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The Compositional Basis of Coffee Flavour

Wenny Bekti Sunarharum

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Abstract

Coffee trade is a billion-dollar industry and coffee remains one of the most traded commodities of economic importance to both the countries producing, and the countries consuming coffee. To ensure demand, it is of great importance to constantly maintain and, where possible, improve its quality.

Flavour is crucial to coffee quality. However, describing coffee flavour is a very complex task as it is influenced by numerous factors from the farm to cup. These factors influence compositional properties of coffee and have a large impact on the perceived flavour.

The ultimate aim of this project was to understand how different components in coffee influence flavour perception. The approach involved an exploration on the sensory and physicochemical profiles of sensorily diverse 26 commercial single-origin 'specialty' coffees. Sensory evaluation of these 26 selected coffees involves assessment on the brews while coffee ground and extracts were analysed for physicochemical profiles. An analytical method was developed and applied for rapid quantification of the targeted volatiles compounds in coffee. The results of sensory and physicochemical evaluation were modelled using a multivariate analysis to explore the relationship between attributes and to know potential sensory markers that could contribute to coffee flavour.

Coffee proved to be a highly challenging matrix to study and required special preparation and presentation of individual samples at consistent temperature during sensory evaluation. Nevertheless, sensory profiles for the diverse range of coffees was achieved. Certain coffees were clearly distinctive: Ethiopian coffees registered *fruity, citrus* and *aromatic spice* sensory profile notes, Australian coffees exhibited *milder* profiles, while India Robusta coffees generally possess *smoky, woody, earthy* and *cereals* profile.

Three analytical stable isotope dilution analysis/gas chromatography-mass spectrometry (SIDA/GC-MS) methods were developed and applied to quantify 27 key volatile compounds which were targeted due to their reported importance to coffee flavour. These methods involved a headspace-solid phase microextraction/gas-chromatography (HS-SPME/GC-MS) of coffee, steam distillation extraction of coffee followed by a HS-SPME/GC-MS and direct liquid injection of a steam distilled extract of coffee to GC-MS.

The 26 medium-roasted single-origin coffees investigated were quite diverse in physicochemical properties. It was clear that some coffees showed distinct physicochemical characters, for example, Robusta coffee contained doubled the caffeine content, higher concentrations of dicaffeoylquinic acids (diCQAs), higher pH, and higher L* value (a measure of *lightness*) but was lower in crude fat and trigonelline than the Arabica coffees.

Principal component analysis (PCA) and partial least squares (PLS) regression were used as multivariate modelling techniques using compositional variables to mathematically predict coffee sensory attributes scoring. The models could be validated ($Rv^2 \geq 0.4$) for sensory attributes: *aroma intensity, citrus*, *earthy*, *sourness*, *bitterness*, *flavour intensity* and *residual*. For example: the prediction of *sourness* using caffeic acid, titratable acidity (TA), L* value and pyrazines; the prediction of *citrus* using ketones and aldehydes. The results indicated that those chemical components could be potential and contribute to certain sensory attributes of coffee.

An additional study was conducted on the evolution of important chlorogenic acids from the green coffee beans to the coffee brews and to know the potential role to coffee flavour, specifically to volatile phenols. Chlorogenic acids were progressively lost during coffee processing with approximately only a fifth of the level in green coffee beans remains in a cup of coffee. The evolution of chlorogenic acids during coffee processing involves biochemical degradation and synthesis pathway that include acyl migration, isomerisation, lactonisation, epimerisation, hydrolysis, and polymerisation mechanism. Since there was no relationship found between the volatile phenols and chlorogenic acids, further investigation on the contribution of chlorogenic acids to coffee flavour will be beneficial.

Since not all important components of coffee have been measured in this study, not every predictive model performed well. However, this is the first study to investigate sensory and physiochemical properties of a broad range of commercially diverse single-origin ‘specialty’ coffees as well as to quantify volatile monoterpenes especially geraniol. This study provided a new knowledge on an Australian coffees and specific coffee types such as Indonesia Luwak (Arabica) and Indian Monsooned Malabar. The present study also provides some interesting insight on coffee flavour. It has shown the potential for further coffee and flavour researches while offering advantages for industrial applications.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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Publications during candidature

Peer-reviewed journal and poster publications:

Sunarharum, W.B.; Williams, D.J.; Smyth, H.E. 2014. Complexity of coffee flavour: A compositional and sensory perspective. Food Research International 62: 315–325 (Review article).

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The aroma of commercial single-origin coffees from around the world (3rd International Congress on Cocoa Coffee and Tea, 22-24 June 2015, University of Aveiro, Portugal 2015).

Exploring 26 single-origin coffees based on non-volatile composition (Tropical Agriculture Conference, TropAg 2015, 16-17 November, Brisbane, Australia).

Oral presentations at conferences, seminars and competitions:

Sensory profiling of coffee from around the world (student oral presentation competition at 46th Annual AIFST Convention, Brisbane July 14-16 2013).

Investigation of chlorogenic acids and caffeic acid content on a range of green, roasted, and brewed Arabica coffee (2014 AIFST Food Science Summer School, Brisbane, 5-7 February 2014).

Why coffee tastes good (National Science Week, Health & Food Science Precinct, 16-24 August 2014).

Why coffee smells good (3MT-QAAFI-UQ Competition, July 2015).

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Contributor	*Statement of contribution*
Wenny B. Sunarharum (Candidate)	Wrote and reviewed paper (90%) Edited paper (65%)
Heather E. Smyth	Wrote and reviewed paper (10%) Edited paper (30%)
David J. Williams	Edited paper (5%)

Contributions by others to the thesis

Contributor	*Statement of contribution*
Australian Research Council	Supporting project through ARC Linkage project funding scheme (project number LP130100376).
Green Cauldron Coffee Australia, Carlini Coffee Company Australia, Kopi Luwak Nusantara Indonesia	Providing commercial coffee samples.
Heather Smyth (Principal Advisor, QAAFI)	Designing the ultimate coffee project. Providing guidance in designing and conducting most of the experiments. Facilitating and providing assistance on data analysis and interpretation on sensory and all compositional data. Review the entire thesis.
David Williams (Co-supervisor, DAF-QLD)	Providing guidance in designing and conducting compositional analysis particularly for caffeine and trigonelline analysis. Providing advice on the non-volatiles data interpretation. Review the thesis.
Glen Fox (Co-Supervisor, QAAFI)	Facilitating and providing assistance on data analysis and interpretation particularly using multivariate model. Review the thesis.
David Edwards	Technical assistance on the HPLC instrument preparation for analysis of chlorogenic acids used in Chapter 5 (p.89), and Chapter 7 (p.125).
Steve Fuller	Undertook analysis of acetaldehyde, acids, furans and sulfur-containing compounds used in Chapter 4 (p.53), and Chapter 6 (p.104).
Steve Appleton	Undertook analysis of crude fat used in Chapter 5 (p.89), and Chapter 6 (p.104).
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“None”

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Keywords

coffee, flavour, sensory, compositional, volatiles, non-volatiles, multivariate, modelling

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List of Abbreviations

CGA	Chlorogenic acids
CQA	Caffeoylquinic acid
diCQA	Dicaffeoylquinic acid
FQA	Feruloylquinic acid
TA	Titratable acidity
GC	Gas chromatography
MS	Mass spectrometry
MSD	Mass selective detector
SIDA	Stable isotope dilution analysis or assay
IS	Internal standard
HS-SPME	Headspace-solid phase micro extraction
SIM	Selective ion monitoring
RF	Response factor
RRF	Relative response factor
LOD	Limit of detection
LOQ	Limit of quantification
HPLC	high performance liquid chromatography
DVB/CAR/PDMS	Divinylbenzene/carboxen/polydimethylsiloxane
OAV	Odour activity value
PVDF	Polyvinyl difluoride
PET/PET/Foil/LLDPE	Polyethylene terephthalate/polyethylene terephthalate/aluminium foil/linear low-density polyethylene
PCA	Principal component analysis
PLS	Partial least square
RMSEC	Root mean square error calibration
RMSECV	Root mean square error cross validation
eV	electron Volt
RT	Retention time
NaCl	Sodium chloride
NaOH	Sodium hydroxide
KH_2PO_4	Phosphate buffer
m/z	Mass to charge ratio

Chapter 1 Introduction

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1.1 Background

Coffee is a very popular brewed beverage that is consumed worldwide. In terms of financial value, it is the most important traded commodity after petroleum. Coffee consumption is increasing across the globe from 139.4 million in 2011 to around 149.2 million of 60 kg bags in 2014 with an annual growth rate of 2.3% since 2011 (ICO, 2015). There is strong global demand for coffee in particular traditional markets such as Canada, the European Union, Japan, Norway, Switzerland, and the United States with potential market growth in new emerging markets such as Algeria, Australia, Russia, South Korea, Turkey, Ukraine and coffee exporting countries (ICO, 2015). These markets certainly demand good quality coffee and therefore the specialty coffee market segment is continually growing. 'Specialty' coffee recognises the effort of many people in the coffee value chain who maintained high standards and excellence from the farm to cup (SCAA, 2014). This premium 'specialty' coffee may include single-origin coffees that attract a high price due to the unique flavours exhibited from the geographical source and consumer demand for provenance and purity. It also includes specific coffee styles offering unique and pleasant coffee flavours.

Good quality coffee flavour has been described as a pleasant sensation, a balanced combination of flavour, body, and aroma in the absence of faults (Mori et al., 2003). Flavour remains the most important consumer parameter and warrants thorough investigation from a sensory and compositional perspective (Mori et al., 2003). Not surprisingly, the composition and sensory properties of coffee has been a target for research for over a century.

The flavour and distinctive sensory qualities of coffee varies enormously across the globe due to influences of genetic strain, geographical location, unique climates, differing agricultural practices and variations in processing method applied. While there is a volume of work on individual or groups of chemical components present in coffee, the likely importance of individual flavour components to certain coffee types, as well as the sensory properties and consumer preferences for coffee, there is limited information available that directly links perceived sensory properties of coffee to specific compositional components. Further, most studies of coffee flavour are limited to identifying key flavour components in a single coffee type, style or geographical location. Not surprisingly, the 'key' flavour compounds identified in one study are often a different set of 'key' flavour components identified by another, depending on the specific coffee studied. It is clear that for a comprehensive understanding of coffee flavour, that includes the spectrum of coffee flavour types, new studies are needed that investigate coffee flavour from a broad perspective so that we may truly understand the compositional drivers of coffee flavour.

1.2 Aims, objectives and approach

The ultimate aim of this research was to understand how different components in a coffee influence flavour perception. This research was especially focussed on what is responsible for, or what could potentially become markers of, certain sensory character in coffee such as *citrus* and *smoky* aromas. This was achieved through profilling a broad range of sensorily diverse commercially available single-origin 'specialty' coffees and exploring relationships using multivariate modelling methods. Firstly, a set of coffees representing the sensory diversity of coffee flavour was selected through sensory pre-screening employing trained panellist. As many as 59 coffees from around the world was screened mostly for the aroma diversity. The selected 26 coffees were further evaluated for their sensory, physical and chemical (physicochemical) properties. Coffee brews were assessed by sensory methods while the coffee grounds were analysed for physicohemical properties. The results were then used to build a multivariate model to get a better understanding on which components of coffee play a role in coffee flavour, and what the nature of that contribution is.

The specific objectives of this projects were:

1. To profile the flavour diversity of commercial medium-roasted coffees through a sensory evaluation using descriptive analysis method.
2. To develop analytical method applicable for comprehensive analysis of coffee flavour using, stable isotope dilution analysis combined with gas chromatography mass spectrometry (SIDA/GC-MS) for targeted aroma compounds.
3. To identify and quantify the targeted volatile compounds in coffee using gas chromatography-mass spectrometry (GC-MS).
4. To explore the non volatiles composition and physical properties in coffee.
5. To model the relationships between sensory, physical and chemical (physicochemical) data in order to get a comprehensive understanding of coffee flavour by means of multivariate data analysis (chemometrics).
6. To study evolution of chlorogenic acids during coffee processing from green, roasted into brewed coffee samples using High Performance Liquid Chromatography (HPLC) as well as investigating the potential relationship to few volatile phenols.

1.3 Significance

Outputs and significance of this research are listed below:

1. Understanding the complexities of coffee sensory analysis.
2. Development of novel analytical methods that can be applied to measure and predict the flavour of broad range of coffees. These methods should be of great benefit to the coffee industry in their efforts to determine quality.
3. Understanding the chemical basis of coffee flavours and the key aroma volatiles contribute to flavour. This may assist the industry to control desirable flavour of their coffee product through processing and farm management techniques
4. Understanding the link between physiochemical and sensory quality. This may contribute to flavour science and the sensory science area.
5. Providing basic information which may facilitate further genetic study and may assist coffee industry with further development of their product.

1.4 Thesis structure

This thesis consists of seven comprehensive chapters.

Chapter 1 (p.1) provides background, aims, objectives, approaches, and justifies the significance of the study.

Chapter 2 (p.5) critically reviewed current researches on coffee.

Chapter 3 (p.30) is the first of five research chapter that reports the sensory profiles of a broad range of coffee and details the methods being implemented in the sensory evaluation.

Chapter 4 (p.53) is dedicated to develop a novel SIDA/GC-MS method specifically developed for identification and quantification of targeted volatile compounds and also confirm the usefulness of the method in profiling coffee volatiles.

Chapter 5 (p.89) profiles the physicochemical (non-volatile) properties related to coffee flavour.

Chapter 6 (p.104) further explores relationship between compositional data (non-volatiles, volatiles), physical data (colour) and sensory data using multivariate model.

Chapter 7 (p.125) investigates chlorogenic acids in coffee and explores the evolution of chlorogenic acids from green coffee beans to roasted coffee beans and brewed coffee while also studying the potential relationship with volatile phenols.

Chapter 8 (p.143) outlines the general conclusion of the thesis, highlighting main findings and recommendations for further research endeavours.

Chapter 2 Literature review

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This chapter presents an updated detail review of the knowledge and current research on coffee flavour. Coffee has been cultivated, produced and consumed around the world for more than five centuries mainly due to its pleasant flavour and physically stimulating properties. It is understood that flavour generation in coffee begins during fruit (cherry) development and continue throughout coffee processing, where the compositional drivers are produced. These compositional drivers that include non-volatile and volatile components could influence coffee quality. Therefore, numerous early researches focused on exploring coffee compositions and/or coffee sensory. Unlike the evaluation of coffee compositional properties, the link between sensory and chemistry is still an under-researched topic. Many early studies typically evaluate a limited range of coffee flavour-types. Therefore, a comprehensive research on sensory versus compositional properties of coffee will be beneficial for a better understanding on coffee flavour.

2.1 General background

Coffee is a very popular brewed beverage that is consumed worldwide. Coffee, a term derived from *kahveh* (Turkish) or *gahweh* (Arabic), is reported to have originally come from Africa (Ethiophia, Kaffa highland), cultivated widely in Yemen, and spread to the Arabs in the Middle East who introduced the processing of coffee into a popular brewed beverage by roasting beans in the sixteenth century (Wasserman et al., 2012). This plant is currently cultivated in more than 70 countries all over the world with 97% of world supply coming from International Coffee Organization (ICO) exporting member countries (Wasserman et al., 2012). The major world producers (Brazil, Vietnam, Colombia, and Indonesia) are also the main exporters (ICO, 2015). World Coffee Outlook (ICO, 2015) reported that exporting and re-exporting activities have been growing consistently in the last five decades.

Considerable growth has been observed after the year 2000 (ICO, 2015). As the world leading importers or consumers, the European Union also plays an important role as the main coffee re-exporters. In 2014/15, they have re-exported almost half of their imported coffee (ICO, 2015). Belgium and Germany mainly re-export green coffee while roast coffee dominates re-exports from Italy, Poland, Sweden and the United States. Other importing countries, particularly Japan, Spain and the United Kingdom mainly re-export soluble coffee.

There are 66 species of genus *Coffea* in *Rubiaceae* family (Illy, 2002) but only two main species of coffee plant (*Rubiaceae* family) are cultivated worldwide (Clarke and Vitzhum, 2001). These commonly cultivated species are *Coffea arabica* (which accounts for 70–80% of world production) and *Coffea canephora* (also referred as Robusta, and accounting for most of the remaining world's production). The Robusta variety is very popular with the instant coffee industry. It has a substantial body, it is relatively harsh, and has an earthy aroma. Robusta contains caffeine from 2.4 to 2.8% by weight (Illy, 2002). Arabica on the other hand, has good cup quality, low caffeine content (below 1.5% by weight), and fine aroma which can be described as flowers, fruit, honey, chocolate, caramel or toasted bread (Illy, 2002, Lashermes and Anthony, 2007). Interestingly, there is a wild coffee species known as *Coffea brassi* (from *Psilanthus brassi* plant) found in Cape York Australia in 2011 and has been mentioned as an Australian native coffee bean (Michael, 2011).

2.2 Flavour perception of coffee

2.2.1 Aroma and taste sensations

Flavour is a complex sensation which can be described as a combination of aroma, taste, texture and mouthfeel (Taylor, 1996) and chemesthesis or trigeminal sensations (Cliff, 1994). The aroma, or odour, is arguably the most important component of coffee flavour.

Many consumers generally perceive and explain taste as what they smell which has led to flavour being sometimes defined as the 'olfactory component of taste' perceived *retronasally* (Petracco, 2001). *Retronasal* perception occurs when food volatiles flow from

the mouth through the back of the throat reaching the nasal cavity through the pharynx (Petracco, 2001) whereby volatiles interact with receptors on the olfactory epithelium, generating olfactory nerve stimulus and signal transmission via the olfactory bulb to the brain which then processes the sensory information as odour recognition (Mombaerts, 2001). *Orthonasal* perception occurs when volatiles are inhaled through the nose and interact with the olfactory system directly (Petracco, 2001). While it has been speculated that the human sense of smell can distinguish more than 10,000 different odourants (Lancet, 1986), the human sense of taste (detected from the tongue receptors) can detect five basic taste sensations, namely sweet, bitter, sour, salty, and umami (savory) (Rawson and Li, 2004). Consequently, the aroma component of flavour for complex products such as coffee is thought to be exceptionally important and is primarily responsible for flavour diversity (Lawless and Heymann, 2010, Murphy et al., 1977).

The sensory properties of coffee have been studied for many years and, with increasing consumption worldwide, interest in coffee flavour and aroma has gained momentum from industry and scientists alike. Recent examples of sensory language that has been used to describe flavour properties of coffee include attributes such as *astringency, body, bitter flavour, burned* aroma, *'typical'*, and *burned* tastes (Bicho et al., 2013a), *sweet-caramel, earthy, roast/sulfur* and *smoky* characteristics (Czerny et al., 1999, Mayer et al., 2000). Further detail on aroma lexicon (term/description) includes *coffee, roasted, burnt/acrid, brown, beany, nutty, cocoa, musty/earthy, floral, fruity, green, ashy/sooty, sweet aromatic, sour aromatic,* and *pungent* (Bhumiratana et al., 2011).

Previous studies have concluded that brewing results in an increase of *sweet-caramel* aroma of Arabica coffee while more *spicy, harsh, earthy* aroma prevailed in Robusta (Blank et al., 1991). Further studies of Arabica and Robusta coffee flavour roasted across three different levels (Bicho et al., 2013b) indicate that the characteristic *odour, astringency, body, bitter flavour, burned aroma,* and *residual, typical*, and *burned* tastes, *citric acid* flavour and aroma accounts for the difference between these two species (Bicho et al., 2013a).

The above-mentioned sensory properties measured through a sensory evaluation involving humans as the assessors. One of the industrial standard measurement of sensory quality (cup quality) of coffee (Teixeira et al., 2005a) is a cup evaluation or coffee

‘cupping’. This method involves trained industry assessors who evaluate the coffee grounds and fresh brew for aroma and flavour with subsequent visual evaluation of the green and roasted beans.

2.2.2 Mouthfeel and chemesthesis

Besides the aroma and taste, texture, mouthfeel and chemesthesis are other components that influences flavour perception and are influenced by food structure interaction with the lining of the mouth during consumption (Taylor, 1996). These sensations typically include properties such as *crunchiness, oiliness, grittiness, viscosity, softness* or *hardness* and also include more complex sensations created from interactions of food components with the surface of the mouth and tongue due to chemical sensitivity of the skin and mucous membranes to *burning, tickling, prickling*, and *cooling* sensations (Cliff, 1994). Importantly, texture, mouthfeel and chemesthesis sensations are not detected via the olfactory system or taste receptor pathways.

Sensory language to describe the texture and mouthfeel of commercial coffee has been developed by research from Japan and Korea and includes terms such as having *body* (viscosity), *astringency, round, smooth, thick, coarse, grainy, rough, oily,* and *sticky,* with overall impressions of being *crisp, pure, non-persistent, clear, sharp, mild, round, soft, delicate, balanced, intense, strong, heavy, hard, light, neutral, monotonous, flat, simple and light, mellow, winey, rich, nippy, piquant, pungent, tangy, acrid, alkaline, easy to swallow* and *refreshing* (Hayakawa et al., 2010, Seo et al., 2009). An Italian study on the sensory classification of espresso developed descriptors such as *thick, lingering, full-mouthed, viscous, resistance to tongue-palate movements, syrupy, consistency, velvety, pasty/doughy, creamy, mouth-coating, smooth, round, clinging/tongue coating, particulate, bulky, rich/heavy* (Navarini et al., 2004).

2.3 The biochemical generation of coffee flavour

The generation of coffee flavour begins in the coffee plant where flavour precursors form as the coffee cherries develop (Figure 2.1). Cherry fruits or coffee berries of the coffee tree are harvested when it is ripe showing through a yellow, red, or purple colour (Wasserman

et al., 2012). The structure of coffee fruits comprises an outer red skin followed by a mesocarp (pulp) and an endocarp (bean) covered by a seed coat (silver skin membrane) in a parchment surrounded by thin mucilage. One cherry generally contains two separated coffee beans, however, when there is only one unseparated bean it is called a ‘Peaberry’ (Mutua, 2000). This ‘Peaberry’ is an abnormal seed produced during fruit development (Ricketts et al., 2004, Wintgens, 2012). Flavour complexity further develops throughout the varying steps of coffee processing and subsequent cup preparation techniques (Figure 2.2).

Figure 2.1 Coffee plants and maturity stage of coffee cherry from green (raw) to purple (fully-ripe)

Coffee from the species *Coffea arabica* and *Coffea robusta* are the two most commonly grown in commercial production and differ distinctly in flavour (Bicho et al., 2013b). Within the *Coffea arabica* species, numerous varieties can be distinguished which suit the myriad environments where coffee is grown around the world. The environmental factors such as geographical origins (Bhumiratana et al., 2011, Costa Freitas and Mosca, 1999), climate, altitude and temperature elevation (Bertrand et al., 2012, Bertrand et al., 2006), shading

(Bosselmann, 2009), and nutritional or fertilizers (Poltronieri, 2011) had been suggested to have an impact on coffee quality.

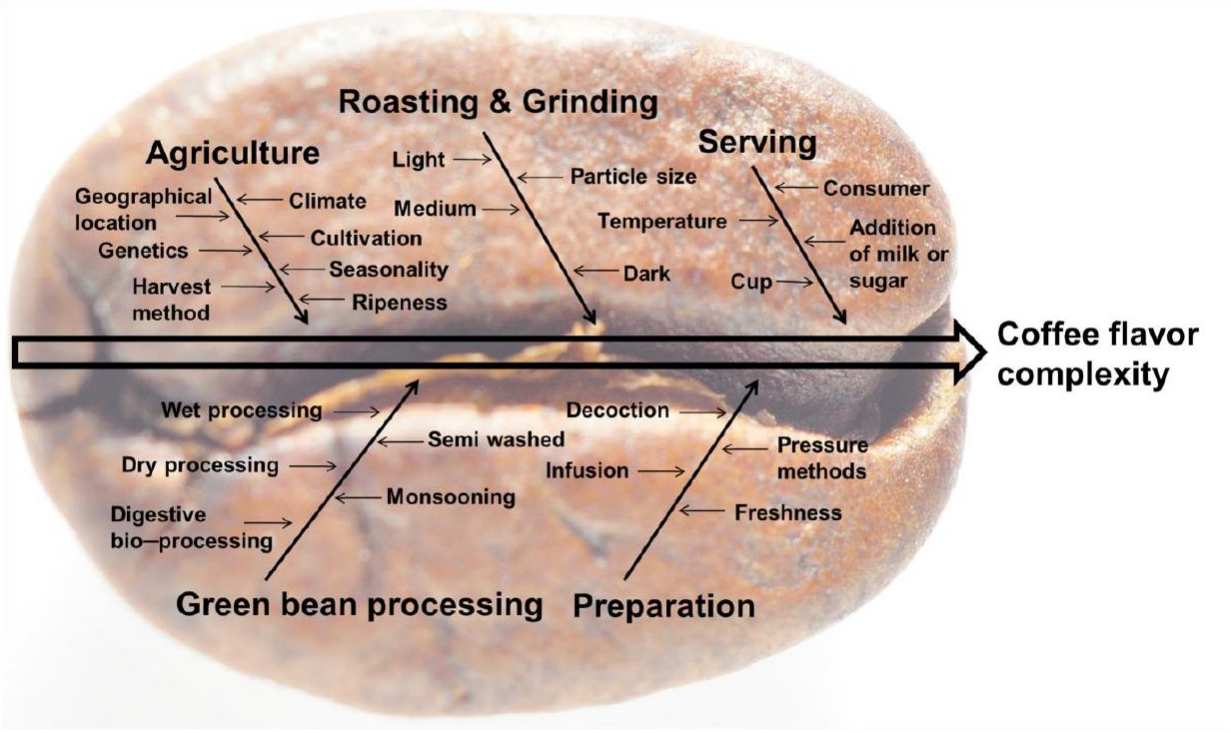

Figure 2.2 Factors that influence coffee flavour complexity from farm to cup (re-used of previously published material with permission from Sunarharum et al. (2014))

In green coffee bean processing, two major processing techniques are applied to the harvested coffee fruits to green bean and include dry processing (natural) and wet processing (washed) (Clarke and Macrae, 1985). Semi-dry (semi-washed) processing is an additional method which comprises components of both the dry and wet processing methods (Teixeira et al., 2005b). The main difference between these methods is the pulping operation as well as the fermentation and washing process (Clarke and Macrae, 1985) and these differences in processing can impact coffee flavour. Dry processing produces a '*hard*' coffee with a *medicinal* flavour (Clarke and Macrae, 1985) while wet processing yields a better quality 'mild' coffee with less *body*, higher *acidity* and more aroma than the dry processing (Mazzafera, 2004). The semi-dry (semi-washed or pulped natural) which is a compromise between the dry and wet method, offers a coffee with intermediate *body* (Duarte et al., 2010). Wet hulled or "giling basah" is another semi-dry

coffee processing commonly applied in some areas in Indonesia. The process is quite similar to pulped natural coffee except that the hulling is performed on a wet (partly dried, moisture 40-45%) coffee parchment yielding a wet-hulled coffee (Ismayadi et al., 2005).

As well as the above-mentioned green coffee bean processes, there are certain ‘specialty’ styles of coffee that are produced using more uncommon processing methods such as a ’digestive bio-processing‘ (e.g. fermentation inside the intestine of Luwak or civet mammals) (Marcone, 2004, Marcone, 2011, Ongo et al., 2012) and monsooning. The latter was developed in India after unique and desirable flavour traits were discovered in coffee beans that had been shipped under humid (monsoon) conditions for an extended period of time (Ahmad et al., 2003).

Further commercial processing of the green coffee beans involves roasting, grinding, and brewing, which are arguably the most important factors contributing to flavour of the coffee beverage. Roasting has the most significant influence on coffee flavour and has been the focus of much research (Buffo and Cardelli-Freire, 2004, Eggers, 2001, Esquivel and Jiménez, 2012). Roasting temperatures can vary and are typically between 180°C to 240°C for periods of between 8 to 15 minutes. During roasting, endothermic and exothermic processes begin from heat transferred to the bean through hot gases or contact with the metal surface of the coffee roaster which reduces water content of the coffee beans, and causes puffing and cooling to produce desirable characteristics. The impact of roasting on flavour comes from the degradation and formation or release of numerous chemical compounds through Maillard reactions, Strecker degradation, break down of amino acids, degradation of trigonelline, quinic acid, pigments, lipids and interaction between intermediate products (Buffo and Cardelli-Freire, 2004, Ribeiro et al., 2009) Importantly, roasting relates directly to cup quality as it converts the *pea-like, green* smell of raw green coffee into the pleasant aromas characteristic of roasted coffee due to a drastic increase of numerous aroma compounds (Czerny and Grosch, 2000, Czerny et al., 1999). Typically, while more complex aromas are formed at a medium roasting level, a light roast produces *sweet, cocoa*, and *nutty* aromas and dark roasting is responsible for *burnt/acrid, ashy/sooty, sour, pungent, coffee*, and *roasted* characteristics (Bhumiratana et al., 2011). While roasting level is a matter of personal preference, certain roasting conditions may better suit coffees of different variety, style, geographical origin or end use,

depending on the aroma characteristics desired in the resulting beans (Bhumiratana et al., 2011). Medium roasting, for example, will express the regional flavours derived from the geographical origin much better than dark roasting, which would mask these characteristics in the coffee beans.

Grinding of roasted bean releases coffee flavour for the purpose of extraction or infusion in coffee beverage preparation (Akiyama et al., 2003) and therefore higher intensities of aroma tend to be perceived after grinding of the roasted beans (Bhumiratana et al., 2011). The grind level and particle size influences the extraction and thus the quality of prepared beverage. Grinding coffee into a very fine level could produce a low volume and *bitter* coffee due to over extraction while too coarse a grind could decrease extraction due to reduction in surface area resulting in a *weak* insipid coffee brew (Andueza et al., 2003a).

It must be acknowledged that the global trend of coffee consumption is toward convenience and health with a growing consumer conscience and interest for origin, variety, brewing and grinding, flavour, packaging, social 'content' and ambience (Ponte, 2002). This has resulted in an increase in specialty and convenience coffee products such as instant (including decaffeinated), but more recently flavoured coffee capsules or pods. The latter might include the addition of other natural or artificial flavour (Petracco, 2001). These products are highly processed and often involve formulation and addition of other ingredients to retain consistency. Consequently, the flavours of these products are outside the scope of this review.

Brewing of coffee is a crucial step in translating coffee flavours from the ground bean into the water matrix of the beverage for consumption. 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, Napoletana) and pressure methods (plunger, moka, espresso) as summarized below (Petracco, 2001)

- Decoction methods: involve contact or continuous reflux of ground coffee in water for certain time and high temperature, extract more intensively and faster than other methods but results in some flavour loss as the common method is through boiling with a direct contact to heat or at high temperature.

- Infusion: conducted by soaking or steeping ground coffee (extra coarse – medium grind) under heated or cold water for a period before filtering, produces a milder coffee than decocted coffee with enhance acidity and flavour.
- Pressure methods: involve the percolation of fluid through a porous medium or a filter by application of high pressure and heat to enhance the body of the beverage such as in an espresso style.

2.4 The compositional drivers of coffee flavour

2.4.1 Non-volatile components and their contribution to coffee flavour

Non-volatile compounds present in roasted coffee beans which may be important to coffee flavour include alkaloids (caffeine, trigonelline), chlorogenic acids, carboxylic acids, carbohydrates and polymeric polysaccharides, lipids, protein, pigments, melanoidins and minerals (Buffo and Cardelli-Freire, 2004, Ribeiro et al., 2009). The occurrence of these components in commercial roasted coffee beans is quite diverse, due to the variability in coffee cultivation and processing (as described previously).

Compared to Arabica, Robusta has been reported to contain a higher caffeine content as green or roasted bean and as instant coffee (Oestreich-Janzen, 2010, Wasserman et al., 2012). Caffeine, a nitrogenous secondary metabolite, is thought to influence the perceived strength, *body* and *bitterness* of a brewed coffee (Clarke and Macrae, 1985). Alkaloids, which in neat form have a bitter flavour, are extractable in water and may give a physiological stimulating effect (Higdon and Frei, 2006).

Trigonelline (*N*-methylpyridinium-3-carboxylate) and its two derivatives (nicotinic acid and *N*-methylnicotinamide) are other alkaloids present in coffee (Buffo and Cardelli-Freire, 2004). Unlike caffeine, these components can be found at higher levels in the Arabica variety compared to other cultivars (Wasserman et al., 2012). They are thought to contribute to the overall aroma perception of both roasted coffee beans and a brewed coffee beverage (Oestreich-Janzen, 2010).

Chlorogenic acids are a family of esters formed between certain *trans*-cinnamic acids (phenolic acids that usually are caffeic acid, ferulic acid and *p*-coumaric acids) and quinic

acid (Clifford, 1985b, Clifford, 1999). These secondary metabolites are present in coffee beans and contribute to the *astringency* (Buffo and Cardelli-Freire, 2004) and *bitterness* of a coffee beverage, and have potential as an antioxidant for human health (Oestreich-Janzen, 2010). Chlorogenic acids are one of the most abundant polyphenols present in plant and plant-based foods and coffee has been reported to be one of the richest sources of chlorogenic acids in the human diet compared to other beverages (Clifford, 1999, Clifford, 2000). A cup of Arabica coffee brew (200 mL) contains 70-200 mg of chlorogenic acid, while in Robusta it may reach 70-350 mg (Clifford, 1999). Due to thermal instability, further processing, particularly roasting of green coffee beans, has been reported to progressively degrade chlorogenic acids (Clifford, 1972) up to 93% for dark roasting (Farah et al., 2006). Chlorogenic acids and quinic acid may form chlorogenic lactones during coffee roasting (Farah et al., 2005a) which contribute to increased *bitterness* of the coffee brew (Ginz and Engelhardt, 2001).

