due to the natural variability of samples, plantations, method of extraction and identification as well as the sample preparation method since this study ground green coffee under liquid N_2 to prevent loss of bioactive components before extraction.

Three methods of assessing of antioxidant activity showed that TPC contents and FRAP assay could be used to evaluate the different methods of coffee drying. To gain a better understanding of the effects of antioxidant activity of

drying methods on various drying conditions, the additional antioxidant activity assays should be investigated in the future.

4.2.2.3 Caffeine contents

Caffeine is an alkaloid compound contributing to the bitter taste of coffee flavor (Perrone *et al.*, 2008). In this study, the contents of this compound were not significantly different in sample subjected to different drying treatments ($p<0.05$) (Table 23).

The values were similar in the heat pump, tray and sun dried samples. This non-significant difference could be explained by the relatively stable structure of caffeine that is very resistant to degradation and release by the drying temperature used in this study (Dong *et al.*, 2017).

The levels of caffeine reported here are in agreement with previous studies, indicated that the caffeine content of Arabica coffee beans ranged from 0.8–1.4 % (Belitz *et al.*, 2009).

Table 23 Sugar and caffeine contents in green coffee subjected to different drying treatments

Sugar profile	Concentration (mg/100 mg green coffee bean)[I]				
	Green coffee drying treatments				
	Heat pump drying			Tray drying (50 ˚C)	Sun drying
	40 ˚C	45 ˚C	50 ˚C		
Sucrose	4.85±0.34[a]	4.43±0.21[b]	4.55±0.40[c]	4.97±0.32[d]	3.96±0.254[e]
Total Sugar	4.8±0.34	4.4±0.21	4.55±0.40	4.96±0.40	3.96±0.40
Caffeine	1.259±0.02	1.199±0.12	1.22±0.10	1.25±0.06	1.21±0.04

[I]Mean± S.D <15% with duplicate analysis. [a,b]values with different letter in a row are significantly different (p<0.05).

4.2.2.4 Sugar contents

The sugar profile of the samples is show in Table 23. Sucrose is normally, the most abundant sugar in green coffee. It plays an important role in the Maillard reaction and Strecker degradation in conversion to fructose and amino acids which continue reacting with the reactive species like amino acids, chlorogenic acids during coffee bean roasting (Belitz *et al.*, 2009; Flament, 2002).

As previous mentioned, mechanical dryer usually provides coffee with less flavor than sun dried coffee due to the metabolic rate of coffee seed produces that is germinating and producing some important aroma precursors (Borém *et al.*, 2014; Kleinwächter & Selmar, 2010). However, considering to the content of total sugar in all heat pump green coffee, drying by heat pump dryer showed the trend to preserve more sugar, comparing with the control (sun drying) even the lack of regenerate of sugars from germination of beans embryo and sugar catabolism from the

metabolic process within the beans (Borem *et al.*, 2014; Kleinwächter & Selmar, 2010). The content of total sugars observed in tray drying at 50 ˚C was the highest (4.97 mg/100 mg green coffee).

Comparing between heat pump drying and tray drying, the higher concentration of sugars indicated that the use of different mechanical drying approach resulting to the variation of main sugars content in green coffee. Due to the removal of water from the coffee beans during mechanical drying, the high content of sugar in tray dried coffee could be explained by the non-enzymatic browning reaction during the long drying time in this study (18 h) because the high drying temperature can enhance sucrose hydrolysis, yielding the amount of glucose and fructose available for Maillard reaction (Borompichaichartkul *et al.*, 2013).

4.2.2.5 Amino acids contents

Coffee beans consist of various amino acids. They have been reported to be susceptible to the processing conditions, depending on the source, and the processing method applied (Dong *et al.*, 2017). The total amino acid contents of all dried coffee samples subjected to different drying treatments were significantly different at p-value< 0.05 (Table 24).

Table 24 Amino acid profile in green coffee subjected to heat pump drying (40, 45 and 50°C), tray drying (50°C) and sun drying

Amino acids	Concentration (mg/100 mg green coffee beans)[a]				
	Green coffee drying treatments				
	Heat pump drying			Tray drying (50°C)	Sun drying
	40°C	45°C	50°C		
Aspartic acid	1.16	1.03	1.27	1.22	1.16
Serine	0.57	0.57	0.58	0.57	0.59
Glutamic acid	2.18[a]	2.11[b]	2.34[c]	2.31[d]	2.17[b]
Glycine	0.65[a]	0.64[a]	0.68[b]	0.66[c]	0.7[d]
Histidine	0.26[a]	0.25[b]	0.27[c]	0.26[a]	0.28[d]
Arginine	0.64[a]	0.63[b]	0.69[c]	0.66[d]	0.67[e]
Threonine	0.41[a]	0.41[a]	0.43[b]	0.41[a]	0.43[b]
Alanine	0.46[a]	0.43[b]	0.47[c]	0.45[d]	0.46[a]
Proline	0.53[a]	0.52[b]	0.54[a]	0.53[a]	0.54[a]
Tyrosine	0.29[a]	0.27[b]	0.3[a]	0.28[a]	0.3[a]
Valine	0.54[a]	0.51[b]	0.57[c]	0.54[a]	0.55[d]
Isoleucine	0.39[a]	0.37[b]	0.4[a]	0.38[c]	0.39[a]
Leucine	0.85[a]	0.82[b]	0.89[c]	0.86[d]	0.86[d]
Lysine	0.65[a]	0.62[b]	0.66[c]	0.66[c]	0.64[d]
Phenylalanine	0.52[a]	0.49[b]	0.56[c]	0.54[d]	0.54[d]
Total Amino acid (mg/100 mg)	10.1[a]	9.67[b]	10.65[c]	9.41[d]	10.28[e]

[I]Mean ±SD<20% with triplicate analysis. [a]different letter in a row are significantly different (p<0.05)

The total amino acid content determined in the samples could be ranked as follows: heat pump drying at 50 °C >Sun drying > heat pump drying at 40 °C > heat pump drying at 45 °C> tray drying at 50 °C. A slightly lower amino acid content was observed in heat pump dried coffee at 45 °C which might be due to the natural variation of green coffee. The concentration of total amino acids in tray dried and sun dried coffee were 9.41 and 10.28 mg/100 g green coffee respectively. Most amino acids

showed a significantly difference between tray dried sample and sun dried coffee. There was also a small difference between amino acid content in heat pump dried and sun dried coffee.

Glutamic acid and Aspartic acid are the main amino acids in green coffee (Belitz *et al.*, 2009). They ranked as follows: heat pump drying at 50 ˚C > tray drying at 50 ˚C > heat pump drying at 40 ˚C > sun drying > heat pump drying at 45 ˚C. The use of heat pump drying resulted in the increase of total amino acid. This may relate to shorter drying time which results in lower rate of proteolytic reaction within the beans in comparison with the longer drying at lower temperature in sun drying.

Considering to the content of essential amino acids; lysine, leucine and phenylalanine by increasing the heat pump drying temperature to 50 ˚C tended to increase the content of lysine, leucine and phenylalanine (0.66, 0.89, 0.56 mg/g green coffee) compared to control sun dried sample.

As mentioned in literatures, the reactive sulfur amino acids, cysteine and methionine play the major role in the development of sulfur volatile compounds via the Strecker degradation to thiols and sulfides group (2-furfurylthiol, methanethiol, methional) that influence mainly coffee like characteristics (Flament, 2002; Holscher & Steinhart, 1992) of coffee beverages.

Table 25 Important free amino acids in green coffee subjected to heat pump drying (40, 45 and 50°C), tray drying (50°C) and sun drying

Amino acids	Concentration (mg/100 mg green coffee bean)[a]				
	Green coffee treatments				
	Heat pump drying			Tray drying (50°C)	Sun drying
	Heat pump drying at 40°C	Heat pump Drying at 45°C	Heat pump Drying at 50°C		
Cysteine	0.002	0.002	0.002	0.001	0.002
Methionine	0.01	0.01	0.01	0.01	0.01

[a]Mean ±SD<10%, with triplicate analysis.

The results on Table 25 show that among heat pump drying treatment, there were no significantly difference observed in the contents of cysteine and methionine. The content of cysteine of heat pump dried sample were equal to sun dried coffee and were slightly higher than tray dried coffee but no significantly difference was observed (p>0.05). The concentration of methionine in all sample were not significant different from each other (p>0.05).

The difference could not be observed in sulfur amino acids of all dried green coffee. This points out that the different drying methods alter the content of some amino acids in green coffee but that of sulfur amino acids. Considering the overall results of amino acids determination, the appropriate drying condition of heat pump drying could be the drying condition at 50 °C due to its benefit in the preservation of amino acids within the shortest time of drying at 11 h.

4.2.2.6 Total Fatty acid contents

The fatty acid profile (% total fatty acids by weight of methyl esters) is presented in Table 26. Eighteen fatty acids were identified in dried green coffee of this study.

The lipid fraction of coffee is not subjected by heat treatment during the roasting process with only minor changes. However, due to the use of heat pump drying, this fraction could change under different drying conditions. Among all free fatty acids, the main ones in coffee are reported to be linoleic acid and palmitic acid (Belitz *et al.*, 2009).

Comparing between dried green samples, the major fatty acid identified in green coffee sample was linoleic acid (C18:2n6) accounting for 37.12% (heat pump drying at 40◦C) to 40.91% (heat pump dried coffee at 50◦C), followed by palmitic acid (C16:0) which ranged from 34.79% (heat pump dried coffee at 50◦C) to 37.45% (sun drying). Despite, the significant difference observed between heat pump dried samples, it was notably that the mechanical drying i.e. heat pump drying and tray drying at 50 ◦C were effective to preserve linoleic acid content more than natural sun drying. However, sun drying still provides a higher content of palmitic acid than the mechanical drying.

The other fatty acids which were detected were stearic acid (C18:0) (6.28%–7.62%), arachidic acid (C20:0) (3.25-4.05%), alpha-linolenic acid (C18:3n3) (1.12-1.46%), and behanic acid (C22:0) (0.71-1.12%). Other fatty acids were detected at percentages of less than 0.5%. The total saturated fatty acids ranged from 46.20% to 49.99%, with the highest percentage in sun dried coffee beans and lowest in heat pump dried bean at 50 ◦C. Considering to contents of monounsaturated fatty acid,

heat pump dried sample (40 and 45 ◦C) were the highest contents of around 8%. For the concentration of unsaturated fat, they ranged from 45.14 to 48.93%. The contents of free fatty acids in this study agrees with the findings of Belitz *et al.* (2009) that reported the major percentage of linoleic acid and palmitic acid in Arabica coffee at 40-45% and 25-35%, with lower amounts of various fatty acids (arachidic, behenic and lignoceric) that are naturally present in the raw coffee waxes in the esterified form, with hydroxytryptamide. The significant difference in some contents may be the effect of different drying treatments.

Lipid oxidation of the main unsaturated fatty acid, linoleic acid, generally produces highly potent aldehyde, hexanal which is an indicator of lipid oxidation and coffee staling (Sanches-Silva *et al.*, 2004; Spadone & Liardon, 1989) in coffee after roasting. Therefore, the differences between linoleic acid content were the main focus in the free fatty acids determination of this study since, it could lead to the generation of different hexanal contents. Linoleic acid in heat pump dried coffee at 40 ◦C was detected to be lowest in this study at 37.12% while the highest content was observed in heat pump sample dried at 50 ◦C (40.91%). The high linoleic acid content had the potential to produce more hexanal than other drying treatments. Heat pump drying at 50 ◦C appeared to preserve more linoleic acid and unsaturated fat more than the other treatments.

Table 26 Fatty acid profile of green coffee samples subjected to heat pump (40, 45 and 50 ˚C), tray dry (50 ˚C) and sun drying treatments

Fatty acid profile	Composition (% of total fatty acids)[I]				
	Condition of drying				
	Heat pump drying			Tray drying 50 ˚C	Sun drying
	40 ˚C	45 ˚C	50 ˚C		
Lauric acid (C12:0)	ND	0.06[a]	0.23[b]	ND	ND
Myristic acid (C14:0)	0.1	0.1	ND	ND	0.12
Pentadecanoic acid (C15:0)	0.04	ND	ND	ND	0.05
Palmitic acid	35.88[a]	35.34[b]	34.79[c]	35.63[a]	37.45[d]
Heptadecanoic acid (C17:0)	0.13[a]	0.12[a]	ND	ND	0.14[a]
Stearic acid (C18:0)	7.62[a]	7.55[a]	6.28[b]	6.78[c]	6.34[b]
Arachidic acid (C20:0)	3.51[a]	3.25[b]	3.44[a]	3.45[a]	4.05[c]
Heneicosanoic acid (C21:0)	0.1[a]	0.09[a]	0.11[a]	ND	0.14[b]
Behanic acid (C22:0)	0.95[a]	0.71[c]	0.84[d]	1.01[b]	1.12[b]
Tricosanoic acid (C23:0)	0.11[a]	0.1[a]	0.12[a]	ND	0.16[b]
Lignoceric acid (C24:0)	0.35[a]	0.29[b]	0.39[a]	ND	0.42[c]
Saturated fat	48.79[a]	47.61[b]	46.2[c]	46.87[d]	49.99[b]
Cis-10-Pentadecenoic acid (C15:1n 10)	0.06[a]	0.05[a]	ND	ND	0.08[b]
Palmitoleic acid (C16:1n 7)	ND	ND	ND	ND	0.04
cis-11-Eicosenoic acid (C20:ln 11)	0.35	0.34	0.33	ND	0.34
Monounsaturated fatty acid	8.05[a]	8.01[a]	6.41[b]	6.13[c]	6.17[c]

Table 26 Fatty acid profile of green coffee samples subjected to heat pump (40, 45 and 50 ◦C), tray dry (50 ◦C) and sun drying treatments (Cont.)

Fatty acid profile	Composition (% of total fatty acids)[I]				
	Condition of drying				
	Heat pump drying			Tray drying	Sun drying
	40 ◦C	45 ◦C	50 ◦C	50 ◦C	
Cis-9,12-Linoleic acid (C18:2n6)	37.12[a]	38.25[b]	40.91[c]	40.66[c]	37.43[a]
Alpha-linolenic acid (C18:3n3)	1.12[a]	1.21[b]	1.58[c]	1.45[d]	1.46[d]
Cis-11,14-Eicosadienoic acid (C20:2)	0.05[a]	0.05[a]	ND	ND	0.08[b]
Polyunsaturated fatty acid	38.29[a]	39.51[b]	42.52[c]	42.11[c]	38.97[d]
Unsaturated fat	46.34[a]	47.52[b]	48.93[c]	48.24[d]	45.14[e]

[I]Mean±SD < 20% from duplicate analysis. [a,b]values with different letter in a row are significantly different (p<0.05).

4.2.3 Odor properties of coffee

After the determination of the effects of heat pump drying on the non-volatile compounds and antioxidant activity of green coffee before conducting the study in aroma development of coffee beverage brewed from difference dried sample, this study was prescreened the samples before further analysis. For this purpose, R-index by similar ranking test was carried out in order to detect the overall difference of aroma by nose with a goal to identify the heat pump drying condition that produces dried beans with the closes R-index to control. Beyond on this finding a further in depth determination of volatile compounds in coffee brews would take place. The R-index values of all green coffee treatments would take place. The results are shown on Table 27.

Table 27 R- index values of coffee brew prepared from samples subjected to different drying treatments

Green coffee treatments	R-index JB[a]
Heat pump drying at 40◦C	54.98
Heat pump drying at 45◦C	53.35
Heat pump drying at 50◦C	31.67
Tray drying at 50◦C	73.33*

[a]Calculated by using John Brown computation against sun dried coffee (control) and expressed as percentage. n = 30 (21 females and 9 males; 18-54 years old). Critical R-index value = 67.10 (n =30, two tailed test). *Significantly different from control.