Acidity, or the *tartness*, is an important attribute of coffee quality in combination with *sweetness, bitterness* and aroma profile. In coffee, *acidity* is often conversely correlated to *sweetness*. Arabica coffee brews are more acidic than Robusta, with pH ranges of 4.85 - 5.15 and 5.25 - 5.40, respectively (Vitzthum, 1976). The acid content of green coffee bean is around 11%, mainly comprising citric, malic, chlorogenic and quinic acids, while roasted bean contains around 6% due to decreases in citric, malic and chlorogenic acids (Ginz et al., 2000, van der Stegen and Duijn, 1987, Urgert et al., 1995). During roasting of coffee beans, these acids form other compounds such as lactones, from the reaction between chlorogenic and quinic (Bennat et al., 1994), and volatile phenols, such as guaiacol and 4-vinylguaiacol from chlorogenic acid degradation (Vitzthum et al., 1990). These breakdown products are volatiles that can influence coffee aroma. Another example is ascorbic acid, a minor organic acids presents in coffee that involved in the formation of furans after roasting (Crews and Castle, 2007, Ribeiro et al., 2009).

While some acids degrade during coffee bean roasting, others increase in concentration including formic, acetic, glycolic and lactic acids. While the first two aliphatic acids increase only up to medium roast before beginning to degrade, the latter two continue to increase during roasting (Ginz et al., 2000, Weers et al., 1995). Dark roasting is the most efficient way to reduce acid content and perceived acidity in coffee (Clifford, 1985a).

Polysaccharides are the major component of coffee beans (see Farah 2012, for instance-44-47%), in the form of arabinogalactans, mannans and cellulose (Bradbury, 2001). Polysaccharides play an important role in retaining volatiles and therefore flavour, and also contribute to the perceived viscosity of the coffee brew (Buffo and Cardelli-Freire, 2004). Other carbohydrate compounds such as glucose and fructose are mainly found in immature beans while higher amounts of sucrose accumulate in mature beans and contribute to perceived coffee *sweetness* (Oestreich-Janzen, 2010, Wasserman et al., 2012).

The lipid fraction of coffee, also known as the coffee oil consists of triglycerides (75%), free and esterified diterpene alcohols (19%), free and esterified sterols (5%), and a small quantity of other lipid types such as tocopherols (Kaufmann and Gupta, 1964, Kaufmann and Hamsagar, 1962). The diterpenes kahweol and cafestol in coffee are often mentioned as having a negative effect on health in relation to cholesterol (Speer and Kölling-Speer, 2001). Roasting of coffee beans results in partial migration of coffee oil to the bean's surface (Savonitti, 2005). While some changes in coffee lipid profile occurs during roasting, sterols and most triglycerides remain unchanged (Maier, 2005). These lipid fractions of the beans are extracted into the coffee brew and provide the *crema* emulsion of espresso coffee that carries flavour volatiles and fat-soluble vitamins, and contributes to perceived texture and mouthfeel of the coffee brew (Oestreich-Janzen, 2010).

Protein content of Arabica is slightly lower than Robusta, even though total amino acid composition is similar (Wasserman et al., 2012). The amino acids content of green bean has an important contribution to flavour development during roasting through Maillard reactions (Liu and Kitts, 2011). Maillard or caramelization reactions occur due to a reaction between the amine group of amino acids or nitrogen-containing compounds and the carboxyl group of reducing sugars, hydroxy-acids and phenols to yield aminoaldoses and aminoketones by condensation (Buffo and Cardelli-Freire, 2004). The resulting product is the brownish colour melanoidins and other components such as several nitrogen and/or sulfur containing heterocyclic compounds which are thought to be important flavour compounds in coffee (Shibamoto, 1983).

Among other minor constituents of coffee are the minerals. Potassium is the major mineral present in roasted coffee, however, manganese, iron, and copper are also present

in smaller amounts and act as important catalysts of certain biochemical reactions which facilitate the production and release of flavour components in coffee bean during processing (Oestreich-Janzen, 2010).

Non-volatiles present in coffee beans and brew are important to the sensory quality of coffee and have been related to positive as well as negative aspects of coffee flavour. Carbohydrates impact on *sweetness, caramel* notes arise from Maillard reactions between sugars and amino acids, and caffeine and chlorogenic acids contribute to *bitterness.* Specifically, trigonelline, 3,4-dicaffeoylquinic acid and, to some extent, caffeine, have been associated with a good cup quality in Brazilian Arabica coffee (Farah et al., 2006). Elevated amounts of chlorogenic acids mainly 5-caffeoylquinic acid, and to some extent feruloylquinic acid and associated oxidation products, are related with poor cup quality and off-flavours such as *harsh medicinal, phenolic* or *iodine-like* flavours (Spadone et al., 1990).

2.4.2 Volatile components and their contribution to coffee

Aroma volatiles produced during coffee bean roasting are arguably the most important quality-determinant of coffee (Andueza et al., 2003a, Baltes and Knoch, 1993, Grosch et al., 2000, Kumazawa and Masuda, 2003) and as such have been of research interest for almost a century with intensive profiling over the past 50 years. Aroma volatiles characterize not only the different cultivars, styles and processing techniques used, but also the geographical origins of the coffee (Costa Freitas and Mosca, 1999). There have been more than 1000 volatiles identified in coffee (Nijssen, 1996) ranging in concentration from part per million (ppm) to part per trillion (ppt) levels, however, only a small number of these are important to the flavour and aroma characteristics of coffee (Buffo and Cardelli-Freire, 2004, Grosch, 2001a). Some authors suggest that as few as 20-30 individual volatiles may be important to the aroma of any single type or style of coffee (Blank et al., 1991, Czerny and Grosch, 2000, Czerny et al., 1999, Deibler et al., 1998, Grosch et al., 2000, Mayer et al., 2000, Mayer and Grosch, 2001, Sanz et al., 2002b, Semmelroch and Grosch, 1996, Semmelroch et al., 1995). In the last decade, research mostly focuses on investigation of specific volatile groups important for coffee aroma such as pyrazines, furans, and thiols (Pickard et al., 2013, Pickard et al., 2014, Bicchi et al., 2011, Petisca et al., 2014, Quintanilla-Casas et al., 2015).

Coffee volatiles are derived from numerous precursors found in the bean and from chemical reactions occurring particularly during roasting, but also during processing and storage (Buffo and Cardelli-Freire, 2004). The generation of aroma compounds has been previously reviewed (Buffo and Cardelli-Freire, 2004, Grosch, 2001b). The main chemical reactions that occur during roasting which generate important aroma volatiles include Maillard reactions (non-enzymatic browning), phenolic acid (Holscher and Steinhart, 1992, Reineccius, 1995, Tressl, 1981); Strecker degradation; breakdown of sulfur amino acids, hydroxy-amino acids, proline and hydroxyproline; degradation of trigonelline, chlorogenic acids and quinic acid, pigments, and lipids; as well as reactions between other intermediate products (Buffo and Cardelli-Freire, 2004, Ribeiro et al., 2009).

Coffee volatile compounds comprise several chemical classes including hydrocarbons, alcohols, aldehydes, ketones, carboxyclic acids, esters, pyrazines, pyrroles, pyridines, other bases (e.g. quinoxalines, indoles), sulfur compounds, furans, furanones, phenols, oxazoles among others. Quantitatively, the top two classes in coffee are furans and pyrazines, while qualitatively, sulfur-containing compounds together with pyrazines are considered the most significant to coffee flavour (Nijssen, 1996). These compounds vary significantly in concentration and sensory potency which makes coffee flavour extremely complex, and explains why different coffee types may exhibit such diverse, unique and specific flavours (Risticevic et al., 2008).

Furans are among the most abundant group of volatiles present in coffee (Grosch, 2001a) and are found in sensorily or aroma active significant concentrations in roasted coffee (Akiyama et al., 2007, Bicchi et al., 2011, Cheong et al., 2013, Gianturco et al., 1964, Ribeiro et al., 2012). They are formed through thermal degradation of carbohydrates, ascorbic acid, or unsaturated fatty acids during roasting (Crews and Castle, 2007, Ribeiro et al., 2009) and range in concentration from 3 - 115 ppb in coffee brew (Kuballa et al., 2005). Volatile furans exhibit malty and sweet roasted aromas (Akiyama et al., 2007, Burdock, 2010, Fors, 1983) with sensory thresholds that are relatively high compared to other groups of coffee volatiles (Burdock, 2010) although due to their high concentrations are still considered of importance to coffee flavour. In relation to health, there is concern over possible negative health impacts of furans and therefore commercial coffee roasting has been optimized to minimize the presence of furans (Bicchi et al., 2011, EFSA, 2004).

Due to their sensory potency, sulfur-containing compounds such as thiols are among the most important contributors to coffee flavour despite their presence at relatively low concentration. Volatile thiols and their influence on coffee sensory quality has been comprehensively reviewed by Dulsat-Serra (Dulsat-Serra et al., 2016). As important example is 2-furfurylthiol, which is reported to have a very low sensory threshold (0.01 ppb) (Semmelroch and Grosch, 1995) and exhibits a strong roasted aroma (Blank et al., 1992). This compound is considered by many as a key impact aroma compound in coffee and has been reported widely in roasted and brewed coffee (Akiyama et al., 2007, Blank et al., 1992, Czerny and Grosch, 2000, Grosch et al., 2000, Holscher and Steinhart, 1992, Mayer et al., 2000, Michishita et al., 2010, Semmelroch and Grosch, 1995). Other thiols, such as 2-methyl-3-furanthiol and 3-methyl-2-butene-1-thiol, are also present in coffee and have very low sensory thresholds while exhibiting meaty characters (Akiyama et al., 2003, Blank et al., 1991, Blank et al., 1992, Grosch et al., 2000). Also belonging to the class of sulfur-containing compounds, 3-methylthiophene (Ribeiro et al., 2009) and 2,4-dimethyl-5-ethylthiazole (Blank et al., 1992) are present in coffee at sensorily significant levels and exhibit *roasted* and *meaty* flavours (Maga, 1975).

Pyrazines are well known class of compounds that arise as a product of roasting various foods and horticultural products including coffee. They are an abundant class of compounds present in coffee, with low sensory threshold concentrations and they are of key importance to the flavour of coffee. Generally, pyrazines have been described as exhibiting *nutty, earthy, roasty, green* aromas (Akiyama et al., 2007, Blank et al., 1991, Czerny et al., 2008, Czerny and Grosch, 2000, Czerny et al., 1996, Holscher and Steinhart, 1992, Semmelroch and Grosch, 1996, Wagner et al., 1999). Ethylpyrazines and ethenylalkylpyrazines have been reported to contribute to the *earthy* aroma characteristic of Robusta (Blank et al., 1991). The volatile 3-isobutyl-2-methoxypyrazine, with an exceptionally low sensory threshold of 0.002 ppb (Belitz et al., 2009) is present at low concentrations in roasted Arabica coffee beans but still have been reported to have a significant impact on roasted Arabica coffee (Czerny and Grosch, 2000). Arguably the other two most important aroma compounds are 2-ethyl-3,5-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine (Akiyama et al., 2003, Blank et al., 1991, Czerny et al., 1999, Grosch et al., 2000, Mayer et al., 2000, Mayer and Grosch, 2001, Semmelroch and Grosch, 1995).

Furanones are generated in coffee mainly via the Maillard reaction and subsequent aldol condensation (Grosch, 2001b). They are a significant group of volatiles in coffee in terms of abundance and potency. Major flavour contributors are thought to be 4-hydroxy-2,5-dimethyl-3(2H)-furanone and 2(5)-ethyl-4-hydroxy-5(2)-methyl-3(2H)-furanone, 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon), and 4-ethyl-3-hydroxy-5-methyl-2(5H)-furanone (abhexon) and are thought to be responsible for the *sweet caramel* aroma of roasted coffee (Akiyama et al., 2007, Akiyama et al., 2003, Blank et al., 1992).

Certain phenolic compounds which generated and released during roasting are thought to be of importance to coffee flavour (Ribeiro et al., 2009), particularly guaiacol, 4-ethylguaiacol and 4-vinylguaiacol which are have a spicy phenolic aroma (Akiyama et al., 2007, Blank et al., 1992, Czerny and Grosch, 2000) and vanillin (Czerny and Grosch, 2000). In roasted Arabica coffee, phenolic compounds range in concentration from 3 to 56 ppm (Cheong et al., 2013, Czerny et al., 1999, Semmelroch and Grosch, 1995) depending on the variety and geographical source. These phenolic compounds arise from thermal degradation of chlorogenic acids (mainly ferulic, caffeic and quinic acids) and their concentration in roasted bean is proportional to the amount of chlorogenic acids present in green bean. Given there are significantly more chlorogenic acids present in green bean of Robusta compared to Arabica, these volatiles are thought to play a key role in the flavour differentiation between these two varieties of coffee (Bicho, 2013, Blank et al., 1991, Sanz et al., 2002a, Semmelroch and Grosch, 1996, Semmelroch and Grosch, 1995).

2.5 Determination of key volatile aroma compounds in coffee

In an attempt to unlock the compositional basis of coffee flavour there have been numerous studies involving the extraction and analysis of the volatile profiles of coffee from green bean to roasted ground bean and brew, from espresso style to instant coffee (Akiyama et al., 2007, Akiyama et al., 2003, Blank et al., 1992, Borém et al., 2013, Czerny and Grosch, 2000, Czerny et al., 1999, Czerny et al., 1996, Grosch et al., 2000, Kumazawa and Masuda, 2003, Mayer et al., 2000, Michishita et al., 2010, Ribeiro et al., 2009, Ribeiro et al., 2010, Semmelroch and Grosch, 1995, Semmelroch and Grosch, 1996). The overriding objective of these studies has been to identify those volatiles that are key contributors to the flavour of coffee. From an extensive review of the coffee

literature, a summary of individual compounds thought to be of greatest important to the flavour of Arabica and Robusta coffee is provided in Table 2.1 together with concentration reported in coffee, aroma description (as a neat compound), aroma threshold concentration, and literature references where studies have indicated the importance of each compound to coffee flavour. All concentrations previously reported were converted here into µg/kg for ease of comparison.

Table 2.1 A summary of important aroma compounds identified in coffee

Key odourants identified in coffee (literature cited)	**Concentration in coffee (µg/kg)***	**Aroma description**	**Aroma threshold (µg/kg)*****
Aldehyde			
2-methylbutanal [1-4]	20,700[2]	-	0.9[26]
2-methylpropanal[5]	-	*buttery oily*[27]	-
3-methylbutanal[1-4,6,7]	18,600[2]	*malty*[11]	0.25[26]
(E)-2-nonenal[8,9]	19[14]	-	0.08[27]
3-methylpropanal[10]	-	-	-
acetaldehyde[1,2,4]	139,000[2]	-	0.7[a,28]
methylpropanal[1,2,4,7]	32,300[2]	-	0.7[26]
p-anisaldehyde[11]	-	*minty*[11]	27[b,29]
phenylacetaldehyde[12,13]	-	*sweet fruity*[12]	-
propanal[1,2]	17,400[2]	-	10[26]
Acid			
2-methylbutyric acid[11,12]	25,000[4]	*sweaty*[14]	10[28]
3-methylbutyric acid[3,8]	18,060-32,180[4,15]	*sweaty*[11]	700[c,30]
Ester			
ethyl-2-methylbutyrate[14]	3.9[14]	*fruity*[14]	0.5[d,14]
ethyl-3-methylbutyrate[14]	14[14]	*fruity*[14]	0.6[d,14]
Furan			
furfural[10,15]	5,880-19,370[15]	-	280[a,28]
2-((methylthio)methyl)furan[12]	-	*smoke-roast*[12]	-
2-furanemethanol acetate[10]	24,520-40,040[15]	-	-
2-methylfuran[10]	-	-	-
5-methyl-2-furancarboxyaldehyde[10,12]	-	-	6000[a,28]
furfurylformiate[13]	-	-	-
furfurylmethyl ether[13]	-	-	-
furfurylformate[10]	4,060-6,420[15]	-	-
furfuryldisulfide[13]	-	-	-
Sulfur-containing compounds			
dimethyl trisulfide[12]	28[19]	*cabbage-like*[28]	0.001[27]
bis(2-methyl-3-furyl)disulphide[11]	-	*meaty*[11]	0.00076[b,29]
methional[3,11,16,17]	213-240[14,22]	*boiled potato-like*[11]	0.2[16]

Key odourants identified in coffee (literature cited)	Concentration in coffee (µg/kg)*	Aroma description	Aroma threshold (µg/kg)***
Thiols			
3-mercapto-3-methylbutylformate[3,7,11,12,16,17]	115-130[16]	*green blackcurrant*[12]	0.0035[5]
2-furfurylthiol[1-5,8,11,12,14,16,17]	1,080-5,080[9,15,16]	*roasty (coffee-like)*[11]	0.01[16]
2-methyl-3-furanthiol[1,11]	60-68[2,4]	*meaty, boiled*[11]	0.007[27]
3-mercapto-3-methylbutylacetate[18]	7.5[18]	*roasty*[18]	-
3-methyl-2-butene-1-thiol[3,19]	13[4]	*amine–like*[19]	0.0003[5]
methanethiol[7,16]	4,550[23]	-	0.02[27]
Thiophene			
3-methylthiophene[10]	-	-	-
Thiazole			
2,4-dimethyl-5-ethylthiazole[11]	-	*earthy, roasty*[11]	-
Furanone			
dihydro-2-methyl-3(2H)-furanone[13,19]	7,580-30,000[15,24,25]	-	0.005[e,31]
2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone[2,3]	16,800[2]	*sweet caramel*[3]	20[7]
3-Hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon)[2,14,11,12,16,17,19]	1.1-1,470[2,14,16]	*sweet caramel*[3]	20[16]
4-Hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol)[2,3,8,11,16,17]	10,930-112,000[2,15,16]	*sweet caramel*[12]	10[16]
5-Ethyl-3-hydroxy-4-methyl-2(5H)-furanone (abhexon)[2,11,16, 19]	85-160[2,16]	*seasoning-like, caramel-like*[11]	7.5[16]
5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone[16]	14,300-17,300[16]	*sweet caramel*[11]	1.15[16]
Ketone			
1-octen-3-one[12]	-	*mushroom-like*[28]	0.0036[b,29]
2,3-hexadione[13]	-	-	-
2,3-butanedione[3,4,5]	48,400-50,800[2,7]	*buttery-oily*[12]	0.3 [a,28]
2,3-pentanedione[3,4,5,6]	3,540-39,600[2,15,7]	*buttery-oily*[12]	20 [a,28]
3,4-dimethylcyclopentenol-1-one[19]	-	*caramel-like, sweet*[11]	-
4-(4'-hydroxyphenyl)-2-butanone[12,20]	1[20]	*sweet fruity*[12] *(raspberry ketone)*	1-10[32]
1-(2-furanyl)-2-butanone[10]	-	-	-
Norisoprenoid			
(E)-*β*-damascenone[3,7,11,14,16]	195-255[2,14,16]	*honey-like, fruity*[11]	0.00075[16]
Phenolic compounds			
guaiacol[2,11,16,17,19]	2,000-28,200[2,15,16,25]	*phenolic, burnt*[11]	2.5[16]

Key odourants identified in coffee (literature cited)	Concentration in coffee (µg/kg)*	Aroma description	Aroma threshold (µg/kg)***
4-ethylguaiacol[10-12,14,16,17,19]	800-24,800[2,14,15,16,25]	*spicy*[11]	25 [a,28]
4-vinylguaiacol[1-3,11,12,14,16,19]	8,000-177,700[2,15,16,25]	*spicy*[11]	0.75 [a,28]
vanillin[11,12,14,16,17]	2,290-16,100[2,14,16]	*vanilla-like*[14]	25[16]
Pyrazine			
2,3-dimethylpyrazine[15]	2,580-6,100[15]	-	800 [a,28]
2,5-dimethylpyrazine[15]	4,550-11,730[15]	-	80 [a,28]
2,3-diethyl-5-methylpyrazine[1-3,11,12,16,17,19]	73-310[2,16]	*nutty-roast*[12]	0.09 [a,28]
2-ethenyl-3,5-dimethylpyrazine[1,2,17,21]	52[2]	*earthy*[21]	0.000012[f,21]
2-ethenyl-3-ethyl-5-methylpyrazine[2,21]	18[2]	*earthy*[21]	0.000014[f,21]
2-ethyl-3,5-dimethylpyrazine[1-4,10,11,12,16]	55-940[2,4,16]	*nutty-roast*[12]	0.04[33]
2-ethyl-3,6-dimethyl-pyrazine[10]	2,570-5,980[15]	-	8.6[33]
2-methoxy-3,5-dimethylpyrazine[14]	1.1[23]	*earthy*	0.006[f,14]
2-methoxy-3,2-methylpropylpyrazine[3,12]	-	*green earthy*[12]	-
2-methoxy-3-isopropylpyrazine[11,19]	2.4[23]	*earthy roasty*[11]	0.002[a,28]
3-ethenyl-2-ethyl-5-methylpyrazine[21]	-	-	-
3-isobutyl-2-methoxypyrazine[2,11,14,17]	59-97[2,14,16]	*peasy*[14]	0.002[27]
6,7-dihydro-5-methyl-5H-cyclopentapyrazine[3,11]	-	*nutty-roast*[27]	-
ethylpyrazine[13]	-	-	4000[a,28]
Pyridine			
pyridine[13]	21,280-65,520[15]	-	77[16]
Pyrrole			
1-methyl pyrrole[13]	-	*negative notes-deffective beans*[6]	-
Terpene			
linalool[11,12]	-	*flowery*[11]	0.17[b,14]
limonene[11]	-	-	4 [a,28]
geraniol[12,20]	-	-	1.1[29]

Re-use of previously published material with permission from Sunarharum et al. (2014). A slight update is provided.

Compounds identified as important contributors, concentration measured and/or aroma description and aroma threshold provided in literatures [1](Grosch et al., 2000); [2](Czerny et al., 1999); [3](Akiyama et al., 2003); [4](Mayer and Grosch, 2001); [5](Holscher and Steinhart, 1992); [6](Ribeiro et al., 2010); [7](Semmelroch and Grosch, 1996); [8](Michishita et al., 2010); [9](Tressl and Silwar, 1981); [10](Ribeiro et al., 2009); [11](Blank et al.,

1992); [12](Akiyama et al., 2007); [13](Ribeiro et al., 2012); [14](Czerny and Grosch, 2000); [15](Cheong et al., 2013); [16](Semmelroch et al., 1995); [17](Mayer et al., 2000); [18](Kumazawa and Masuda, 2003); [19](Blank et al., 1991); [20](Akiyama et al., 2008); [21](Czerny et al., 1996); [22](Balzer, 2001); [23](Grosch, 2001b); [24](Gianturco et al., 1964); [25](Silwar et al., 1987); [26](Milo and Grosch); [27](Belitz et al., 2009); [28](Burdock, 2010); [29](Czerny et al., 2008); [30](Salo, 1970); [31](Barrett et al., 1983); [32](Larsen and Poll, 1992); [33](Buttery and Ling, 1997).

* Authors report compound concentration within the range indicated, these concentrations relate to roasted Arabica and Robusta coffee grounds or beans (weight/weight) (not coffee brew). Where no concentration is listed, none could be found in the literature. Extractions of the compounds were performed by using solvent such as dichloromethane, diethyl ether, methanol, pentane, hexane or water or a combination. Most of the quantifications were performed by SIDA in combination with a High Resolution GC-MS except for the compounds furfural, 2-furanemethanol acetate, furfurylformate, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2-ethyl-3,6-dimethyl-pyrazine, and pyridine, where 5-methyl-2-hexanone and alkanes standards were used in combination with a GC-MS/FID (Flame ionisation detector).

** Aroma description sourced only from coffee literature.

*** Where two or more aroma thresholds were found, the lowest is presented. All aroma thresholds concentrations were determined in water except: [a] matrix unknown; [b] threshold measured by first diluting compounds in ethanol in a defined concentration and then dissolved in water, for linalool as R-linalool; [c] in ethanolic solution 9.5%; [d]threshold in cellulose; [e] in ale; [f]in air.

Investigation of coffee volatiles involves an extraction or isolation step to separate volatiles from the sample, followed by analysis to detect and identify or quantify the compounds. Generally, the isolation technique was developed based on compounds solubility and volatility (Chaintreau, 2001). Extraction methods such as liquid-liquid extraction (LLE), solid phase extraction (SPE), Soxhlet extraction, supercritical fluid extraction (SFE) and solid phase micro extraction (SPME) are based on compounds solubility, while steam distillation tecnique, vacuum transfer and headspace extraction are based on compound's volatility (Chaintreau, 1999).

As a conventional method, steam distillation involves gaseous water and solvents that flow through a modified glass distillation apparatus such as Markham still, which is widely applied in flavour analysis such as for volatiles acids isolation in wines and beers (Ault, 1968, Zoecklein et al., 1999). Another variation of this technique is the simultaneous distillation extraction (SDE) that was developed for the first time in the 60s for the analysis of hop oil in beer (Nickerson and Likens, 1966). This SDE method has been continuously improved and became popular for volatile isolation with the benefit of saving time and solvents (Chaintreau, 2001). However, the major drawback of SDE is the build up of sample residue (Hinshaw, 2003) and artifact formation, particularly due to Maillard or Stecker reaction (Schieberle, 1995, Weurman et al., 1970, Engel et al., 1999) and therefore certain compounds such as furaneol could be discriminated (Pickenhagen et al., 1981). Further improvement was made by the introduction of solvent-assisted flavour

extraction (SAFE) method with the application of vacuum distillation and a low boiling temperature (Engel et al., 1999) which is applicable for a wide range of samples such as beer, orange juice, and potato snacks (Engel et al., 1999, Majcher and Jeleń, 2009).

Solid phase microextraction (SPME) became the method of choice for many coffee aroma volatiles analysis in recent decades (Akiyama et al., 2007, Akiyama et al., 2003, Bicchi et al., 2000, Bicchi et al., 2002, Bicchi et al., 2011, Bicchi et al., 1997, Caprioli et al., 2012, Costa Freitas et al., 2001, Jeleń et al., 2012, Ribeiro et al., 2011, Ribeiro et al., 2010, Ryan et al., 2004) mainly due to its sensitivity, rapidness, and solvent-free properties (Pawliszyn, 1997). An extraction from the headspace (HS) of samples has also been reported as able to provide the most accurate composition of flavours (Caprioli et al., 2012).

The separation and quantification method of the extracted volatiles commonly utilises a gas chromatographic (GC) separation due to its sensitivity and selectivity to resolve traces compounds in a short time (Hinshaw, 2003). The separation is based on partitioning or distribution of a sample between a moving (mobile) phase (gas), and a fixed or stationary phase (Wittkowski and Matissek, 1993). In combination with a GC, chemical detection of coffee flavour compounds can be conducted such as via a flame ionisation detector (FID) (Ribeiro et al., 2010, Ribeiro et al., 2012) or mass selective detector (MSD) (Akiyama et al., 2007, Akiyama et al., 2008). However, coupling a GC to a Mass Spectrometer (MS) has been reported to provide several advantages in flavour research due to its high sensitivity detection and the faster scan rates required for the GC peaks identification and traces analysis (McNair and Miller, 1998).

Mass spectrometry identification is based on ionisation of the molecules. In this system, molecules are converted into ions in the gas phase before separation based on mass-to-charge ratio (*m/z*) (Mellon et al., 2000). Mass spectrometry analysis using SIM will overcome problems associated with co-elution of isotope labelled standards from analytes (IOFI, 1997) while also offer advantages in quantification of compounds that present in trace amounts (Bicchi et al., 2011) because it selects and monitors only few selected m/z ratios, increasing sensitivity (IOFI, 2012).

The concentration of analytes or target compounds present in the sample can be quantified by comparison of the ratio between the peak area of these compounds and the internal standard/s. Determination of response factor (RF), a ratio between concentration

and area of analyte per corresponding labelled standard, makes it possible of a single labelled isotope standard to be used for its homologues such as the utilisation of d_4-furan for identification and quantification of furan and 2-methylfuran (Bicchi et al., 2011). The presence of four deuterium atom in d_4-furan has been reported to yield a higher molecular ion of this isotope (*m/z* 72) as compared to the molecular ion of furan (*m/z* 68) while the compound 2-methylfuran shows heavier molecular ion (*m/z* 82) (Bicchi et al., 2011). Therefore, the labelled standard is distinguishable from the targets using SIM.

Studies on coffee flavour have relied on a variety of methods to determine the relative and likely importance of individual volatiles to the flavour of coffee. These methods include: direct comparisons of relative compound concentrations with sensory detection thresholds (Czerny et al., 2008); application of odour activity values (OAVs) (Acree et al., 1984, Semmelroch and Grosch, 1995) whereby the ratio of concentration of each compound with its sensory threshold is calculated and likely odour-activity of components are ranked (Grosch, 2001a); calculation of odour spectrum values (OSV) where likely activity of odour compounds are ranked independent of concentration using gas chromatography-olfactometry (GC-O) methods (Acree et al., 1984); and application of other GC-O methods such as aroma extract dilution analysis (AEDA) (Blank et al., 1991, Blank et al., 1992) or Charm analysis (Grosch, 2001a).

Given the extensive differences in the genetics, cultivation, processing and geographical origins of coffee, it is not surprising that coffee exhibits a broad range of flavour and aroma types. Consequently, while there has been extensive investigation on the key aroma volatiles of coffee, individual studies often report different sets of key volatiles that are representative of the particular sample of coffee studies. Further, the different methods used for measuring volatile compound composition in coffee may also result in differences in ranking of key volatiles of any particular coffee sample. This is especially apparent in the case of detecting components at trace levels, such as the more recent detection of potent 3-mercapto-3-methylbuthyl acetate (Kumazawa and Masuda, 2003), 1-(3,4-dihydro-2*H*-pyrrol-2-yl)-ethanone and 4-(4-hydroxyphenyl)-2-butanone (Akiyama et al., 2007) in Arabica coffee brew.

In a study of roasted and brewed Arabica coffee as many as 13 potent odourants have been identified as key aroma contributors of roasted coffee based on AEDA experiments

(Blank et al., 1992). 2-Ethyl-3,5-dimethylpyrazine was found to be important for roasted and brewed coffee while 3-mercapto-3-methylbutyl-formate, 2-furfurylthiol, and (E)-*β*-damascenone were important to the roasted powdered coffee while methional, sotolon, and 4-hydroxy-2,5-dimethyl-3(2H)-furanone and vanillin were important contributors to the brew (Blank et al., 1992). Another study on roasted Arabica and Robusta coffee quantified 14 and subsequently 22 important compounds and highlighted methional, trialkylated pyrazines, guaiacol, 4-vinylguaiacol, 4-hydroxy-2,5-dimethyl-3(2H)-furanone as key aroma volatiles (Semmelroch and Grosch, 1996, Semmelroch and Grosch, 1995). A geographical comparison between blends and varieties of medium roasted Arabica coffee from four countries identified 28 potent odourants which were thought to be key to the aroma of those coffees (Mayer, 1999). Different geographical origins and roasting resulted in different concentrations of important compounds identified (Mayer, 1999). The compounds 2,3-butanedione, 2,3-pentanedione, 3-isobutyl-2-methoxypyrazine, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 4-vinylguaiacol, 4-ethylguaiacol, 2-furfurylthiol, 3-mercapto-3-methylbutylformate and 3-methyl-2-buten-1-thiol were found to be affected by origins while propanal, 2(5)-ethyl-4-hydroxy-5(2)-methyl-3(2H)-furanone, guaiacol, 4-ethylguaiacol, 2-furfurylthiol, 3-methyl-2-buten-1-thiol and methanethiol were affected by roasting (Mayer, 1999).

A study using flavour omission and aroma model experiments was carried out with 27 coffee odourants dissolved in an oil/water mixture (Czerny et al., 1999) using previously quantified compounds from the headspace of roasted Arabica coffee powder (Mayer et al., 2000). The findings confirmed the importance of 2-furfurylthiol, some alkylpyrazines, furanones and phenols, methional and 3-mercapto-3-methylbutyl formate as key drivers of Arabica coffee (Mayer et al., 2000).

Most of the research conducted on identifying key odour-contributing volatiles dates back to the 90s. In the past decade, there have been only a handful of studies investigating importance of coffee volatiles. These studies have focused on topics such as investigating key aroma changes in green coffee (Scheidig et al., 2007), fingerprinting coffee flavour (Huang et al., 2007), and discrimination of volatiles in defective coffee (Toci and Farah, 2008). More recent studies of coffee composition focus on coffee, health or bioactive compounds (Lee et al., 2015, Azeredo, 2011, Yeretzian et al., 2012) and investigating

compositional markers for processing such as roasting (Kučera et al., 2016, Yang et al., 2016).

2.6 Relationship between sensory properties and composition of coffee

Compositional data only, is not enough to explain the importance of key compounds and importantly, the nature of their contribution, to coffee flavour. Similarly, sensory information of coffee aroma properties, in the absence of good quality chemical data, cannot be used to explain what's causing specific sensory attributes. Good quality and comprehensive research that matches these properties in coffee to explain the compositional basis of coffee flavour is still limited.

To fully understand the correlation between sensory (consumer data) and the sensory descriptive analysis results or physicochemical measurements, researchers may use a multivariate data analysis tools known as chemometrics (Resurreccion, 1988, Wold and Sjostrom, 1998). Commonly applied methods are a principal component analysis (PCA) and a partial least squares (PLS) regression which are widely used for a food analysis and are useful in identifying compounds accounting for specific aroma nuances in complex systems such as coffee.

Despite the wide application of chemometrics, correlating compositional data with sensory attributes is a complicated task and can be problematic if the methodology used to collect the information is not suitably comprehensive with a degree of accuracy and precision. Consequently, there are few studies to date that correlate physicochemical and sensory attributes of coffee by means of a multivariate tools in understanding coffee flavour. A recent application of PCA has successfully discriminated aroma characteristics of Arabica coffee from three different origins and different roasting level (Bhumiratana et al., 2011) as previously explained in Section 2.3 p.8. PCA was also applied to successfully describe sensory effects of additives on the quality of stored Colombian coffee brews (Pérez-Martínez et al., 2008). Further, PLS has been applied to correlate sensory data to volatile chromatogram profiles resulting in adequate predictions of *acidity, cleanliness, overall quality, bitterness, body*, and *flavour* of Brazillian Arabica coffee (Ribeiro et al., 2012).

Two recent studies were devoted to understanding espresso coffee flavour by exploring the sensory and compositional linkage (Kerler et al., 2014, Charles et al., 2015). The former study (Kerler et al., 2014) reported a good correlation for several sensory attributes even though the cause-effect relationship could not be proven. Several correlations were found to be counterintuitive such as in case of bitter compounds that did not strongly correlated to *bitterness* (Kerler et al., 2014). The second study (Charles et al., 2015) investigated different roasting and sugar levels using temporal dominance sensations (TDS) and nosespace (NS) analysis. The findings mentioned the significant effect of roasting with more dominant of *burnt*-related attributes. The addition of sugar suppressed *bitterness, sourness*, and *roasted, burnt* attributes while improving *caramel, nutty* and *overall flavour* that showed potential taste-smell interaction in flavour perception.

The studies that have correlated physicochemical and olfactory or sensory panel data typically analysed a limited range of coffee flavour-types (Akiyama et al., 2008, Bhumiratana et al., 2011, Pérez-Martínez et al., 2008, Ribeiro et al., 2012, Ribeiro et al., 2009, Kerler et al., 2014, Charles et al., 2015). Certainly there is scope for future research efforts to model coffee flavour more comprehensively in terms of the range of sensory properties exhibited in Arabica coffees from around the world.

2.7 Conclusion

Complexity of coffee flavour arises from numerous influences from cultivation to processing and preparation. Variations in these influences cause differences in the formation of flavour and aroma components in the green and roasted coffee bean and subsequent brew. From the compositional point of view, the volatiles and non-volatiles have a great influence on flavour perception and consumer acceptance and enjoyment of coffee.

Knowledge on the chemical composition of coffee flavour is important, but reliable measurement and ranking of aroma components in coffee in the absence of good quality sensory information cannot effectively describe the importance, or the nature of contribution, of individual or groups of flavour components in coffee. Further, the coffee matrix itself interacts with volatiles and has a large impact on the perceived flavour

assessed through a sensory study. Thus, matching or creating a comprehensive link on all components of coffee flavour and sensory quality will lead to a deeper understanding of coffee flavour. For example elucidating what compounds cause the *nutty, cocoa, caramel*, *fruity*, or '*coffee-type*' flavour which can then be subsequently tracked back to individual processes involved in their formation. Understanding on flavour will aid the coffee industry to control desirable flavour outcomes of coffee through processing or other farm management technique.

Chapter 3 Profiling the sensory diversity of coffee flavour

Chapter 3 is submitted for publication.

This chapter describes the selection of coffee samples and profiling of sensorily diverse commercial single-origin ‘specialty’ coffees from around the world. The method implemented in the sensory evaluation and the challenges faced are detailed in this chapter. The results achieved in this chapter provided sensory data used to build a comprehensive flavour model (detailed in Chapter 6, p.104).

Additional supporting documents not to be included in the submitted manuscript can be found in Appendix A-C (p.170-174).

3.1 Introduction

Coffee trade activities have been growing consistently in the last five decades with a considerable growth experienced from the year 2000 (ICO, 2015). Coffee is a billion dollar industry and it remains one of the most important traded commodities of economic importance to both the countries producing, and the countries consuming, coffee. To ensure demand, and for companies to improve their competitive edge, it is of great interest to constantly maintain and, where possible, improve coffee quality.

Flavour, made up of taste and importantly aroma, is crucial to coffee quality. Describing coffee flavour is a very complex task as it is influenced by numerous factors from the farm to cup (Sunarharum et al., 2014). Not surprisingly, the diversity of flavour types available commercially in specialty coffee is extraordinarily wide-ranging and in truth a simple description of generic “coffee flavour” is impossible. Throughout the last decade, there has been much research investigating the compositional basis of coffee flavour using advanced technology and instrumentation, with non-volatile (Ginz et al., 2000, Ginz and Engelhardt, 2001, Farah et al., 2005a, Farah and Donangelo, 2006, Oestreich-Janzen, 2010, Liu and Kitts, 2011, Bartel et al., 2015) and volatile compounds (Andueza et al., 2003a, Baltes and Knoch, 1993, Grosch et al., 2000, Kumazawa and Masuda, 2003, Mestdagh et al., 2014) being explored as potential drivers or markers of coffee quality.

To better understand and explore coffee flavour, considerable effort must be focused on objectively measuring coffee sensory properties using a scientific approach. Sensory evaluation is conducted to characterise sensory properties of products through measuring human responses with minimum bias (Lawless and Heymann, 2010) and therefore is the most rigorous approach for any coffee quality assessment.

Sensory evaluation to assess the quality of a coffee brew or an espresso coffee (EC) is commonly conducted through a cup-test ("coffee cupping") method. There are cupping procedures and standards developed for industrial purposes by various coffee associations and companies worldwide such as by the Specialty Coffee Association of America (SCAA). While these methods do vary depending on the organization that developed them, they all tend to follow a similar overarching protocol. In the SCAA industry cupping method, the usual sensory procedure is to assess at least 5 covered cups per coffee starting from the beans for roast colour and defect, prior to evaluating ground coffee for aroma (or fragrance). The brew is typically prepared using 55 g of coffee per liter of water or the ratio of 8.25 grams (whole bean) coffee (±0.25 grams), to 5.07 fluid ounces (150 mL) water (SCAA, 2012). The tasting could be conducted by 1 expert for small to medium coffee operations up to a group of trained cuppers rating a few sensory attributes and evaluating perceptions of overall quality (Turer, 2010). The results obtained from the coffee cupping technique have been demonstrated to be different to that obtained by conventional sensory descriptive analysis (Di Donfrancesco et al., 2014) and in truth falls short of fully describing the perceived sensory qualities of a coffee. This is likely to be due to the fact that coffee cupping lacks a common language of flavour descriptions as well as a distinct lack of consistency in the vocabulary used (Di Donfrancesco et al., 2014). Furthermore, the limited number of assessors and lack of repetition means that cupping data can be inherently biased and is not necessarily reproducible.

Several studies have been conducted that utilize more rigorous scientific sensory methods for coffee sensory evaluation (for examples see Bhumiratana et al., 2011, Masi et al., 2015, Sanchez and Chambers, 2015) although these are not easily or directly comparable. The published sensory studies of coffee are relatively limited in the range of different coffees that were assessed, typically only 2-10 different coffee types are evaluated, such as coffees from different origins, different processing trials and/or coffees obtained commercially. Not surprisingly, panellist fatigue is a major issue in coffee evaluation

together with sample carry-over effects from consuming a strongly flavoured hot beverage. This naturally limits the number of samples that can be evaluated in any one session and prolongs the duration and expense of any sensory trial. A substantial amount of work in coffee sensory evaluation has been conducted in the previous decades by SCAA resulting in a well-known coffee flavour wheel that can be used as reference in describing coffee flavour. Extensive studies were successfully performed by the World Coffee Research Organisation (WCR, 2016) on coffee sensory lexicon development involving 105 coffee samples from 13 countries. The results of the current study will enrich previous coffee research by including unique and specific coffee types such as Australian coffee, Monsooned and Luwak coffee.

There is also considerable variation in published literature on the preparation methods used for coffee presentation, such as different brewing procedures. The flavour attributes developed and described by any one study are very much influenced by the specific preparation or brewing procedures applied. The most common preparation methods have included the espresso technique including the use of bar machines and capsules (Andueza et al., 2003b, Barron et al., 2012, Masi et al., 2015, Parenti et al., 2014, Iamanaka et al., 2014, Kerler et al., 2014, Charles et al., 2015) drip or filter coffee (Bhumiratana et al., 2011), or infusion into a hot pot (Di Donfrancesco et al., 2014). No studies have reported specifically the French press method, a direct-infusion technique, which is one of the most common brewing methods for domestic consumption. Further no previous studies have been published that seek to provide a holistic overview of the diversity of coffee flavours present commercially, linked with physicochemical properties.

This research was carried out to profile, compare and describe the broad range of sensory properties that are present in globally diverse commercial specialty coffees using objective sensory methods. These results will provide a foundation for understanding the sensory diversity of coffee flavour expressed commercially and will provide a basis for future research seeking to explore the importance, and nature of the sensory contribution of various flavour components found in coffee.

3.2 Methodology

3.2.1 Materials

A total of 59 (1 kg) and 26 (3 kg) commercial single-origin, medium-roasted, 'specialty' coffee samples were obtained from five Australian coffee companies, traders or roasters. The Indonesian Luwak coffees were sourced directly from a coffee producer in Indonesia. On arrival, samples were sub-sampled (~110 g) and sealed under vacuum in pouches made from four layer laminated polyethylene terephthalate/polyethylene terephthalate/aluminium foil/linear low-density polyethylene (PET/PET/Foil/LLDPE) material and stored at -30°C, before removing to -20°C a week prior to the assessments. All coffees were examined within 7 months of purchase and roasting.

3.2.2 Sample preparation

On the day of any sensory evaluation session, coffee beans were thawed overnight prior to grinding (CLIMAX Coffee Grinder, Climax Engineering, Melbourne, Australia). The ground coffees (50-60% that passes through 20 mesh Endecotts sieve or 850 µm) were kept in sealed airtight containers until used. Grinding was conducted 20 to 30 minutes prior to sensory assessment as per the SCAA guidelines (SCAA, 2012).

For sensory screening sessions, ground coffee samples (±1 g) were weighed into small capped 22 ml plastic cups labelled with a three digit blinding code.

For sensory descriptive analysis, ground coffee samples were weighed into 900 mL French press plungers (PYREX) for brewing (38.5 g for 700 mL brew) following the brewing standard of SCAA (SCAA, 2012) of 55 g/litre. Boiling RO water (±95°C) was poured 10 min prior to sessions followed by steeping (4 min). The brews were decanted into a hot glass jug, covered with aluminium foil and kept at ±70°C in a water bath (no more than 10 minutes) until serving. Samples (40-50 mL) were presented to the panel in 118 mL single layer paper cups that had been covered with a Pyrex watch glass, immediately after pouring.

3.2.3 Sensory evaluation

Panellists were selected from a pool of experienced sensory panellists that involves staff and students of the Health and Food Sciences Precinct, Brisbane, Australia. They were

exposed to several hours of training sessions prior to formal sessions as per conventional descriptive techniques and their acuity tested. Practice and formal evaluation sessions were held in a purpose-build sensory evaluation laboratory which was temperature controlled (22^0C) with day-light equivalent lighting, and was equipped with 12 isolated sensory booths.

For sensory screening, at least six individuals attended each session and panellists were asked to individually evaluate and describe the aroma of no more than six ground coffee samples per session, nine sessions in total (each of 45 min duration). The SCAA coffee flavour wheel was used as a reference during these preliminary sessions. However, panellists were also asked to use their own words to describe coffee flavour. Subsequent discussions were held to identify the spectrum of major aroma types among the 59 coffees screened, with a view to select and shortlist 26 coffees that represented the broadest range of aroma types possible. Coffee samples that were shortlisted were of very high quality and were, importantly, fault free. These selection criteria of coffee samples were based on the commercial coffee supplier. However, re-assessment on the coffee samples were performed upon receive based on the visual appearance such as the package was sealed properly, not wet or too oily, no contamination, uniformity of colour, and no off flavour. Based on this selection, certain coffees were excluded as faulty. Certain selected coffee such as El Salvador (El Angel) has been replaced with El Salvador (San Emilio) due to seasonal unavailability.

Conventional sensory descriptive analysis was the method used to evaluate the 26 shortlisted coffee samples (Lawless and Heymann, 2010). Seven male and seven female panellists, aged 25-56 years old (mean age of 44) attended 26 training sessions prior to 26 formal assessments. The training involved: familiarizing the panellists with the samples; adoption of a tasting protocol together with palate cleansers; and development of descriptive terms, attribute definitions, associated sensory reference standards and attribute scales on a paper-based questionnaire for rating during formal sessions. Each coffee was presented at least twice during training. A total of 17 sensory attributes (12 aroma, and five in-mouth flavour attributes) were selected and are presented together with definitions and sensory reference standards in Table 3.1. Towards the end of training, practice sessions were held which mimicked formal evaluation, to verify the suitability of the method and to assess panellist performance, prior to formal assessment.

During the formal evaluations, a total of three samples of coffee brew and one 'palate primer' (half strength coffee of a generic brew) were served per set. Three replications of each sample were assessed and were presented within sets according to a balanced presentation design. Samples were randomly allocated within each replicate. There were 26 tasting sets in total, each set presented in a 30 minute session. In each session, panellists were instructed to re-acquaint themselves with the attribute definitions and sensory reference standards, before assessing the aroma across all samples served within a set. The aroma assessment of each sample was conducted in two sniffs. Subsequently, panellists were asked to sip the 'palate primer' before assessing the in-mouth taste and flavour of the samples using two sips (sample temperature at ~40°C). Attributes were scored using an unstructured 15 cm line scale anchored from none (0) to high (15) according to the paper-based questionnaire. The panellists were required to refresh their senses with fresh air between samples and to cleanse their palate first with lightly sparkling, then with filtered still water.

Table 3.1 Definition of sensory attributes and corresponding reference standards.

Aroma	Definition	Sensory reference standards*
aroma attributes		
aroma intensity	The overall aroma intensity of the sample.	-
citrus	A citrus peel, tamarind-like aroma, reminiscent of crushed coriander seed.	Grated lemon peel (0.01 g)
fruity	A fruity poached-pear and jammy almost blackcurrant aroma	Blackcurrant jam (Woolworth Select):poached pear = 2:1 (0.5 g)
boiled vegetables	A boiled root vegetable aroma, carrot and sweet potato-like.	Boiled carrot:sweet potato= 1:2 in 500 mL water (1 g)
aromatic spice	An aromatic spice aroma, crushed black pepper, pine needle-like, some sweet spice.	Chopped dry pine needles (0.05 g) mixed with freshly crushed black peppercorn (0.005g)
woody	Aroma of wood, freshly cut/sawn wood, woodchips.	Wood mixtures (fresh sawn wood:woodchips=2:1, pre-soaked in water) (1.5 g)
earthy	A wet-earth, earthy, musty, dank aroma, almost mushroom-like or wet paper.	Wet earth (1 g)
nutty	Aroma of roasted nuts, like the pellicle of hazelnuts, almost popcorny.	Crushed hazelnut:roasted almond (Woolworth Select)= 3:1 (0.5 g)
cereal	An oaty chaff-like aroma, bran-like, dried hay, slightly malty nougat caramelly, sweet drinking chocolate.	Allbran cereal (Kellogs) 0.5 g

Aroma	Definition	Sensory reference standards*
toasted	A toasty aroma, not burnt and with some bready notes.	Freshly toasted white bread (Sunbless) (1 g)
smoky	Aroma of smoke, from smoky to intensely charred, acrid burnt when high.	Burnt/charred European beech wood (0.001g)
dark chocolate	Aroma of dark chocolate, cocoa-like.	Chopped Lindt 85% dark chocolate block (0.2 g)
in-mouth flavour attributes		
sourness	A tart sour taste when the sample is in the mouth.	0.1 g/100 mL citric acid solution
bitterness	The bitter taste experienced with the sample in the mouth.	0.8 g /500 mL caffeine solution
astringency	An astringent drying roughness experienced on the roof of the mouth and cheek pouches.	0.1 g/100 mL tannic acid solution
flavour intensity	The overall flavour intensity of all flavours and tastes experienced when the sample is in the mouth.	-
residual	The length of time the flavour/mouthfeel remains on the tongue and mouth after swallowing or tasting	-

*Each sensory reference standard was presented to each panellist in a 22 mL plastic cup covered with a lid.

3.2.4 Measurement of pH and colour

Brewed coffee samples from the sensory evaluation were analysed in duplicate for pH using an auto-titration apparatus (702 SM Titrino Metrohm AG CH-9101 Herisau, Switzerland completed with a 728 Metrohm stirrer) and colour (CIE L*a*b* colour space) using a CR 310 photometer (Konica Minolta Imaging, Dietikon, Switzerland).

3.2.5 Statistical analysis

The results from paper questionnaires were manually entered into Microsoft Excel and exported into either SENPAQ v6 (Qi Statistic, Reading, UK) or XLSTAT version 2014 (Addinsoft, New York, USA) for data analysis. Panel performance analysis was carried out by calculating the F-ratio (or also mentioned as F-value), probability (*p*-value), mean squared error (MSE) as well as Pearson's correlation coefficient of each panellists' mean score with panel mean (Tomic, 2007, Tomic, 2010, Næs et al., 2010, Kermit and Lengard, 2005). Descriptive statistics calculated for all sensory attribute scores included minimum, maximum, mean, standard deviation (SD) and the coefficient of variation (CV). Factors and interactions effects were analysed using Mixed Model Analysis of Variance (three way

and two way ANOVA) applied on the raw data set (26 products x 14 panellists x three replicates) for each attribute to determine significant differences (α=0.05). Further post-hoc mean separation Tukey-Kramer HSD was performed for the significantly different attributes based on two-way ANOVA, with a confidence interval of 95%. Where the ranking test by Tukey-Kramer HSD found no significant different, Duncan multiple comparison was performed. Principal component analysis using a correlation matrix was performed to examine the structure of the data and identify potentially responsible factors for differentiation and sample grouping.

3.3 Results and discussions

To explore and describe the spectrum of coffee flavours available in specialty coffee, it was important to conduct an initial screening of a vast array of coffees to ensure the final selection of coffees comprised the broadest possible range of sensory types commercially available. The 59 coffees screened were initially selected based on advice from coffee industry experts and suppliers, as well including samples from various regions of production and samples processed using different techniques and styles. As it was important to maximize the regional and processing distinctions and nuances in flavour of all coffees assessed, rather than to purely compare coffees based on roast flavour, only medium roasted samples were selected, in the belief that in these coffees the flavour is fully developed (Illy and Viani, 2005). The aroma of ground coffee was assessed during screening in order to minimize panellist fatigue and to simplify the appraisals by avoiding the need for control over brewing and temperature during presentation. This method proved to be very effective and repeatable with coffee aromas of the ground beans remaining relatively consistent over an extended period of time at room temperature. Further, the aroma of the ground beans is thought to be a good indicator of the aroma of the coffee brew. It has been recently stated to be critical to consumer liking (Fisk et al., 2012) and is a standard practice used by industry during ‘coffee cupping’ (SCAA, 2012).

The 26 coffees selected represented the complexity and variability among the aroma descriptions given by the sensory panel for the 59 ground coffees. The selected coffees are presented in Table 3.2 together with region of production, processing treatment, pH (n=2) and colour measurement (n=2) (L*value represents lightness). All the coffees

selected were *Coffea arabica* (hereon mentioned as Arabica) except for one Indian sample which was *Coffea canephora* cv.Robusta (hereon mentioned as Robusta). The Robusta coffee was both the highest in pH (pH 5.7) and the lightest in colour (L*value 45) compared to the Arabica coffees. A number of coffees with varying processing treatments were included such as washed, natural, semi-dry (pulped natural or wet-hulled), natural-washed/mixture. Of interest, two quite unique and specialty styles of Arabica coffee were included in the selection, namely, the Indonesian Luwak coffee (two different types including a 'Peaberry' bean), and an Indian Monsooned Malabar style.

Luwak coffee is highly valued coffee type produced from beans that have been through a fermentation process in the intestine of a Kopi Luwak (or Indonesian civet cat) (Marcone, 2004). After passing through the intestine, the beans are collected via the droppings, washed and prepared for roasting. Fermentation of coffee by any means is thought to potentially improve coffee quality. Fermentation processes for coffee may include an animal's bio-digestive tract, such as a Luwak (Marcone, 2004) or elephant (Main, 2014), or may be conducted *in vitro*, using the naturally available microflora on the coffee cherries (de Melo Pereira et al., 2014, Evangelista et al., 2014, Evangelista et al., 2015, Avallone et al., 2001, Silva et al., 2000). 'Peaberry' coffee comes from isolating a certain bean morphology from the coffee crop. A 'peaberry' bean is a rounder-intact and un-split bean often eliminated since it is considered as defect in a bulk coffee mixture, but is believed to have specific characters when processed separately from the common split coffee beans. Monsooned coffee comes from a process involving fermentation or curing of coffee beans. Unique to the Malabar Coast this method involves exposing the beans to high humidity monsoonal conditions (Ahmad et al., 2003) which allows the green coffee beans to absorb moisture, followed by sun-drying process, and occurs in cycles for several weeks.

Based on initial coffee screening, the coffees selected expressed a representative broad range of aroma dimensions important for this study. Some coffees contain elements that might be described in a typical coffee such as *nutty*, *chocolaty*, *sweet caramelly*, *malty*, and *smoky aroma*. Interestingly, some of the coffees exhibited more diverse aromas described as *curry*, *popcorn*, *berries*, *blackcurrant*, *citrus*, *floral* and *alliaceous* (or like the pungency of *onion-garlic*). The multi-dimensional sensory diversity in the selected samples made for an excellent set of coffees to include for sensory profiling.

Table 3.2 Coffee samples selected for sensory descriptive analysis

Coffee samples (details)[a]	Region	Processing treatment [a]	pH	L*value
Australia NQ1 (north Queensland)	Asia-Pacific	washed	5.2	39.5
Australia NQ2 (north Queensland)	Asia-Pacific	natural	5.2	38.4
Australia NSW (K7, New South Wales)	Asia-Pacific	natural	5.1	41.6
Bolivia (Verde)	South America	washed	5.3	41.2
Brazil1 (Yellow Bourbon)	South America	natural	5.2	40.6
Brazil2 (Super)	South America	semi-dry (pulped natural)	5.1	41.6
Colombia (Supremo)	South America	washed	5.1	41.5
Costa Rica (Strictly High Grown)	Central America	washed	5.3	39.7
Dominican Republic (Barahona)	Caribbean	natural-washed mixture	5.2	38.3
El Salvador (San Emilio)	Central America	semi-dry (pulped natural)	5.5	43.4
Ethiopia1(Sidamo Guji)	Africa	washed	5.4	39.6
Ethiopia2 (Harrar)	Africa	natural	5.3	40.4
Guatemala1 (Antigua-Acate)	Central America	washed	5.2	39.2
Guatemala2	Central America	washed	5.3	38.8
Honduras	Central America	washed	5.3	42.3
India Monsooned Malabar	Asia-Pacific	dry-monsooned	5.5	42.3
India Robusta[b]	Asia-Pacific	natural	5.7	45.7
Indonesia Java	Asia-Pacific	semi-dry (wet-hulled)	5.3	39.3
Indonesia Luwak1 (Sumatra Gayo)	Asia-Pacific	Luwak fermentation	5.1	38.8
Indonesia Luwak2 (Sumatra Gayo ‘Peaberry’)	Asia-Pacific	Luwak fermentation	5.2	39.6
Indonesia Sumatra (Gayo Supreme)	Asia-Pacific	semi-dry (wet-hulled)	5.4	38.8
Nicaragua (Strictly High Grown)	Central America	washed	5.4	39.9
Papua New Guinea1 (Kimel)	Asia-Pacific	washed	5.2	39.9
Papua New Guinea2 (Purosa)	Asia-Pacific	washed	5.2	39.4
Peru (Cafe Feminino)	South America	washed	5.4	39.8
Rwanda (Inzovu)	Africa	washed	5.3	39.6

[a] information collected from the commercial suppliers

[b] Robusta coffee, others Arabica species.

3.3.1 Optimization of preparation and protocol for sensory evaluation of coffee

Quantitative sensory descriptive analysis was the method employed to profile the 26 coffees in this study. This technique involves a training phase and vocabulary development as well as a formal evaluation phase. During training, coffee preparation and sensory assessment methods were optimized. First of all, the vessel for presentation was assessed by comparing single layer paper cups, double layer paper cups, porcelains/ceramics, and porcelain and paper cups combined by logging temperature during conditions mimicking the coffee session (with Pyrex lid on and off). A single layer paper cup was found to be the most adequate and simplest approach to retain coffee temperature during serving and sensory evaluation. Using this cup type, coffee brew temperature was decreased at the rate of ±2.5°C/min from the ideal serving temperature at 70°C, resulting in the temperature of ±40-45°C when tasting commenced. Brewing temperature, water to coffee ratio, steeping time, serving temperature and sensory assessment time were all carefully controlled to avoid temperatures that were either too hot or too cold thereby affecting the results. Where high temperatures would burn the palate, colder brew temperatures would be perceived differently due to the loss of some aromatic compounds important to coffee quality. It is important to assess sensory attributes of coffee brew while it is hot and fresh. Immediate consumption, less than 30 min after preparation, is typically suggested (Arvidson et al., 2006).

The use of a palate primer coffee prior to tasting samples was found to be useful in preparing the palate before tasting. Limiting samples to a maximum of three per session (plus the primer) was found to minimise fatigue. Given the challenge in preparing hot brew for 14 panellists it was impractical to balance the presentation order across the entire replicate given the need to prepare a fresh ‘batch’ of each coffee brew individually. Thus, samples were balanced within the set of three samples within a replicate and then randomised across each of the three replicates.

Considering the fatiguing nature of the beverage, not only sample sets were limited, but panellists were also given recuperation time between samples to refresh their senses and palate. Nevertheless, many panellists advised that they still struggled when assessing coffee flavour, mainly due to the change in aroma over time added to the multidimensional complexity of the aroma. Certainly, this observation can be explained by previous reports

where it was shown that coffee odour composition changed with time soon after extraction (Baggenstoss et al., 2008, Manzocco et al., 2011). In addition, the samples being medium roasted meant that they were at maximum complexity in terms of flavour (Illy and Viani, 2005) which creates competition and overlapping of the olfactory receptor sites (Bhumiratana et al., 2011, Laing, 1994, Jinks and Laing, 2001) contributing to the difficulty in discrimination of coffee aroma properties. Coffee assessment requires the panellist to focus and concentrate particularly hard on the assessment of the beverage and this in itself was extremely fatiguing.

It is understood that interaction between liquid food systems like coffee and saliva as well as brewing techniques and sipping volume may influence volatile partitioning and aroma release of coffee in the mouth (Genovese et al., 2009, Genovese et al., 2014, Genovese et al., 2015). While in this study sipping volume was discussed with the panel, and a protocol agreed upon, it was difficult to control given the natural human variation, and logically, some variation is expected. Every effort was made to control for the variation in coffee preparation, serving and assessment protocol to produce quantitative and qualitative sensory information of high quality.

3.3.2 Evaluation of the quality and validity of the coffee descriptive sensory data

Assessing panel performance, along with determining the effects of factors such as replicate and panellists on the attribute scores, is an important part of determining the quality and validity of sensory data (Kermit and Lengard, 2005, Næs et al., 2010). Given the complex nature of the coffee beverage, a thorough evaluation of panellist's performance was a necessary requirement of this study. Further, panellist feedback during training and formal evaluation suggested that, for many of panellists, the scoring of attributes for any one coffee sample was a real challenge due to the samples complexity and changing aroma over time. Prior to evaluating differences between the coffee samples, the quantitative sensory data generated from the formal sessions was subjected to careful analysis to ascertain both panel performance in general and to further analyse the effects of panellists, products, replications and interactions on the scoring of sensory attributes.

The results of a Mixed Model Analysis of Variance are shown in Table 3.3. Based on the model (Table 3.3), the scoring of each attribute was found to be significantly different (α=0.05) across the samples except for attributes *woody*, *cereal*, *toasted* and *dark chocolate*. While significant differences were also observed between panellists for each attribute, this is regularly found in sensory evaluation (Næs et al., 2010, Masi et al., 2015). There were no significant differences in the overall scoring of attributes between replicates, with the exception of *aroma intensity*, evidence of the careful control exercised to ensure consistency between sessions and replicates.

Table 3.3 Results of a mixed model analysis of variance showing interaction effects of factors on each sensory attribute (F ratio and significance shown) (26 samples x 14 panellists x three replicates)

Sensory attributes	Samples	Panellists	Replicates	Samples x Panellists	Samples x Replicates	Panellists x Replicates
aroma attributes						
aroma intensity	3.6	40	4.0	1.1 ^{ns}	1.1 ^{ns}	1.4 ^{ns}
citrus	2.5	16	1.6 ^{ns}	0.9 ^{ns}	0.8 ^{ns}	1.3 ^{ns}
fruity	2.5	13	0.8 ^{ns}	1.2	1.3 ^{ns}	1.4 ^{ns}
boiled vegetables	3.0	10	0.7 ^{ns}	0.9 ^{ns}	0.8 ^{ns}	1.4 ^{ns}
aromatic spice	1.9	21	1.6 ^{ns}	1.0 ^{ns}	1.0 ^{ns}	1.7
woody	1.0 ^{ns}	21	0.4 ^{ns}	1.2	1.4	0.9 ^{ns}
earthy	1.7	14	0.3 ^{ns}	1.2	1.5	1.3 ^{ns}
nutty	1.5	27	0.4 ^{ns}	1.2 ^{ns}	1.0 ^{ns}	0.8 ^{ns}
cereal	0.9 ^{ns}	14	0.2 ^{ns}	1.0 ^{ns}	0.9 ^{ns}	1.5
toasted	0.5 ^{ns}	19	0.4 ^{ns}	1.1 ^{ns}	1.1 ^{ns}	2.1
smoky	2.1	23	0.4 ^{ns}	1.3	1.0 ^{ns}	1.6
dark chocolate	0.8 ^{ns}	12	1.7 ^{ns}	1.0 ^{ns}	0.9 ^{ns}	1.1 ^{ns}
in-mouth flavour attributes						
sourness	3.8	23	1.9 ^{ns}	1.1 ^{ns}	1.0 ^{ns}	1.5
bitterness	3.8	53	2.4 ^{ns}	1.0 ^{ns}	1.1 ^{ns}	1.0 ^{ns}
astringency	2.5	14	1.6 ^{ns}	1.2 ^{ns}	1.2 ^{ns}	1.6
flavour intensity	2.1	15	1.0 ^{ns}	1.4	1.7	2.0
residual	2.8	30	1.1 ^{ns}	1.2	1.7	1.5
Degrees of freedom	25	13	2	325	50	26

^{ns} non significance (α=0.05).

There was a significant difference observed for the interaction between samples by panellists only for *fruity*, *woody, earthy, smoky, flavour intensity* and *residual*. This indicates the panellists scored the coffee for these attributes in different ways leading to a

low interaction score. Interaction of sample by replicate was significant only for *woody*, *earthy*, *flavour intensity* and *residual* indicating variation for almost all attributes for each sample across replicates was minimal. Interaction of panellist by replicate was not significantly different for the majority of attributes, with the exception of *aromatic spice*, *cereal*, *toasted*, *smoky*, *sourness*, *astringency*, *flavour intensity* and *residual,* indicating that the sensory panel scored most replicates in the same way.

A summary of results for panel performance including discrimination ability, consistency and reproducibility, is shown in Table 3.4. This evaluation indicated there were one non-reproducible and four non-discriminating panellists in the sensory panel. One panellist was also in disagreement with the rest of the panel for *earthy* aroma. However, there was no individual panellist consistently disagreeing with the group for the rating of attributes even though they may have used the scale differently from normal occurrence in sensory studies. Excluding data from poorly performing panellists had no detrimental effect on the data set and differences between samples, therefore data from all panellists were used in further analyses.

Table 3.4 Summary of sensory performance evaluation (26 samples x 14 panellists x three replicates)

Panellists	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Discrimination ability	1	0	1	2	2	0	3	2	3	0	0	1	3	6
Repeatability	15	12	17	13	11	12	17	7	4	8	16	16	17	14
Consistency	17	14	17	12	10	15	17	8	4	9	16	16	17	10
Total	**33**	**26**	**35**	**27**	**23**	**27**	**37**	**17**	**11**	**17**	**32**	**33**	**37**	**30**

The higher total score means better performance.

3.3.3 Sensory diversity expressed in 26 diverse specialty commercial coffees

With acceptable panel performance, the average sensory scores for each of the 26 specialty coffees were calculated (an average of three replicates x 14 panellists for each coffee for each attribute). The resulting coffee sample attribute scores (from a scale of 0-15) are summarized in Table 3.5 including mean score for each attribute, minimum, maximum, standard deviation (SD) and coefficient of variation (CV%).

With the exception of flavour, the maximum average score for attributes were typically low with respect to the scale (0-15) with the majority being below a score of 6 on the line scale.

This is mainly due to the large amount of 0 scores, whereby panellists have missed particular aroma attributes entirely due to the challenges of an elusive and changing sample headspace. *Citrus*, *earthy* and *smoky* were rated very low in comparison to other attributes, potentially due to only few panellists detecting and consistently rating these aroma attributes in the coffee samples. Nevertheless, these attributes were still rated at significantly different levels across the samples. In general, the flavour attributes showed a much smaller diversity (mostly <20%) than the aroma attributes which varied across samples from as little as 9% to as much as 44% CV. Not surprisingly, the aroma variation for coffee is far more diverse and broad than the variation in coffee taste.