The application of R-index by similar ranking indicated that only R-index of tray dried coffee (73.33) was significantly different from control (95% level of confidence) when compared with critical R-index value (67.10, critical value 17.10) while the rest showed the same similar trend of aroma by nose with control. Consider heat pump dried coffee samples, the sample dried at 50 ºC had the closest similar odor properties to control with an R-index at 31.67 (value ≤ 50%). Therefore, this treatment had been selected for further analysis of aroma components. However, the sensory evaluation of coffee brew still has a weakness related to the type of panelists. Increase the number of panelists to 30 would make the results more reliable, some of panelists may have difficulty in distinguishing a large number of samples and with sometimes confuse to small differences. The use of a trained panel could overcome these problems.

Hence to confirm the difference in the perception of aroma in selected sample, heat pump dried coffee at 50 ˚C was compared with the conventionally dried samples (sun drying and tray draying). Therefore, in the next section of the study, the multiple techniques combined with stable isotope dilution assay (SIDA) have been applied to quantify the selected potent odorants of coffee brew that had been identified in previous sections on volatile analysis.

4.2.4 Volatiles profile of coffee

4.2.4.1 Target key volatile compounds in coffee brew

The propose of this part of research is to apply the use of Stable isotope dilution assay- headspace solid- phase microextraction- gas chromatography/mass spectrometry (SIDA-HS-SPME/GC-MS) technique in order to accurately investigate the effects of heat pump drying on selected 40 key odorants

which contribute to flavor properties of coffee beverages according to previous research (Belitz *et al.*, 2009; Semmelroch *et al.*, 1995; Semmelroch & Grosch, 1996).

The high accuracy instrument, GC-MS system (7890A; Agilent Technologies; California, USA) coupled with Time-of-Flight Mass spectrometer (TOF-MS) (Pegasus 4D; LECO®; Michigan, USA) was used for the precise quantification of selected key volatiles in coffee brew in trace amounts at ppt level.

A total of 40 volatile compounds were monitored in this part following on the finding in part 1 of this study and the reported in previous research. The list of monitored compounds is showed on Appendix A, Table 31. Before calculate the concentration of target volatile compounds, the identification of them were included retention index, mass spectra, and reference standard compounds. The values of Odor Threshold, concentration and Odor Activity Value of 40 selected compounds are shown in Table 28.

The results show that there were 25 key volatiles, having OAV>1 indicating a significant difference in volatile profile of heat pump dried, tray dried and sun dried coffee brew ($p< 0.05$). Most of the volatile profile of the dried sample and control were similar to each other, especially in heat pump dried and sun dried sample.

The rankings of the OAVs showed their variation based on the differences in the detected level of odorants concentrations. It appears that most of compounds are in pyrazine groups. Their contribution of nutty aroma was already been described (Flament, 2002; Pickard *et al.*, 2013). However, they play a less important role to the characteristics of coffee brew in this study since their OAV values are lower than 1, confirming their margined role in comparison with other odorants.

Consider the results, (*E*)-β-damascenone, the Strecker aldehydes (2-methylpropanal and 3-methylbutanal) and 2-furfurylthiol were the compounds showing the highest OAVs in every dried coffee brews. The result of calculated OAV of 3-methylbutanal agrees with the results of previous studies by Semmelroch & Grosch (1996). It also confirms the important role of 3-methylisomer via it highest OAV in this study among other key odorants even it contrasts with the study of Ho *et al.* (1993) on the lesser importance of 3-methyl isomer to coffee aroma contribution than the 2-methyl isomer. The OAV of 3-methylbutanal, the potent odorant responsible for malty note of coffee that belongs in sweet/caramel like group attribute note of coffee (Belitz *et al.*, 2009) was the highest in heat pump dried coffee brew at 5935 with the concentration detected at 2077.3 µg/L. The concentration and the OAV of this odorant in tray dried and sun-dried coffee were significantly different from heat pump dried coffee (p-value<0.05).

In addition, its concentration was unexpectedly higher than the prior detection in Arabica coffee (550 µg/L; Semmelroch & Grosch, 1996) and was closer to the value determined in Robusta coffee at 925 µg/L. 3-methylbutanal generally forms via the pyrolysis of leucine and the degradation of leucine from the react of polyphenol oxidase (Flament, 2002) as part of the Maillard reaction network. The pattern of how 2-methylbutanal is generated is similar to that of 3-methylisomer, except its reactive amino acid is isoleucine.

The intensity of the Maillard products and the relation to the composition of the roasted coffee was previously discussed that their diversity can be broaden including the composition of the precursors in green bean as well as the influence from many involved factors like the natural variance of green beans, reaction

temperature, time, pressure, pH and moisture content (Ho *et al.*, 1993; Ledl and Schleicher, 1990).

In this study, leucine contents in heat pump dried sample and others were significantly higher than isoleucine which confirms this claim to the more generation of 3-methyl isomer than another isomer. Besides, the detection of 2-methylbutanal in roasted coffee described by Guyot *et al.* (1988) indicates its decreasing content while roasting when the green beans were obtained from more mature of coffee cherries. Combing the comment from Guyot *et al.* (1988) and the method used for coffee roasting in this experiment, the difference in mechanism of the chemical reaction generated while roasting from coffee precursors may also have led to a higher content of 3-methyisomer than 2-methylisomer. This was probably due to the fact that the cherries were harvested at the most appropriate period to produce mature fruit.

A total of 9 alkylpyrazines were identified in this study, most of them in different concentration in heat pump dried samples than in those subjected to other drying treatments. However, based on the OAVs calculation in Table 4.20, only group of 2,6-diethylpyrazine, 3-ethyl-2,5-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine influenced the overall characteristics of coffee flavour given that their calculated OAV was above>1.

2-ethyl-3,5-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine have been earlier mentioned on their important role of potent odorants in coffee brew (Pickard *et al.*, 2013; Pickard *et al.*, 2014) and their contribution to coffee odor characteristics was confirmed in this study.

2,3-diethyl-5-methylpyrazine showed the highest impact to the odor profile of sun-dried coffee brew with the high concentration at 9.5 µg/L compared to mechanically dried coffee. Its concentration, again is comparable to the concentration of Robusta coffee brew (9.3 µg/L) reported by Semmelroch & Grosch (1996). 2,3-diethyl-5-methylpyrazine is a diethyl methylpyrazine formed from the heating between serine and threonine with and without sucrose (Flament, 2002). It is most quite clear whether a marginally higher content of serine and threonine would be sufficient to contribute to a significantly higher 2,3-diethyl-5-methylpyrazine content in sun-dried coffee. Other physical factor may be involved in the process of formation of this compound.

One important factor that influences the development of aroma components in coffee brew observed in this study is the coffee preparation methods (Gloess *et al.*, 2013). According to Pickard *et al.* (2014), they found that coffee brews prepared by 6 brewing methods had a similar profile of alkylpyrazines, but different alkylpyrazines contents. The brewing methods influence to the extraction of those pyrazines with the extraction yield depending on the method used. In their study, the coffee pad and espresso machines gave the highest yields of extracted alkylpyrazines (82%) due to the influence of pressure when coffee is brewed with hot water. The extraction efficiency is increased with pressure in coffee machine (Andueza *et al.*, 2002).

Considering influence of these parameters on the volatile extraction kinetics during brewing, the variability of extracted key odorants from coffee beds to the brew can be easily explained since highly polar volatile are easily extracted

at the beginning of brewing but for lower polar odorants (also having a lower volatility), more time to the extraction from the coffee bed is required. These factors need further studies to explain the extraction yields of odorants group like alkylpyrazines and the diversity of volatile extraction to coffee brew.

The results from phenolic group showed a very large range of OAV values. The OAV of 4-ethylguaiacol were around 3 which indicates that it is less important than guaiacol (~150) and 4-vinylguaiacol (~351) in all dried samples.

The concentration of *m*-cresol, the phenolic compound responsible for the phenolic, medicine, wood like among of coffee ranges in this study between 509.75-677.07 µg/kg which is remarkably higher than *p*-cresol (19.93-27.49 µg/kg), the smoke like odor. In contrast to the finding in this study, the amount of *p*-cresol in previous studies were found to be higher than *m*-cresol at the concentration around 1.3 ppm in Arabica coffee (Flament, 2002; Tressl *et al.*, 1978). Volatile phenolics are usually derived from lignin as well as the decomposition of chlorogenic acids during Maillard reaction. Since the quantities and type of them depend on nature of samples, roasting and brewing methods, this fact might well explain the higher content of *m*-cresol detected in this study.

The intensity of most impact character volatiles of coffee like sulfur compounds that are very low in their concentration might change in time and temperature which may result in their loss by evaporation, oxidation, degradation and interaction with other volatiles or non-volatile in coffee brew matrix (Weerawatanakorn *et al.*, 2015) while the brews were left at room temperature during performance of sensory evaluation or during determination of low-boiling point key odorants. To avoid such effects, before the sulfur volatile analysis with the rapid SIDA-HS-SPME-GC/MS,

the brews were cooled immediately on ice bath, and cysteine was added to the coffee brew to prevent a rapid covalent binding of volatile thiols to nonvolatile components in coffee, mainly hydroxyhydroquinone (Mestdagh *et al.*, 2014; Müller & Hofmann, 2007; Sun *et al.*, 2018). Published results helped to find the true concentration and Odor Activity Value (OAV) of the target sulfur compounds in this study.

Addition of cysteine has previously demonstrated its ability in thiol release to avoid of 2-furfurylthiol in free form to shift the stage to the bound form from the binding with the non-volatile hydroxyhydroquinone, melanoidin molecules when the brews reach their room temperature (Rowe, 2009). Cysteine acts as a competitive binder with the hydroxyhydroquinone, thus results in the release of the 2-furfurylthiol in bound form with coffee matrix to the free form (Rowe, 2009). The results from the precise quantification of SIDA-HS-SPME-GC/MS in table 4.20 showed that 2-furfurylthiol contents of selected heat pump dried coffee and sun dried coffee were not dissimilar to each other (p-value = 0.207). It appears that the concentration of 2-furfurylthiol in tray dried coffee was significantly different from heat pump dried coffee (p-value =0.01) and sun dried coffee.

Hence, 2-furfurylthiol was the sulfur key volatile significantly discriminating coffee produced from heat pump drying, tray drying and sun drying. The concentration of free 2-furfurylthiol detected in heat pump dried coffee, sun dried and tray dried coffee were precisely quantified using labelled internal standard at 64.3, 60.5 and 54.4 µg/L respectively. The OAV of 2-furfurylthiol was highest in heat pump dried sample at 6304 and in sun dried sample at 6154. In contrast to 2 dried coffee, the OAV of 2-furfurylthiol in tray dried coffee was lower to 5542. The concentration and OAV

of 2-furfurylthiol confirmed its usefulness in distinguishing between coffee samples subjected to different drying treatments.

2-Furfurythiol had been previously detected their concentration in coffee brew without the addition of the competitive binder (l-cysteine) at 19.1 µg/L (Arabica coffee) and 39 µg/L (Robusta coffee) (Semmelroch & Grosch, 1996). In previous report in the concentration determined after the addition of l-cysteine without the use of stable isotope dilution assay (SIDA), the quantitated amounts using external calibration curve in Arabica coffee were at 11.34 µg/L (Yunnan) and 15.33 µg/L (Columbia), for Robusta coffee, it was 21.02 µg/L (Sun *et al.*, 2018). Our concentrations detected in this study were higher than the previous identified due to the addition of the binder to release all free 2-furfurylthiol to the brew and the precisely quantified by the robust SIDA-HS-SPME-GC/MS. According to Weerawattanakorn *et al.* (2015) and Poisson *et al.* (2009), that reported about the possible pathway of 2-furfurylthiol formation, many reactions had been claimed, one of that mentioned about the ability of cysteine to further degrade and yield the sulphide to react with 2-furaldehyde from arabinose degradation during the occurrence of Maillard reaction during roasting.

Methional, 3-(methylthio)propanal, a potent odorant of coffee produces the cooked-potato like note. Being a product from the Strecker degradation of methionine and α-dicarbonyl compounds during roasting (Di. *et al.*, 2003; Semmelroch & Grosch, 1996), it was quantified in all coffee brew treatments. This study found that the quantified content of methional in sun dried coffee, heat pump dried coffee and tray dried coffee were 31.28, 13.1 and 16.3 respectively with the high OAV at 156.4, 65.5 and 81.5. They were higher than previously reported in coffee brew (5.7 µg/L;

Semmelroch and Grosch, 1996). Study of (Czerny & Grosch, 2000) found that its concentration could be from 22 ppb (green bean) to 213 ppb (roasted bean) via the quantitation using a labeled internal standard and multidimensional high resolution GC-MS which agreed with the study of Semmelroch *et al.* (1995) for the identified amount of methional in roasted coffee at 240 ppb. This odorant is heat labile and can be degraded easily to methanethiol (Flament, 2002). Considering from the low OAV of methanethiol in this study when compared to previous studies (1050; Semmelroch & Grosch, 1996), it might be assumed that methional in this study was not degraded rapidly due to the sample preparation manner that the beans were frozen at -70 ˚C, 1 hour prior grinding in order to prevent the volatiles from evaporating prior to extraction with water.

3-mercapto-3-methylbutylformate, a sweaty-catty note of coffee odorants, forming from the biogenic precursors prenyl diphosphate, hydrogen sulfide and formic acid was quantified using 1-heptanethiol as internal standard in this study due to the unavailability of labelled IS, 1-heptanethiol has been considered to be chosen due to its belong to the same class of sulfur volatile, a close selected ion (m/z) and retention time while eluting in a SAC-5^{TM} column. The concentration of this odorant in this study was around 2 µg/L which is slightly lower than previously described (5.5 µg/L; Semmelroch & Grosch, 1996) but somehow still in accordance in the range of detectable concentration. In this experiment, this volatile compound showed its usefulness in the discrimination of heat pump dried coffee from tray dried and sun dried coffee.

High boiling point volatiles with the rapid analysis, LLE technique were determined in order to maintain the integrity of the test to observe the effects of heat pump drying on aroma components of coffee. Maltol (sweet-caramel like), Furaneol® (caramel like), sotolon (spicy like) and abhexon (spicy-like) are well known on their antioxidative properties and for being the high boiling point volatile group, previously mentioned at a trace level, very low odor threshold (for some of them) and their presence in the characterization of Arabica coffee, as they are the high boiling point odorants. To quantify components rapidly, this study used an inexpensive method, liquid-liquid extraction using dichloromethane as a solvent coupled with the application of low cost internal standard (IS) (ethyl maltol) to quantify these contents in the three dried coffee samples. Ethyl maltol was chosen as an applied IS due to its quite similar physicochemical properties to Furaneol® and the close retention time when subject to GC-MS system. The response factors of these components were precisely quantitated in the coffee matrix of control treatment (sun drying).

Furaneol® (a caramel like sweet flavor) had been claimed to have the most considerable impact on food aroma due to its very low flavor threshold in water, which could be low to 0.04 mg/kg (Weerawattanakorn *et al.*, 2015), it had been reported as the distinct aroma characteristic of Arabica coffee (Blank *et al.*, 1992). This compound is formed by thermal degradation of fructose, pyrolysis of D-glucose or 1-deoxy-1-piperidino-D-fructose, and heating of amino acids with rhamnose (Weerawattanakorn *et al.*, 2015). The results on table 4.20 show the highest Furaneol® contents were highest in sun dried coffee (8447 µg/L), followed by heat pump dried coffee (8053 µg/L) and tray dried coffee (5508 µg/L). This odorant is the high boiling point odorant that showed the clearly difference between tray dried coffee from sun

dried and heat pump dried coffee (p<0.05). The OAV of Furaneol®, determined in three samples were within the range of 518-844.7.

Sotolon (spicy, curry like), another important potent odorant of coffee brew is a compound formed from the -oχo butyric acid and pyruvate during roasting of coffee (Blank *et al.*, 1992), heat pump dried coffee at 50 ˚C provides the highest concentration at 11.40 µg/L followed by sun dried coffee and tray dried coffee at 10.60 and 9.40 µg/L respectively. The non significant difference in the content of this volatile are shown in Table 4.20 (p>0.05).

Abhexon is another compound claimed on its low threshold, belongs to spicy note group of coffee and formed via the same reaction as sotolon except the reaction is usually self-occurance (Blank *et al.*, 1992) while Maltol (sweet caramel like) has been previously mentioned on its antioxidative activity on the inhibition of c5 and c6 acids formation from the corresponding aldehydes when comparing the activity with 2,6-di-tert-butyl-4-methylphenol and α-tocopherol (Singhara *et al.*, 1998). Despite the significant difference between samples (p<0.05), the OAV of maltol was less than 1, confirming the low importance of this odorant to the overall aroma difference of coffee. The OAV of abhexon in sun dried coffee and heat pump dried coffee were > 1 (1.72 and 1.32) while for tray dried coffee, it was less than 1. The OAV of sun dried coffee and heat pump dried coffee showed its contribution to the flavor of those 2 coffee and confirmed the similar overall aroma between sun dried and heat pump dried coffee, despite their lower OAV when compared to the more important odorants. The concentration of Furaneol®, sotolon, abhexon and maltol in this study is in accordance with the range reported in literatures (4500-13000 µg/L, 33-77 µg/L, 4.4-8.7 µg/L and 29210 µg/L) (Cheong *et al.*, 2013; Semmelroch & Grosch,

1996) but with slightly differences due to the sample preparation steps, coffee species, location. For the further discrimination of three coffees based on volatile profile, 25 key odorants were selected based on their contribution to the significant differences from univariate analysis (one-way ANOVA) and their OAV (more than 1) in the conduct of PCA analysis to relate the relationship and separate coffees based on volatile qualities. According to the PCA score plot in Fig 4.10, the first two principal components; PC1 (34.33%) and PC2 (20.51%) responsible for the overall cumulative variance between coffee samples at 54.84%. The score plot of PC2 against PC1 also separated and segregated coffee samples from a distinct volatile characteristic to a clear cluster with a boundary of 95% confidence. The distances of each cluster correlate to the proximity in the expression of significant volatile constituents where sun dried coffee was a significant discriminant on the positive side of PC1 axis while the other dried samples were separated on the negative side of this PC axis. The visualization of PC2 against PC1 showed the close distance and relationship between heat pump dried coffee and tray dried coffee. This indicates to the sharing of same aroma profiles between two mechanical dried sample which relate to same species, origin and the use of mechanical drying. The axis of PC1 showed a closer distance between heat pump dried and sun dried coffee than tray dried coffee which confirmed the similarity of volatile profile determined by SIDA-HS-SPME/GC-MS and the one-way ANOVA analysis. Although the results provided by PCA showed the separation of sun dried from heat pump dried coffee clusters, the sensory test from R-index similar ranking test indicated that the consumer could not separate these two coffees based on the overall difference of the aroma by nose. This might relate to the lack of significant difference in the content of 2-furfurylthiol between samples. As 2-furfurylthiol has been claimed as the major

impact key volatile compound in coffee, its very low threshold and concentration might conceal the significance of other odorants and the resulting non-significantly result. The distance between tray dried and sun dried coffee also confirmed the difference in key volatile concentration detected by One-way ANOVA and agrees with the ability of consumer to discriminate this coffee from others. Regardless of the different drying methods, the three dried coffees were characterized by the high positive coefficients (>0.7) of trimethylpyrazine, dimethyl trisulfide, 2-ethyl-3,5-dimethylpyrazine, 3-ethyl-2,5-dimethylpyrazine, 2,6-diethylpyrazine, 2,3-diethyl-5-methylpyrazine and methional in PC1. The direction of the eigen vectors of these volatiles also indicated the dominant characteristic of sun dried coffee via the high concentration of these volatiles that can clearly distinguish it from mechanically dried coffees. The direction of eigen vectors and negative coefficients of 2-furfurylthiol (-0.054) to the PC1 showed the distinct characteristics of heat pump dried coffee that was different from tray dried coffee while the eigen vectors of guaiacol and 4-ethylguaiacol were related to volatile quality of tray dried coffee.

Therefore, the result from PCA analysis showed it as a successful tool in disclosing variations in coffee characteristics that could be caused by drying methods based on volatiles profiles as well as confirmed the result of One-way ANOVA analysis and R-index similar by ranking test.

Table 28 Odor Threshold, concentration and Odor Activity Value of selected 40 key odorants in coffee brew prepared from dried coffees

No.	Selected volatiles	Odor Threshold in water[II] (μg/L)	Concentration (μg/L)[I]			Odor Activity Value (OAV)		
						OAV calculated		
			Sun drying	Heat pump drying (50 ◦C)	Tray drying at 50 ◦C	Sun drying	Heat pump drying (50 ◦C)	Tray drying at 50 ◦C
1	Methanethiol	0.2[III]	23.40[ac]	23.80[b]	23.70[ab]	117	119	118.50
2	Propanal	10[III]	996.40[a]	1072.10[b]	1009.50[a]	100	107.21	100.95
3	2-Methylpropanal	0.7[III]	2810[a]	2439.90[b]	1820.50[c]	4014.28	3485.57	2600
4	2-Methylbutanal	1[IV]	1793.50[a]	1433.28[b]	1296.85[b]	1793.50	1433.28	1296.9
5	3-Methylbutanal	0.35[III]	1368.90[a]	2077.30[b]	1625.39[c]	3911	5935	4643.9
6	2,3-Butanedione	15[V]	1732.25[a]	1483.29[b]	1983.05[c]	115	99	132.03
7	2,3-Pentanedione	30[III]	1931.49[a]	1657.56[b]	1419.33[c]	64.38	55.25	47.31
8	Hexanal	10[V]	1.99[a]	1.50[b]	2.10[a]	0.19	0.15	0.21
9	2-Methylpyrazine	105000[VI]	794.64[a]	766.64[b]	685.56[c]	0.007	0.007	0.006
10	2-Ethylpyrazine	21000[VI]	702.03[a]	589.43[b]	618.16[c]	0.03	0.03	0.03
11	2,6-Dimethylpyrazine	1500[VII]	1240.76[a]	3122.49[b]	1712.20[c]	0.82	2.08	1.14
12	2,3-Dimethylpyrazine	2500[VII]	931.94[a]	1424.46[b]	856.19[c]	0.37	0.57	0.34

Table 28 Odor Threshold, concentration and Odor Activity Value of selected 40 key odorants in coffee brew prepared from dried

No.	Selected volatiles	Odor Threshold in water[II] (µg/L)	Concentration (µg/L)[I]			Odor Activity Value (OAV)		
						OAV calculated		
			Sun drying	Heat pump drying (50 ◦C)	Tray drying at 50 ◦C	Sun drying	Heat pump drying (50 ◦C)	Tray drying at 50 ◦C
13	Nonanal	1[VII]	1.57[a]	0.75[b]	0.75[b]	1	0.7	0.7
14	Dimethyl trisulfide	0.01[IV]	0.14[a]	0.11[b]	0.11[b]	14	11	11
15	Trimethylpyrazine	91[VIII]	72.07[a]	52.99[b]	53.53[b]	0.79	0.58	0.58
16	2,6-Diethylpyrazine	6[VII]	85.75[a]	53.42[b]	52.04[b]	14.61	8.90	8.67
17	3-Ethyl-2,5-dimethylpyrazine	8.6[c]	141.82[a]	96[b]	89[b]	16.49	11.16	10.35
18	2-Furfurylthiol	0.01[V]	61.54[a]	63.06[a]	55.42[b]	6154	6306	5542
19	1-Octen-3-ol	1[III]	2.74[a]	2.51[b]	2.45[b]	2.74	2.51	2.45
20	2-Ethyl-3,5-dimethylpyrazine	2[IX]	37.99[a]	25.77[b]	25.97[b]	18.99	12.88	12.98
21	Acetic acid	50000[IV]	24587	25009	22750.30	0.49	0.50	0.46
22	Methional	0.2[VI]	31.28[a]	13.01[b]	16.30[c]	156.4	65.05	81.5

Table 28 Odor Threshold, concentration and Odor Activity Value of selected 40 key odorants in coffee brew prepared from dried coffees (Cont.)

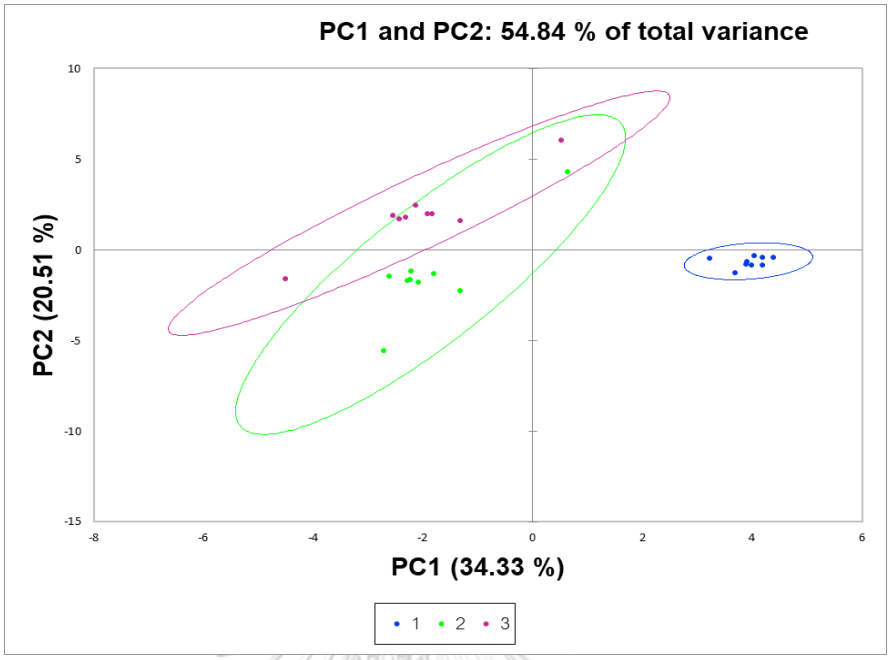
No.	Selected volatiles	Odor Threshold in water[II] (µg/L)	Concentration (µg/L)[I]			Odor Activity Value (OAV)		
						OAV calculated		
			Sun drying	Heat pump drying (50 ◦C)	Tray drying at 50 ◦C	Sun drying	Heat pump drying (50 ◦C)	Tray drying at 50 ◦C
23	2,3-Diethyl-5-methylpyrazine	0.09[III]	9.50[a]	4.50[b]	4.40[b]	105.56	50	48.89
24	3-Mercapto-3-methylbutylformate	0.0035[VII]	1.59[a]	1.98[b]	1.74[a]	454.28	565.71	497.14
25	2-Isobutyl-3-methoxypyrazine	0.005[III]	4[a]	4.2[a]	3[b]	800	840	600
26	Linalool	6[X]	13.26	13.97	13.12	2.21	2.33	2.17
27	3-Methylbutanoic acid	560[V]	2720.17[a]	3146.78[b]	2979.38[c]	4.85	5.62	5.33
28	(*E*)-β-Damascenone	0.00075[V]	2.87[a]	2.98[b]	3[b]	3826.67	3973.33	4000
29	Guaiacol	2.5[IX]	336.14[a]	355[b]	387[c]	134.46	142	154.8
30	2-Phenylethanol	1000[XI]	109.35[a]	71.24[b]	70.27[b]	0.11	0.07	0.07
31	Maltol	9000[IV]	6839.1[a]	7680.51[b]	7289.02[c]	0.76	0.86	0.81
32	Furaneol®	10[V]	8447[a]	8053[a]	5508[b]	844.7	805.3	550.8
33	4-Ethylguaiacol	50[V]	152.38[a]	151.54[a]	178.91[b]	2.54	3.06	3.52
34	*p*-Cresol	550[XII]	27.49[a]	19.93[b]	27.04[a]	0.04	0.04	0.05

Table 28 Odor Threshold, concentration and Odor Activity Value of selected 40 key odorants in coffee brew prepared from dried coffees (Cont.)

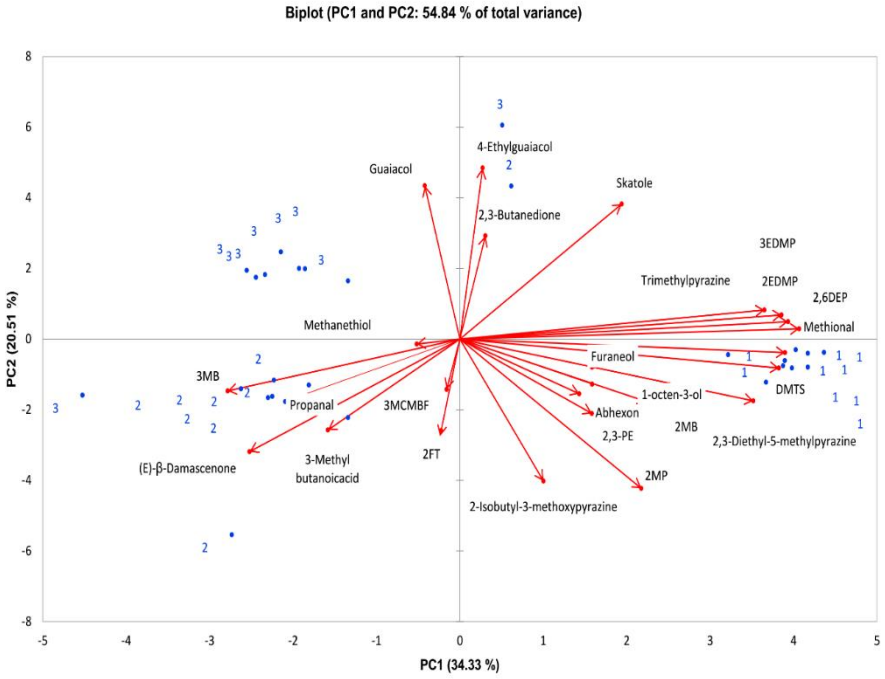
No.	Selected volatiles	Odor Threshold in water[II] (µg/L)	Concentration (µg/L) [I]			Odor Activity Value (OAV)		
						OAV calculated		
			Sun drying	Heat pump drying (50◦C)	Tray drying at 50◦C	Sun drying	Heat pump drying (50◦C)	Tray drying at 50◦C
35	*m*-Cresol	68[XIII]	509.75[a]	610.15[b]	677.07[c]	7.50	8.97	9.96
36	4-Vinylguaiacol	20[V]	7013.15	7014.45	7021.35	350.67	350.72	351.6
37	Sotolon	0.3[V]	10.93	11.24	9.4	36.43	37.467	31.33
38	Abhexon	8.7[IX]	14.92[a]	12.92[a]	8.73[b]	1.714	1.48	1.003
39	Indole	90[XIV]	118.9	121.74	115.97	1.321	1.28	1.28
40	3-Methylindole (skatole)	3[XIV]	5.91[a]	4.74[b]	5.81[a]	1.97	1.58	1.94

[I]Odorants no. 2-13, 15-17, 19-21, 23, 25-40 were identified and Calculated based on peak area detected on Stabilwax® column and 1-2 14, 18, 22, 24 were identified and calculated via SAC-5™ column with relative S.D < 30% from triplicate analysis. [II]Odor detection thresholds in water reported in literatures. [III]Semmelroch & Grosch, 1996. [IV]Rychlik *et al.* (1998). [V]Guth and Grosch (1993). [VI]Koehler *et al.* (1971). [VII]Guadagni *et al.* (1972). [VIII]Czerny &Wagner (1995). [IX]Semmelroch *et al.* (1995). XButtery *et al.* (1990). [XI]Schieberle (1991). [XII]Buttery *et al.* (1998). [XIII]Maga (1978). [XIV]Moss *et al.* (1993). [abc]Significance difference at $p < 0.05$ from One-way ANOVA (LSD).