Table 3.5 Summary of the descriptive analysis scores for the aroma and flavour of 26 coffee samples)

Sensory attributes	Min. [a]	Max. [a]	Mean [b]	SD	CV (%)
aroma attributes					
*aroma intensity**	6.3	9.4	8.0	0.7	9
*citrus**	0.7	3.2	1.6	0.6	39
*fruity**	0.8	5.2	2.6	0.9	36
*boiled vegetables**	1.5	4.5	3.1	0.8	27
*aromatic spice**	1.7	5.0	3.0	0.7	24
woody	3.1	6.7	4.6	0.7	16
*earthy**	0.5	3.8	1.6	0.7	44
*nutty**	1.8	1.8	2.8	0.7	25
cereal	2.6	2.6	3.4	0.5	14
toasted	2.5	3.8	3.0	0.3	11
*smoky**	0.6	3.1	1.7	0.6	38
dark chocolate	2.0	4.0	2.8	0.5	17
in-mouth flavour attributes					
*sourness**	4.8	8.2	6.9	0.9	13
*bitterness**	4.8	8.7	6.7	1.0	15
*astringency**	2.8	6.4	4.7	0.9	18
*flavour intensity**	6.0	9.6	8.0	0.9	11
*residual**	4.7	8.5	7.0	1.0	14

* Indicates attributes that are significantly different (α=0.05)

[a] n = 3 replicates x 14 panellists

[b] n = 3 replicates x 14 panellists x 26 coffees

(scale of 0-15 cm).

With a focus on aroma diversity, PCA on the standardised mean data for 26 coffee samples was used to explore differences between samples, to identify groupings of similar samples, and to identify relationships between the 12 aroma attributes (Figure 3.1 and Figure 3.2).

The first three principal components (PCs) explained 67% of variation in the data set for the aroma scores for 26 coffee samples demonstrating a depth in complexity in the sensory data that cannot be easily visualized in just three dimensions. Samples were separated across PC1 (28%) according to samples that were scored highly for *citrus* and *fruity,* from those with higher scores for *boiled vegetables*, *earthy*, *woody* and *nutty*. Indeed strong positive correlation was found between attributes *citrus* and *fruity* and attributes *earthy* and *woody* (r>0.60, Pearson's correlation). Principal component 2 (21%) separated samples with high *aroma intensity*, a*romatic spice* and *smoky* scores from those that were lower in *aroma intensity* with more *dark chocolate* aromas. *Aromatic spice* and *aroma intensity* were also positively correlated with each another, as were attributes *nutty* and *cereal* (r>0.60, Pearson's correlation). Principal component 3 (18%) separated coffees that were rated higher for *cereal*, *toasted*, *nutty*, and *smoky*, from coffees that had higher *woody* scores. Further principal components, PC4 (7%) and PC5 (6%) were also explored visually, but are not presented given the low variation explained.

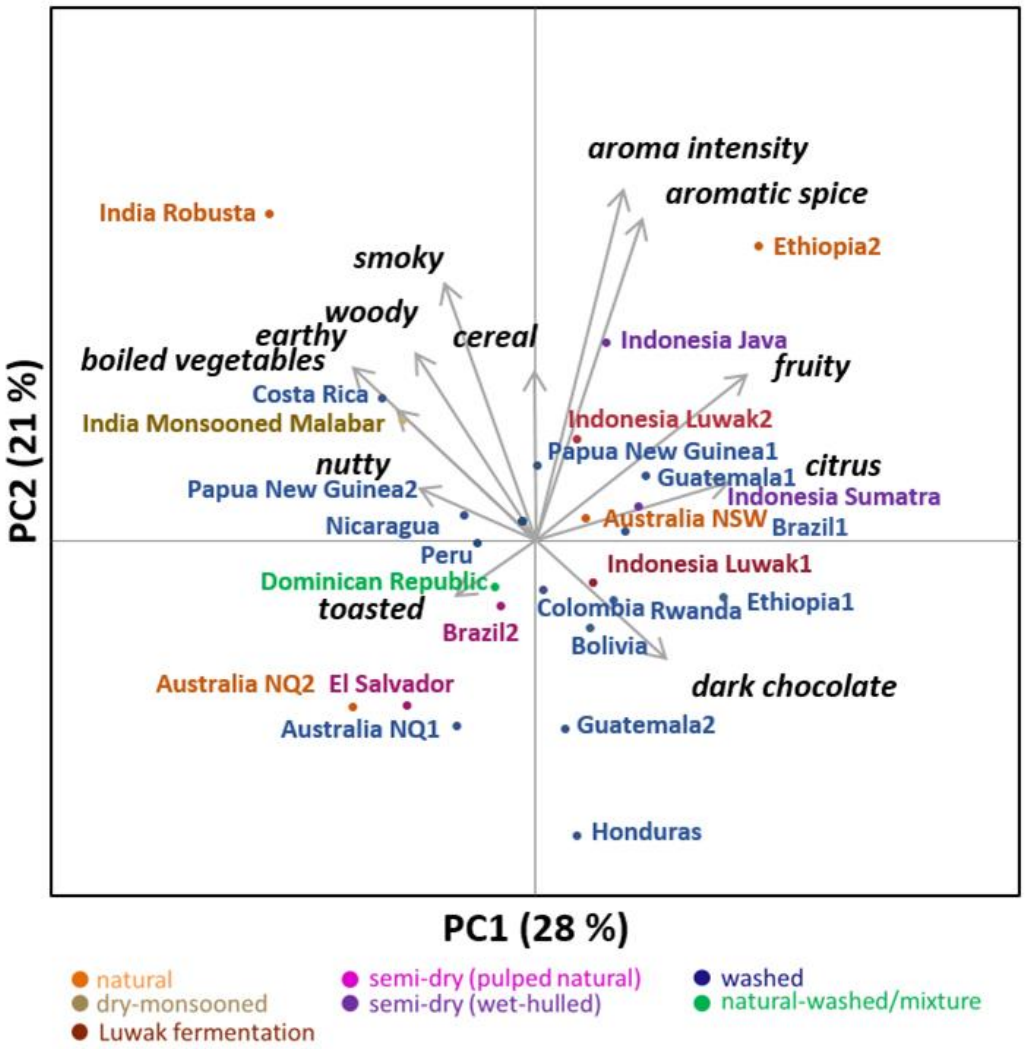

Figure 3.1 PCA bi-plot of aroma attribute scores for 26 coffees, PC1 (28%) versus PC2 (21%)

Overall, the samples did not group according to the bean processing technique applied and did not group according to country of origin or region of production. This is not surprising given the samples were specifically chosen to represent a range of different flavour types and were not selected to be representative of particular regions or processing treatments.

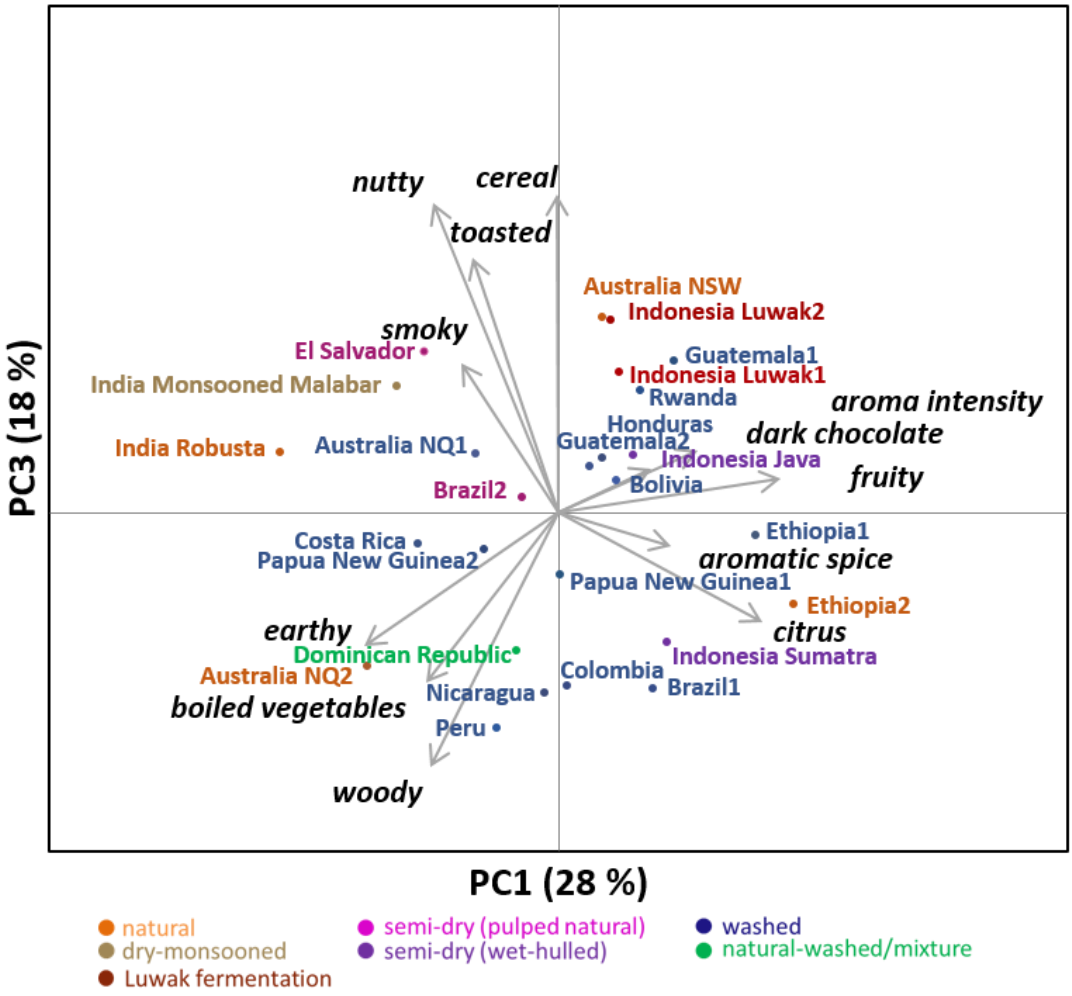

Figure 3.2 PCA bi-plot of aroma attribute scores, PC1 (28%) versus PC3 (18%)

The Indian Robusta coffee had an aroma profile that was very distinctive from other coffees mainly due to significantly higher scores for *smoky*, *woody*, *earthy* and *cereal* characteristics. The Robusta species is known for having much more *spicy, earthy*, and *harsh* (Blank et al., 1991, Czerny et al., 1996, Illy, 2002) flavours than Arabica making it less desirable to many consumers. The *spicy* aroma of Robusta has also been defined by some authors as *smoky*, and related to the odour perceived for certain volatile phenols such as guaiacols (Czerny et al., 2008). Other published work on the sensory properties of Robusta have also found this species displays *woody* aroma characteristics (Maeztu et al., 2001)

The other stand out coffees according to the PCA was the Ethiopian (2) and the Honduras coffee. The Ethiopian (2) coffee was highly *aromatic spice*, *fruity*, *citrus* and very high in *aroma intensity*. This coffee was produced using a 'natural' process. 'Natural' coffees are made by drying coffee cherries under sunlight or in mechanical dryer before removing the dry pulp mechanically, which is also referred to as dry processing. This style of processing is thought to produce coffees with pleasant aromas (Teixeira et al., 2005b, Mori et al., 2003) and *sweet* (Mori et al., 2003) but sometimes 'naturals' are reported to exhibit a 'hard' *medicinal* flavour (Clarke and Macrae, 1985). The *fruity, citrus* and *aromatic spice* notes are thought to be retained from the sweet cherry pulp and are influenced by fermentation process. Sun drying may take several weeks whereby natural fermentation may occur to yield ethanol and carboxylic acids which may correspond to certain *medicinal, acidic* and *fruity*, *citrus* characters in the brew (Silva et al., 2000). The limitation of the 'natural' process is the potential of undesirable fermentation (Teixeira et al., 2005b) and spoilage that may occur due to prolonged fermentation resulting from poor drying and high humidity conditions (Silva et al., 2000) that impact the quality and indeed the microbiological safety of coffee.

The Honduras coffee was very low in *aroma intensity* and was scored relatively low for all the other aroma attributes. This coffee was produced using a washed processing method where a substantial amount of water is utilized. Typically the washed process involves soaking under water, pulping and mucilage removal that significantly reduces undesired fermentation processes (Teixeira et al., 2005c) and results in higher quality coffee (Farah, 2012) that is softer and milder (Balzer, 2001). Improper 'wet' processing can lead to uncontrolled fermentation due to microbial contamination with the creation of poor cup quality and the production of 'stinker' beans (Teixeira et al., 2005b).

The coffees that represented the widest diversity in the sensory data set were also plotted using a typical sensory profile cobweb plot (Figure 3.3). These included the Australian NQ2, Ethiopia 2, Indonesia Java, Honduras, Brazil 1, India Robusta, Indonesia Luwak 1. As can be seen from both the PCA (Figure 3.1) and the cobweb plot (Figure 3.3), the India Robusta coffee was easily distinguished from the Arabica coffees.

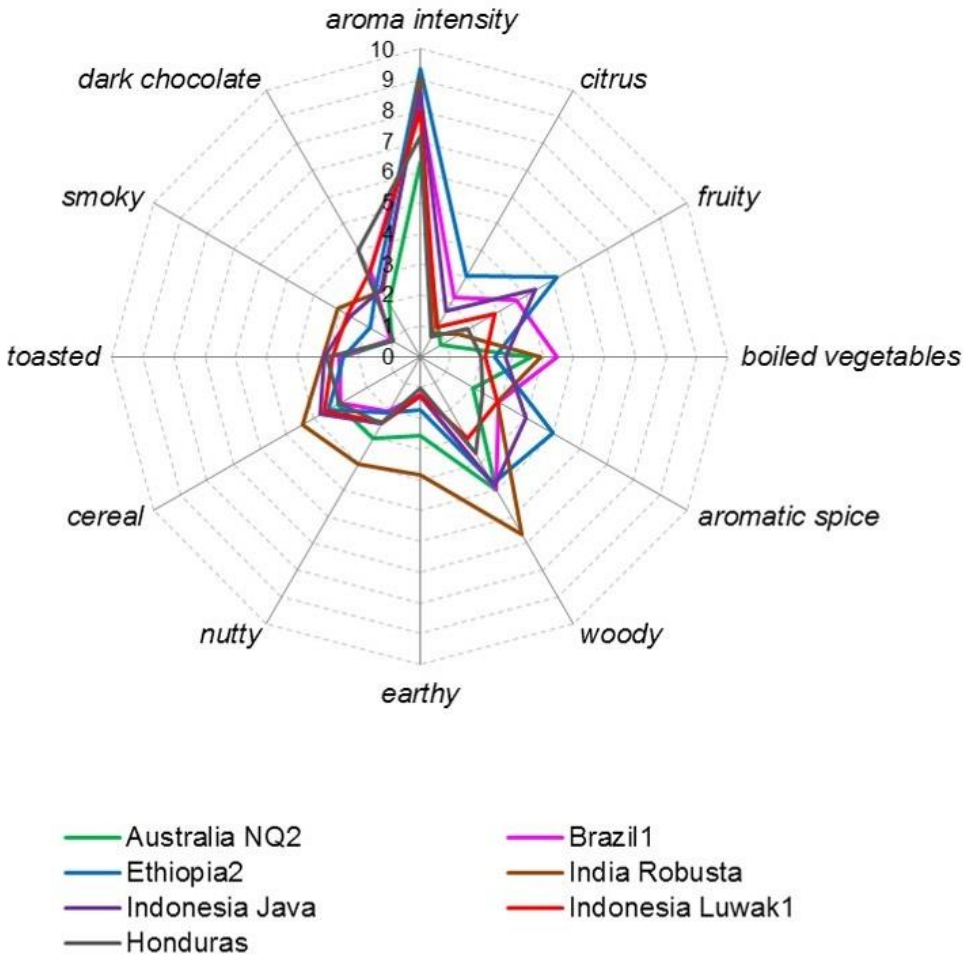

Figure 3.3 Selected coffees representing diversity on aroma profiles

Some unique and specific coffee styles were included in this study, which have either none, or very limited published sensory profile data. These coffees included the Australian coffee samples (Australia NQ1, NQ2 and NSW) from producers in north Queensland and northern New South Wales, and those with unique processing methods including the Luwak Arabica coffees (Indonesia Luwak 1 and the 'Peaberry' Indonesia Luwak 2) and the monsooned coffee (India Monsooned Malabar). The Australia NQ1 and NQ2 coffees (Figure 3.1 and 3.2) were more *woody*, *earthy* with *boiled vegetables* aroma, while the Australia NSW was found to be richer in aroma intensity, *citrus*, *fruity, aromatic spice, nutty, cereal*, and with *dark chocolate* notes.

Interestingly, the Indonesia Luwak coffees (Indonesia Luwak 1 and the 'Peaberry' Indonesia Luwak 2) were not too dissimilar from one another. Both Luwak coffees were

found to be mid-range in terms of *aroma intensity* with *cereal*, *toasted*, *nutty, smoky, dark chocolate, citrus, fruity* and *aromatic spice* dominating the profile. Closer comparisons between the two Luwak coffees revealed that the Indonesia Luwak (2) (which is a Sumatra Luwak Gayo 'Peaberry' coffee) tended to have a stronger aroma characters than the Indonesia Luwak (1) (Sumatra Luwak Gayo non-peaberry). The 'Peaberry' coffee bean type is a round small unseparated bean and the production of this coffee is highly regulated by specific standards as its thought to yield a higher quality coffee (ICO, 2013). 'Peaberry' coffee is specifically labelled for particular premium specialty coffee markets compared to ordinary Luwak coffee. To the authors' knowledge, this is the first scientific publication of an objective sensory descriptive profile of the aroma of Luwak Arabica coffee. Previous published work on Indonesian Luwak Robusta and African civet coffee, was limited to flavour attribute evaluation using an industrial cupping method (2004).

Interestingly, the India Monsooned Malabar Arabica coffee had a milder but not too dissimilar profile to the India Robusta coffee despite the very different processing technique employed, with *boiled vegetables, toasted, dark chocolate, citrus* and *fruity* notes being more prominent. This monsooned coffee has previously been reported to express *musty* aromas, *good body*, *low acid*, *syrupy* (CBI, 2014), *sweet, musk candy, caramel, nutty* (Stewart, 2008), and *cheesy* (Ahmad et al., 2003).

In terms of in-mouth flavour profiles, the Indian Robusta was rated as the least *sour* coffee, but with relatively high *bitterness, astringency, flavour intensity* and *residual* sensation while the Ethiopian coffees (Ethiopia 1 and 2) were scored relatively high in *sourness*. Comparatively, the monsooned coffee scored low for *sourness*, indicating the reduction of acids during the monsooning process. Both Indonesian Luwak coffees (Sumatra Gayo) were high in *sourness* but still less sour and generally less intense in flavour attributes than the non-Luwak (Indonesia Sumatra Gayo). In agreement to Marcone (2004), the fermentation process in Luwak (civet) animal's intestine might contribute to unique milder flavours due to leaching or formation of certain organic acids as well as other biochemical reactions supported by digestive enzymes. The Indonesia Java coffee scored the highest in *flavour intensity* while the Australian NSW coffee was found to have less intense flavour but is still higher than the northern Queensland coffee counterparts.

Assessment of in-mouth flavour attributes was reported by the panel to be a real challenge, particularly in differentiating the attributes *sourness* and *bitterness*, and *sourness* or *bitterness* and *astringency*. However, high positive correlations were observed between most of the in-mouth flavour attributes (r>0.80).

To some extent, *flavour intensity* was also found to correlate to *aroma intensity* and aromatic spice (r>0.60, Pearson's correlation), indicating the specific influence of these two attributes to overall flavour of the coffee brew both *orthonasally* and *retronasally*. The attribute *aroma intensity* was also found to correlate positively to *bitterness,* and *residual, astringency* (r>0.60, Pearson's correlation). Another interesting finding was that *sourness*, without any significant correlation to other flavour attributes, was perceived to have a positive correlation to *fruity* (r>0.60) and to lesser extent to *citrus*. Again, this implies the influence of aroma inhaled *orthonasally* through the nose to the flavour perceived *retronasally* while drinking coffee.

While varietal differences and individual panellist physiology are known to influence the perception and scoring of in-mouth flavour attributes, the brew preparation also can have an influence, specifically where sub-optimal extraction occurs. Consistent grind size, coffee to water ratio, temperature and time might still result in different extraction properties. Therefore, beans with different composition require unique brewing conditions to optimize extraction. In addition, differences in processing alters the compositional properties of coffee beans, particularly acids, lipids, proteins, sugars and polysaccharides, etc. which relate directly to in-mouth flavour. For example, natural coffee (dry processing) was reported to yield higher total soluble solids in the brew (Teixeira et al., 2005c) which might translate to the strong body and the in-mouth profiles. By contrast, increased exposure to water, such as in wet processing, semi-dry processing, monsooning or Luwak fermentation is thought to reduce total soluble solids resulting in less body, less acid and milder in-mouth flavours. All the aforementioned factors might contribute to the challenges of in-mouth flavour perception in coffee.

3.4 Conclusion

This study is the first to incorporate sensory and physicochemical properties of a broad range of sensorily diverse specialty coffees. Through trained panel quantitative sensory descriptive analysis, 12 aroma and five in-mouth flavour attributes were developed and defined. These attributes represent the major differences that may be found in commercial specialty coffee of medium roast level. Certain coffees were clearly distinctive, such as the *fruity*, *citrus* and *aromatic spice* notes for the Ethiopian coffees, the milder Australian coffees with *citrus, fruity* and *earthy* attributes. Interestingly, despite the much higher price tag, the specialized processing treatments such as the monsooning process and Luwak fermentation did not result in specific sensory attributes that were considerably different from other coffee types. This data will provide the foundation for further research in understanding the compositional basis of coffee aroma.

Coffee flavour perception remains a multi-dimensional and complex human experience. Brewed coffee proved to be an extremely challenging and dynamic matrix to study, and careful panel training, together with strict sample control was needed during assessments to achieve good quality sensory data. Nevertheless, further improvements can always be made. There are many factors to consider when studying the sensory aspect of coffee flavour which opens up a multitude of opportunities for further research in this area.

Chapter 4 Profiling key coffee flavour volatiles using novel SIDA/GC-MS methods

In the previous chapter (Chapter 3, p.30), the sensory diversity of selected commercial single-origin ‘specialty’ coffees were profiled. This chapter further explores the key volatiles profiles of these coffees using targeted approach. A novel stable isotope dilution analysis or assays/gas chromatography-mass spectrometry (SIDA/GC-MS) to suit the purpose of this study was developed and applied for rapid evaluation of targeted volatiles in coffees. The method development, the challenges faced and the coffee volatiles profiles are also detailed in this chapter. The results achieved in this chapter provided the basis of the volatiles data used to build a comprehensive flavour model (detailed in Chapter 6, p.104).

4.1 Introduction

Aroma volatiles generated during coffee roasting are arguably the most important quality-determinant of coffee (Andueza et al., 2003a, Baltes and Knoch, 1993, Grosch et al., 2000, Kumazawa and Masuda, 2003). It has been the subject of intense research efforts over the past few decades with over 1000 volatile compounds identified (Nijssen, 1996) ranging in concentration from part per million (ppm) to part per trillion (ppt) and has been comprehensively reviewed (Sunarharum et al., 2014). Of the volatiles found in coffee, only as few as 20-30 compounds are thought to be important to the perceived aroma of any single coffee (Blank et al., 1991, Blank et al., 1992, Grosch et al., 2000, Czerny et al., 1999, Deibler et al., 1998, Mayer et al., 2000, Mayer and Grosch, 2001, Sanz et al., 2002a, Semmelroch and Grosch, 1996, Semmelroch et al., 1995).

Instrumental analysis of coffee volatiles is usually achieved using gas chromatography coupled with mass spectrometry detection (GC-MS) (for examples see: (Bicchi et al., 1997, Budryn et al., 2011, Caprioli et al., 2012, Pickard et al., 2014, Becalski et al., 2016)). Instrumental determination and quantification of the detected volatiles in coffee is possible by the utilisation of internal standards and development of external standard addition calibration equations (Wittkowski and Matissek, 1993, Ismail and Nielsen, 2010). The

limitation of this technique for coffee is largely due to competition effects and the complex nature of the coffee matrix (Coleman, 1996). Isotopically labelled compounds as internal standards, such as those used in a Stable Isotope Dilution Analysis, or Assay (SIDA), provides an advanced tool for use in overcoming some of the issues arising from the volatile analysis of complex matrices. The internal standards used with SIDA are generally deuterium (^{2}H or *d*) or carbon 13 (^{13}C) labelled and will be discriminated against target compounds through MS fragmentation since molecular fragments are unique and specific for the labelled standard and the targeted analyte (Bicchi et al., 2011).

The SIDA method was first introduced by Schieberle & Grosch (Schieberle and Grosch, 1987) and has since been applied in research with other sample matrices such as in wine, olive oil, popcorn, peanuts, beverages, and other food commodities (Siebert et al., 2005, Rayne and Eggers, 2007, Crump et al., 2014, Roland and Schneider, 2015, Dierkes et al., 2011, Schieberle, 1995, Chetschik et al., 2010, Hawthorne et al., 1992, Cervino et al., 2008), and to measure caffeine in beverages. The SIDA approach has also been used for coffee volatile analysis (Semmelroch and Grosch, 1995, Pickard et al., 2013, Mayer, 1999, Bicchi et al., 2011, Petisca et al., 2014). Less than a 30 individual aroma compounds in total have been quantitatively measured in coffee so far across a range of methods combining GC-MS with either solvent extraction or liquid-liquid extraction (Semmelroch and Grosch, 1995, Pickard et al., 2013, Pickard et al., 2014), static headspace analysis (SHA) (Mayer, 1999), or headspace-solid phase micro extraction (HS-SPME)/GC-MS for furans (Bicchi et al., 2011, Petisca et al., 2014). A maximum of 12 compounds have been analysed in one assay in any one study of coffee aroma volatiles. With so few compounds analysed in any one assay previously, and given the diverse and complex nature of coffee aroma, it is unlikely that any previously published method has captured all (or even most) of the key volatile compounds of importance to coffee aroma.

In flavour chemistry, the sampling techniques most often coupled with SIDA/GC-MS are either some form of liquid-liquid sample extraction or headspace (HS) solid phase microextraction (SPME). Solid phase microextraction offers a sensitive, rapid, and solvent-free approach (Pawliszyn, 1997) and compared to other extraction methods is often preferred due to its simplicity which enables high throughput of samples. Nevertheless, in coffee flavour research, very few published studies report the use of HS-SPME coupled

with SIDA and GC-MS for volatiles analysis. These include the determination of furan in coffee (Bicchi et al., 2011), 14 important volatiles in Colombian Arabica and Indonesian Robusta coffees (Semmelroch et al., 1995), the analysis of alkylpyrazine in 10 commercial ground coffees (Pickard et al., 2013) and effect of different key brewing parameters on the alkylpyrazines of two Arabica coffee beverages (Pickard et al., 2014). The small range of targeted compounds in these previous studies limits the usefulness of these methods to quickly and efficiently analyse larger numbers of coffees for a comprehensive range of volatiles of likely importance to coffee. It would be beneficial to develop a high throughput method to analyse a broad range of important coffee volatiles which can be applied in research to better understand the spectrum of coffee flavour types available commercially and to be used in genetic population studies for plant breeding purposes.

The objective of this study was to develop a rapid, accurate and precise analytical method suitable for high sample throughput which utilises a combination of SIDA and GC-MS to simultaneously evaluate a broad range of important aroma volatiles of coffee. Such a comprehencive method could then be applied to more sufficiently evaluate the range and diversity of coffee volatile flavour.

4.2 Methodology

4.2.1 Materials and samples

For aroma volatiles analysis, reference pure compounds methylpropanal, 2,3-butanedione, 3-methylbutanal, 2-methylbutanal, ethyl-2-methylbutyrate, 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, D-limonene, 2-ethyl-3,5(6)-dimethyl-pyrazine, 2,3-diethyl-5-methylpyrazine, (E)-2-nonenal, 3-isobutyl-2-methoxypyrazine, linalool, geraniol, guaiacol, 4-vinylguaiacol, acetaldehyde, furfural, 5-methylfurfural, 3-methylbutyric acid, methional, 2-furfurylthiol, and 3-mercapto-3-methylbutylformate were purchased from Sigma Aldrich (Sydney, Australia); 2,3-pentanedione was purchased from Organics, Aromatic; 4-ethylguaiacol was purchased from TCI (Japan); *β*-damascenone was a gift from Dr. Dimitra Capone of The Australian Wine Research Institute (Adelaide, Australia). Commercially available internal Isotope labelled standards of corresponding compounds (deuterium labelled) d_6-2,3-butanedione, d_2-3-methylbutyraldehyde-2,2, d_5-2,3-pentanedione-1,1,1,4,4, d_9-ethyl (±) 2-methylbutyrate, d_3-linalool (d3-vinyl),

d_3-2-isobutyl-3-methoxypyrazine, d_3-2-methoxyphenol, d_5-4-ethyl- 2-methoxyphenol, d_4-acetaldehyde, d_3-3,4,5-furfural, d_9-3-methylbutyric acid and d_2-methional were purchased from CDN Isotopes (SciVac Pty Ltd, NSW). The d_5-D-limonene was synthesised by Anh et al (unpublished work) as a mixture fractions 9:1 and 1:1 of d_6-terpinolene and d_5-limonene. Sodium chloride (NaCl) salt, analytical grade ethanol, methanol, sulfuric acid (H_2SO_4), dichloromethane (CH_2Cl_2), sodium sulfate (Na_2SO_4), were supplied by Sigma Aldrich (Sydney, Australia). Positive ion EI mass spectra at 70 eV for all deuterated standards are shown in Table 4.1.

Samples of 26 commercial single-origin, medium-roasted, 'specialty' coffee samples (hereon mentioned as roasted coffees) being investigated includes 25 samples of Arabica coffees and one sample of Robusta coffee supplied by commercial coffee companies and roasters in Australia and Indonesia (see Chapter 3, p.39 for details). 4-Layer laminated polyethylene terephthalate/polyethylene terephthalate/aluminum foil/linear low-density polyethylene (PET/PET/Foil/LLDPE) pouches were used to repackage these samples under vacuum (±110 g) before storing them under -20°C until further use.

The coffee sample used for validation was a commercial single-origin coffee (Australia NSW) that was milled and bulked before further N_2 flushing for ±1 h to devolatilise (i.e. to reduce the concentration of volatiles). This coffee was stored under vacuum in an aluminium foil bag overnight before analysis.

A model coffee (phosphate buffer adjusted at pH 5.3) was also used for calibration and validation.

Table 4.1 Positive ion electron impact mass spectra of polydeuterated standards at 70 eV

Isotope standards	***m/z (%)***
d_6-2,3-butanedione	92 (M^+, 21), 91 (3), 56 (1), 54 (1), 53 (1), 47 (2), 46 (100), 45 (18), 44 (7), 43 (4), 40 (1)
d_2-3-methylbutyraldehyde-2,2	88 (M+, 6), 73 (30), 69 (2), 59 (40), 58 (59), 57 (4), 51 (2), 47 (12), 46 (100), 45 (50), 44 (19), 43 (72), 42 (48), 41 (51), 40 (23), 39 (25), 38 (4), 37 (2)
d_5-2,3-pentanedione-1,1,1,4,4	105 (M+, 19), 104 (34), 60 (3), 59 (48), 58 (4), 47 (4), 46 (100), 45 (11), 44 (7), 38 (1)

d_9-ethyl (±) 2-methylbutyrate	139 (M+, 2), 121 (8), 112 (6), 108 (5), 107 (60), 94 (37), 93 (12), 92 (3), 80 (2), 79 (19), 78 (2), 67 (5), 66 (100), 65 (9), 64(6), 60 (4), 58 (4), 53 (8), 46 (31), 42 (8)
d_5-D-Limonene*	141 (M^+, 45), 126 (25), 124 (12), 123 (23), 112 (22), 111 (18), 108 (10), 99 (18), 98 (39), 97 (23), 96 (18), 95 (28), 94 (35), 93 (100), 92 (53), 91 (18), 83 (11), 82 (11), 81 (23), 80 (26), 79 (40), 78 (12), 77 (20), 73 (79), 72 (40), 71 (35), 69 (11), 68 (91), 67 (68), 53 (22), 41 (20)
d_3-linalool (d3-vinyl)	157 (M+, 1), 139 (8), 124 (29), 109 (12), 96 (82), 95 (30), 85 (37), 74 (100), 69 (46), 55 (45), 53 (10), 43 (58), 41 (45), 39 (16)
d_3-2-isobutyl-3-methoxypyrazine	169 (M+, 2), 168 (3), 155 (2), 154 (20), 128 (9), 127 (100), 126 (4), 119 (2), 109 (4), 98 (7), 97 (9), 95 (18), 94 (10), 92 (2), 83 (7), 81 (8), 80 (4), 68 (3), 54 (6), 53 (8), 52 (3), 43 (5), 41 (8), 40 (5).
d_5-4-ethyl- 2-methoxyphenol	157 (M^+, 38), 139 (100), 140 (10), 124 (9), 96 (9), 95 (4)
d_4-acetaldehyde	49 (M^+, 1), 48 (48), 47 (3), 46 (21), 45 (1), 44 (4), 42 (2), 31 (1), 30 (100), 29 (2), 26 (2), 20 (3)
d_3-3,4,5-furfural	100 (M^+, 6), 99 (100), 98 (99), 97 (1), 71 (2), 70 (11), 54 (5), 53 (2), 52 (2), 51 (2), 50 (2), 44 (2), 43 (12), 42 (54), 41 (36), 40 (22), 39 (5), 38 (12), 37 (1), 36 (2), 30 (10), 29 (13), 28 (6), 27 (1), 26 (2)
d_9-3-methylbutyric acid	94 (M^+, 2), 93 (15), 79 (1), 74 (3), 66 (4), 65 (6), 64 (25), 63 (100), 62 (1), 51 (1), 50 (27), 49 (17), 48 (3), 47 (2), 46 (42), 45 (20), 44 (12), 43 (1), 42 (15), 41 (1), 40 (1), 34 (9), 33 (2), 32 (1), 31 (1), 30 (18), 29 (3), 28 (2)
d_2-methional	108 (M^+, 2), 107 (2), 106 (35), 105 (1), 91 (1), 48 (100), 80 (1), 79 (1), 78 (28), 77 (4), 76 (1), 63 (4), 62 (3), 61 (28), 60 (5), 58 (3), 57 (3), 56 (2), 51 (1), 50 (8), 49 (11), 47 (26), 46 (11), 45 (22), 44 (2), 43 (8), 42 (4), 41 (1), 40 (2), 39 (1), 38 (1), 37 (1), 36 (2), 35 (9), 34 (1), 33 (1), 32 (2), 31 (9), 30 (8), 29 (26), 28 (15), 27 (11), 26 (2)

4.2.2 Calibration and validation

Accuracy and precision of the measurement of each target compound was determined through preparation of a standard addition calibration equation in model coffee and a real coffee matrix against the relevant stable isotope internal standard. A Stock standard mixtures in ethanol were prepared and further serially diluted (1/10) in a model coffee (phosphate buffer adjusted at pH 5.3), miliQ water or dichloromethane to certain

concentration ranges between 0, 0.01 and 50000 µg/L. Calibration curves were constructed by using a minimum of six calibration points of standard solutions spiked with known amount of the isotope standards. The isotope labelled internal standards solutions were prepared in ethanol unless otherwise stated. Concentrations of these isotope solutions for spiking were optimised to suit the developed analytical methods. Table 4.2 presents concentration of isotope labelled internal standards (IS) and the solvents used for calibration and analysis of particular methods including headspace solid phase micro extraction /gas chromatography-mass spectrometry (HS-SPME/GC-MS), steam distillation extraction/HS-SPME/GC-MS, and steam distillation extraction/liquid injection/GC-MS.