(B)


PC1 and PC2: 54.84 % of total variance
PC2 (20.51 %)
PC1 (34.33 %)
10
5
0
-5
-10
-15
-8
-6
-4
-2
0
2
4
6
1
2
3

(A)


Biplot (PC1 and PC2: 54.84 % of total variance)
PC2 (20.51 %)
PC1 (34.33 %)
4-Ethylguaiacol
Guaiacol
Skatole
2,3-Butanedione
3EDMP
Trimethylpyrazine
2EDMP
2,6DEP
Methanethiol
Methional
Furaneol
3MB
1-octen-3-ol
DMTS
Propanal
3MCMBF
Abhexon
2,3-PE
2MB
2,3-Diethyl-5-methylpyrazine
(E)-β-Damascenone
3-Methyl
butanoicacid
2FT
2MP
2-Isobutyl-3-methoxypyrazine

(B)

Figure 20 (A) PCA score plot (PC 2 against PC 1) from the concentration of 25 significant key volatile compounds of three dried coffee brew; (B) PCA biplot (PC 2 against PC 1) of three dried coffee brew: (1) Sun dried coffee; (2) Heat pump dried coffee; (3) Tray dried coffee.

; 2MB= 2-methylbutanal, 3MB = 3-methylbutanal, 3MCMBF =3-Mercapto-3-methylbutylformate, 2FT = 2-Furfurylthiol, 2MP = 2-Methylpropanal, 2,3-PE = 2,3-Pentanedione, DMTS = Dimethyl trifulside, 2,6-DEP = 2,6-Diethylpyrazine, 2EDMP = 2-Ethyl-3,5-dimethylpyrazine, 3EDMP = 3-Ethyl-2,5-dimethylpyrazine.

4.2.4.2 Calibration of rapid Furaneol® determination in Arabica coffee

Furaneol® has been widely reported as one of key odorants of Arabica coffee. It has been reported that this compound is unstable to the presence of air, heat and at high pH of aqueous solution (Chen *et al.*, 1996; Hirvi *et al.*, 1980; Liu & Yang, 2008; Roscher *et al.*, 1997; Weerawatanakorn *et al.*, 2015). According to result of a stability study of this compound in an aqueous buffer solution at pH 2-8, its degradation was pH dependent, with its optimum stability was detected at pH 3.5, and the rate of decomposition follows first-order kinetics (Hirvi *et al.*, 1980; Roscher *et al.*, 1997). Hence, in order to accurately and precisely quantify this compound, stable isotope dilution assays (SIDA) with direct solvent extraction-solvent assisted flavor evaporation (DSE-SAFE) technique is commonly used (Engel *et al.*, 1999). However, available SIDA with DSE-SAFE technique is expensive due to the requirement of special equipment, a high cost of labeled internal standard, numerous steps and spending several hours in neutral/acid/base fractionation in volatile analysis to precisely isolate Furaneol® in the stable pH from the complexity of coffee matrix containing around 1000 volatiles. Thus in previous section, the use of the LLE technique with dichloromethane as solvent was applied for Furaneol® determination and ethyl maltol was chosen as an IS due to its similar physicochemical properties to Furaneol®.

The aim of this part of research was to calibrate the use of an inexpensive method coupled with the application of low cost internal standard (IS) and the conventional method, (SIDA) coupled with direct solvent extraction-solvent assisted flavor evaporation (DSE-SAFE). Two common injection techniques used to quantitate high boiling point components in trace level; on-column and cold-splitless programmed□temperature (PTV) injection were to be used to confirm the accuracy of the Furaneol® determination described in section 4.2.4.1. The calibration of Furaneol® determination was performed in duplicate using the same batch of sun dried coffee (control). The comparison of Furaneol® contents and OAV determined in two extraction and two injection techniques are showed on Table 29.

Table 29 Concentration and Odor Activity Value (OAV) of Furaneol® determined in sun dried coffee with multiple injection techniques[a]

Arabica coffee produce by sun drying (control)					
Concentration (µg/L)			OAV		
SAFE extraction with SIDA		LLE extraction with ethyl maltol	SAFE extraction with SIDA		LLE extraction with ethyl maltol
Injection techniques			Injection techniques		
on-column	coldsplitless	coldsplitless	on-column	coldsplitless	coldsplitless
12070±200.74	13710±870	12860±62.27	1207	1371	1286

[a]Mean±SD < 10% from duplicate analysis

The concentration of Furaneol® determined by SIDA couple with DSE-SAFE and injected to on-column and cold-splitless mode were 12070 and 13710 µg/L respectively. The concentration of Furaneol® determined by liquid-liquid extraction and injected by cold-splitless injection was 12080 µg/L. The statistical analysis by LSD test confirmed the non significance difference between the extracted samples which the difference in Furaneol® content between LLE and SIDA with DSE-

SAFE being less than 10 percent (p >0.05). The OAV of all Furaneol® from multiple injection technique ranged between 1207 and 1370.

As previously described, cool-on-column injection has been used widely for the injection to GC-MS to minimize mass discrimination and provide optimal quantitative accuracy, particularly for the quantitation of high-boiling point volatile in trace level (see section 2.4.2).

Hence, from the mentioned benefits, the PTV injection in cold -splitless type is suitable for the quantitation of heat sensitive extracts containing trace level of high boiling point volatiles and it was selected as a main injection technique to quantify Furaneol® of coffee brew in section 4.2.4.1. Due to the claims about cool on-column capillary injection on its high precision of result in comparison with conventional injection, on-column injection delivers the extract with the slow injection to oven at a temperature of less than 40°C. This prevents the re-condensation of solvent during injection and loss of the sensitive material (Zrostlikova *et al.*, 2001). Hence, this technique as well as the prementioned cold-splitless PTV injection are very suitable for the comparison and validation of the accuracy of determination of high boiling point volatiles at trace level (ppm level or less in the final injected sample). The use of these two techniques will indicate the accuracy of Furaneol® content in extract determined by LLE technique that excludes the pre-cleaning step in the rapid analysis. If LLE extraction proves comparable to the conventional techniques used to quantitate Furaneol®, the result from the use of SIDA to quantitate the amount of Furaneol in coffee in both cool-on-column and cold-splitless should not differ from LLE. Besides, the result provided by cool-on-column with the accurate standard deviation would indicate the reliability of ethyl maltol as IS while compared with

SIDA coupled with SAFE extraction and on-column injection.

Therefore, the result of SIDA coupled with SAFE extraction and two conventional techniques confirmed the precision and accuracy of the rapid method, LLE, to determine Furaneol® and high-boiling point volatile compounds in coffee without the use of labelled IS with DSE-SAFE that usually takes several days and also involves a high cost of the labeled IS.

CHAPTER V

CONCLUSION AND SUGGESTIONS

The aim of the study was to investigate the overall impact of processing methods on the quality of coffee, mainly green beans. The study was divided in two parts. The conclusions of the findings in part 1 and part 2 of the study are presented in sections 5.1 and 5.2 respectively.

5.1 Effects of post-harvest processing practice on quality of coffee

5.1.1 Maturity of coffee cherries (ripe *vs* unripe)

In this section, the goal was reached to use of volatile compound profile and antioxidant activity to differentiate ripe and unripe green semi-dry processed coffee. The findings of study will be used for the quality control of green coffee after harvest. There were 18 and 5 compounds identified only in green beans from ripe and unripe berries, respectively. For the antioxidant activity, FRAP value could be used as quality marker in order to evaluate the selection process of green coffee.

5.1.2 Processing methods and the authenticity of green coffee quality

The study in this section had been done for the propose to apply rapid analysis methods; volatile analysis by head space solid phase micro extraction-gas chromatography/mass spectrometry (HS-SPME/GC-MS), antioxidant activity determination and electronic tongue (E-tongue) evaluation for the identification of authenticity of green coffee in each process. A total of 12 key volatiles were identified as having potential to be used as markers with the propose of distinguishing civet green coffee from the other processes. In terms of antioxidant activity determination, DPPH

radical scavenging assay could not distinguish civet coffee from other kinds of beans. The result of E-tongue could discriminate three processed coffee samples to clear cluster groups via the first principal component (PC1) and the third principal component (PC3). Therefore, total phenolic content, antioxidant activity by FRAP, volatile profile and E-tongue could be used as quality markers in order to evaluate the quality of green beans processed by different methods.

5.1.3 Processing methods, subdivided processing, locations of processing, countries of processing, animal species and coffee species. Effects on volatile compounds of roasted coffee

The results in this part of the study showed that HS-SPME/GC-MS could identify 20 key odorants that indicate the difference of the volatile profile of roasted coffee influenced by the processing method chosen (civet and non-civet process), subdivided processing, location area of processing, coffee species and confirmed the relationship between these 20 key odorants and the separation by PCA analysis with 2-dimensions. Volatile profile indicates to the special characteristics (authenticity) of weasel coffee and free-caged civet coffee from Thailand. 2-Furfurylthiol was found to be the most character impact compounds with the highest OAV determined in all coffee samples which indicates the difference between roasted coffee of different coffee species and subjected to different processing methods (e.g. processing using digestion by civet).

5.1.4 Processing methods, subdivided processing, locations of processing, countries of processing, animal species and coffee species. Effects on sensorial properties of coffee brew

Rapid E-tongue showed the potential to differentiate between coffee brewed samples that had been subjected to different processing condition (Civet cat *vs*. Weasel *vs*. normal wet-process), coffee species (Robusta *vs*. Arabica), country of processing (Thailand *vs*. Indonesia *vs*. Vietnam) and location of processing (Doitung *vs*. Doichang *vs*. Loei). From three experiments focusing on overall difference, consumers were able detect the differences between different processes (civet, weasel and normal wet processes) and feeding conditions (caged and free-range). Different coffee species (Robusta and Arabica), the countries of processing and location of processing (Doitung and Doichang) could also be perceived by the consumers. Results of E-tongue and sensory evaluation by panelists could be correlated to each other via the use of GPA analysis. The results endorse the claim that the E-tongue is a promising tool for quality control and product development.

5.2 Effects of heat pump drying on physicochemical properties of Arabica coffee

5.2.1 Physical properties

In term of moisture content and water activity values, it was find out in this part that heat pump drying was effective in coffee drying with the shortest drying time 11 h at 50 ˚C compared with the conventional tray drying at 50 ˚C (18 h) and natural sun drying (72 h). All dried coffee has the value of water activity of less than 0.6 which is safe range to prevent the growth of microorganisms. The result showed that there were no difference observed in a_w of all green coffee.

Among drying treatments compared in this study, heat pump drying at 50 ˚C had the shortest drying time that could giving coffee to the safe moisture content of ≈10% w.b. The result of color assessment showed that coffee samples dried by heat pump and tray drying resulted in a brightener shade of green coffee than sun dried samples.

5.2.2 Chemical properties of coffee

The finding in this research indicates that the use of heat pump drying significantly altered the chemical components of green coffee and gave the closest contents of sugar, caffeine and most amino acids to sun dried coffee.

A high content of 3,4-diCQA, 3,5-diCQA, 4,5-diCQA and a lesser total CGA were found in all heat pump dried samples and compared with sun dried coffee. Heat pump drying at 50 ˚C seems to be most effective to address the research propose of obtaining coffee with high content of 3,4-, 3,5- and 4,5-dicaffeoylquinic acid, isomer of CGA, indicating a good quality of coffee. The use of lower temperature in heat pump drying was also resulting in higher CGA content than tray drying at 50 ˚C.

Three determination of antioxidant activity elucidated that total phenolic contents and FRAP assay could differentiate heat pump dried coffee from other dried coffee samples. The coffee sample subjected to heat pump drying at 50 ˚C gave the highest total phenolic content and reducing antioxidant power.

The result of this section indicated that heat pump drying could be an alternative method to dry coffee with shorter time and preserving important coffee chemical compounds.

5.2.3 Odor properties of coffee

The application of R-index by similar ranking indicated that only R-index of tray dried coffee was significantly different from control (95% level of confidence) when compared with critical R-index value while all condition of heat pump drying showed the same trend of detection of aroma by nose as the control (sun drying). Consider among heat pump dried coffee, heat pump drying at 50 °C had the same R-index value as the control.

5.2.4 Volatiles profile of coffee

Heat pump dried coffee showed an increase in the abundance of aldehydes, ketones, furans, esters, and phenols in comparison with tray dried and sun dried coffee. The results also showed that there were 25 key odorants that could be used in differentiating three dried coffee based on volatile profiles from the analysis of one-way ANOVA combined with PCA analysis. Some of the target key volatiles determined in this study show a concentration and OAVs both higher and lower, than previously described in literatures. 2-furfurylthiol was the most important key volatile indicating a significant difference between heat pump dried coffee and the conventional tray dried and sun dried coffee. The PCA analysis also confirmed the result of one-way ANOVA and indicated the clear difference between dried samples and those subjected to the other drying treatments. The determination of high boiling point volatiles using the rapid method namely liquid-liquid extraction (LLE), with no labelled isotopically internal standard showed to be successful in quantitation of the odorant group with the highest OAV. Furaneol® was detected among the other high boiling point odorants. Hence, Furaneol® was the selected compound to be considered for the comparison of LLE extraction with other common techniques.

After the comparison of results from rapid low cost method and the use of SIDA with multiple injection techniques in this part of study, it was confirmed that LLE was precise and accurate to determine Furaneol® in coffee without the use of SIDA couple with DSE-SAFE. The latter usually take a few days and are more expensive because of the isotope used in this common method. Hence, the use of LLE and using ethyl maltol as an IS can be an alternative low cost and feasible method for the Furaneol® determination.

5.3 Suggestion for further research

- Sensory descriptive analysis of roasted coffee in part 1 and coffee brew in part 2 will provide more understanding in the change and difference of sensory attributes of coffee which are affected by harvest practices.
- Aroma recombination and omission test should be performed to confirm the importance of key odorants in coffee.
- Determination of the effects of storage temperature and time on the development of aroma precursors in parchment coffee.

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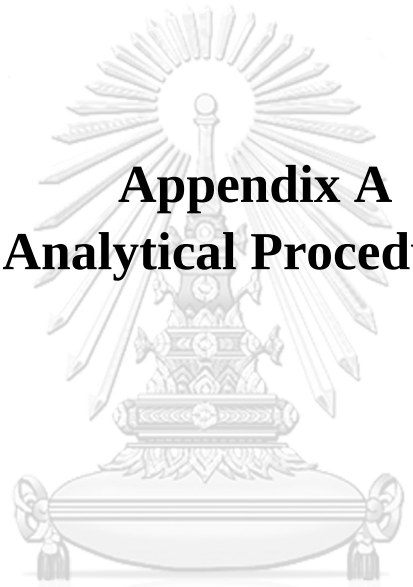
APPENDICES

Appendix A
Analytical Procedures



A.1 Determination of volatile compounds in section 3.2.1.2 and 3.2.2.2

The head space solid phase microextraction- gas chromatography/ mass spectrometry (HS-SPME/GC-MS) conditions used in the study were previously validated in preliminary experiments. The SPME fiber, 1 centrimetre used was triple phase 50/30 µm fiber (DVB/CAR/PDMS; Supelco Co., Bellefonte, PA, USA).

One gram of each coffee powders was put into 60 mL SPME vial with a PFTE/silicone septum cap (Supelco Co., Bellefonte, PA, USA). 2µL of 3.4 µg/mL, 2-methyl-3-heptanone was spiked into each SPME vial containing coffee sample as an internal standard. The sample vials were incubated at 60 °C in water bath for 15 min to reach sample headspace equilibrium. Then, the fiber was exposed in the headspace above coffee powder for 15 min to reach equilibration temperature.

For compound desorption, the fiber was placed into the GC injection port (split mode at ratio of 10:1) and heated to 250 °C for 5 min. To verify the volatile profile, samples were analyzed in triplicate. In addition, to avoid contamination, a blank test was performed before each analysis at the same chromatographic conditions of the sample analyses. The GC-MS (6890 GC/5973 mass selective detector (MSD); Agilent

Technologies, Santa Clara, CA, USA) was used to identify and quantify volatile compounds of green coffee beans. Separations were performed using RTX-5 capillary column (10 m x 0.18 mm i.d. x 0.2 µm film thickness; Restek Crop., Bellefonte, PA, USA). The oven was programmed from 40 to 240 °C at a rate of 50 °C/min with initial and final holding times of 1.5 and 6.5 min, respectively. Helium was used as carrier gas at constant rate of 0.4 mL/min. The MSD conditions were as follows: transfer line temperature, 230°C; ionization voltage, 70 eV.

To identify volatile compounds, the retention indices (RI) were calculated for each compound against reference standards (n-alkane, C_5-C_{26}). Identification was done based on comparison RI values and mass spectra of volatile compounds against NIST 08 mass spectrum database. A compound was considered to be tentatively identified if no authentic standard was available for comparison. The individual volatile compounds (identified and unidentified peaks) were tentatively quantified based on their peak areas relative to that of the internal standard. Due to the unavailability of commercially authentic standards, the R_f were assumed to be 1.00 in the present study (Cheong *et al.*, 2013; Gokbulut & Karabulut, 2012).

A.2 Determination of volatile compounds in section 3.2.3.1

The head space solid phase microextraction-gas chromatography/mass spectrometry (HS-SPME/GC-MS). The SPME fiber, 1 centrimetre used was triple phase 50/30 µm fiber (DVB/CAR/PDMS; Supelco Co., Bellefonte, PA, USA).

One gram of each roasted coffee powders was put into 60 mL SPME vial with a PFTE/silicone septum cap (Supelco Co., Bellefonte, PA, USA). 2µL of 3.4 µg/mL, 2-methyl-3-heptanone was spiked into each SPME vial containing coffee sample as an internal standard.

The sample vials were incubated at 60 °C in water bath for 15 min to reach sample headspace equilibrium. Then, the fiber was exposed in the headspace above coffee powder for 15 min to reach equilibration temperature. For compound desorption, the fiber was placed into the GC injection port (split mode at ratio of 10:1) and heated to 250°C for 5 min.

To verify the volatile profile, samples were analyzed in triplicate. In addition, to avoid contamination, a blank test was performed before each analysis at the same chromatographic conditions of the sample analyses.

The GC-MS (6890 GC/5973 mass selective detector (MSD); Agilent Technologies, Santa Clara, CA, USA) was used to identify and quantify volatile compounds of green coffee beans. Separations were performed using RTX-5 capillary column (10 m x 0.18 mm i.d. x 0.2 µm film thickness; Restek Crop., Bellefonte, PA, USA). The oven was programmed from 40 to 240 °C at a rate of 6 °C/min with initial and final holding times of 5 and 30 min, respectively. Helium was used as carrier gas at constant rate of 0.4 mL/min.

The MSD conditions were as follows: transfer line temperature, 230°C; ionization voltage, 70 eV. To identify volatile compounds, the retention indices (RI) were calculated for each compound against reference standards (n-alkane, C5-C26). Identification was done based on comparison RI values and mass spectra of volatile compounds against NIST 08 mass spectrum database. A compound was considered to be tentatively identified if no authentic standard was available for comparison. The individual volatile compounds (identified and unidentified peaks) were tentatively quantified based on their peak areas relative to that of the internal standard. Due to the unavailability of commercially authentic standards, the Rf were assumed to be 1.00 in the present study (Cheong *et al.*, 2013; Gokbulut & Karabulut, 2012).

A.3 HPLC analysis of chlorogenic acids from green coffee in section 3.3.2.2

A.3.1 Separation of Chlorogenic acids

The separation of chlorogenic acids from green coffee extracts was performed on an Acquity UPLC® CSH™ Phenyl-Hexyl (1.7 µm, 2.1 × 100 mm) column connected to an UltiMate® 3000 Standard LC System (Thermo Fisher Scientific Inc., MA, USA) coupled with a Q Exactive plus quadrupole-orbitrap mass spectrometer (Thermo Fisher Scientific Inc.).

Three caffeoylquinic acids (3-CQA, 4-CQA, and 5-CQA), two feruloylquinic acids (4-FQA and 5-FQA), and three dicaffeoylquinic acids (3,4-diCQA, 3,5-diCQA, and 4,5-diCQA) were detected by negative ion electrospray ionization (ESI-) and by a photodioarray detector (DAD) monitored from 200 to 600 nm.

The mobile phase consisted of acetonitrile with 0.1% formic acid (A), and 0.5% formic acid (B). Linear gradient elution was programmed as follows: 0-1 min, 90% B;

1-10 min, 90-60% B; 10-12 min, 60-20% B; 12-15 min, 20-20% B; 15-16 min, 20-90% B; 16-19 min, 10-90% B. Flow rate was set at 0.3 mL/min.

A.3.2 HPLC-MS identification of chlorogenic acids

Neochlorogenic acid (3-CQA), chlorogenic acid (5-CQA), and cryptochlorogenic acid (4-CQA) were identified by matched RT and fragments referred to purchased standard compounds. Although other chlorogenic acid derivatives are not commercially available, in this study they could be characterized by ultrahigh mass accuracy through selected ion monitoring (SIM 353.0878 for CQAs, 367.1035 for FQAs, 515.1120 for diCQAs) with isolation window of 5 ppm.

The isomers were further identified by their fragments referred to literature (Matsui *et al.*, 2007). The fragments of nine chlorogenic acid derivatives in this study are shown in Fig A-1 to Fig A-9. The instrumentation linked a mass detector after a DAD enabled to obtain chromatograms represented to mass spectrometry and absorption spectrometry with a time delay of 0.04 min. Therefore, the identified peaks in a mass chromatogram can be referred to chromatogram acquired by DAD through matched retention time and could be integrated via the DAD chromatogram combined with the molar extinction coefficients as described by Farah *et al.* (2005b). The LC/MS response factors of each chlorogenic acid derivatives can be obtained by the ratio between the mass responses to the true concentrations previously computed from the area of DAD chromatogram corrected by molar extinction coefficients. LC/MS response factors are showed in Table A-1. The chlorogenic acids contents in samples were obtained by the related mass spectra response of each chlorogenic acids to chlorogenic acid corrected with response factors. 5-CQA and other CQA derivatives were quantitated in each sample through calibrated standard compound.

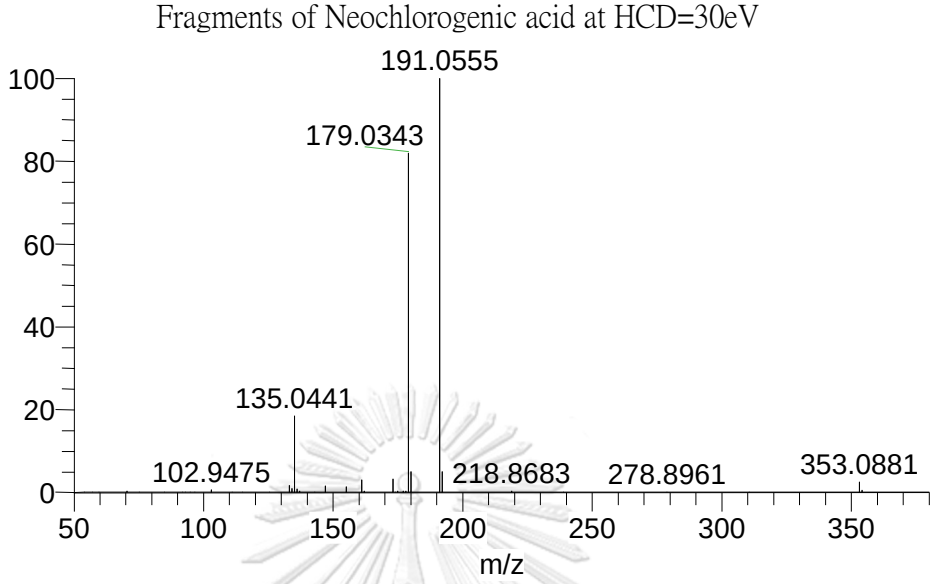


Figure 21 Fragment of Neochlorogenic acid (3-CQA

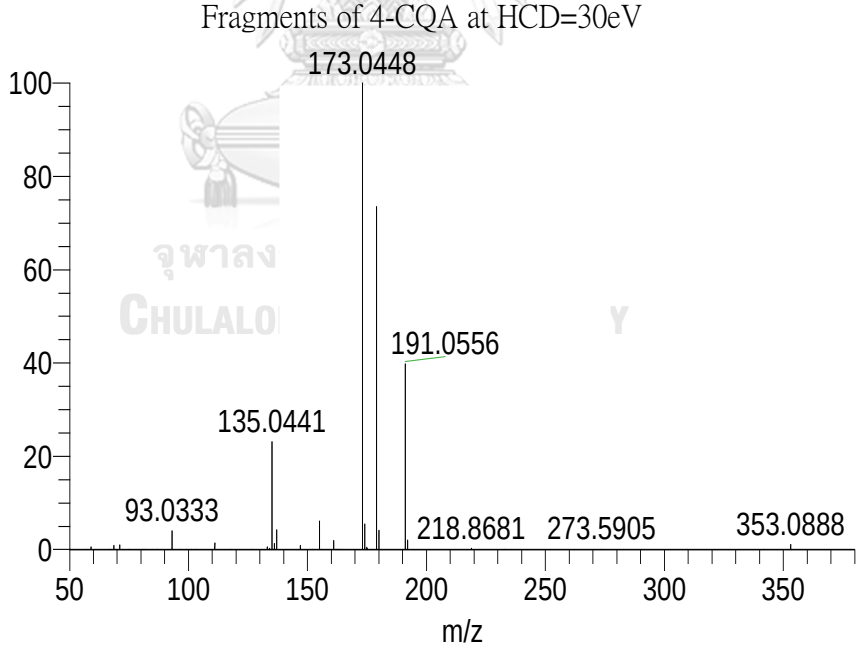


Figure 22 Fragment of 4-CQA

Fragments of chlorogenic acid at HCD=30eV

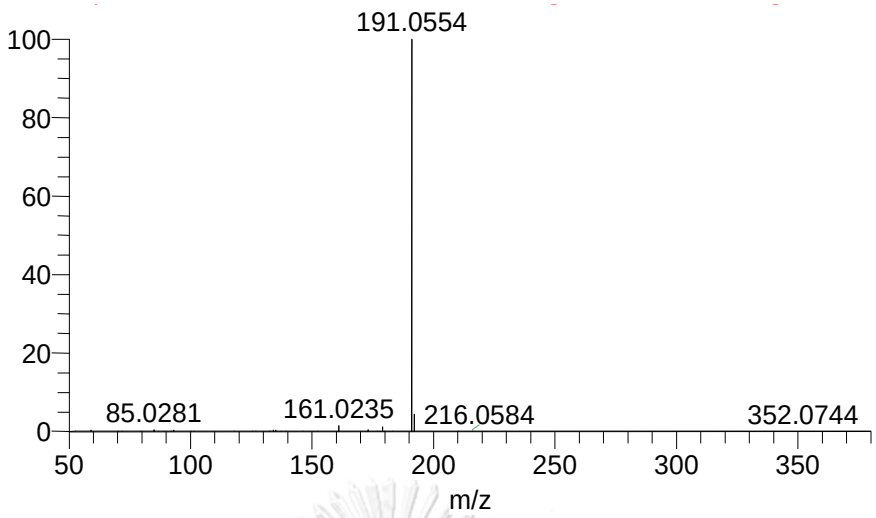


Figure 23 Fragment of Chlorogenic acid (5-CQA)