Table 4.2 Effective 'coffee concentration' of isotope labelled internal standards (IS) added to the coffee samples prior to analysis

Isotope labelled internal standards (IS)	Concentration (µg/kg)	GC-MS method
d_6-2,3-butanedione	500	HS-SPME/GC-MS
d_2-3-methylbutyraldehyde-2,2	500	HS-SPME/GC-MS
d_5-2,3-pentanedione-1,1,1,4,4	500	HS-SPME/GC-MS
d_9-ethyl (±) 2-methylbutyrate	50	HS-SPME/GC-MS
d_5-D-limonene*	53	HS-SPME/GC-MS
d_3-linalool (d3-vinyl)	50	HS-SPME/GC-MS
d_3-2-isobutyl-3-methoxypyrazine	50	HS-SPME/GC-MS
d_5-4-ethyl- 2-methoxyphenol	500	HS-SPME/GC-MS
d_4-acetaldehyde	5272**	Steam distillation extraction/HS-SPME/GC-MS
d_3-3,4,5-furfural	6274**	Steam distillation extraction /HS-SPME/GC-MS
d_9-3-methylbutyric acid	5294**	Steam distillation extraction HS-SPME/GC-MS
d_2-methional	25240***	Steam distillation extraction /Liquid injection/GC-MS

Approximate concentrations were shown, but each was measured (and account for) exactly

*d_5-D-limonene was a in a mixture with d_6-terpinolene

**10 mL of 0.01 N H_2SO_4 containing d_4-acetaldehyde, d_3-3,4,5-furfural, d_9-3-methylbutyric acid was added prior to steam extraction

***100 µL of d_2-methional in methanol was added prior to steam extraction

Others in ethanol, directly added in a 20 mL SPME vials.

The method was validated by spiking two concentration of standard solutions in a model coffee and a real coffee at least. Precision and accuracy were determined as the coefficient of variation (CV%). Limit of detection (LOD) and limit of quantification (LOQ) were determined based on linearity of the calibration curve while also considering signal-to-noise ratio.

4.2.3 Sample preparation

Roasted coffee beans (±10-14 g in total per sample) were milled using Retsch Mixer Mill 400 ball mill at a maximum frequency of 30 cycles/s for 20 s, bulked and homogenised prior to extractions. When the samples could not be directly analysed, ground coffee were stored at -20°C under a nitrogen atmosphere in securely capped 30 mL brown glass vials until time of extraction.

4.2.4 Extraction

For the analysis of aldehydes, ketones, esters, pyrazines, phenols, terpenes and norisoprenoids, ground coffee was directly weighed (±2.00 g) into a 20 mL HS-SPME vial (Merck, Australia) before adding a 2 mL saturated aqueous NaCl solution, a 2 mL miliQ water, a magnetic stir flea (5 x 2 mm) and a 100 µL of stable isotope solutions mixture in known concentration (Table 4.2). Samples were prepared fresh every day of analysis. Prepared sample in vials were clearly labelled and analysed within 24 h. In a rare case that analysis could not be conducted within 24 h, samples were stored at 4°C (maximum of 18 h) and were gradually equilibrated to room temperature prior to analysis.

For the analysis of acetaldehyde, acids and furans, ground coffee (±2.00 g) was added to the Markham semi-micro distillation unit before adding a 10 mL of an internal standard in 0.01 N H_2SO_4 containing d_9-3-methylbutyric acid, d_3-furfural-3,5,4, and d_4-acetaldehyde (refer to Table 4.2). Steam distillation extraction was performed at 1 atm using 0.1 N H_2SO_4 and the extract was collected in a 20 mL HS-SPME vial containing 1 mL of 0.1 N H_2SO_4 to reach the volume of ±11 mL. A 2 g NaCl was added before the vial was securely capped. The contents were mixed by Vortex mixer to dissolve NaCl and the vial was stored at -20°C until time of analysis. At the time of analysis, the coffee extracts were removed from -20°C storage, equilibrated to room temperature and mixed thoroughly by vortex.

For the analysis of sulfur-containing compounds, ground coffee (± 4.00 g) was added to the Markham semi-micro distillation unit before adding a 100 uL d_2-methional (refer to Table 4.2) in methanol as an internal standard. Steam distillation extraction was performed at 1 atm using deionized water and the extract was collected in a 20 mL HS-SPME vial containing 4 mL of dichloromethane to reach the volume of ±20 mL. The vial was mixed (Vortex mixer) for 60 s on maximum speed and the solution (solvent + water) was allowed to separate for 10 min. The lower dichloromethane layer was removed and dried through 3 g of sodium sulphate in a Pasteur pipette into a micro Kuderna–Danish concentrator tube followed by a 0.5 mL dichloromethane rinse. The extract volume was reduced to approximately 0.4 mL and further concentrated to 0.2 mL using a gentle steam of nitrogen and transferred to a sample vial fitted with a reduced volume liner. The vial was then securely capped and stored at -20°C until time of analysis. The conditions are summarised in Table 4.3.

4.2.5 Instrumental conditions and sampling techniques

The samples were analysed by an Agilent 6890N gas chromatograph (GC) and an Agilent 5975 series Mass Selective Detector (MSD) unit (Agilent Technologies Inc., California, USA) with a MPS-2 multipurpose sampler (Gerstel, Germany) installed, or a Shimadzu GC-2010 gas chromatograph coupled with a Shimadzu GCMS-QP2010S mass selective detector (MSD) with a Combi-PAL autosampler (CTC Analytics, Zwingen, Switzerland). For both GC instruments, the interface and ion source temperatures were set to 250 °C (MS, 70 eV) and the MSD was set to selective ion monitoring (SIM). The carrier gas was helium (BOC gasses, ultra high purity). The GC column type and temperature program was optimised.

All of HS-SPME sampling was performed using a 10 mm long gray/plain divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30-μm SPME fibre (Supelco Inc., Bellefonte, PA.) fibre. PTV injection port for SPME sampling was equipped with a 0.75- mm I.D. borosilicate glass SPME inlet liner (Sigma-Aldrich Co., LLC). HS-SPME method was optimised for amount of sample, salt, water addition, fibre incubation time and temperature, desorption time and temperature. Table 4.3 details the GC-MS instrumental data that were optimised for each analytical method. SIM conditions are further detailed in Table 4.4.

Table 4.3 Details of methods used for coffee volatile analysis including target analytes, instrument, column type, method parameters and extraction conditions.

Parameter	***aldehydes, ketones, esters, pyrazines, phenols, terpenes and norisoprenoids***	***acetaldehyde, acids and furans***	***sulfur-containing compounds***
volatiles extraction from samples	direct HS-SPME	Steam, 1 atm, 10 mL of condensate collected into 1 mL 0.1 N H_2SO_4	Steam, 1 atm, approx 15 mL of condensate collected into 4 mL of dichloromethane
instrument	Agilent 6890N GC Agilent 5975 MSD (Agilent Technologies Inc., California, USA)	Shimadzu GC-2010 GC Shimadzu GCMS-QP2010S (MSD) (Shimadzu Corporation, Kyoto, Japan)	Shimadzu GC-2010 GC Shimadzu GCMS-QP2010S (MSD) (Shimadzu Corporation, Kyoto, Japan)
autosampler	MPS-2 multipurpose sampler (Gerstel, Germany)	Combi-PAL autosampler (CTC Analytics, Zwingen, Switzerland)	Combi-PAL autosampler (CTC Analytics, Zwingen, Switzerland)
column type	ZB-5ms, 60 m × 0.25 mm, 0.25-μm film thickness (Phenomenex, California, USA)	DB-Wax, 60 m× 0.25 mm i.d., 0.25 μm (Agilent Technologies Inc., California, USA)	DB-Wax, 60 m× 0.25 mm i.d., 0.25 μm (Agilent Technologies Inc., California, USA)
inlet liner	0.75- mm I.D. borosilicate glass SPME inlet liner (Sigma-Aldrich Co., LLC)	Agilent Crosslab Ultra Inert 1.25mm	Agilent Crosslab Ultra Inert 1.25mm
mode	splitless	split	splitless
injector temperature	200°C	250°C	250°C
Carrier gas	Helium	Helium	Helium
linear velocity	29 cm/s	32 cm/s	27.9 cm/s
flow rate	1.3 mL/min	1.57 mL/min	1.2 mL/min
inlet pressure	75.7 kPa	166.4 kPa	132.1 Kpa
total flow	70.3 mL/min	43.9 mL/min	34.1 mL/min
initial oven temp	40°C held for 2 min	40°C held for 1 min	40°C held for 1 min
1st ramp	5 °C/min to 170°C	2 °C/min to 135°C	4 °C/min to 150 °C

Parameter	*aldehydes, ketones, esters, pyrazines, phenols, terpenes and norisoprenoids*	*acetaldehyde, acids and furans*	*sulfur-containing compounds*
2nd ramp	20 °C/min to 240°C and held for 10 min	25 °C/min to 250°C and held for 6.9 min	30 °C/min to 250°C and held for 5 min
transfer line	250°C	250°C	250°C
split ratio	n.a.	25:1	n.a.
split vent opened	30 s	n.a.	60 s
injection type	SPME	SPME	liquid injection
SPME fibre type	10 mm long gray/plain DVB/CAR/PDMS 50/30-*μ*m (Supelco Inc., Bellefonte, PA.)	10 mm long gray/plain DVB/CAR/PDMS 50/30-*μ*m (Supelco Inc., Bellefonte, PA.)	na
liquid injection volume	na	na	1 uL
NaCl in SPME vial	2 mL (saturated)	2 g	n.a.
water addition	2 mL	na	n.a.
Sample in vial	2 g (coffee)	10 mL (steam extract) + 1 mL 0.1 N H_2SO_4	0.2 mL (concentrated extract)
SPME equilibration time and temp	50°C, 5 min	60°C, 20 min	n.a.
agitation	250 rpm	n.a.	n.a.
HS-SPME extraction time	15 min	30 min	n.a.
SPME extraction temp	50°C	60°C	n.a.
SPME desorption time	10 min	2.5 min	n.a.
ion source	250°C, 70 eV	250°C, 70 eV	250°C, 70 eV
quadrupole temp	150°C	n.a.	n.a.
MSD mode	SIM	SIM	SIM
Solvent delay	4 min	3 min	6.5 min
total run time	41.5 min	60 min	36.8 min
sample amount and dilution factor	n.a.	2 and 10	4 and 1

Parameter	*aldehydes, ketones, esters, pyrazines, phenols, terpenes and norisoprenoids*	*acetaldehyde, acids and furans*	*sulfur-containing compounds*
Data collection and identification	Enhanced ChemStation software MSD ChemStation G1701EA revision E.02.02	Shimadzu GCMS solution Version 4.20	Shimadzu GCMS solution Version 4.20
	NIST-05 library database G1033A revision D.05.01	NIST Mass Spectral Library	NIST Mass Spectral Library

na: not applicable

4.2.6 Quantification of targeted volatiles

The selected SIM ions were used to assist identification and quantification of the targeted volatiles in coffees. Response factors were calculated for all calibration levels used for quantification as the ratio between concentration and peak area of analyte against those of the corresponding labelled standard (Bicchi et al., 2011). The concentration of the targeted volatiles in the samples was determined following the equation used by Bicchi et al. (2011):

$$C_{analyte} = \left(\frac{m_{labeled}}{m_{coffee}} x \frac{A_{analyte}}{A_{labeled}} \right) xRF$$

where,
$m_{labeled}$ is the amount of corresponding labelled compounds added to the sample analysed;
m_{coffee} is the amount of coffee analysed;
$A_{analyte}$ is the area of analyte;
$A_{labeled}$ is the area of corresponding labelled compounds;
RF is the response factor.

4.2.7 Statistical analysis

Mean, standard deviation and CV (%) were calculated on the model coffee data set for calibration and method validation purposes. General descriptive statistical analysis and analysis of variance (ANOVA) were performed on a triplicate analysis of coffee samples. Where a significant difference observed, further post-hoc analysis of Tukey-Kramer HSD at 95% confidence interval was performed. Principal component analysis was performed to explore diversity and to know relationships between volatile compounds measured. All statistical analysis was performed using XLSTAT version 2015 (Addinsoft, New York, USA).

4.3 Results and discussion

The objective of this work was to develop a robust and high throughput method for measuring key coffee volatiles for application in experiments to evaluate and profile the diversit of coffee flavour. To select the targets for method development a thorough review of literature was conducted to summarise the knowledge and rank those coffee volatiles thought to be of greatest importance to coffee aroma (refer to table presented in Sunarharum et al. (2014)). The top 30-50 ‘most important’ compounds were evaluated in

terms of availability of standards and suitability for GC-MS method development. Standards of the target compounds were purchased, along with a corresponding stable isotope (where available), and initial trials were conducted to identify those volatiles that would be suited more to direct HS-SPME or other sample preparation method where a pre-extraction step may be required.

The compounds for which methods were successfully developed are shown in Table 4.3, together with the sample preparation technique most suited for each compound. Importantly, compounds that would have been highly desirable to include as targets, but were not targeted due to various challenges and limitations, included furanone such as sotolon and abhexone, certain ketone such as 4-(4-hydroxyphenyl)-2-butanone, certain sulfur-containing compounds including thiophene, thiazole, pyridine, pyrrole, as well as some pyrazines including the ethenylpyrazines (Table 2.1). These compounds are know to be important to the flavour of certain coffee types, however, access to standards, complications due to the reactive nature of many of these compounds present at trace levels, together with project time and resourse limitations, meant that they were not targetted in this instance.

4.3.1 Analytical method development

A combination method of SIDA and GC-MS was successfully achieved for the quantitative measurement of 27 key aroma volatile compounds in coffee. The first attempt was made to analyse many compounds simultaneously. While it would have been ideal to include all compounds in a one-step analysis, the nature of the coffee matrix and the individual analytical requirements for each compound, leading to the need of developing three different methods. The author of this thesis was directly involved in developing the first of the three methods involving direct HS-SPME/GC-MS analysis of 20 compounds including aldehydes, ketones, esters, pyrazines, phenols, terpenes and norisoprenoids. Technical assistance was provided to develop the other two analytical methods involving steam distillation extraction/HS-SPME/GC-MS (for analysis of four compounds including acetaldehyde, acids and furans) and a steam distillation extraction/liquid injection/GC-MS method (for analysis of three sulfur-containing compounds). All methods were applied by the author, to measure the coffees in this study.

Selected ion monitoring (SIM) was used to identify the analytical targets in all the methods employed. The target quantitation and qualifier ions selected for each analyte and labelled internal standard are shown in Table 4.4, together with each analytes retention time.

In the study, it was also observed that during a 'run' of samples, the retention times of a few compounds, such as methylpropanal and 2,3-butanedione, were observed to occasionally drift. These early eluting compounds, mainly methylpropanal, had a retention time (RT=4.547) that is close to the solvent (ethanol). Therefore ethanol may have been interfering in the column chromatography and resulting separation of methylpropanal at very low concentrations. Retention time drift was also observed for a few other compounds at different concentration levels. This can present problems when the targeted compound co-elutes closely with other compounds or indeed with their corresponding labelled isotope internal standard. The interference of some ions might have occurred at eluting peaks due common ions but the most stable ions were chosen to eliminate this problem. For example, *m/z* 41 was used for identification of methylpropanal, and *m/z* 44 was found to better separate the d_2-3-methylbutyraldehyde-2,2 from its analogue at a low concentration.

Table 4.4 Analytical parameters for target volatiles and corresponding internal standards as applied for the analysis of coffee

Analyte	**RT (min)**	**Target ion *m/z***	**Qualifier ions *m/z* (%)**	**Internal standards**	**RT (min)**	**Target ion *m/z***	**Qualifier ions *m/z* (%)**
aldehydes, ketones, esters, pyrazines, phenols, terpenes and norisoprenoids							
methylpropanal	4.5	41	43 (220), 72 (95), 39 (41)	d_2-3-methylbutyraldehyde-2,2	5.8	59	43 (180), 58 (135), 46 (270)
2,3-butanedione	4.8	43	86 (14), 42 (7), 44 (2)	d_6-2,3-butanedione	4.8	46	45 (18), 43 (4), 92 (21)
3-methylbutanal	5.8	44	41 (214), 43 (94), 58 (82)	d_2-3-methylbutyraldehyde-2,2	5.8	59	43 (180), 58 (135), 46 (270)
2-methylbutanal	6.0	57	41 (168), 58 (74), 39 (26)	d_2-3-methylbutyraldehyde-2,2	5.8	59	43 (180), 58 (135), 46 (270)
2,3-pentanedione	6.6	57	100 (45), 42 (3), 43 (198)	d_5-2,3-pentanedione-1,1,1,4,4	6.4	59	46 (210), 105 (41)
ethyl-2-methylbutyrate	10.8	102	57 (130), 85 (60), 74.10 (50)	d_9-ethyl (±) 2-methylbutyrate	10.6	66	107 (60), 94 (37), 46 (31)
2,5-dimethylpyrazine	12.9	108	42 (50), 39 (18), 40 (3)	d_3-2-isobutyl-3-methoxypyrazine	21.4	127	154 (20), 95 (18), 94 (10)
2,3-dimethylpyrazine	13.2	108	67 (79), 40 (16), 42 (18)	d_3-2-isobutyl-3-methoxypyrazine	21.4	127	154 (20), 95 (18), 94 (10)
D-limonene	16.9	68	93 (74), 67 (74), 79 (37)	d_5-D-limonene	16.8	93	68 (91), 73 (79), 67 (68)

Analyte	**RT (min)**	**Target ion *m/z***	**Qualifier ions *m/z* (%)**	**Internal standards**	**RT (min)**	**Target ion *m/z***	**Qualifier ions *m/z* (%)**
2-ethyl-3,6-dimethyl-pyrazine	18.4	135	136 (74), 42 (21), 39 (14)	d_3-2-isobutyl-3-methoxypyrazine	21.4	127	154 (20), 95 (18), 94 (10)
2-ethyl-3,5-dimethylpyrazine	18.6	135	136 (77), 56 (33), 39 (18)	d_3-2-isobutyl-3-methoxypyrazine	21.4	127	154 (20), 95 (18), 94 (10)
guaiacol	18.8	109	124 (46), 81 (36), 53 (12)	d_5-4-ethyl- 2-methoxyphenol	24.3	139	157 (38), 140 (10), 124 (9)
linalool	19.1	71	93 (115), 41 (78), 43 (62)	d_3-linalool (d_3-vinyl)	19.1	74	96 (82), 43 (58), 41 (45)
2,3-diethyl-5-methylpyrazine	20.8	150	135 (78), 149 (70), 56 (29)	d_3-2-isobutyl-3-methoxypyrazine	21.4	127	154 (20), 95 (18), 94 (10)
(E)-2-nonenal	21.0	41	43 (81), 55 (94), 70 (90)	d_2-3-methylbutyraldehyde-2,2	5.8	59	43 (180), 58 (135), 46 (270)
3-isobutyl-2-methoxypyrazine	21.5	124	41 (61), 93 (32), 68 (19)	d_3-2-isobutyl-3-methoxypyrazine	21.4	127	154 (20), 9(18), 94 (10)
geraniol	23.6	69	41 (61), 93 (32), 68 (19)	d_3-linalool (d_3-vinyl)	19.1	74	96 (82), 43 (58), 41 (45)
4-ethylguaiacol	24.4	137	152 (38), 122 (11), 91 (11)	d_5-4-ethyl-2-methoxyphenol	24.3	139	157 (38), 140 (10), 124 (9)
4-vinylguaiacol	25.5	150	135 (57), 107 (21), 77 (21)	d_5-4-ethyl-2-methoxyphenol	24.3	139	157 (38), 140 (10), 124 (9)
β-damascenone	27.4	69	121 (82), 105 (32), 190 (22)	d_3-linalool (d_3-vinyl)	19.1	74	96.10 (82), 43 (58), 41 (45)

Analyte	RT (min)	Target ion *m/z*	Qualifier ions *m/z* (%)	Internal standards	RT (min)	Target ion *m/z*	Qualifier ions *m/z* (%)
acetaldehyde, acids and furans							
acetaldehyde	3.8	29	41 (4)	d_4-acetaldehyde	3.8	30	48 (48)
furfural	33.2	96	95 (98), 67 (13)	d_3-3,4,5-furfural	33.1	99	98 (99), 70 (11)
5-methylfurfural	33.0	110	109 (88), 53 (81), 81 (15)	d_3-3,4,5-furfural	33.1	99	98 (99), 70 (11)
3-methylbutyric acid	45.9	60	43 (52)	d_9-3-methylbutyric acid	45.4	463	46 (42)
sulfur-containing compounds							
methional	22.5	104	76 (84)	d_2-methional	22.4	106	78 (86)
2-furfurylthiol	21.8	114	53 (247), 81 (406)	d_2-methional	22.4	106	78 (86)
3-mercapto-3-methylbutylformate	24.7	69	75 (13), 102 (22)	d_2-methional	22.4	106	78 (86)

% is relative to target ion of the compound.

Table 4.5 Calibration parameters achieved for target volatiles in a model coffee matrix

Targeted volatile compounds	Concentration in coffee (µg/kg)[citation]*	Aroma threshold (µg/kg) [citation]*	Calibration range (µg/kg) (1/10 dilution)**	Actual working range*** (µg/kg)	Calibration equation	R^2	RRF#	CV(%)#
aldehydes, ketones, esters, pyrazines, phenols, terpenes and norisoprenoids								
methylpropanal	32,300[1]	0.7[11]	0, 10-10000	11-11310	y = 0.0162x2 + 0.7345x - 0.0576	0.9978	0.63	8.7
3-methylbutanal	18,600[1]	0.25[11]	0, 50 -10000	56-11100	y = 1.0774x + 0.0048	0.9954	1.4	3.1
2-methylbutanal	20,700[1]	0.9[11]	0, 0.2-10000	0.2-11420	y = 0.1926x2 + 7.8241x - 0.9328	0.9991	5.9	3.9
(E)-2-nonenal	25[3]	0.08[12]	0, 0.2-10000	0.2-10890	y = 38.965x - 8.2509	0.9938	0.02	6.2
2,3-butanedione	48,400-50,800[1,2]	0.3 [a,13]	0, 10-20000	12-24300	y = 1.9993x - 0.5118	0.9898	1.8	4.1
2,3-pentanedione	3,540-39,600[1,7,2]	20 [a,13]	0, 5-20000	6-24040	y = 0.9033x + 0.0907	0.9956	1.5	4.5
ethyl-2-methylbutyrate	3.9[6]	0.5[d,6]	0, 0.01, 50000	0.01-58000	y = 4.3788x + 2.0072	0.9969	0.66	5.0
2,5-dimethylpyrazine	4,550-11,730[7]	80 [a,13]	0, 50-20000	62-24720	y = 0.0184x - 0.1581	0.9902	0.01	4.9
2,3-dimethylpyrazine	2,580-6,100[7]	800 [a,13]	0, 50-20000	57-22980	y = 0.01x + 0.031	0.9945	0.01	3.0
2-ethyl-3,5-dimethyl-pyrazine	55-330[1,3, 4]	0.04[16]	0, 0.01-50000	0.01-54350	y = 0.2402x + 0.033	0.9988	0.07	5.0
2-ethyl-3,6-dimethyl-pyrazine	2,570-5,980[7]	8.6[16]	0, 0.1-50000	0.1-54350	y = 0.2469x + 0.022	0.9989	0.28	5.5
2,3-diethyl-5-methylpyrazine	73-95[1,3]	0.09 [a,13]	0, 0.01-50000	0.01-58000	y = 0.8511x + 0.3671	0.9955	0.12	5.7
3-isobutyl-2-methoxypyrazine	59-97[1,3,6]	0.002[12]	0, 0.01-50000	0.01-54550	y = 4.663x + 3.0715	0.9943	1.2	4.8
D-limonene (as limonene in ref)	1,080-1,380[17]	4 [a,13]	0, 0.01-10000	0.01-12740	y = 0.0013x2 + 1.7641x - 2.5017	0.9876	3.0	16

Targeted volatile compounds	Concentration in coffee (µg/kg)[citation]*	Aroma threshold (µg/kg) [citation]*	Calibration range (µg/kg) (1/10 dilution)**	Actual working range*** (µg/kg)	Calibration equation	R^2	RRF#	CV(%)#
guaiacol	2,000-17,970[1,3,7, 10]	2.5[3]	0, 50-50000	54-53650	y = 0.6404x + 0.0251	0.9933	0.72	4.8
4-ethylguaiacol	800-24,800[1,6,7,3,10]	50[3]	0, 2-50000	3-66400	y = 0.377x + 0.1594	0.9958	0.43	2.4
4-vinylguaiacol	8,000-64,800[1,3,7, 10]	20[3]	0, 200-50000	230-57550	y = 5E-05x2 + 0.0007x + 0.0015	0.9559	0.002	14
linalool	780-1310[17]	0.17 [b,6]	0, 1-20000	1-10630	y = 8.9044x - 4.1877	0.9671	1.50	4.5
geraniol	-	1.1[14]	0, 50-5000	58-11540	y = 0.0214x2 + 6.486x - 0.9061	0.996	0.32	21*
β-damascenone (as E- *β*-damascenone in ref)	195-255 [1,3,6,	0.00075[3]	0,0.05-100	0.05-101.92	y = 48.114x + 0.0051	0.9771	3.0	4.3
acetaldehyde, acids and furans								
acetaldehyde	139,000[1]	0.7[a,13]	0, 3800-22820	3800-22820	y = 1.000143x	0.9985	0.99	2.8
furfural	5,880-18,370[7]	280[a,13]	0, 5380-32280	5380-32280	y = 1.076896x	1.0000	1.1	0.24
5-methylfurfural	-	6000[a,13]	0, 4610-27660	4610-27660	y = 2.281358x	0.9997	0.99	1.6
3-methylbutyric acid	18,060-32,180[4,7]	700[c,15]	0, 4370-26190	4370-26190	y = 1.411605x	0.9995	0.99	3.2
sulfur-containing compounds								
methional	213-240[6,8]	0.2[3]	0, 100-6000	100-6000	y = 0.9861x + 0.0032	0.9985	1.00	2.3
2-furfurylthiol	1,080-5,080[3,9,7,	0.01[3]	0, 1000-20000	1000-20000	y = 2.1611x - 0.4681	0.9964	1.8	27*

Targeted volatile compounds	Concentration in coffee (µg/kg)[citation]*	Aroma threshold (µg/kg) [citation]*	Calibration range (µg/kg) (1/10 dilution)**	Actual working range*** (µg/kg)	Calibration equation	R^2	RRF#	CV(%)#
3-mercapto-3-methylbutylformate	130[3]	0.0035[5]	0, 100-3000	100-3000	y = 10.9692x - 0.0796	0.9995	10	8.1

* As previously summarised by Sunarharum et al. (2014) with a slight updates, concentrations were converted to µg/kg for ease of comparison. [1](Czerny et al., 1999); [2](Semmelroch and Grosch, 1996); [3](Semmelroch et al., 1995); [4](Mayer and Grosch, 2001); [5](Holscher and Steinhart, 1992); [6](Czerny and Grosch, 2000); [7](Cheong et al., 2013); [8](Balzer, 2001); [9](Tressl and Silwar, 1981); [10](Silwar et al., 1987); [11](Milo and Grosch); [12](Belitz et al., 2009); [13](Burdock, 2010); [14](Czerny et al., 2008); [15](Salo, 1970); [16](Buttery and Ling, 1997); [17]in coffee brew (Piccino et al., 2014).

Authors report compound concentration within the range indicated, these concentrations relate to roasted Arabica and Robusta coffee grounds or beans (weight/weight), otherwise stated. Where no concentration is listed, none could be found in the literature. Extractions of the compounds were performed by using solvents such as dichloromethane, diethyl ether, methanol, pentane, hexane or water or combination between few different solvents (refer to Table 2.1 for details)

Where two or more aroma thresholds were found, the lowest is presented. All aroma thresholds were determined in water except: [a] matrix unknown; [b] threshold measured by first diluting compounds in ethanol in a defined concentration and then dissolved in water, for linalool as R-linalool; [c] in ethanolic solution 9.5%; [d]threshold in cellulose.

**coffee diluted ten times.

***as linearity range observed from calibration curve, the lowest actual working range showed the lower limit of quantification (LOQ) of the compounds, signal-to-noise ratio >10.

#relative response factor (RRF) was calculated as an average of response factors across developed calibration range. CV(%) for RRF is also presented.

4.3.1.1 Method validation

The precision, accuracy and repeatability of the method was determined in model coffee and was validated in a real (de-volatilised) coffee matrix. Table 4.5 presents the calibration parameters for target volatiles in model coffee. A linear calibration function was achieved for each compound throughout the concentration range shown with the exception of methylpropanal, 2-methylbutanal, D-limonene, 4-vinylguaiacol and geraniol, for which a quadratic calibrations were a better fit. The quadratic calibration demonstrate that these compounds may have a different behaviour on the SPME fibre (DVB/CAR/PDMS) or GC ZB5-ms column due to varying partition coefficient and extraction kinetics. This could be related to selectivity of the coating polymers of the SPME fibre (Siebert et al., 2005) and GC column, as well as polarity of the compounds (Mestdagh et al., 2014).

In a real coffee matrix, while some of targeted compounds showed good calibration performance, it seems that some other compounds such as 2,3-butanedione, methylpropanal, 2-methylbutanal, D-limonene, 4-vinylguaiacol, 2,5-dimethylpyrazine and 2,3-dimethylpyrazine showed a poor performance (R^2<0.90). The coffee matrix is very complex and therefore finding an ideal model matrix representing the coffee matrix for calibration purpose would still be a challenge. Previously, water (Bicchi et al., 2011) or soft drink made of fruit juice concentrate resembling coffee-matrix in lipid, carbohydrate and protein (Pickard et al. 2014) has been used previously. The application of the current method may require re-validation should different conditions or samples be applied.

The developed methods were generally acceptable for most of the compounds with some exceptions. The average coefficient of variation (CV) (shown in Table 4.6) for aldehydes, ketones, esters, pyrazines, phenols, terpenes and norisoprenoids compounds group was calculated based on at least triplicates spiked addition of standards to a model coffee matrix or an actual coffee matrix (two concentrations x three or six reps) while the data was missing (analysis on spiked standard was not performed) for acetaldehyde, acids and furans, as well as sulfur-containing compounds. Generally, average CV<20% was observed in both model coffee and in an actual coffee matrix, except for geraniol and 2,3-dimethylpyrazine. Nevertheless, in general, the CV of most of the compounds fell below an acceptable range within 20% (FDA, 2001). The CV of maximum of 20% was taken as an arbitrary acceptance level for the concentrations of individual compounds on

each single-origin coffee sample. Variation of CV>20% could possibly occur in natural coffee samples due to separation of the lipid layer in the coffee brew. Volatile kinetics and effect of fat content on reducing release rate of certain volatiles in the emulsion system has been previously reported (Frank et al. 2012, Mestdagh et al., 2014, Itobe et al., 2015).

The developed calibration range covers the reported concentration range of each compound in coffee while also considering their individual sensory thresholds. For example, the largest calibration range developed was for ethyl-2-methylbutyrate, 2-ethyl-3,5-dimethyl-pyrazine, 2,3-diethyl-5-methylpyrazine, and 3-isobutyl-2-methoxypyrazine, with an approximate concentration range of 0.01 to 50000 µg/kg. This range mostly covers the aroma threshold of each of the compounds except for 3-isobutyl-2-methoxypyrazine, which was reported to have a very low aroma threshold of 0.002 (Belitz et al., 2009). The compound *β*-damascenone used the lowest calibration range (0.05-101.92 µg/kg) since it was previously reported to have a much lower aroma threshold at 0.00075 (Semmelroch and Grosch, 1995) and was expected to be found in coffee at low concentration.

Table 4.6 Average of coefficient of variation (CV%) of the targeted volatile compounds (aldehydes, ketones, esters, pyrazines, phenols, terpenes and norisoprenoids) measured in model coffee (two concentrations x six replicates) and coffee matrix (two concentrations x three replicates)

Targeted volatile compounds	**Model coffee**	**Coffee matrix**
	CV (%)	**CV (%)**
2,3-butanedione	3.4	13
methylpropanal	6	15
3-methylbutanal	2	8.6
2-methylbutanal	2.4	5.5
(E)-2-nonenal	4.6	12
2,3-pentanedione	3	16
ethyl-2-methylbutyrate	3.7	3.6
2,5-dimethylpyrazine	4.3	7.6
2,3-dimethylpyrazine	2	30*
2-ethyl-3,6-dimethylpyrazine	2.9	9
2-ethyl-3,5-dimethylpyrazine	2.8	7.6
2,3-diethyl-5-methylpyrazine	2.7	6.2
3-isobutyl-2-methoxypyrazine	2.4	3.7
D-limonene	2.2	6.2

Targeted volatile compounds	Model coffee	Coffee matrix
	CV (%)	CV (%)
guaiacol	1.8	5.6
4-ethylguaiacol	2.3	4.8
4-vinylguaiacol	9	4.3
linalool	3.2	3.7
geraniol	22*	28*
β-damascenone	3.8	7.8

Average CV was presented, CV>20% were indicated in asterix (*).

Generally, the calibration range targeted suited the aroma threshold and concentration range found in coffee even though some compounds might have been present in coffee samples at a higher concentration than the developed range, such as for some aldehydes (methylpropanal, 3-methylbutanal, 2-methylbutanal, acetaldehyde), ketones (2,3-butanedione and 2,3-pentanedione), *β*-damascenone, 3-methylbutyric acid and furfural. However, the calibration functions for these compounds were thought to give acceptable accuracy and were adequately used for quantification at higher concentration ranges (double or triple) even though a slightly decreased R^2 value might be expected.

There was less sensitivity (poor peak shape at a low concentration) observed on the detection of 2,3-dimethyl pyrazine and 2,5-dimethyl pyrazine as compared to the other pyrazines. In contrast, the detection of certain compounds such as D-limonene, ethyl-2-methylbutyrate, (E)-2-nonenal, 3-isobutyl-2-methoxypyrazines and 2,3-diethyl-5-methylpyrazine was better at lower concentrations. Perhaps this is due to reduction of ethanol (solvent) during dilution and therefore less ethanol interference on the SPME fibre. The quantification of (E)-2-nonenal was found to be affected by a high background noise recorded on the chromatogram due to changes in the coffee matrix of certain coffee types.

The limit of quantification (LOQ) of some compounds such as those belongs to phenols, acetaldehyde, acids, furans and sulfur-containing compounds was high, indicating less sensitivity of the method (or the GC column) to quantify them at a low concentration. However, it would not be a problem for coffee analysis since those compounds were usually present in a very high concentration in coffee. For example, 4-vinylguaiacol with lower LOQ at 230.2 µg/kg typically present in coffee at between 8,000-64,800 µg/kg

(Czerny et al., 1999, Semmelroch et al., 1995, Silwar et al., 1987, Tressl and Silwar, 1981, Cheong et al., 2013).

At a very high concentration (approximately 50000 µg/kg), D-limonene showed a very large peak area, which meant the labelled isotope could not be identified due to a large common ion interference. Other compound such as (E)-2-nonenal were also found to have a wide peak shape, making quantification at the 50000 µg/kg level impractical. Different behaviour was observed for guaiacol, where two peaks (split peak) were formed at a concentration of 10000 µg/kg and higher due to GC inlet overload, even though the compound could be still be quantified based on the selected ion *m/z* 109. Regarding the compound geraniol, even though sensitivity increased with concentration, the peak was found to be tailing mainly at a very high concentrations. It may be that, apart from overloading the GC inlet, analyte carry-over on the fibre or column may also explain the unusual behaviour of certain analytes at very high concentration. It may be that an alternate column would be more suited to these compounds or some other pre-preparation step or different extraction technique was required. However, this method was designed to measure as many compounds as possible in one single step, so a compromise was necessary to achieve reasonable calibration functions and statistic for each compound.

4.3.1.2 The benefits of selective ion monitoring (SIM)

Selective ion monitoring (SIM) in mass spectrometry applied for the analysis of coffee volatiles, offered many advantages in comparison to scan mode. Firstly, using mass spectral ion peaks from selected ions from SIM were found to increase precision in analysis compared to peaks obtained in full scan (*m/z* 35-350). Figure 4.1 depicts a typical chromatogram of coffee volatiles evaluated comparing peaks from scan and SIM, the specific example being d_5-4-ethyl-2-methoxyphenol, 4-ethylguaiacol and 4-vinylguaiacol as shown. The selection of specific ions aids in identification and quantification of target analytes as it focuses only on those ion peaks of interest from the otherwise very complex chromatogram of coffee. Indeed the SIDA method itself relies on quantification using selected ions whereby co-elution of the labelled isotope standard and analyte often occurs and each compound can only be distinguished due to their distinctive ion fragments. This is because the deuterium labelled isotope contains a deuterium atom in place of hydrogen atom, thus ion fragments typically have a larger mass and a distinctive ion. For example,

d_3-2-isobutyl-3-methoxypyrazine could be discriminated against 3-isobutyl-2-methoxypyrazine using *m/z* 127 and *m/z* 124, respectively. The presence of three deuterium atoms in the H position on the methyl group (OCH_3 → OCD_3) increased the weight of the molecular ion from *m/z* 124 for 3-isobutyl-2-methoxypyrazine to *m/z* 127 (d_3-2-isobutyl-3-methoxypyrazine). This was also the case for for d_5-4-ethyl-2-methoxyphenol, consisting 5 deuterated hydrogen (*m/z* 157) in the structure, differentiating it from the analyte compound, 4-ethyl-2-methoxyphenol (4-ethylguaiacol, *m/z* 152).

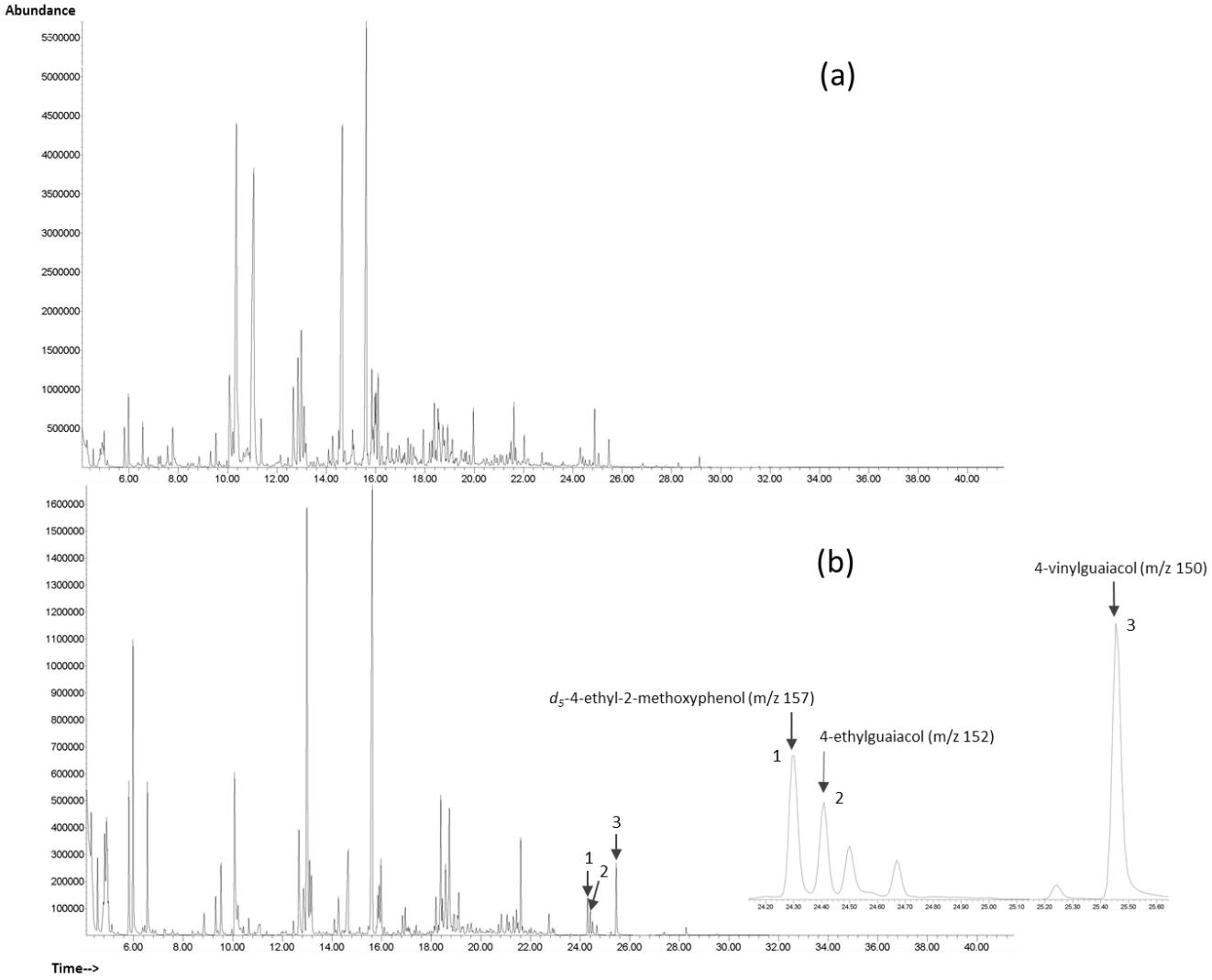

Figure 4.1 A typical coffee gas chromatogram trace of a direct HS-SPME in (a) full scan, and (b) SIM

In this study, where no isotopically labelled standard could be purchased, another labelled standard, from a similar chemical class, was used for identification and quantification of compounds. For example, 2,2-d_2-3-methylbutyraldehyde was used to measure and to quantify target aldehyde compounds 3-methylbutanal, 2-methylbutanal, (E)-2-nonenal. Similarly, d_3-linalool (d_3-vinyl) was used to build calibrations for terpenes linalool, geraniol and *β*-damascenone; d_5-4-ethyl-2-methoxyphenol was used to quantify all the target phenolic compounds; and d_3-2-isobutyl-3-methoxypyrazine was used to quantify all of target the pyrazines. While this was less than ideal, as usually SIDA required the internal standard to be identical to the target analyte except for the label, custom synthesis for all isotope labelled compounds was not possible in all instances. Further, not all matching isotopes could be used to quantify the corresponding analyte due to other co-elution issues and lack of unique ions as was the case for d_3-2-methoxyphenol and 2-methoxyphenol (guaiacol). In this instance, instead of using d_3-2-methoxyphenol, d_5-4-ethyl-2-methoxyphenol was used for quantification of guaiacol.

To ensure robustness of the calibrations, the ratio response factor (RRF) of the compounds in model coffee was calculated (Table 4.4). Nevertheless, accuracy was optimum when identical isotopes analyte pairs were used for calibration. In SIDA, it is expected that the labelled analogue of a given target analyte will behave chemically and physically in an identical way in the matrix and during extraction and analysis, thus giving an ideal relative ratio to rely on for analysis.

In rare cases during analysis, the compound 3-methylbutanal co-eluted with acetic acid when the acid concentration was high which caused complications in calibration. The same phenomenon was observed for ethyl-2-methylbutyrate that often co-eluted with methylbutyric acids during analysis of real coffees. Even though ion interference, co-elution or partial co-elution with few compounds occurred, selections of specific ions through SIM proved particularly useful. As an example, the specific ion of 3-methylbutanal (*m/z* 44) and ethyl-2-methylbutyrate (*m/z* 102) along with their few other qualifier ions were sufficient for identification and quantification purpose of these targeted compounds.

The analytical method was optimised during preliminary method development. The optimisation parameters explored included the addition of water (1-2 mL), saturated salt solution (1-2 mL), weight of coffee sample (1-2 g), incubation time (5-15 min), incubation

temperature (40-60°C), desorption time (600-800 s), GC column types (DB wax and ZB5ms) and column length (30 m and 60 m), and GC temperature programming (initial GC temperature and temperature ramps). The resulting method proved to be an optimised method which was a compromise between certain analytes to enable a large range of compounds to be measured in a single step. The addition of 2 mL of water and 2 mL of saturated salt solution was found to be optimum to allow homogenisation of the coffee-water matrix and the release of volatile compounds. While a DB-wax column (high polarity) performed better than the ZB-5ms in the separation of polar compounds, co-elution of some early eluting compounds occurred with the DB-wax such as between 2-methylbutanal, 3-methylbutanal and 2,3-butanedione, and also between guaiacol and geraniol. The ZB-5ms GC column offered a better separation of those compounds, and the 60 m length of this column type performed better than the 30 m. The limitation of the ZB-5ms column used in this analysis was that some of the acids, furanones, thiols (sulfuric compounds) and a few of the target furans could not be sufficiently detected and had to be quantified using another approach. The alternative method which targeted acetaldehyde, furans, acids and sulfur-containing compounds involved solvent extraction of the volatiles from coffee samples using sulfuric acid or dichloromethane to eliminate matrix influences. Besides trapping the extracted volatiles, the sulfuric acid improves the separation process due to its role as a catalyst (Olmsted et al., 1929, Zeitsch, 2000). Similarly, dichloromethane is also used to trap the volatiles and increase effectiveness of the extractions (Blanch et al., 1993, Gu et al., 2009, Xu et al., 2016).

The compromise methods developed for analysis of coffee were suitable for the purpose of the current study to rapidly identify and quantify of a range of target volatile compounds. These methods were further applied to 26 roasted coffees (Arabica and Robusta) samples to explore coffee volatile flavour diversity and to demonstrate the usefulness of the methods.

4.3.2 Application of analytical methods to measure key aroma volatiles in commercial single-origin coffees

A total of 27 volatile compounds, of known importance to coffee flavour, were measured in 26 roasted coffees. The results of this analysis are summarised in Table 4.7. The level (mean data) of each volatiles evaluated in each coffee samples can be viewed in Appendix

D (p.175) and Appendix E (p.177). Table 4.8 presents the odour activity value (OAV) calculations for the volatiles measured in the 26 coffees. The OAV was calculated for each volatile compounds as the ratio of each compounds concentration to the odour threshold with the purpose of highlighting the likelihood of importance to the odour of the coffees (Grosch, 2001b). All compounds were measured above their reported sensory thresholds in all of the coffees, demonstrating the suitability of the target list of volatiles for quantification. Of the 27 compounds measured, only (E)-2-nonenal was not detected in a few of the samples, including the Australia NQ1, Australia NSW and Bolivia coffees, and geraniol, one of important monoterpenes, was not found in Ethiopian coffees (Ethiopia 1 and Ethiopia 2).

As shown in Table 4.7, generally, major volatiles present in high concentration are furfural, 2,5-dimethylpyrazine, 3-methylbutyric acid, acetaldehyde, and 5-methylfurfural. Quantitatively, (E)-2-nonenal, ethyl-2-methylbutyrate, and β-damascenone present in a very low amount while geraniol was hardly detected and quantified in most of the coffee samples. Varietal differences resulted in key variations in volatile composition such as a higher concentration of aldehydes, ketones, terpenes, and lower concentration of phenols and pyrazines in Arabica compared to Robusta.

Concentrations of some compounds such as 2,3-butanedione, *β*-damascenone, (E)-2-nonenal, ethyl-2-methylbutyrate were found to be lower than reported previously (Czerny and Grosch, 2000, Czerny et al., 1999, Semmelroch and Grosch, 1995, Semmelroch and Grosch, 1996) while others are in the range or above previously reported concentrations in coffee, such as for 2,5-dimethylpyrazines and 4-ethylguaiacol (Cheong et al., 2013).

The current study provides an insight on the concentration of several key aroma volatiles present in single-origin roasted coffees worldwide. Some of the compounds being investigated, particularly the volatile terpenes, have not previously been quantified accurately although their presence and peak abundance have been reported previously (Del Terra et al., 2013, Gonzalez-Rios et al., 2007, Mondello et al., 2005, Akiyama et al., 2008). Mostly found in coffee flowers and berries, terpenes are known to remain in the roasted coffee beans and corresponding brews (Stashenko et al., 2013, Emura et al., 1997, Akiyama et al., 2008). As known contributors the boquet of wines and beers, these

monoterpenes are also likely to influence coffee flavour. Indeed, linalool is reported to contribute to a *woody* or *flowery* note in coffee depending on concentration (Bonnländer et al., 2006). While previous reports of terpenes in coffees are very limited, this is one of few quantitative report on the concentration of volatile monoterpenes in coffee, namely D-limonene, geraniol and linalool. To the author knowledge, this is the first quantitative report on geraniol concentration in coffee. It is also the first report on the volatile profiles of specific specialty coffee types such as Indonesian Luwak Arabica coffee, India Monsooned Malabar coffee and Australian coffee.

Table 4.7 Summary of the analysis of targeted volatile compounds in 26 roasted coffees

Targeted aroma compounds	**Arabica (Robusta*)**				
	Min.	**Max.**	**Mean**	**SD**	**CV (%)**
Aldehydes					
methylpropanal	1008	5497	2591 (2317)	998	39
3-methylbutanal	1703	6708	3613 (3091)	1229	34
2-methylbutanal	6620	2696	1462 (1399)	452	31
(E)-2-nonenal	n.d	31	3.3 (2.0)	6.3	193
acetaldehyde	33818	75752	58958 (49054)	8609	15
Acids					
3-methylbutyric acid	37930	133972	69536 (50700)	20906	30
Esters					
ethyl-2-methylbutyrate	0.8	8.3	1.9 (1.0)	1.5	81
Furans					
furfural	53912	153818	101224 (40823)	22321	22
5-methylfurfural	31752	64091	50417 (26337)	7389	15
Ketones					
2,3-butanedione	6287	35392	18980 (12946)	6322	33
2,3-pentanedione	5647	29488	13058 (4807)	4863	37
Norisoprenoids					
β-damascenone	1.3	5.9	3.2 (4.0)	1.1	34
Phenols					
guaiacol	1731	4357	3011 (4162)	744	25
4-ethylguaiacol	178	546	281 (824)	72	26

Targeted aroma compounds	Arabica (Robusta*)				
	Min.	Max.	Mean	SD	CV (%)
4-vinylguaiacol	14013	28878	21994 (29075)	2941	13
Pyrazines					
2,5-dimethylpyrazine	42145	142217	92469 (77216)	30303	33
2,3-dimethylpyrazine	6520	22157	13223 (12238)	4611	35
2-ethyl-3,6-dimethylpyrazine	3521	16757	8601 (12167)	3229	38
2-ethyl-3,5-dimethylpyrazine	637	2460	1385 (1961)	490	35
2,3-diethyl-5-methylpyrazine	52	259	135 (251)	48	36
3-isobutyl-2-methoxypyrazine	5.8	115	20 (1.0)	20	103
Sulfur-containing compounds					
2-furfurylthiol	1265	4691	2929 (4337)	903	31
methional	851	1879	1386 (794)	261	19
3-mercapto-3-methylbutylformate	208	982	476 (233)	173	36
Terpenes					
linalool	90	485	192 (44)	94	49
geraniol	n.d.	71	68 (n.d.)	3.0	4.0
D-limonene	408	743	510 (391)	70	14

Concentrations of compounds were presented in µg/kg dry matter
*mean value of single Robusta sample presented in brackets

Table 4.8 Odour activity value (OAV) of key aroma compounds measured in 26 commercial specialty coffees

Targeted aroma compounds	Aroma descriptors [citation]*	Aroma threshold (µg/kg) [citation]*	OAV**	
			Arabica	Robusta
Aldehydes				
methylpropanal	*fruity, malty*[1]	0.7 [7]	3738	3310
3-methylbutanal	*malty*[2]	0.25 [7]	14673	12363
2-methylbutanal	*buttery-oily*[3]	0.9 [7]	1642	1555
(E)-2-nonenal	*buttery-oily*[3]	0.08 [8]	35	22
acetaldehyde		0.7[a,9]	85295	70952
Acids				
3-methylbutyric acid	*sweaty*[2]	700[c,10]	101	73

Targeted aroma compounds	Aroma descriptors [citation]*	Aroma threshold (µg/kg) [citation]*	OAV**	
			Arabica	Robusta
Esters				
ethyl-2-methylbutyrate	*fruity*[4]	0.5[d,4]	3.6	2.2
Furans				
furfural	*woody, bready*[5]	280[a,9]	366	148
5-methylfurfural	*caramel, bready*[5]	6000[a,9]	8.5	4.4
Ketones				
2,3-butanedione	*buttery-oily*[6]	0.3 [a,9]	64148	43153
2,3-pentanedione	*buttery-oily*[6]	20 [a,9]	657	240
Norisoprenoids				
β-damascenone	*honey-like, fruity*[2]	0.00075 [11]	4394	5837
Phenols				
guaiacol	*phenolic, burnt*[2]	2.5 [11]	1194	1665
4-ethylguaiacol	*spicy*[2]	50 [11]	5.5	17
4-vinylguaiacol	*spicy*[2]	20 [11]	1089	1458
Pyrazines				
2,5-dimethylpyrazine	*nutty*[5]	80 [a,9]	1160	965
2,3-dimethylpyrazine	*nutty*[5]	800 [a,9]	17	15
2-ethyl-3,6-dimethylpyrazine	*nutty*[6]	0.04 [12]	1001	1415
2-ethyl-3,5-dimethylpyrazine	*nutty-roast*[6]	8.6 [12]	34678	49032
2,3-diethyl-5-methylpyrazine	*nutty-roast*[6]	0.09 [a,9]	1488	2788
3-isobutyl-2-methoxypyrazine	*peasy*[4]	0.002 [8]	9686	696
Terpenes				
linalool	*flowery*[2]	0.17 [b,4]	1114	259
geraniol	*floral, fruity*[5]	1.1 [13]	19	0
D-limonene	*citrus, orange*[5]	4 [a,9]	127	98
Sulfur-containing compounds				
2-furfurylthiol	*roasty (coffee-like)*[2]	0.01 [11]	296767	439154
methional	*boiled potato-like*[2]	0.2 [11]	7036	4022
3-mercapto-3-methylbutylformate	*green blackcurrrant*[6]	0.0035 [14]	136435	67370

* Aroma description and sensory threshold provided in literatures as previously summarised by Sunarharum et al. (2014) with updates [1](Arctander,1969), [2](Blank et al., 1992); [3](Michishita et al., 2010),[4](Czerny and Grosch, 2000),[5](Mosciano, 2016), [6](Akiyama et al., 2007), [7](Milo and Grosch); [8](Belitz et al., 2009), [9](Burdock, 2010), [10](Salo, 1970), [11](Semmelroch et al., 1995), [12](Buttery and Ling, 1997), [13](Czerny et al., 2008), [14](Holscher and Steinhart, 1992).

Where two or more aroma thresholds were found, only two is presented. Aroma thresholds concentrations based on literatures were presented in µg/kg to ease comparison. All aroma thresholds were determined in water except: [a] matrix unknown; [b] threshold measured by first diluting compounds in ethanol in a defined concentration and then dissolved in water, for linalool as R-linalool; [c] in ethanolic solution 9.5%; [d]threshold in cellulose.

** Odour activity values were calculated by dividing the concentration (mean data) by the respective aroma threshold (reported in the literature).
n.a. not available.

Based on the odour activity values calculated (Table 4.8), all 27 volatiles measured were found to be odour active (OAV>1) except for geraniol that was not detected in most of coffee samples. Compounds with a large OAV indicate that those compounds were measured in coffee many times above their reported sensory detection threshold.

The six major odour active compounds (i.e. those with the largest OAV) in sequence were 2-furfurylthiol, 3-mercapto-3-methylbutylformate, acetaldehyde, 2-ethyl-3,5-dimethylpyrazine, and 3-methylbutanal. Sulfur-containing compounds, mainly 2-furfurylthiol, was the most odour active compounds in coffee. However, 2-furfurylthiol was of greater importance to Robusta coffee than that of Arabica coffees. This is also true for phenols that were found to be more important to Robusta aroma than to Arabica. The compound *β*-damascenone that exhibits a *honey-like*, *fruity* aroma was found to have a considerably high OAV in the coffees (OAV=4000+), even though it had been previously reported as not important in coffee odour (Semmelroch and Grosch, 1995).

In order to further explore diversity amongst coffee samples, to determine potential groupings between samples and to study relationships between the volatile compounds, a principal component analysis (PCA) was performed on the standardised mean of volatiles data (n=26 coffees x three replicates) (Figure 4.2 and Figure 4.3).

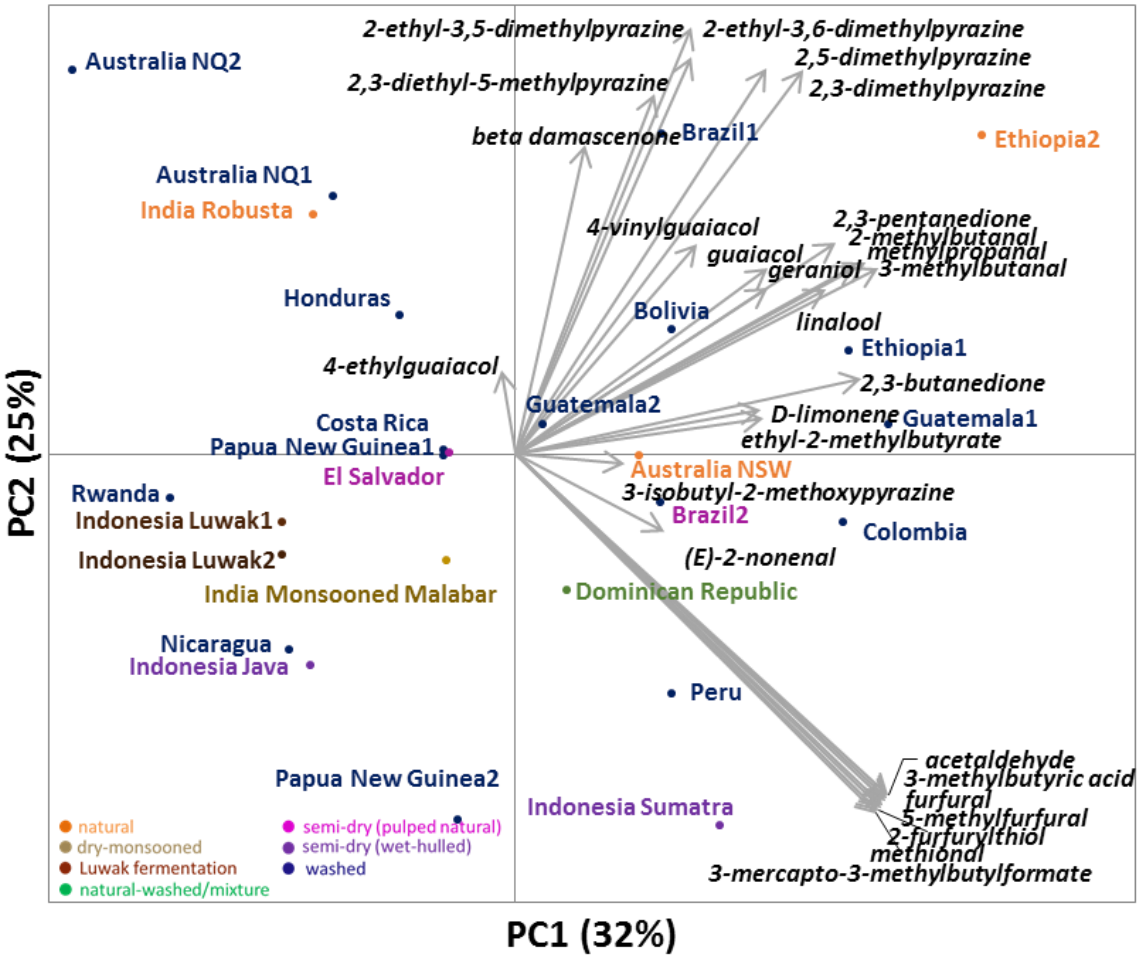

Figure 4.2 PCA bi-plot of volatile compounds measured in commercial single-origin roasted coffees (26 samples x three replicates), PC1 (32%) versus PC2 (25%)

The first three principal components (PCs) explain 71% of variation in the data set for the 27 volatile compounds measured in 26 coffee samples. Samples were separated across PC1 (32%) according to samples that were higher in aldehydes (3-methylbutanal, 2-methylbutanal, methylpropanal, (E)-2-nonenal), terpenes (D-limonene, linalool, geraniol) groups and ester (ethyl-2-methylbutyrate). Principal component 2 (25%) separated samples with higher content of pyrazines (2,5-dimethylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-3,6-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine) from those that were lower in pyrazines but higher in furans (furfural, 5-methylfurfural) and sulfur-containing compounds (2-furfurylthiol, methional, 3-mercapto-3-methylbutylformate mainly due to pyrazines (and sulfur-containing compounds). Principal component 3 (14%)

separated compounds mainly due to phenol content (4-ethylguaiacol, 4-vinylguaiacol and guaiacol) to that of higher in ketones (2,3-butanedione, 2,3-pentanedione) and aldehydes.

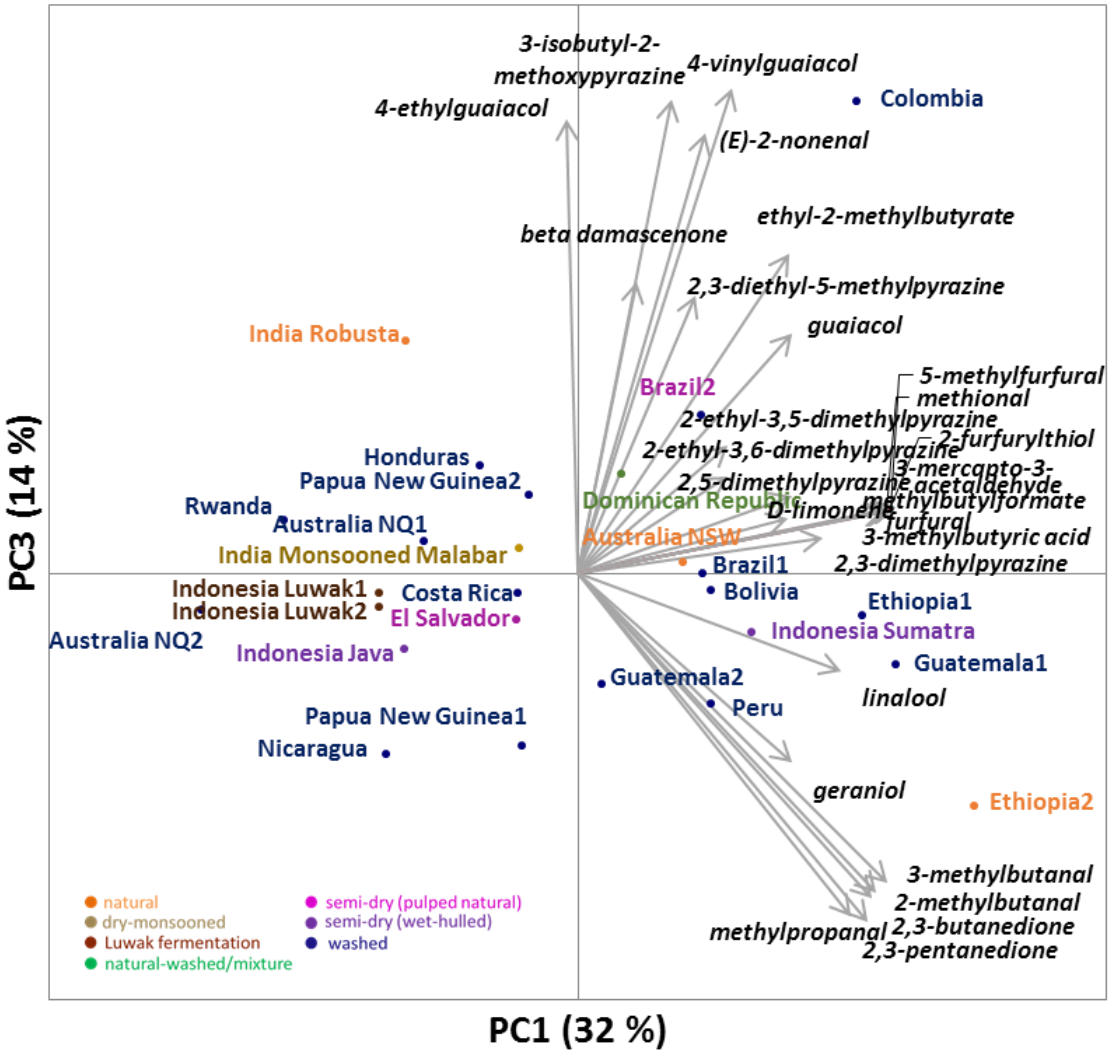

Figure 4.3 PCA bi-plot of volatile compounds measured in commercial single-origin roasted coffees (26 samples x three replicates), PC1 (32%) versus PC3 (14%)

As expected, the coffee samples were quite diverse in terms of volatile profile. In terms of clustering, coffees from certain origins such as Indonesia Luwak (Luwak1 and Luwak2), Australia northern Qld (Australia NQ1 and NQ2), and potentially Ethiopia (Ethiopia1 and Ethiopia2) appeared to be grouped, indicating similar volatile profiles in those coffees in terms of the compounds measured. Certain volatiles may indeed be discriminating markers of the origin of coffee, for example, terpenes, ketones and aldehydes were higher and characteristic of the Ethiopian coffees (Ethiopia1 and Ethiopia2).

Compounds belonging to similar functional groups were highly correlated, for example the pyrazines or aldehydes (Figure 4.2 and Figure 4.3). However, there were some exceptions to this, for example 3-isobutyl-2-methoxypyrazine, which was not clustered with the other pyrazines possibly due to its particularly low concentration.

Interestingly, a few compounds that were highly correlated may be involved in synergism or antagonism behaviours as reported in previous works (Grosch, 2001b, Laska et al., 1990). For example, linalool, geraniol and D-limonene were highly correlated as shown in the PCA bi-plot and with a high correlation coefficient (Pearson's correlation r>0.70) (Appendix F, p.179).