Fragments of 3-FQA at HCD=30eV

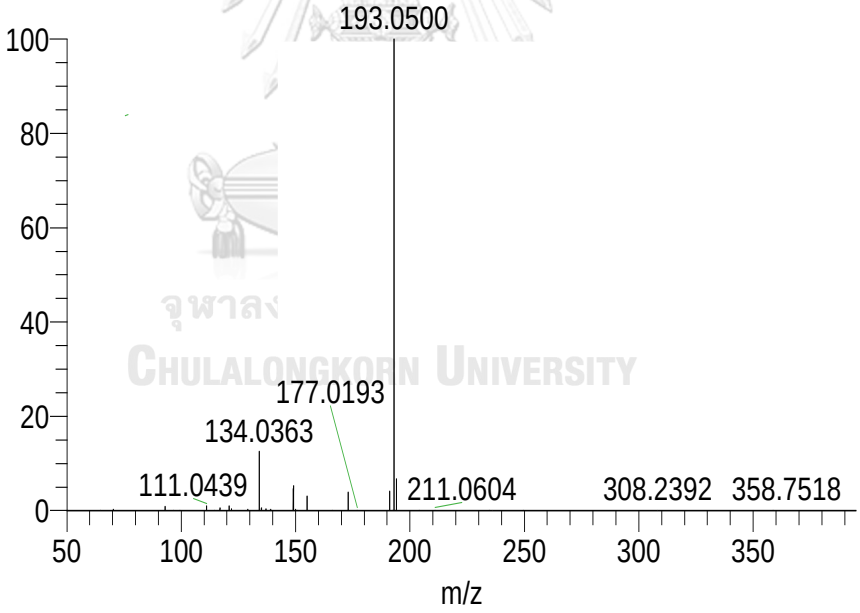


Figure 24 Fragments of 3-FQA

Fragments of 4-FQA at HCD=30eV

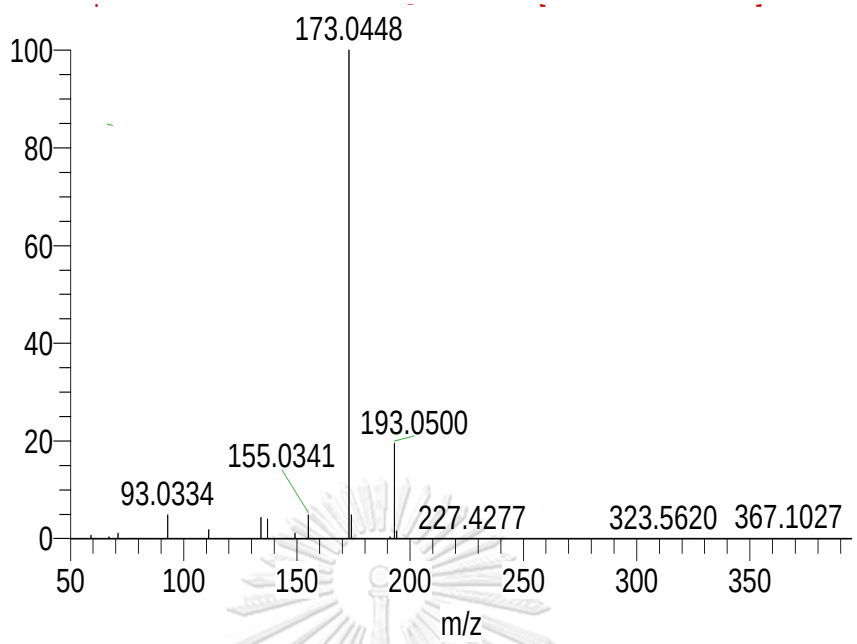


Figure 25 Fragment of 4-FQA

Fragments of 5-FQA at HCD=30eV

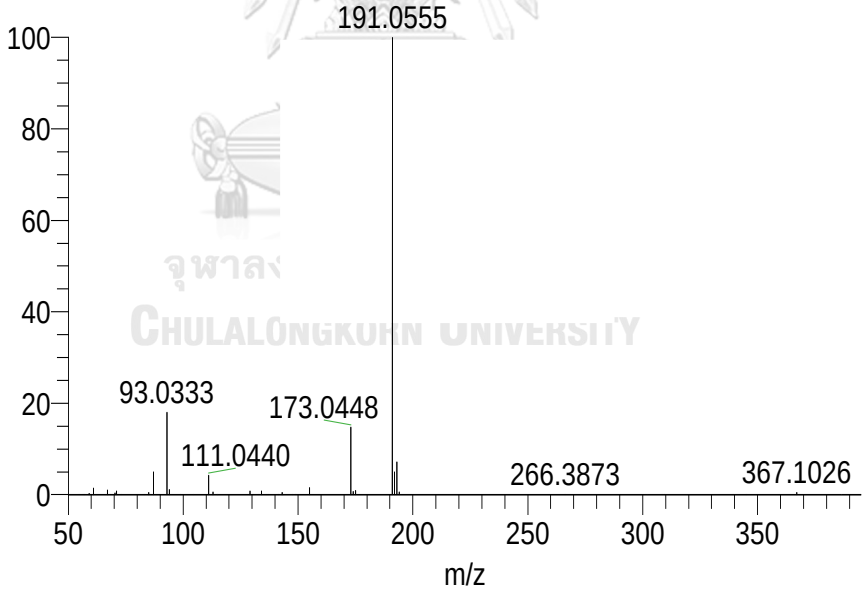


Figure 26 Fragment of 5-FQA

Fragments of 3,4-diCQA at HCD=30eV

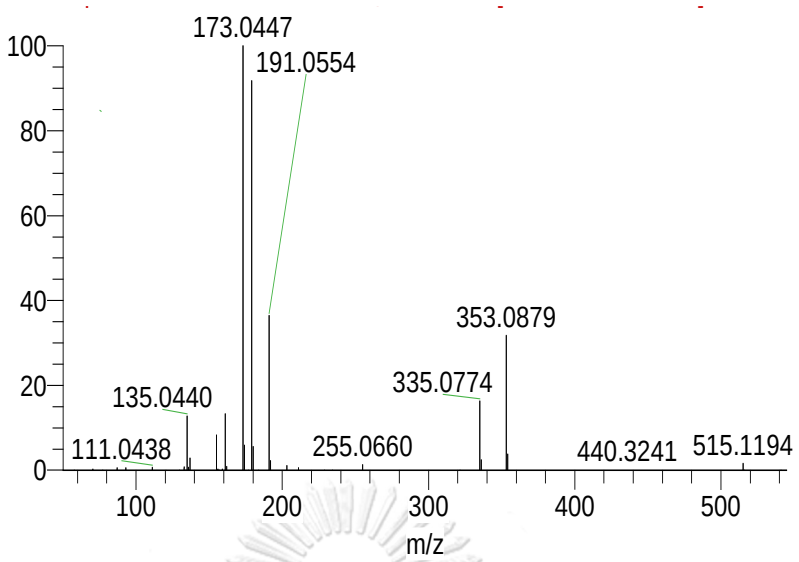


Figure 27 Fragment of 3,4-diCQA

Fragments of 3,5-diCQA at HCD=30eV

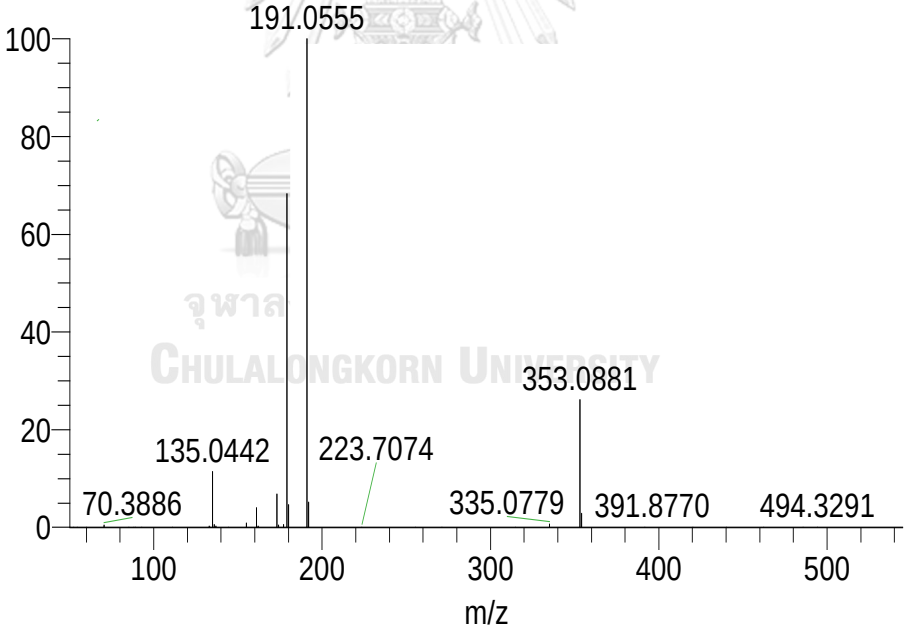


Figure 28 Fragment of 3,5-diCQA

Fragments of 4,5-diCQA at HCD=30eV

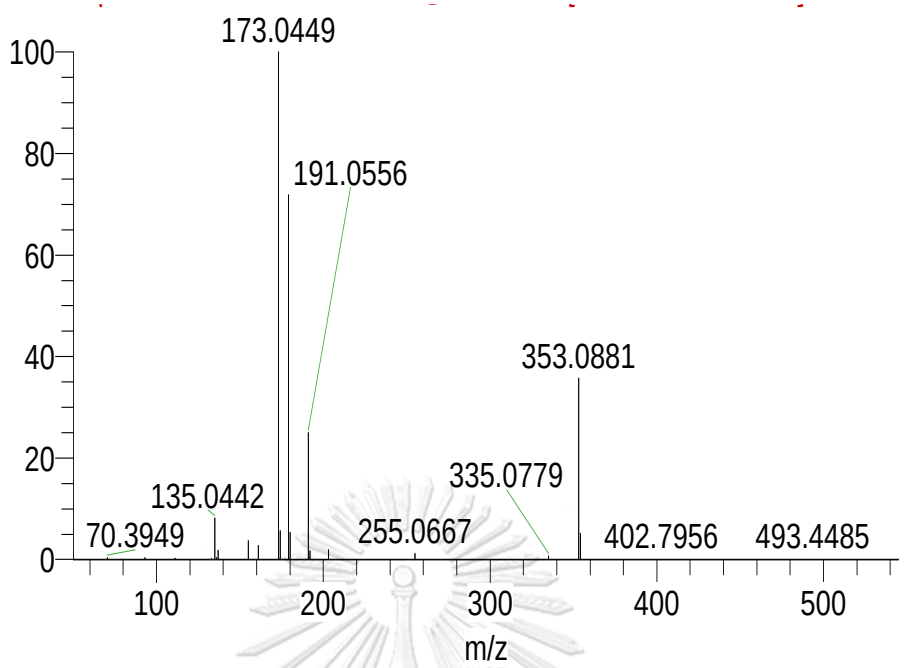


Figure 29 *Fragment of 4,5-diCQA*

Table 30 Response factors of chlorogenic acid

Chlorogenic acid	ε	Response factor
5-CQA	1.95 (330nm)	1.084
4-CQA	1.8 (330nm)	0.800
3-CQA	1.84 (330nm)	1.000
4-FQA	1.95 (325nm)	0.596
5-FQA	1.93 (325nm)	0.905
3,4-diCQA	3.18 (330nm)	1.361
3,5-diCQA	3.16 (330nm)	1.520
4,5-diCQA	3.32 (330nm)	1.304

A.4 Determination of target key volatile compounds in coffee brew in section 3.3.3.1

A.4.1 Determination of low-boiling point volatile compounds and sulfur compounds

Three drying treatments were selected from the results of R-index similar by ranking test. In order to prepare coffee brew for the determination of low-boiling point volatiles and sulfur compounds. All roasted coffees were prepared by weighing a given amount of sample in a Mason glass jar of 4 oz. They were then vacuumed and sealed to prevent any evaporation of volatile within beans shell.

Headspace-solid phase microextraction (HS-SPME) coupled with stable isotope dilution assay (SIDA) were used to determine the target key volatiles that defining an important coffee characteristic in order to determine the effect of drying methods. Before analysis, the labelled internal standards (IS) of selected aroma-active compounds were prepared individually in dichloromethane (~1 mg/mL) instead of methanol to avoid decomposition.

Sample were frozen at -70 ˚C, 1 h prior to grinding with Hamilton Beach Fresh-Grind Coffee Grinder to obtain a fine particle size with a sieve range 1.77-2.36 mm in order to preserve the volatile integrity. To prepare coffee brew, a modification of steep cup methodology (Lyman *et al.*, 2003) was used in this study to control the constant parameters in order to prepare coffee brew following gold standard cup of SCAA.

About 8.5 grams of coffee powder were brew with 150 mL of hot odorless water (98-100 ˚C) for 5 min. Coffee brews were then cooled immediately in an ice bath to stop the chemical reaction during brewing. For the case of sulfur compounds determination after the cooling step, l-cysteine (900 mg) was added to prevent the binding between thiols and complex matrix of coffee and the labelled internal standard was added of in known concentration (20 µl).

Brew samples were then stirred for 10 min and centrifuged at 3500 rpm to remove coffee particles. An aliquot of 2 ml of coffee brew was transferred to 20 mL screw top headspace vials equipped with Teflon coated stir bar and a Teflon-lined silicon closure. For the determination of other target low-boiling point volatiles, after this step, a known concentration of labelled IS of other targeted volatiles were spike through Teflon-lined silicon septum and then well mixed prior further analysis.

The sample vial was analysed using a CombiPal autosampler coupled to 7890 N GC-MS system (Agilent Technologies) by incubated at 60°C for 10 min at the agitator speed 250 rpm. 2 cm of SPME fiber (50/30µm DVB/ Carboxen™/ PDMS StableFlex™; Supelco, Bellefonte, PA) was exposed into HS of the sample vials, and the HS extracted volatiles for 30 min at the same temperature.

Volatile compounds were desorbed from the SPME fiber in to GC-MS system (7890A; Agilent Technologies; California, USA) coupled with Time of Flight Mass Spectrometer (TOF-MS) (Pegasus 4D; LECO®; Michigan, USA) by hot splitless injection (260 ◦C; 4 min valve-delay). Two different phase of capillary columns; Stabilwax® (30 m x 0.25 mm id x 0.25 µm film thickness; Restek; Pennsylvania, USA) and SAC™-5 (30 m x 0.25 mm i.d. x 0.25 µm df; Supelco, Bellefonte, PA) were used to separate analysed volatiles. Helium was used as the carrier gas at a constant rate of 1 mL/min. Oven temperature was programmed from 40 ◦C to 225 ◦C at 4 ◦C/min with initial and final holding times of 5 min and 30 min respectively.

The MSD conditions were as follows: Capillary direct interface temperature, 260 ◦C; ionization energy, 70 eV; mass range, 35 to 300 amu; electron multiplier voltage (Autotune+ 200V); scan rate, 5.27 scans/s. Data acquisition was performed using the simultaneous full scan at 35-300 m/z.

Identification of volatiles was performed via the full identification comprising comparison of mass spectra, calculation of retention index (RI) using alkane C5-C30 and identification with the reference standard of the investigated compound. The RI of each compound was calculated using the retention time (RT) of that compound compared against the RTs of a series of standard n-alkanes C5-30 (Van Den Dool & Dec-Kratz, 1963). Key odorants were positively identified based on comparison of their RI values, and mass spectra against those of authentic standard compounds to avoid erroneous identifications.

A compound was considered to be tentatively identified if no authentic standard was available for comparison. In this case mass spectra were compared against those in the NIST2008 mass spectral database, and retention indices were compared to literature values.

List of target volatiles, isotopically internal standards used and their concentration, Selected ion and Response factor are shown in Table 31. Example of response factors determination are given in Section A.4-A.7

The calculation for peak area of the selected ion target analytes was performed using Chroma TOF software (version 3.34) The mass of a target analytes was calculated from the mass ratio, area ratio, and GC-MS response factor (R_f).

R_f of all target compounds in appendix A were calculated in the standard mixture of labeled and unlabeled compound in known concentration dissolved in dichloromethane that was prepared in the ratio of 1:10, 2:10, 5:10, 10:10, 10:5, 10:2, 10:1 and analysed via GC-MS system (6890N/5973N; Agilent Technologies; California, USA) using the same condition as described above except using the hot split mode at 260 ◦C.

For 3-mercapto-3-methyl-butylformate, since its internal standard was 1-heptanethiol, the calibration curves were determined by SPME-GC-MS as already described. The response factor of this compound was determined against 1-heptanethiol spiked in sun dried coffee brew which represent the same matrix with coffee and could help in determining the recovery loss of 3-mercapto-3-methylbutylformate when analysed with SPME-GC-MS technique. Different approximate mass ratio of the compound prepared in methanol were spiked into 2 ml coffee brew prior to analysis.

R_f was calculated as the inverse of slope of the calibration plot of area ratio versus mass ratio, that was determined by analysis of five levels of standard compound (unlabelled) against the isotope compound (labelled) and internal standard (1-heptanethiol). The R^2 values of linear regressions obtained for calibration plots were greater than 0.9 for all compounds.

The mass of a target compound was calculated from the mass ratio, area ratio, and GC-MS response factor (R_f)

The concentration of all target volatiles was calculated using equation A1 and A2:

$$R_f = \frac{[\text{area of ion}_t/\text{area of ion}_i]}{[\text{mass}_t/\text{mass}_i]} \qquad \text{(A1)}$$

$$\text{Concentration}_t = \text{concentration}_i \times R_f \times \frac{[\text{area of ion}_t/\text{area of ion}_i]}{[\text{mass}_t/\text{mass}_i]} \qquad \text{(A2)}$$

Where i = labelled internal standard, t = target compound

A.4.2 Determination of high-boiling point volatiles via rapid method

Furaneol®, abhexon, sotolon and maltol were selected for the determination of the high boiling point volatiles in this study due to their contribution of coffee aroma characteristics as mentioned in previous studies. Coffee brews of selected three treatments were prepared as mentioned in A.4.1. A modification method of Cheong *et al.* (2013) was applied for volatile analysis.

150 mL of coffee brew were extracted with 25 mL of dichloromethane using ethyl maltol as internal standard and by liquid-liquid extraction. A known amount of ethyl maltol, prepared in methanol was spiked as an IS before extraction. The suspension was stirred using a vortex mixer at 200 rpm at 25 ◦C for 30 min. Subsequently, the extract was centrifuged and water removed by drying with Na_2SO_4. The solvent was removed under a purified nitrogen stream until the volume was reduced to approximately 2 mL.

The concentrated extract was dark brown. The sample was stored at -70 °C before being subject to GC-MS analysis. Two µL of extract were injected into the GC-MS system consisting of a 6890N GC/5973N mass selective detector (Agilent Technologies, Inc.)

Stabilwax® (30 m × 0.25 mm i.d. × 0.25 µm df; Restek) capillary column was used to separate every volatile fractions. Volatile fractions were injected using a CIS4 (Gerstel GmbH & Co. KG, Germany) programmable temperature vaporization (PTV) inlet in the cold-splitless mode (-50 °C for 0.1 min, then ramped at 12 °C/sec and held at 260 °C).

GC oven temperature was programmed from 35 to 225 °C at 4 °C/min with initial and final hold times of 5 and 20 min, respectively. The flow rate of helium carrier gas was 1 mL/min. The mass spectra were recorded in full scan mode (35-350

a.m.u., scan rate 4.45 scans/s, interface temperature 260 °C, and ionization energy 70 eV).

R_f of Furaneol®, abhexon and sotolon were determined by GC-MS as previously described. The response factors of these compounds were determined against ethyl maltol spiked in coffee brew (sun dried coffee), the same matrix with analyzed coffee sample in this study. Different approximate mass ratio between standard compound and ethyl maltol prepared in methanol were spiked into coffee brew prior to analysis.

R_f was calculated as the inverse of slope of the calibration plot of area ratio versus mass ratio, that was determined by analysis of five levels of standard compound against the internal standard (ethyl maltol). The R^2 values of linear regressions obtained for calibration plots were greater than 0.9 for all compounds. The retention index (RI) of each compound was calculated using the retention time (RT) of that compound compared against the RTs of a series of standard n-alkanes C5-C30 (Van Den Dool & Kratz, 1963). The odorants were positively identified based on comparison of their RI values, and mass spectra against those of authentic standard compounds to avoid erroneous identifications as described by (Molyneux & Schieberle, 2007). For quantification, the mass ions for internal standard and target compounds were chosen on the basis of uniqueness and relative intensity as showed in Table A2. The peak areas for selected ions were integrated using MSD ChemStation software (Agilent Technologies, Inc.). The mass of a target analytes was calculated as previous described. The concentration of all target volatiles was investigated following the previous equation showed in previous section A.4.1.

A.5 Calibration of Furaneol® determination

In order to calibrate the accuracy of Furaneol® content which was determined by the method mentioned above, the use of stable isotope dilution analysis (SIDA) and direct solvent extraction-solvent-assisted flavor evaporation (DSE-SAFE) were applied to determine Furaneol® concentration to make the comparison and ensure the correctness of rapid method. Sun dried coffee from the same batch as in previous section was used in this test as control to determine the accuracy of Furaneol® determination. Coffee brew was prepared following the same procedure as mentioned in A.4.

Volatile compounds of coffee brew were isolated using DSE technique using known amount of $^{13}C_2$-Furaneol® as internal standard. An aliquot of 150 mL of coffee sample was placed in 250-mL centrifuge Teflon bottle were extracted with dichloromethane (25 ml). This mixture was stirred for 30 min to achieve equilibrium between the internal standards and analytes. After centrifuging, the solvent layer was collected and the residue was extracted two more times with 25 mL of dichloromethane (25 mL solvent contact time for each extraction).

The combined solvent extracts were concentrated to 50 mL using a Vigreux column (40 ˚C) prior to SAFE. Volatile compounds were collected after distillation at 5 x 10^{-5} ~ 9x10^{-5} Torr for 3 h as previously described (Engel *et al.*,1999). The SAFE apparatus was kept at 40 ˚C with a circulating water bath.

The SAFE distillate was then fractionated to neutral/basic (NB) fraction and acidic (AC) fractions (Potsatchakul *et al.*, 2008). The aroma extract was fractionated with aqueous 0.5 M NaH_2CO_3 (3 × 20 mL) into acidic (aqueous phase) and neutral-basic (organic phase) fractions. The aqueous layer was acidified to pH 2 with aqueous 4 N HCl, and extracted with diethyl ether (3 × 20 mL). Each fraction was washed with

saturated aqueous NaCl (2 × 15 mL), concentrated to 10 mL by Vigreux column distillation, and dried over 2 g of anhydrous Na_2SO_4. The ether extract was further concentrated to 200 μL, and kept at -70 °C until analysis.

Two μL of extract were injected into the GC-MS system consisting of a 6890N GC/5973N mass selective detector (Agilent Technologies, Inc.).

Stabilwax® (30 m × 0.25 mm i.d. × 0.25 μm df; Restek) capillary column was used to separate the volatile fractions.

For this calibration, volatile fractions were injected in two modes. First was the cool on-column injection mode (±3 °C oven tracking mode). The initial oven temperature was 35 °C. After 5 min, the oven temperature was increased at 4 °C/min to the final temperature 225 °C, and held for 20 min. The flow rate of helium carrier gas was 1 mL/min. The mass spectra were recorded in full scan mode (35-300 a.m.u., scan rate 5.27 scans/s, interface temperature 280 °C, and ionization energy 70 eV) Second was the injection using a CIS4 (Gerstel GmbH & Co. KG, Germany) programmable temperature vaporization (PTV) inlet in the cold-splitless mode (-50 °C for 0.1 min, then ramped at 12 °C/sec and held at 260 °C).

GC oven temperature was programmed from 35 to 225 °C at 4 °C/min with initial and final hold times of 5 and 20 min, respectively. Other conditions were as follows: MSD interface temperature, 260 °C; ionization energy, 70 eV; mass range, 35-350 a.m.u; EM voltage, Autotune + 165 V; scan rate, 4.45 scans/s. The concentration of analyte and their odor activity value were calculated as section A.2.

Table 31 Selected ion (m/z) and response factors (R_f) used in stable isotope dilution analysis and internal standard method analysis

No.	compound	ion[a]	Labeled internal standard	ion[b]	R^{2c}	R_f[d]
1.	Methanethiol	47	[$^2H_3$]-Methanethiol	51	1.00	0.597
2.	Propanal	58	[$^2H_3$]-Propanal	61	0.99+	1.7
3	2-Methylpropanal	72	[$^2H_2$]-2-Methylpropanal	74	1.00	0.597
4	2-Methylbutanal	86	[$^2H_2$]-2-Methylbutanal	88	0.99+	0.518
5	3-Methylbutanal	86	[$^2H_2$]-3-Methylbutanal	88	0.99+	0.58
6	2,3-Butanedione	86	[$^2H_2$]-3-Methylbutanal	88	0.99+	0.072
7	2,3-Pentanedione	100	[$^{13}C_2$]-2,3-Pentanedione	102	0.99+	0.692
8	Hexanal	72	[$^2H_4$]-Hexanal	76	0.99+	0.156
9	2-Methylpyrazine	94	[$^2H_3$]-2-Methylpyraizine	97	0.99+	1.013
10	2-Ethylpyrazine	108	[$^2H_5$]-2-Ethylpyraizine	113	0.99+	1.514
11	2,6-Dimethylpyrazine	108	[$^2H_3$]-2,6-Dimethylpyraizine	111	0.99	1.267
12	2,3-Dimethylpyrazine	108	[$^2H_3$]-2,3-Dimethylpyraizine	111	0.981	1.097
13	Nonanal	114	[$^2H_4$]-Nonanal	116	0.99+	0.440
14	Dimethyl trisulfide	126	[$^2H_6$]-Dimethyl trisulfide	132	0.99+	0.518

Table 31 Selected ion (m/z) and response factors (R_f) used in stable isotope dilution analysis and internal standard method analysis (Cont.)

No.	compound	ion[a]	Labeled internal standard	ion[b]	R^{2c}	R_f[d]
15	Trimethylpyrazine	122	[$^2H_5$]-3-Ethyl-2,5-dimethylpyrazine[e]	141	0.99+	0.68
16	2,6-Diethylpyraizine	135	[$^2H_{10}$]-2,6-Diethylpyraizine	144	1	0.694
17	3-Ethyl-2,5-dimethylpyrazine	135	[$^2H_5$]-3-Ethyl-2,5-dimethylpyrazine	141	0.99+	0.68
18	2-Furfurylthiol	114	2-[α-$^2H_2$]-furfurylthiol	116	0.99+	1
19	1-Octen-3-ol	57	[$^2H_3$]-1-Octen-3-ol	60	0.99+	0.703
20	2-Ethyl-3,5-dimethylpyrazine	135	[$^2H_5$]-3-methy-2,5-dinethylpyrazine[e]	141	0.99+	0.68
21	Acetic acid	60	[$^2H_3$]-Acetic acid	63	0.99+	0.504
22	Methional	104	[$^2H_3$]-Methional	107	0.99+	1.18
23	2,3-Diethyl-5-methylpyrazine	150	[$^2H_3$]-2,3-Diethyl-5-methylpyrazine	153	0.99+	1.088
24	3-Mercapto-3 methylbutylformate	102	1-Heptanethiol[e]	98	0.97	0.692
25	2-Isobutyl-3-methoxypyrazine	124	[$^2H_2$]-2-Isobutyl-3-methoxypyrazine	127	0.99+	1.67
26	Linalool	121	[$^2H_2$]-Linalool	123	0.99+	1.09
27	3-Methylbutanoic acid	87	[$^2H_2$]-3-Methylbutanoic acid	89	0.99+	0.860

Table 31 Selected ion (m/z) and response factors (R_f) used in stable isotope dilution analysis and internal standard method analysis (Cont.)

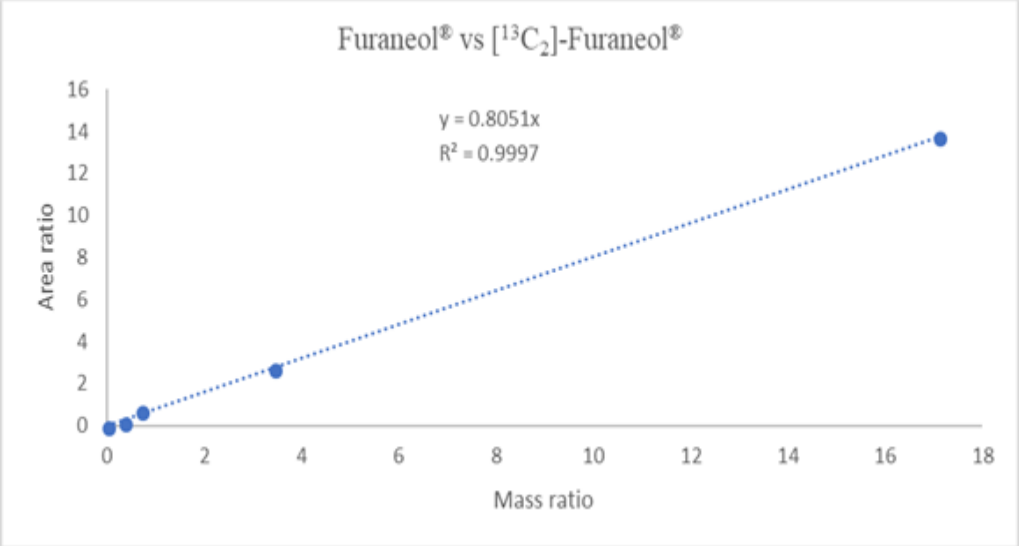
No.	compound	ion[a]	Labeled internal standard	ion[b]	R^{2c}	R_f[d]
28	(*E*)-β-Damascenone	190	[$^2H_4$]-(*E*)-β-Damascenone	194	1.00	0.721
39	Guaiacol	124	[$^2H_2$]-Guaiacol	127	0.99+	0.922
30	2-Phenylethanol	122	[$^{13}C_2$]-2-Phenylethanol	124	0.99+	1
31	Maltol	126	Ethyl maltol[e]	140	0.9567	2.57
32	Furaneol®	128	Ethyl maltol[e]	140	0.957	17.89
33	4-Ethylguaiacol	152	[$^2H_5$]-4-Ethylguaiacol	157	0.99+	1.07
34	*p*-Cresol	108	[$^2H_3$]-*p*-Cresol	111	0.99+	0.869
35	*m*-Cresol	108	[$^2H_8$]-*m*-Cresol	115	0.99+	0.443
36	4-Vinylguaiacol	150	[$^2H_3$]-4-Vinylguaiacol	154	0.99+	0.891
37	Sotolon	128	Ethyl maltol[e]	140	0.9589	1.57
38	Abhexon	142	Ethyl maltol[e]	140	0.9144	2.18
39	Indole	117	[$^2H_4$]-Indole	121	1.00	0.451
40	3-Methylindole (skatole)	131	[$^2H_3$]-3-Methylindole	134	1.00	0.909
41	Furaneol®	128	$^{13}C_2$-Furaneol®	130	0.99+	1.24

[a]Numbers corresponding to those in Table 4.20. [b]Selected ion used for quantitation. [c]Coefficient of determination for calibration plot. [d]Response factor. [e]Isotope unavailable, a structurally similar compound was used as internal standard.