The developed method proved to be readily applicable to measure important volatiles in coffee and provided an added benefit in measuring many important compounds comprehensively in a minimum number of instrumental runs. The methods developed were suitably high throughput with a potential to be adopted and applied by industry or for research enabling rapid and accurate screening of many samples. Nevertheless, further work can be done to improve upon these methods and to add additional compounds of importance that were not encompassed by the methods.

4.4 Conclusion

Analytical methods using SIDA coupled with GC-MS were developed and validated. The analysis involved three different preparation methods to optimise for the diversity of the compounds tested and provide a rapid and comprehensive set of analyses for coffee flavour. The analytical methods were applied to measure 27 compounds in 26 commercial, single-origin coffees. The profiles of the coffees analysed were quite diverse and, interestingly, certain volatile compounds showed potential as discriminating markers of coffee country of origin. Authentication of coffee using volatile profiles may indeed be a topic for future research.

This method was sufficiently sensitive and able to produce calibration functions with high coefficient of determination (R^2) across the calibration range in most cases. It was demonstrated to be generally applicable to the analysis of coffee volatiles with some

limitations such as missing compounds including furanones, pyridine, ethenylpyrazines and some other compounds, as well as complexity of coffee matrix as described earlier.

Further work may be needed to ensure the methods are suitably validated for specific types of coffee. Nevertheless, this method provides an improvement in the comprehensive, accurate and precise analysis of coffee volatile flavour compared to previously published methods. This method provides a valuable tool for use in future coffee research and where industrial application requires rapid evaluation of several volatiles in many samples.

Most importantly, this is the first study that successfully quantify monoterpenes, particularly geraniol and measure volatiles profile of some interesting very limitedly published coffee samples including Australian coffees and specific coffee types such as Indonesia Luwak (Arabica) and Indian Monsooned Malabar.

The volatiles data collected will provide the basis for modelling against sensory data to establish the compositional basis of aroma that will be addressed in Chapter 6 (p.104).

Chapter 5 Profiling physicochemical properties related to coffee flavour

Previous chapters (Chapter 3, p.30 and Chapter 4, p.53) provided sensory and volatiles data of the selected commercial single-origin 'specialty' coffees important for this study. This chapter further explores the physicochemical profiles of these coffees including the non-volatile component and physical characteristic. The output of this chapter provided the basis of the physicochemical data used to build a comprehensive flavour model (detailed in Chapter 6, p.104).

5.1 Introduction

Coffee is a popular beverage consumed worldwide due to its desirable aroma and flavour. Flavour quality of coffee relates to bean composition which includes volatile and non-volatile. Bean composition is influenced by several factors including genetics, geographical growing location, agricultural practices, and post-harvest treatment and processing (Bhumiratana et al., 2011, Costa Freitas and Mosca, 1999, Bicho et al., 2013a). (Bertrand et al., 2012, Bertrand et al., 2006, Bosselmann, 2009, Poltronieri, 2011).

The non-volatile compounds of roasted coffee beans thought to be important to coffee flavour has been thoroughly reviewed by Buffo & Cardelli-Freire (Buffo and Cardelli-Freire, 2004) and more recently by Sunarharum et al. (2014). These compounds include chlorogenic acids, caffeine, trigonelline, carboxylic acids, carbohydrates and polymeric polysaccharides, lipids, protein, pigments, melanoidins and minerals (Buffo and Cardelli-Freire, 2004, Ribeiro et al., 2009). Certain compounds such as caffeine, chlorogenic acids, trigonelline are of particular interest due to their potential health benefit and/or their relevance to coffee quality (Oestreich-Janzen, 2010, Clarke and Macrae, 1985, Higdon and Frei, 2006, Buffo and Cardelli-Freire, 2004)

In the current investigation, 26 sensorily diverse single-origin coffee beans were examined with the objective to obtain quantitative data on the diversity of the coffees' physical and chemical properties, which could be related through data modelling to the sensory qualities of coffee thereby enhancing our understanding of coffee flavour. This is the first study that

encompasses such a diverse range of coffee flavour types covering many global regions linked with their physicochemical profiles.The coffees were carefully selected to represent a broad spectrum of commercial coffee flavour-types as well as including some unique coffees such as Australian coffee and specific coffee styles such as most notably Monsooned and Luwak coffee for which there is limited published data.

The volatile compounds measured in the coffees have been discussed previously (see Chapter 4, p.53), thus the current chapter deals with the measurement of targeted non-volatile compounds only. Both samples of roasted bean and the corresponding green beans were analysed for comparison. The non-volatile compounds and physical components studied were chlorogenic acids, caffeic acid, caffeine, trigonelline, crude fat, pH, TA (titratable acidity), and lightness (L*value).

5.2 Methodology

5.2.1 Materials

Samples of 26 commercial single-origin, medium-roasted, specialty coffee samples (hereon mentioned as roasted coffees) and the corresponding green coffee beans being investigated includes 25 samples of Arabica coffee and one sample of Robusta coffee. Coffees were provided by five commercial coffee companies and roasters located in Australia and Indonesia (see Chapter 3, p.39 for details).

A 4 layer laminated polyethylene terephthalate/polyethylene terephthalate/aluminum foil/linear low-density polyethylene (PET/PET/Foil/LLDPE) pouch was used to re-pack each coffee bean sample (±110 g) under vacuum before storing at -20°C until required.

5.2.2 Non-volatile analysis

All analyses were conducted in duplicate unless otherwise specified.

5.2.2.1 Analysis of chlorogenic acids and caffeic acid in coffee

Materials

Standard 5-caffeoylquinic acid (5-CQA) was obtained from Sigma–Aldrich (Sydney, Australia) as was the acetonitrile and 2-propanol; while 3,4-dicaffeoylquinic acid (3,4-diCQA), 3,5-dicaffeoylquinic acid (3,5-diCQA), and 4,5-dicaffeoylquinic acid (4,5-diCQA) were obtained from CFM Oskar Tropitzsch (Marktredwitz, Germany).

Sample preparation

Coffee beans were removed from -20°C to -80°C a day prior to analysis. Samples were removed from -80°C and directly milled into powder. Approximately 4-5 g coffee beans were milled using a Retsch Mixer Mill 400 under 30/s frequency at 20 s and 40 s for roasted coffees and green coffee beans, respectively.

Extraction

Samples (0.05 g green coffee powder or 0.5 g roasted coffee powder) were extracted using 25 mL of methanol/water (70/30 v/v) in 50 mL falcon tubes and rotated for 18 h (±15 min) at 4°C in the dark following a method modified from Ky (1997) and Campa et al. (2005). Bisulfite was not added during the extraction process since preliminary trials indicated the addition of bisulfite was not significant to the recovery of compounds being analysed (α=0.05). The coffee extracts were then treated with Carrez reagents I & II (each of 25 µL). These mixtures were vortexed for 5 s and let stand for 10 min before centrifugation (1500 rpm, 2 min). Clear supernatant was decanted into 50 mL volumetric flasks and further 20 mL methanol/water (70/30 v/v) was added followed by vortex mixing for 5 s and further centrifugation at 1500 rpm for 2 min. The supernatant was decanted and made up to 50 mL using methanol/water (70/30 v/v) and mixed. Extracts were then filtered into high performance liquid chromatography (HPLC) vials through a 0.45 µm polyvinyl difluoride (PVDF) syringe filter and stored at -80°C for HPLC analysis. Extractions were conducted in triplicates.

Instrumental conditions

Filtered samples were analysed using a Shimadzu HPLC system consisting of a system controller (SCL-10Avp), degasser (DGU-14A), low pressure gradient switching valve (FCV-10ALvp), pump (LC-10ATvp), auto-sampler (SIL-20ATHT), column oven (CTO-10Avp), photodiode array detector (SPD-M10Avp), and equipped with LabSolutions software. Reverse phase-HPLC was conducted using a C18 column (Phenomenex Gemini, 3 µm, 4.6 x 150 mm) with matching guard column at 30°C.

The mobile phase consisted of 1% v/v formic acid in water: acetonitrile: 2-propanol (70:22:8 v/v/v). Separation of the phenolic acids was achieved by isocratic elution at a flow rate of 0.75 mL/min. The injection volume was 10 µL and monitoring was performed at 325 nm.

Identification and quantification were accomplished by using a linear calibration curve constructed by utilising five concentrations of standard mixtures dissolved in methanol and ranging in concentration from 12.5 µg/L to 200 µg/L (triplicate injections were undertaken).

5.2.2.2 Analysis of caffeine and trigonelline in coffee

Materials

Standard compounds of caffeine and trigonelline hydrochloride were purchased from Sigma-Aldrich (Sydney, Australia). Analytical grade methanol and KH_2PO_4 were purchased from Thermo Fisher Scientific (Melbourne, Australia).

Sample preparation

Coffee beans were prepared and milled using a Retsch Mixer Mill 400 following the abovementioned procedures for chlorogenic acids and caffeic acids (Section 5.2.2.1 p.91).

Extraction

Each coffee sample was extracted based on the method applied by Casal et al. (1998) with slight modification. Briefly, samples (2 g) of roasted coffee powder were weighed into a 50 mL erlenmeyer flasks containing magnetic stirrers then boiled using 20 mL of MiliQ water for 2.5 min. After that, the extracts were allowed to sit for 2.5 min before transferring

into 100 mL volumetric flasks. The boiling cycle was repeated two times prior to mixing all extracts together in the 100 mL volumetric flask and diluted to the volume mark with MiliQ water. The extracts were then cooled down in an ice bath before filtering into HPLC vials using 0.45 µm syringe filter. The filtered extracts in HPLC vials were stored at -80°C before testing. Analyses were conducted in duplicates.

Instrumental conditions

The HPLC conditions followed that of Casal et al.(1998, 2000) using Spherisorb S5 ODS2 (0.46 x 25.0 cm), with the guard column µBondapak C18 (10 µm) from Waters Associates. Injection volume was 20 µL with gradient (A) phosphate buffer (KH_2PO_4) 0.1 M (pH 4.0), (B) methanol at 0 min (7% B), 4 min (9% B), 6 min (25% B), 13 min (29% B), 21 min (50% B), 35-40 min (7% B). Four calibration points were used to create linear calibration curve in order to quantify the compounds (caffeine: 0.05-500 µg/mL; trigonelline: 0.15-450 µg/mL). UV detection was performed at 265 nm.

5.2.2.3 Analysis of crude fat in coffee

Crude fat content of the dry samples were performed gravimetrically following AOAC Procedure Soxhlet method 960.39 (AOAC, 2007). A weighed amount of ground coffee sample was introduced into a Soxhlet thimble and then extracted for at least six hours on the Soxhlet extractor with diethyl ether as the solvent. The collected fat was then weighed and the result expressed as weight percent.

5.2.2.4 Analysis of pH and titratable acidity

Sample pH and TA (titratable acidity, end point pH 8.20) were analysed by means of an auto-titration apparatus (702 SM Titrino Metrohm AG CH-9101 Herisau, Switzerland with an attached 728 Metrohm stirrer). Hot boiling water was added into coffee powder (coffee to water ratio=1:10) and the extract was used for pH determination and titratable acidity analysis.

5.2.2.5 Analysis of moisture and colour

Moisture content of coffees was determined as per the AOAC Official method 979.12 (AOAC, 2007). Vacuum Oven Method II. Moisture data was used for calculation of the chemical compositions in dry matter. Colour (lightness, L*value) was measured using a

CR 310 photometer (Konica Minolta Imaging, Dietikon, Switzerland). Calibration was performed following the manual for CR 310 photometer. Calibration channel 00 was performed using a white calibration plate for CR 310 at the start of measuring session. Recalibration of channel 00 to the white calibration plate automatically adjusts calibration of other channels (01 through 19) as necessary.

5.2.3 Statistical analysis

Analysis of variance (ANOVA) and further post-hoc multiple comparison Tukey-Kramer HSD at 95% confidence interval were performed using XLSTAT version 2015 (Addinsoft, New York, USA). Principal component analysis multivariate exploration was performed using XLSTAT version 2015 (Addinsoft, New York, USA) and the Unscrambler® X (CAMO, Oslo, Norway, AS).

5.3 Results and discussion

The objective of this work was to measure selected physical and non-volatile chemical properties important to flavour in a set of 26 sensorily diverse single-origin coffee samples in both green coffee beans and roasted coffees at a medium level.

The target properties measured were chlorogenic acids, caffeic acid, caffeine, trigonelline, crude fat (lipids), pH, titratable acidity and lightness (L*value). Sucrose was not the subject of detailed investigation as preliminary trials, indicated it was below the limit of detection in the roasted beans even though it was found in higher concentrations in the green coffee beans.

5.3.1 Physical analysis and non-volatile profiles of green coffee beans

The green coffee beans (n=26) of corresponding roasted coffees studied in this project were also collected at the time of purchase and tested for a range of non-volatile properties, namely for 5-CQA, 3,4-diCQA, 3,5-diCQA, 4,5-diCQA, caffeic acid, caffeine, trigonelline, pH, and L*value. Mean data for these green coffee beans can be viewed in the Appendix G (p.181). Generally, green coffee contained significantly higher concentration of chlorogenic acids, caffeic acid and trigonelline, while also being higher in pH and L*value than the roasted coffees. However, caffeine concentration of the green

coffee beans and the roasted coffees was found to be not significant (α=0.05). The statistical data could be found in Appendix J (p.186). Results from the green coffee beans were not used in the modelling of sensory properties (Chapter 6, p.104) but some measurements such as the chlorogenic acids concentrations will be discussed further in Chapter 7 (p.125).

5.3.2 Physical analysis and non-volatile profiles of roasted coffees

The physicochemical properties of roasted coffees were investigated. Table 5.1 provides a summary of the results for each physical attribute and chemical component measured on the roasted coffee samples including minimum, maximum, mean, standard deviation (SD), coefficient of variation (CV%) and significant difference (α=0.05), calculated from raw data across all 26 coffee samples (26 samples x three replicates or 26 samples x two replicates). Mean data of the measured non-volatiles physicochemical attributes for each of roasted coffees can be viewed in the Appendix H (p.183) and Appendix I (p.185).

All physicochemical properties were significantly different across the coffee samples tested (α=0.05) (Table 5.1). The diCQA isomers (hereon mentioned as diCQAs) tended to have greater variability than 5-CQA with 3,5-diCQA having the most variation amongst samples (CV>20%). However, 5-CQA showed predominance with the highest concentration in agreement with previous studies (Clifford, 1979, Clifford, 2006, Duarte et al., 2010).

The caffeic acid concentration of roasted coffees ranged between 1.3 mg/g and 2.3 mg/g dry matter (Table 5.1). This phenolic acid is believed to react with quinic acid in the chlorogenic acids formation pathway (Clifford, 1999) and it was previously reported to contribute to *astringency* and *bitterness* (Dadic and Belleau, 1973, Callemien and Collin, 2009).

Table 5.1 Summary of results for physicochemical components measured in commercial single-origin roasted coffees (26 samples x two replicates or three replicates[a])

Attributes	Min	Max	Mean	SD	CV (%)
5-CQA*[a]	9.5	16	13	1.8	14
3,4-diCQA*[a]	0.9	1.7	1.2	0.2	14
3,5-diCQA*[a]	0.5	1.2	0.8	0.2	25
4,5-diCQA*[a]	0.6	1.2	0.8	0.1	15
caffeic acid*[a]	1.3	2.3	1.8	0.2	10

Attributes	Min	Max	Mean	SD	CV (%)
caffeine*	9.6	25	13	2.5	20
trigonelline*	5.8	10	8.1	0.9	11
crude fat*	6.7	18	14	2.0	14
titratable acidity*	1.1	1.9	1.6	0.2	10
pH*	5.0	5.7	5.3	0.1	2.7
L*	38	46	40	1.7	4.3

5-CQA, 3,4-diCQA, 3,5-diCQA, 4,5-diCQA, caffeic acid, caffeine, trigonelline (in mg/g dry matter), crude fat (%), titratable acidity (mL NaOH/g), data rounded to one decimal only

[a]Three replicates analysis for each of roasted coffees were performed, others in two replicates

*Significant difference of attributes amongst coffee samples (α=0.05).

Acidity of coffee was measured by measuring pH and titratable acidity. The pH assesses the hydrogen ion activity and is calculated as a negative logarithm of the molar concentration of hydrogen ions while titratable acidity measures total acid concentration (Covington et al., 1985, Sadler and Murphy, 2010). The latter was measured at the end point pH 8.2 and expressed in mL NaOH/g. The pH of the roasted coffees measured ranged from 5.0 to 5.7 with a mean pH of 5.3. Consistent to previous study (Vitzthum, 1976), the Arabica coffees were also found to be more acidic than the Robusta.

The titratable acidity of coffee being investigated ranged between 1.1 mL NaOH/g and 1.9 mL NaOH/g. Difference in the acidity of coffee brew is believed to be the result of differences in the nature or geographical conditions (Rodrigues et al., 2007) and processing including roasting (Ginz et al., 2000, Maier, 2005, Rodrigues et al., 2007).

It was revealed the tested coffees also exhibited a wide range of crude fat content (6.7%-18.0%) with the lowest fat content found in the India Robusta coffee. This is particularly interesting, since lipid could be one of the reasons why Arabica coffees have a higher perceived quality and is preferred by specialty coffee consumers over Robusta. Coffee lipids include triglycerides, diterpene alcohols, sterols, tocopherols (Kaufmann and Gupta, 1964, Kaufmann and Hamsagar, 1962), which act as a medium to carry volatiles and fat-soluble vitamins as well as influencing the texture and mouthfeel of the subsequent brew (Oestreich-Janzen, 2010). In this study, crude fat was measured to obtain a representation of lipid content in coffee. However, it should be noted that this crude fat represents a crude mixture of all fat-soluble components obtained from the ether extraction of coffee samples.

For colour measurement, an L*value (darkness-lightness) was measured as it provides an indication of roasting degree. Roasting the green coffee beans reduced the L*value (thus increasing darkness) due to the Maillard browning reactions (Akiyama et al., 2003). Since all coffees in this study were roasted to a medium level, it would be expected that they exhibit a similarity in colour (L*value). However, this was not the case as the roasted India Robusta coffee was found to have a significantly higher L*value than others, suggesting it was more lightly roasted. Possibly this is due to the different physical characteristics of Robusta green coffee beans, which were comparatively smaller and harder than the others therefore requiring specific roasting adjustments to reach the second crack or the finish line of the entire roasting process. This indicates that different coffees samples are naturally unique; meaning each different batch of coffee beans will need specific roasting profiles to achieve an arbitrary 'medium' roasted level. Different roasting parameters may also be required for coffees of different species or origin (Carlin, 2013). In addition, different roasters could apply different roasting techniques contributing to the difference in L*values thereby resulting in different compositional properties.

In order to further explore the variations present in the data set as well as comparing different samples with one another, principal component analysis was performed on the physicochemical (non-volatile) data of the 26 coffee samples. Principal component analysis bi-plots were initially generated with raw data for each coffee sample and replicate for each of the 11 variables measured to visualise the grouping of replicates and look for outliers. Subsequently, the standardised mean data of all 11 non-volatile variables for each sample were used to build the PCA bi-plots given in Figure 5.1 and Figure 5.2. Figure 5.1 presents PC1 vs PC2 of all the non-volatiles components being evaluated in this study while Figure 5.2 presents the PC1 vs PC3 (n=26).

The first three principal components (PC's) explained 70% of variation in the data. Principal component 1 (34%) separated coffees on the left of Figure 5.1 which containing lower crude fat concentrations but higher L*value, higher caffeine content and higher pH, from those on the right of Figure 5.1. Principal component 2 (23%) separated coffees that contained higher titratable acidity, caffeic acid, trigonelline and chlorogenic acid concentrations (the top of Figure 5.1) from those with lower concentrations of these components (samples in the bottom of Figure 5.1). Principal component 3 (13%)

separated coffees with higher concentration in trigonelline, caffeic acid and 5-CQA (the top of Figure 5.2) from samples which lower in those components but higher in diCQAs content (in the bottom of Figure 5.2).

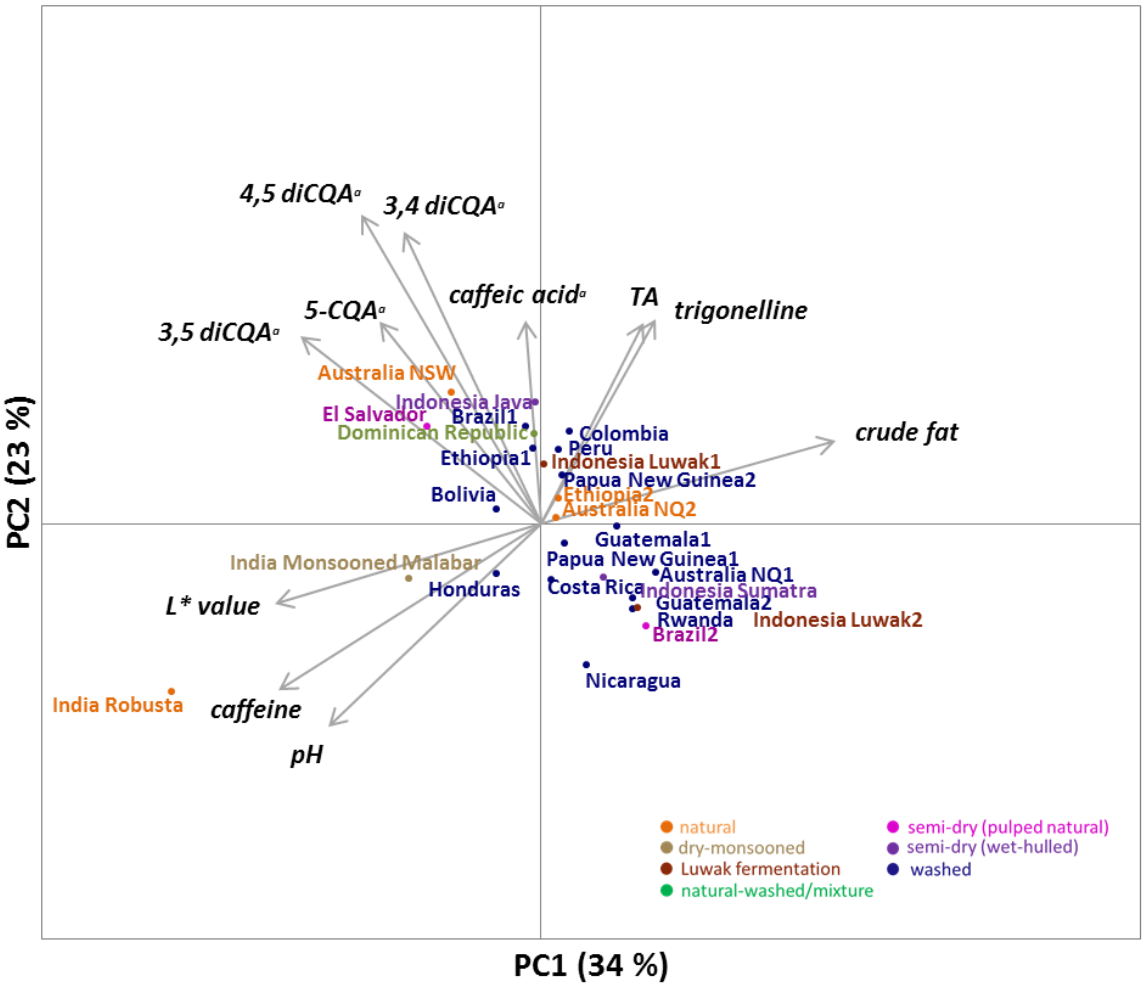

Figure 5.1 PCA bi-plot of physicochemical components measured in commercial single-origin roasted coffees (26 samples x two replicates or three replicates[a]), PC1 (34%) versus PC2 (23%)

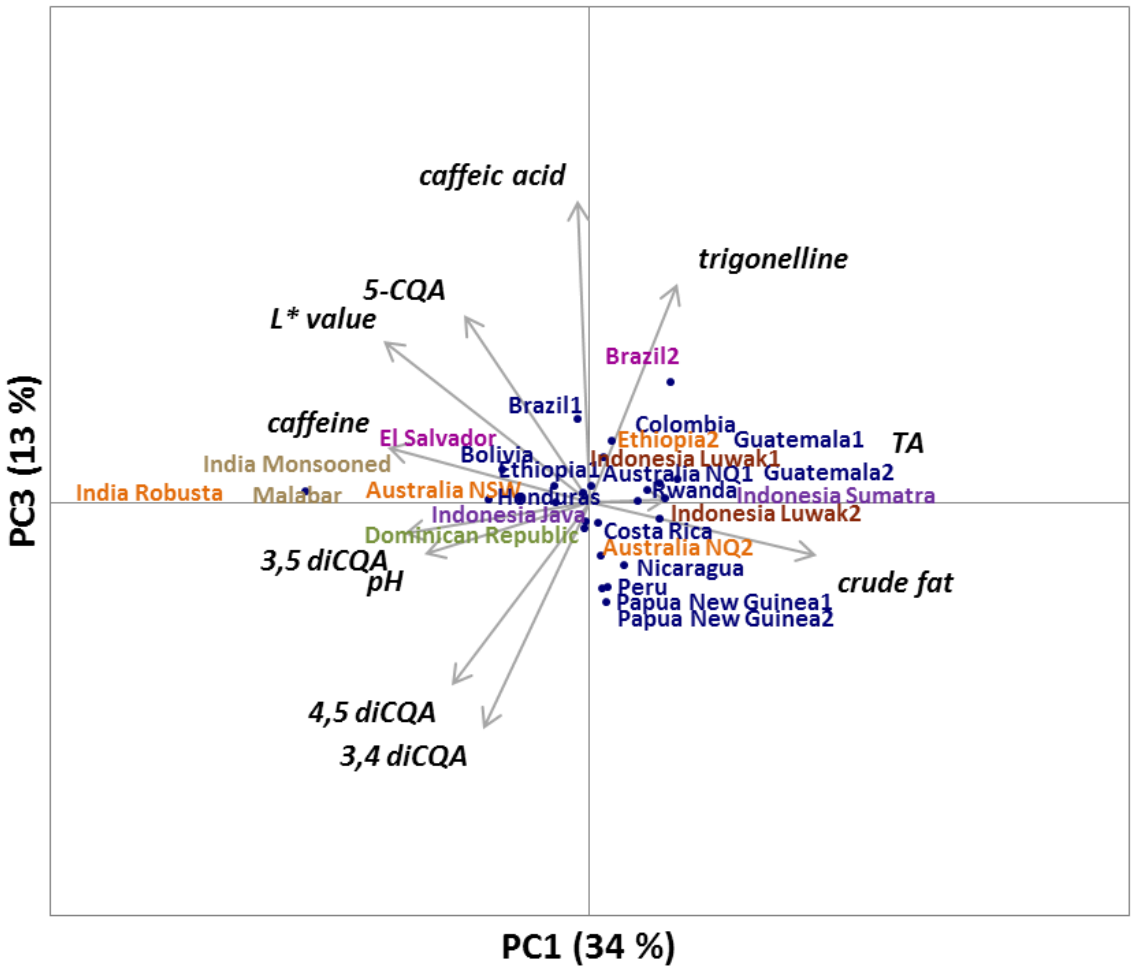

Figure 5.2 PCA bi-plot of physicochemical components measured incommercial single-origin roasted coffees (26 samples x two replicates or three replicates[a]), PC1 (34%) versus PC3 (13%)

The Indian coffees such as the Indian Robusta and the Indian Monsooned Malabar showed some similarities regardless of different species and processing. However, the India Robusta was quite different probably due to the doubled caffeine content, lower trigonelline and higher pH than the Arabica species. Comparison between Robusta and Arabica coffees had been reported elsewhere showing similar trends to the current results (Vitzthum, 1976, Bicho et al., 2011).

It was also revealed from the PCA plot (Figure 5.1) that pH was inversely correlated to titratable acidity meaning that the higher pH, the lower titratable acidity. However, this

relationship is not causal since pH is a combination between titratable acidity and conjugate base (Sadler and Murphy, 2010). Difference in the acidity of coffee brew is believed to be the result of differences in the nature or geographical conditions (Rodrigues et al., 2007) and processing such as roasting (Ginz et al., 2000, Maier, 2005, Rodrigues et al., 2007).

While the India Robusta coffee is important to sensory diversity, it is clear that this sample is an outlier that has dominated the visualisation of samples in the PC bi-plots due to high caffeine, pH and L*value. Therefore, the data was further examined after excluding the India Robusta coffee to focus on differences in the Arabica samples. Figure 5.3 and Figure 5.4 present PCA bi-plot of PC1 versus PC2 and PC1 versus PC3 constructed using a new data set (n=25), respectively.

The spread of the Arabica samples was clearer in the plot after eliminating the influence of the India Robusta coffee with the first three PC's explaining 73% of variation of the dataset. Principal component 1 (39%) separated coffees with higher 5-CQA and diCQAs level than other samples while PC2 (21%) separated coffees that contained more crude fat and caffeic acid with lower pH and L*value. Principal component 3 (13%) separated coffees that possessed higher pH and concentrations of caffeine but contain less trigonelline.

The Australian NSW coffee could be distinguished across PC1 due to lower level of 5-CQA and diCQAs as compared to that of El Salvador and Ethiopia 2 coffees. Principal component 2 separated India Monsooned coffee with its high pH and low fat from the Guatemala 1 coffee which contained the highest crude fat. It is believed that the monsooning process is also responsible for washing out some lipids from the green coffee beans. Interestingly, the Australian coffees were somewhat clustered together due to low caffeine content in PC3 while the two coffees from northern Queensland (Australia NQ1 and NQ2) seemed to have similar trigonelline and 5-CQA profiles differing from the Australian NSW coffee.

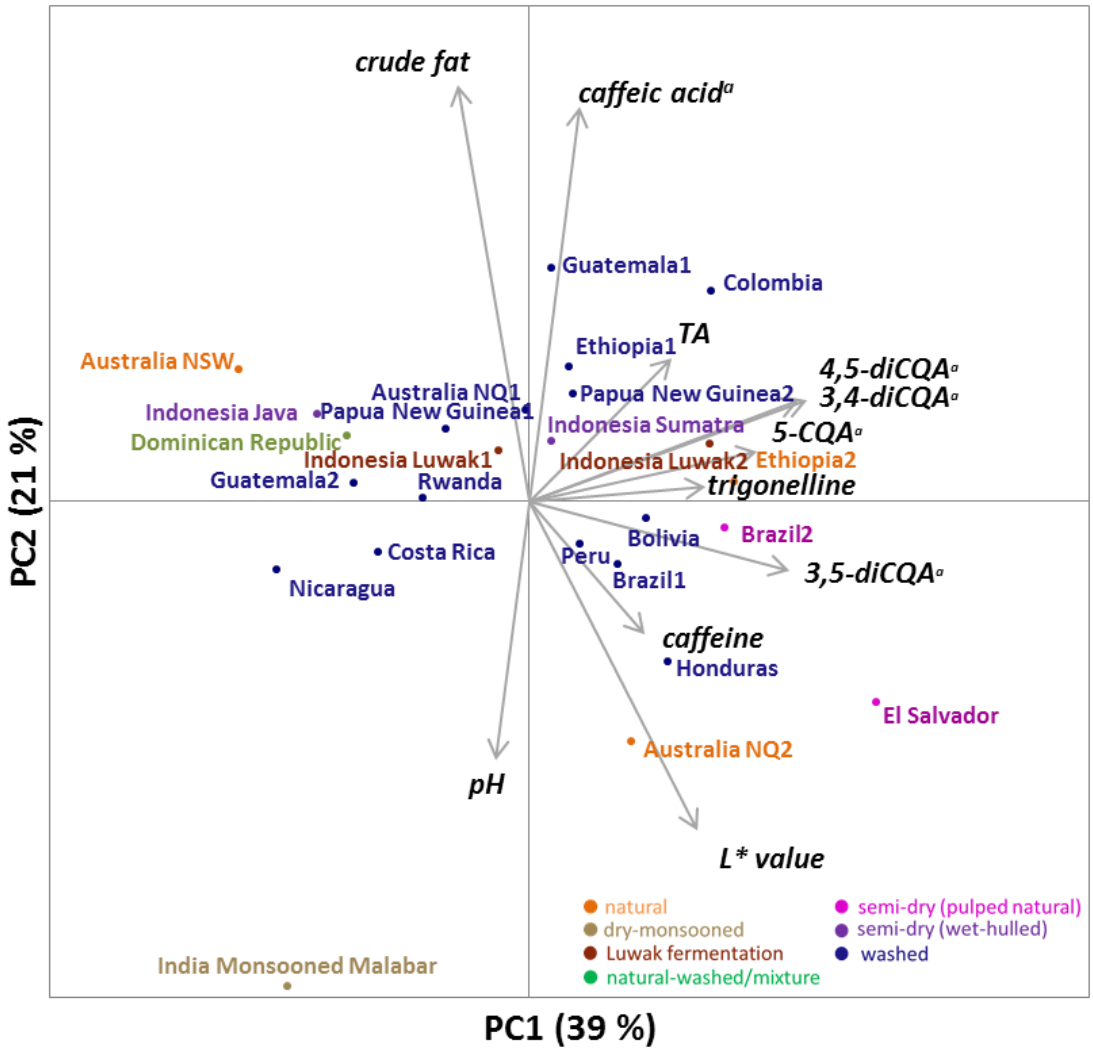

Figure 5.3 PCA bi-plot of physicochemical components measured in commercial single-origin roasted coffees (25 samples x two replicates or three replicates[a]), PC1 (39%) versus PC2 (21%)

No obvious clustering was identified based on the different styles of green coffee beans processing. Although, this is not surprising given it was not the objective of this study to choose samples that were representative of the particular processing treatments. There are many other factors involved during the processing and distribution chain which are likely to have caused the variation observed across the different coffees. It is clear, however, that genetic factors such as species differences (i.e. Arabica or Robusta) plays an important role in the variation observed in this non-volatile data set. Finally, processing is considered, but not terroir.