A.6 Response Factor of Furaneol® vs [$^{13}C_2$]-Furaneol®

Stock solution	Concentration (µg/µl)
Furaneol®	4
[$^{13}C_2$]-Furaneol®	1.17

Volume ratio	Mass ratio Label/unlabel (128/130)	Area ratio Label/unlabel (128/130)
1/10	0.341880342	0.169752294
1/5	0.683760684	0.725397607
1/1	3.418803419	2.727734096
5/1	17.09401709	13.76176588
10/1	34.18803419	20.22851601

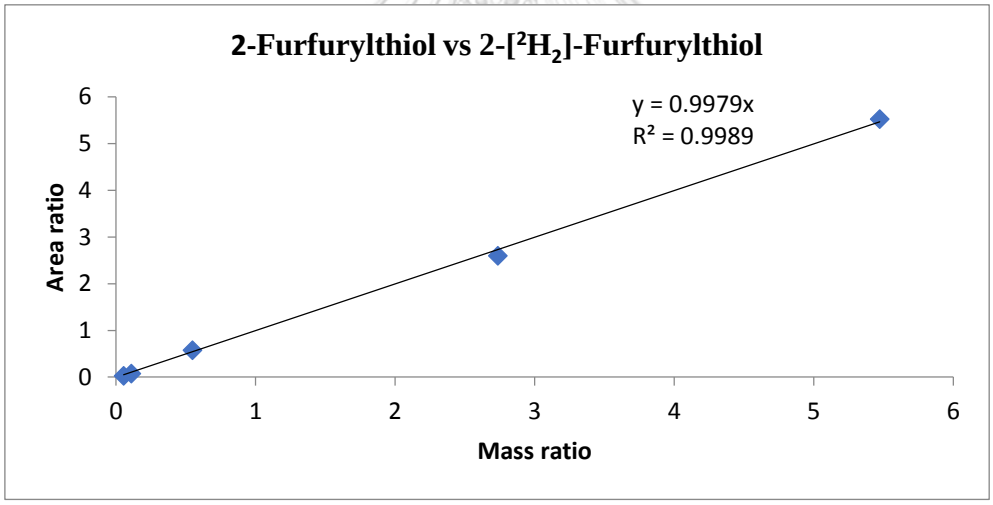


Response Factor (R_f) =1/slope =1/0.8051 = 1.24

A.7 Response Factor of 2-Furfurylthiol vs 2-[$^2H_2$]-Furfurylthiol

Stock solution	Concentration (µg/µl)
2-Furfurylthiol	2500
2-[$^2H_2$]-Furfurylthiol	4568.5

Volume ratio	Mass ratio Label/unlabel (114/116)	Area ratio Label/unlabel (114/116)
1/10	0.054722557	0.032905071
1/5	0.109445113	0.07911926
1/1	0.547225566	0.577334397
5/1	2.736127832	2.599307805
10/1	5.472255664	5.523983083

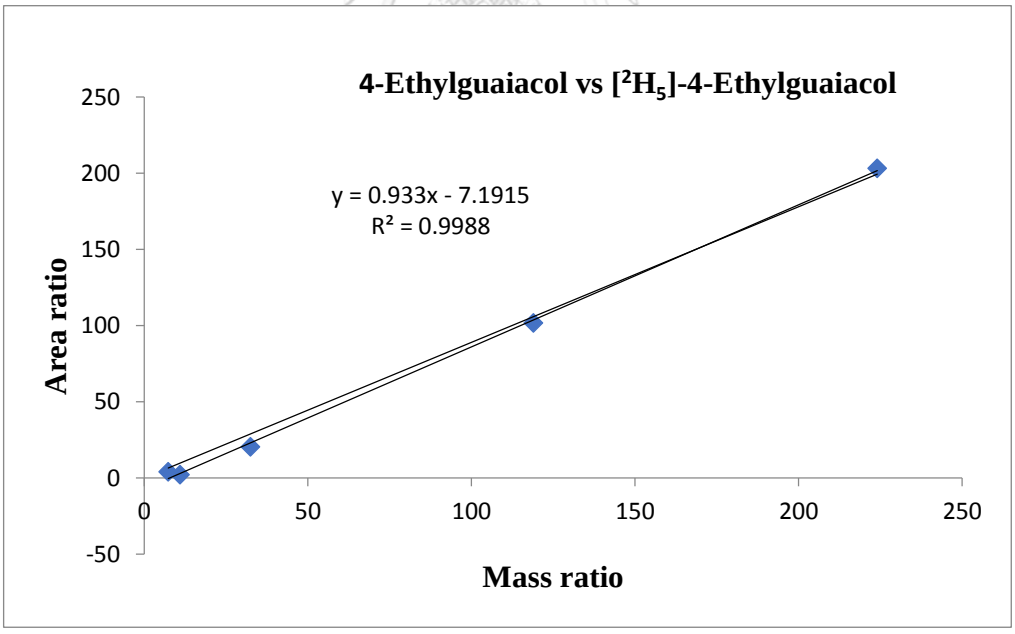


Response Factor (R_f) =1/slope =1/0.9979 = 1.002

A.8 Response Factor of 4-Ethylguaiacol vs [$^2H_5$]- 4-Ethylguaiacol

Stock solution	Concentration (µg/µl)
4-Ethylguaiacol	0.5
[$^2H_5$]- 4-Ethylguaiacol	0.0246

Volume ratio	Mass ratio Label/unlabel (152/157)	Area ratio Label/unlabel (152/157)
1/10	10.90548035	2.032520325
1/5	7.276375845	4.06504065
1/1	32.50650227	20.32520325
5/1	118.9591856	101.6260163
10/1	224.0006978	203.2520325

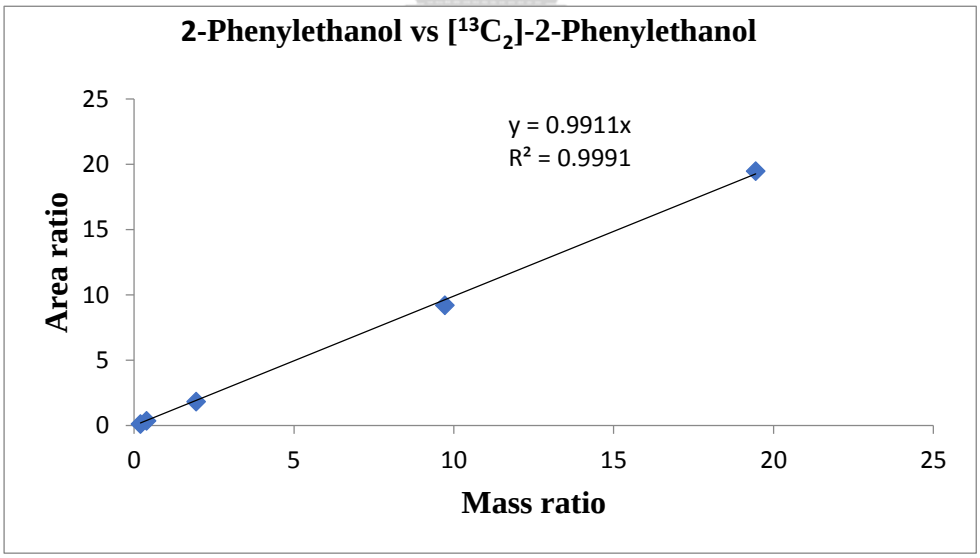


Response Factor (R_f) =1/slope =1/0.933 = 1.07

A.9 Response Factor of 2-Phenylethanol vs [$^{13}C_2$]-2-Phenylethanol

Stock solution	Concentration (µg/µl)
2-Phenylethanol	2.1
[$^{13}C_2$]-2-Phenylethanol	1.08

Volume ratio	Mass ratio	Area ratio Label/unlabel (122/124)
	Label/unlabel (122/124)	
1/10	0.194444444	0.104600978
1/5	0.388888889	0.353089404
1/1	1.944444444	1.833511628
5/1	9.722222222	9.206268069
10/1	19.44444444	19.49601789



Response Factor (R_f) =1/slope =1/0.9911 = 1.

A.10 Standard curve of gallic acid for determination of total phenolic content (TPC) in green coffees with concentration of standard gallic acid, 5-80 mg/L in ethanol

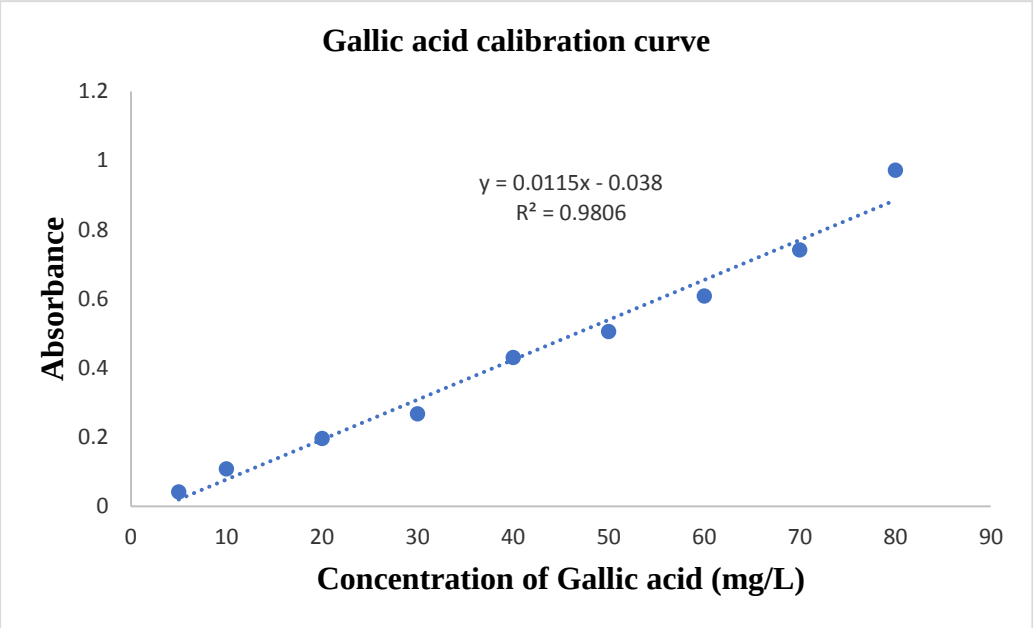


Figure 30 Standard curve of gallic acid for total phenolic content (TPC) determination

A.11 Standard curve for determination of FRAP assay in green coffees with concentration of Trolox in ethanol, 100-800 mg/L

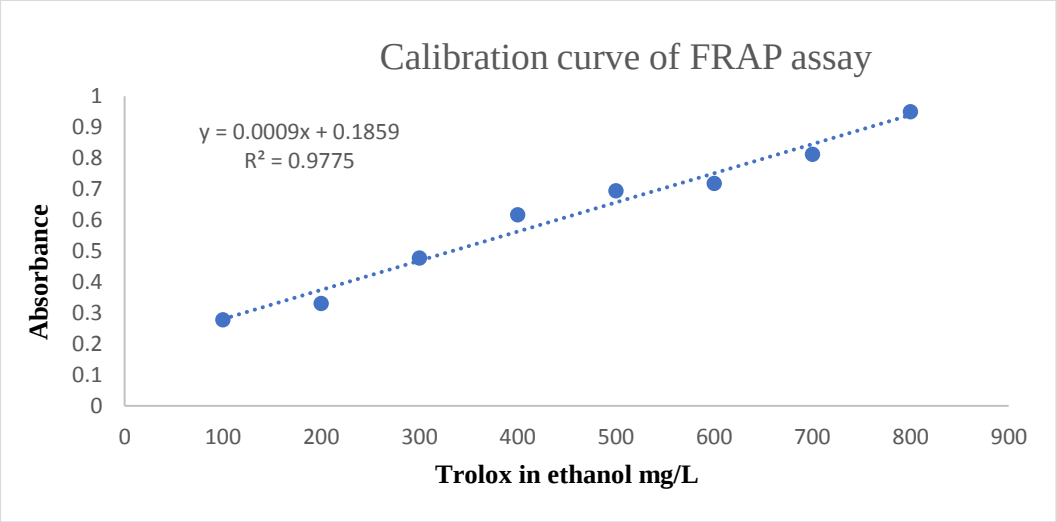


Figure 31 Standard curve of Trolox in ethanol for FRAP assay

Appendix B

Consent form of sensory evaluation and ballots

B.1 IRB Approval letter

Consent form

INFORMED CONSENT FORM FOR SENSORY EVALUATION PANELISTS

"Difference Testing of coffee"

You are invited to participate in a study involving sensory evaluation of Coffee beverages. The goal of this research is to determine and gain the better understanding of the aroma difference between coffee produced from difference post harvesting methods. The products will be evaluated using a ranking test and analyzed by the R-Index method. The R-Index method is a difference test used to determine if a significant difference is detected between the samples. You will be presented with a sample labeled as "noise". You will review this sample and when you are familiar, will be presented with a set containing 5 samples. You will put the samples in order from most similar to the "noise" to least similar to the "noise".

A complete list of ingredients is available for review. All foods and ingredients have been designated as safe for food use by their respective manufacturers and are commonly found in commercially available foods. The University of Illinois does not provide medical or hospitalization insurance coverage for participants in this research study nor will the University of Illinois provide compensation for any injury sustained as a result of participation in this research study, except as required by law. You are free to withdraw from the study at any time for any reason and it will have no effect on your grades at, status at, or future relations with the University of Illinois. The experimenter(s) also reserve the right to terminate the participation of an individual subject at any time.

You will be participating in one, 20-30 minute sessions. You are free to withdraw at any time during the course of the study.

Your participation in this study is confidential. The researchers will keep the responses confidential, and any publications or presentations of the results of the research will only include information about group performance. Data gathered from the entire project will be summarized in the aggregate, excluding references to any individual responses. The aggregated results of our analysis will be for journal articles and conference presentations. Again, your input is very important to us and any information we receive from you will be kept secure and confidential.

You are encouraged to ask any questions about this study before, during, or after your participation. However, specific questions about the samples that could influence the outcome of the study will be deferred to the end of the experiment. Questions can be addressed to Dr. Keith Cadwallader (217-333-5803, cadwlldr@illinois.edu) and Fareeya Kulapichitr (217-898-5295, fareeya@illinois.edu). You may also contact the IRB Office (217-333-2670, irb@illinois.edu) for any questions about the rights of research subjects. If you live outside the local calling area, you may also call collect.

I understand the above information and voluntarily consent to participate in the study described above.

I have been offered a copy of this consent form. ☐ Yes ☐ No

I am 18 years of age or older. ☐ Yes ☐ No

Signature Date

B.2 Questionnaire for overall difference test of coffee in section 3.2.4.2

Overall difference test of coffee brew

No. of Experiment

Date .....Time.....

Age ..... Gender (.....) Male (.....) Female

No. of Panelist.....

Suggestion

Please fill in your number, date, time and code of samples on the blank space of ballot

In this sensory section, you will receive **pair of coffee brew samples**. Before evaluate samples, please re-read the question on this ballot again.

After completely read the questions, please evaluate samples and follow the instruction in each section of ballot. If you have a question, please do not hesitate to ask.

Please drink water to rinse your mouth before evaluate next pair of coffee brew samples.

Code of sample 1..... **Code of sample 2**.....

Section 1. Please evaluate the pair of samples by taste them from the left side to the right side and after completely evaluate the pair, please make the right checked ✓ on words that exact to describe your judgement the most

☐ Different ☐ Not different

Section 2. After completely test the pair of coffee brew, please confirm the confidence on your answer in section 1 by make the right checked ✓ on "Sure" or "Not-sure"

☐ Sure ☐ Not-sure

Note for suggestion

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VITA

Miss Fareeya Kulapichitr was born on August 22, 1987 in Bangkok, Thailand. She obtained her B.Sc. in Food Technology, Faculty of Science, Mahidol University in 2010 and M.Sc. in Food and Nutrition for Development, Institute of Nutrition, Mahidol University, 2012. While studying M.Sc., She received the supporting fund scholarship for Thesis Research from the Office of the National Research Council of Thailand (NRCT). Since 2012, after graduating, she has worked as Research and Development Officer (R&D), Coffee and Counter Bar Products Developer at DoiKham Food Products Company for 5 month.

In 2013, She began to study at Department of Food Technology, Faculty of Science, Chulalongkorn University. In 2016, she received the scholarship from Thailand Research Fund (TRF) in catagory of Royal Golden Jubilee Scholarship for PhD.program to exchange at Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign to do a research in coffee aroma 1 year.

Publications

Lopetcharat, K., Kulapichitr, F., Suppavorasatit, I., Chodjarusawad, T., Phatthara-aneksin, A., Pratontep, S., Borompichaichartkul, C. (2016). Relationship between overall difference decision and electronic tongue: discrimination of civet coffee. Journal of Food Engineering, 180, 60- 68.

Kulapichitr, F, Borompichaichartkul, C, Pratontep, S, Lopetcharat, K, Boonbumrung, S, Suppavorasatit, I. (2017). Differences in volatile compounds and antioxidant activity of ripe and unripe green coffee beans (Coffea arabica L. 'Catimor'). Acta Horticulturae, 1179. 261-268.

Kulapichitr, F., Suppavorasatit, I., Borompichaichartkul, C., Chodjarusawad, T., Phatthara-aneksin, A., Pratontep, S. and Lopetcharat, K. (2017). Impact of processing method and growing location on overall differences of brewed coffee using electronic tongue and sensory panel. Acta Horticulturae, 1179, 269-276.

Award

Kulapichitr, F., Suppavorasatit, I., Borompichaichartkul, C., Cadwallader, K.R. An alternative low cost and feasible method for determination of Furaneol® in coffee.The 2nd International Flavor and Fragrance Conference in Wuxi, China (May 28-31) (3rd Place in poster competition).