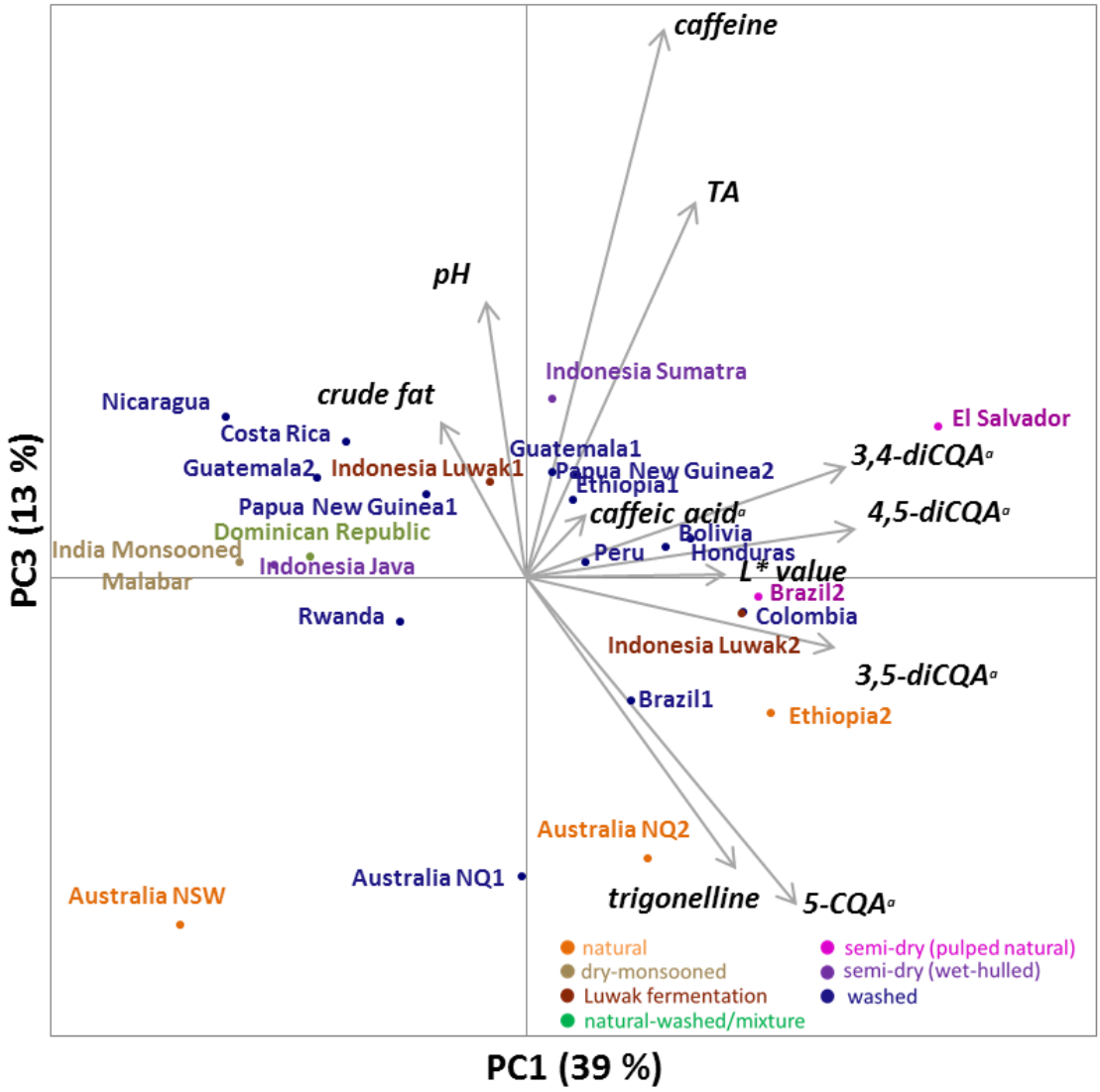

Figure 5.4 PCA bi-plot of physicochemical components measured in commercial single-origin roasted coffees (25 samples x two replicates or three replicates[a]), PC1 (39%) versus PC3 (13%)

It should be noted that the coffees being studied were commercially roasted by different commercial roasters. This creates another dimension for potential variability due to differences in roasting parameters and standards applied. A study by Farah (2012) reported that samples of the same coffee roasted to the same level may differ in chemical composition due to different roasting conditions such as the amount of coffee in the roaster, temperature, roasting time, and speed of hot air circulation used. In the present research, the roasters are highly likely to have roasted each coffee differently accounting

for variations in the green coffee bean characteristics such as shape, bean size, starch and other compositional considerations. Nevertheless, in reality, this represents the true situation that consumers of commercially available premium single-origin coffees experience.

5.4 Conclusion

The 26 roasted coffees investigated in this study exhibited greatly diverse physicochemical properties. The India Robusta coffee displayed a distinct profile as compared to the Arabica species. The Arabica data was best explored using multivariate methods without the influence of the Robusta sample data. For many of the samples, namely the Australian and the Luwak coffees, this is the first investigation into the non-volatile composition of these unique coffee types. Interestingly, there was no specific clustering of samples according to region or style or processing. Given the complex and diverse nature of commercial coffee production and processing, this is perhaps not surprising.

While non-volatile profiles are meaningful to explore and interpret in themselves, it is the comparison of this data, together with volatile profiles, to the sensory profiles of the coffees that will facilitate an in-depth understanding of the role that each of these components play in coffee quality. This is indeed the topic of this thesis and such models will be detailed in Chapter 6 (p.104).

Chapter 6 Modelling the compositional basis of coffee flavour

This chapter explores the relationships between sensory and physicochemical data obtained from the previous chapters (Chapter 3, p.30; Chapter 4, p.53, and Chapter 5, p.89), using multivariate modelling technique. The results identified components that could be potential markers, or could be responsible for coffee flavour.

6.1 Introduction

The measurement of compositional data alone cannot effectively describe the importance of individual, or groups of, flavour components to the quality of coffee flavour. Similarly, sensory information, without compositional data, cannot explain the potential cause of a specific sensory character perceived in a coffee. Detailed assessment linking these two properties of coffee is needed to truly explain the compositional basis of coffee flavour.

To fully understand the correlations between sensory data and physicochemical measurements in a food or beverage matrix, multivariate data analysis tools, also known as chemometrics, could be used (Resurreccion, 1988, Wold and Sjostrom, 1998). Commonly applied methods are principal component analysis (PCA) and partial least squares (PLS) regression which are widely used in studies of food composition and are useful in identifying compounds that might account for specific aroma nuances in complex systems such as coffee (Ribeiro et al., 2012, Bhumiratana et al., 2011, Pérez-Martínez et al., 2008). However, correlating compositional data with sensory attributes is a complicated task and can be problematic if the methodology used to collect the information is not suitably comprehensive. Such models are mathematical relationships only and must be validated with further experiments such as sample reconstitution and spiking experiments (Guth and Grosch, 1999, Czerny et al., 1999, Grosch, 2001b, Frank et al., 2011, Liu et al., 2012).

Multivariate methods have been used to explore coffee flavour in very limited number of previous studies. A recent application of PCA successfully discriminated aroma characteristics of Arabica coffee from different origins and different roasting levels

(Bhumiratana et al., 2011). Principal component analysis was also applied successfully to describe sensory effects of additives on the quality of stored Colombian coffee brews (Pérez-Martínez et al., 2008).

Partial least squares regression modelling has been applied to correlate sensory data to volatile chromatographic profiles resulting in adequate predictions of *acidity*, *cleanliness*, *overall quality*, *bitterness*, *body*, and *flavour* of Brazillian Arabica coffee (n=53) (Ribeiro et al., 2012). In that study, models were created based on volatile peak area data (not concentration data) to predict sensory attribute scores which were generated using industrial cupping protocols and not specifically for sensory aroma attributes from conventional descriptive testing using scientific methods.

Collectively, most of the previous works that used multivariate data analysis to relate physicochemical and olfactory or sensory panel data have typically utilized semi-quantitative compositional methods at best, or have studied just a limited range of coffee samples (n<10) with relatively similar flavour profiles (for examples see: (Akiyama et al., 2008, Bhumiratana et al., 2011, Pérez-Martínez et al., 2008, Ribeiro et al., 2012, Ribeiro et al., 2009, Kerler et al., 2014, Charles et al., 2015).

The present research aims to go beyond previous research attempts in this area by targeting a larger sample set of commercially diverse coffee flavour-types (26) previously screened from 59 single-origin medium-roasted coffees from around the world and by applying highly-regarded sensory descriptive methods together with a more comprehensive, accurate and precise compositional analysis approach from that which has been used in previous studies. Principal component analysis and partial least squares regression modelling were used to explore the relationship between physical properties, sensory and compositional data of 26 roasted Arabica and Robusta coffees to provide a more detailed insight into the compositional basis of coffee flavour. This chapter details the approach, method and results of the PCA and PLS modelling employed.

6.2 Methodology

The sensory and physical and compositional data used for multivariate modelling were developed as described in previous chapters of this thesis. Data were exported from Microsoft excel into XLSTAT version 2015 (Addinsoft, New York, USA) and The Unscrambler® X (CAMO, Oslo, Norway, AS) for further analysis. After initial statistical evaluation (as described in previous chapters) the data were prepared for multivariate analysis using mean scores of replicated measurements for both sensory and compositional data.

Principal component analysis was plotted on a standardised data (1/SD) prior to PLS regression to evaluate data structure and interpret relevant information (in XLSTAT software). Prediction of sensory properties was performed using PLS regression (PLS2 and PLS1) with The Unscrambler® X software. Partial least square 2 (PLS2) presented a general overview relationship between all physicochemical (X) and all sensory attributes (Y) in the dataset while PLS1 applied to predict each individual sensory attribute (Y). Partial least square 2 (PLS2) regression was first constructed using mean data of sensory scores assigned by the panel as dependent variables (Y) and mean data of physicochemical measurements as independent variables (X) to simultaneously describe relationships between X's and Y's. The data were weighted by 1/SD to give each variable an equal influence on the models. Further, PLS1 for specific individual sensory attributes (Y) was performed, followed by Martens uncertainty test, to obtain the best prediction models. Calibration models were developed with full cross validation. The statistics used for evaluating models were mainly coefficient of determination for calibration (Rc^2) and for validation (Rv^2), root mean square error of calibration (RMSEC), root mean square error of cross validation (RMSECV), optimum number of components used (c_{opt}), number of iterations or recalculation based on Marten's uncertainty test for each of sensory attribute (Y), and the number of X-variables used in the prediction.

6.3 Results and discussion

6.3.1 Relationship between sensory and physicochemical properties of coffee

Multivariate analysis was performed on the dataset using mean data of sensory scores, physical measurements and compound concentrations. Prior to multivariate analysis, variables that were found to be not statistically significant (α= 0.05) between samples (by ANOVA) were identified.

Prior to partial least square modelling, the data were explored using PCA to see potential clustering of samples, relationships between all the variables and to explore differences between the samples. Principal component analysis bi-plots were generated on the standardised mean data for 26 coffee samples using 55 variables as presented in Figure 6.1 (PC1 versus PC2) and Figure 6.2 (PC1 versus PC3).

Based on the PCA, 53% of variation in the data set could be explained in the first three PCs indicating a complex set of data. Principal component 1 (22%) separated samples that scored high for *fruity*, caffeic acid, crude fat and titratable acidity to those in the left quadrant, with higher scores for *nutty, earthy, caffeine*, high L*value, pH and 4-ethylguaiacol (Figure 6.1).

Principal component 2 (18%) separated samples contained higher concentration of pyrazines, trigonelline, *β*-damascenone, 5-CQA and 3,5-diCQA from those that had lower concentrations of these compounds. Principal component 3 (13%) differentiated coffees that had higher concentrations of caffeine, guaiacols, 3,4-diCQA, 4,5-diCQA, pH, L*value and had high scores for *earthy, woody, smoky, cereal* attributes, from coffees that received higher scores for *dark chocolate, sourness* and had higher concentrations of trigonelline and crude fat (Figure 6.2).

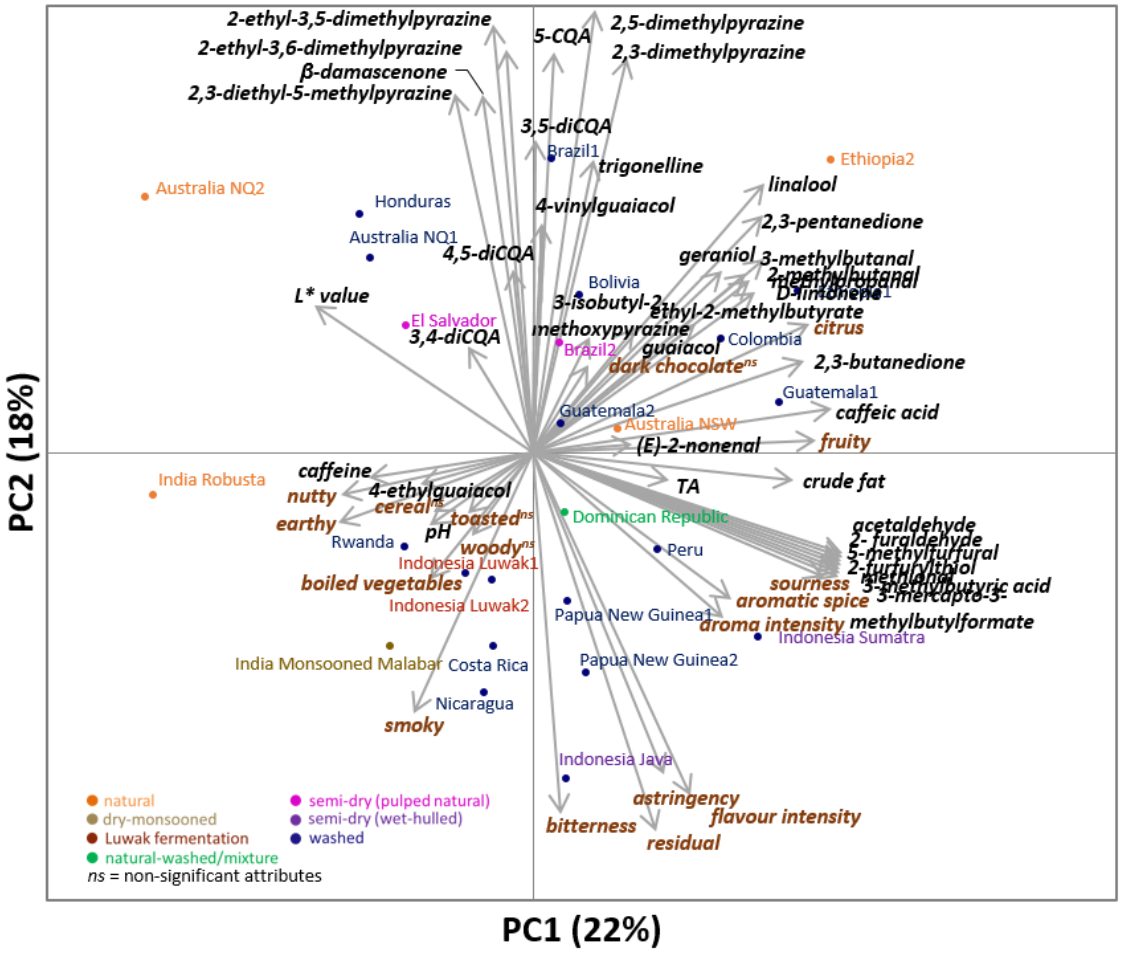

Figure 6.1 PCA bi-plot of 26 coffee samples based on 55 variables, PC1 (22%) versus PC2 (18%)

The bi-dimensional PCA plot revealed there was extensive variable redundancy which might create distortion and overfitting of the multivariate model. In order to avoid those problems, the dataset needed to be simplified. One way to simplify the data set is by grouping independent variables (X) that are found to be collinear, and have shared similarities in chemical structure, odour contribution and where synergies may be created in coffee aroma. Thus, the data set was simplified by generating new 'grouped variables', particularly for volatile compounds, by calculated a new single variable using a method previously reported by Aznar et al. (2003) and Smyth (2005). This method involved firstly identifying collinear compounds that were of similar chemical structure and similar odour contribution. The concentration of each compound in the group is then divided by its respective reported sensory (aroma) threshold and then adding collinear and similar

compounds together. In this way, the sensory contribution of each compound to the group is taken into account. For example, for pyrazines that were closely correlated to each other (r>0.70) were grouped together and a new single variable calculated. Other groups identified included: aldehydes (3-methylbutanal, 2-methylbutanal and methylpropanal), furans (furfural and 5-methylfurfural), a sulfur-containing compound group (methional, 2-furfurylthiol and 3-mercapto-3-methylbutylformate), phenolic aromatics (4-vinylguaiacol, guaicol and 4-ethylguaiacol) and also terpenes (linalool, geraniol and D-limonene). These groups are further detailed in Table 6.1.

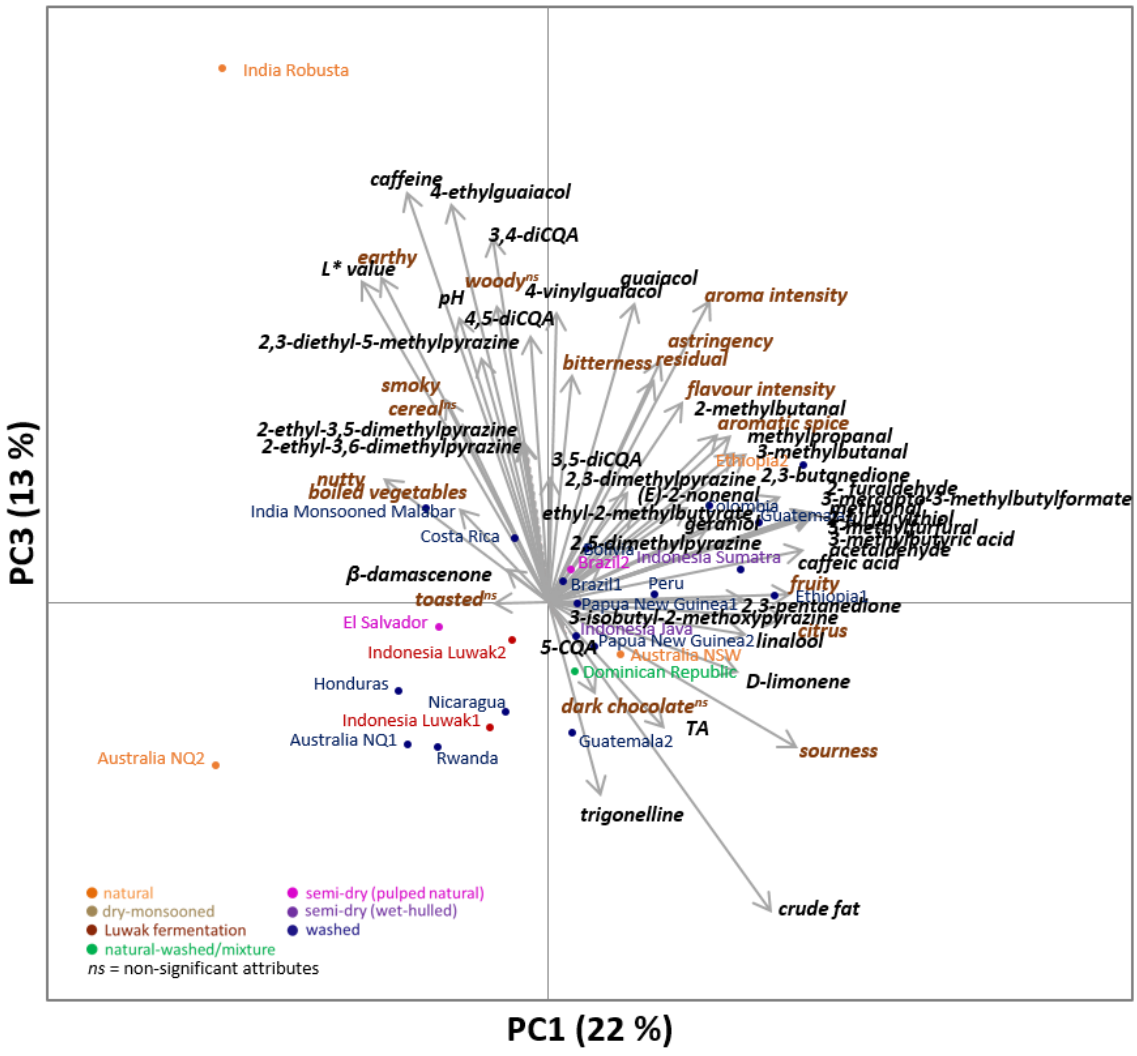

Figure 6.2 PCA bi-plot of 26 coffee samples based on 55 variables, PC1 (22%) versus PC3 (13%)

Besides volatile compounds, the non-volatile and high collinear chlorogenic acids were grouped (3,4-diCQA, 3,5-diCQA and 4,5-diCQA), but sensory threshold concentrations were not used and compound concentrations were simply added together.

Table 6.1 Grouping of highly correlated and structurally similar compositional variables

New group (number of compounds)	Attributes or compounds included in the group
ketones (2)	2,3-butanedione; 2,3-pentanedione
aldehydes (3)	methylpropanal; 3-methylbutanal; 2-methylbutanal
furan aldehydes (2)	furfural; 5-methylfurfural
terpenes (3)	linalool; geraniol; D-limonene
pyrazines (5)	2,5-dimethylpyrazine; 2,3-dimethylpyrazine; 2-ethyl-3,6-dimethylpyrazine; 2-ethyl-3,5-dimethylpyrazine; 2,3-diethyl-5-methylpyrazine
sulfur-containing compounds (3)	2-furfurylthiol; methional; 3-mercapto-3-methylbutylformate
phenols (3)	guaiacol; 4-ethylguaiacol; 4-vinylguaiacol
diCQAs (3)	3,4-diCQA, 3,5-diCQA, 4,5-diCQA

To further simplify the dataset, and reduce the potential of over fitting models, all variables which were found to be non-significantly different across the coffees were removed (including: *woody, cereal, toasted* and *dark chocolate*).

Principal component analysis bi-plots were again constructed using the simplified dataset containing the grouped variables and excluding variables which did not significantly vary (X=35). Furthermore, data were thoroughly explored with the inclusion or exclusion of the potential sample outliers based on previous PCA bi-plots (see Figure 6.1 and Figure 6.2) such as the India Robusta and the Australia NQ2 coffees, which separated far distance from other coffee samples. While the India Robusta coffee was a good sample for showing diversity of coffee flavour, it has highest leverage and residual. It was also indeed a different coffee species that might cause detraction of the multivariate models. Therefore, PCA bi-plots were constructed using standardised mean data without the India Robusta

sample (n=25) presented in Figure 6.3 and Figure 6.4 while the bi-plots with the India Robusta sample (n=26) could be found in Appendix K (p.187) and Appendix L (p.188).

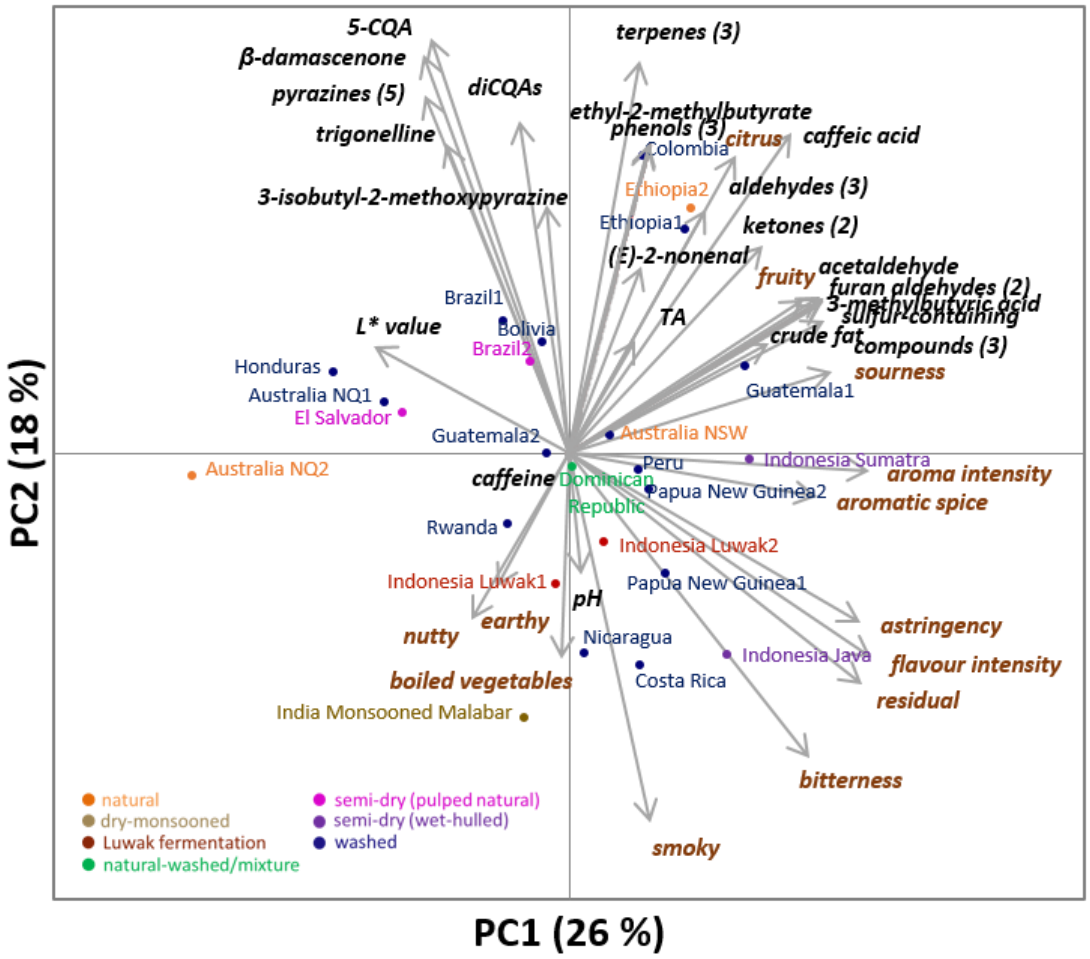

Figure 6.3 PCA bi-plot of 25 coffee samples based on 35 variables, PC1 (26%) versus PC2 (18%)

The first three PCs explains 54% of variation in the dataset. It seems that there were some potential clustering of samples roughly based on region of origin such as Ethiopia (Ethiopia 1 and 2), Brazil (Brazil 1 and 2), Papua New Guinea (Papua New Guinea 1 and 2) and Indonesia (Indonesia Luwak 1 and 2). Coffees from northern Queensland (Australia NQ 1 and 2) showed similar profiles but differed from the Australian coffee produced in northern New South Wales (Australia NSW). However, this section focuses on the relationship between variables and is not concerned with further exploration of sample differences.

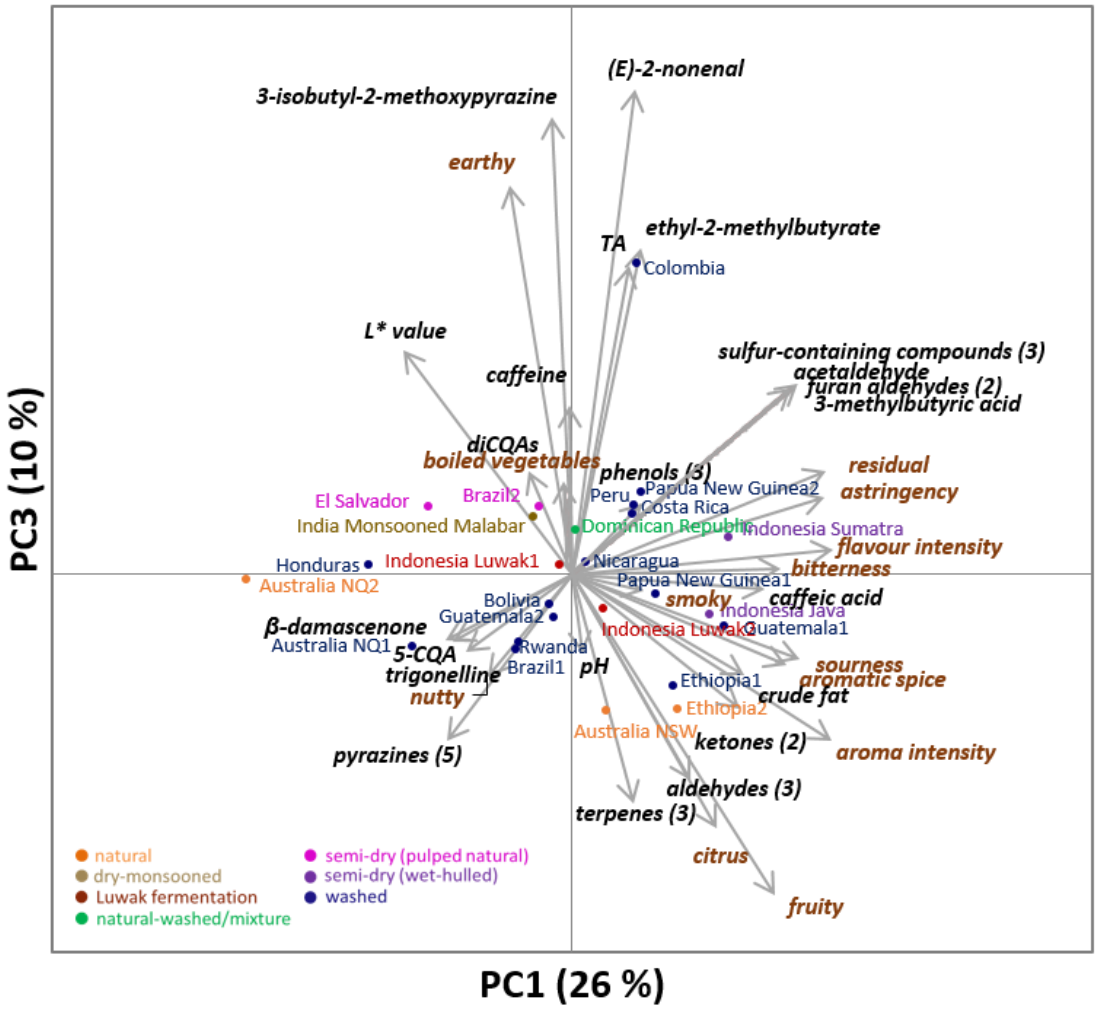

Figure 6.4 PCA bi-plot of 25 coffee samples based on 35 variables, PC1 (26%) versus PC3 (10%)

As can be seen from the bi-plot, PC1 and PC2 also showed some good relationships between sensory variables such as *sourness*, *citrus* and *fruity*, as shown by the close distance between each other in the PCA bi-plots on the up-right quadrant (Figure 6.3). *Earthy, nutty* and *boiled vegetables* attributes also appeared to have a potential relationship as also between *aromatic spice* and *aroma intensity*, as well as amongst the rest of in-mouth flavour attributes such as *bitterness, flavour intensity*, *residual,* and *astringency*. This trend could be explained partly due to potential influence of certain aroma to the intensity of particular taste or flavour attributes (Schifferstein and Verlegh, 1996) such as between *citrus, fruity* and *sourness*. Except for *sourness*, the perceived intensity of in-mouth flavour attributes were closely related, perhaps related to challenge in

flavour perception mechanisms. It is suggested there may be potential synergistic effects between in-mouth flavour attributes, similar to that reported for aroma perception characteristics (Laska et al., 1990). Alternatively, a stronger tasting coffee overall may indeed influence a panellist to inflate other taste scores rather than truly consider each attribute in isolation. For example, when a panellist tastes a coffee as strongly *bitter* or *astringent*, they may automatically also attribute a higher score for *residual* and/or *flavour intensity*. The challenges and fatiguing nature of coffee sensory evaluation has been previously detailed in Chapter 3 (p.30).

Some physicochemical and sensory attributes were positively correlated in the PCA including attributes *sourness* and *citrus* and compositional variables such as acids (caffeic acid and 3-methylbutyric acid) and titratable acidity. These were not correlated with chlorogenic acids as may have been expected. Furthermore, based on the PCA bi-plots, it can be seen that there were some inverse correlations such as between the sulfur-containing compounds, furan aldehydes and L*value (a measure of *lightness*). Since lower L*value represents a higher/darker degree of roasting, these result showed that the darker the roast (or the lower L*value), the higher the concentration of sulfur-containing compounds and furans. This is sensible give the knowledge that furans are formed through thermal degradation of carbohydrates, ascorbic acid, or unsaturated fatty acids during roasting (Crews and Castle, 2007, Ribeiro et al., 2009) while sulfur compounds are derived via direct degradation of amino acids or interaction with reduced sugars during roasting (Ribeiro et al., 2009).

While some of the relationships observed were similar to previous works such as between *citrus, fruity* and terpenes, aldehydes (Plotto et al, 2004, Lukić et al 2012), several relationships seemed inconsistent with previous studies. For example, the inverse relationship between sensory attributes *smoky, bitterness, astringency, residual, flavour intensity* and concentrations of trigonelline, 5-CQA, diCQAs, *β*-damascenone, and pyrazines. In other words, the higher content of those compounds in coffee might be related to lower scores for *smoky, bitterness, astringency, residual, flavour intensity.* Caffeine may have been expected to relate positively to those same sensory attributes, however, no relationship was observed. The results showed relationships between attributes were quite complex to explain since contribution of derivatives or intermediate products to certain sensory properties might be more dominant. For an example,

contribution of chlorogenic acids to *bitterness* and *smoky* is related mostly to the chlorogenic lactones and chlorogenic acids degradation products formed during roasting as reported by Farah et al. (2005b) and not directly related to the chlorogenic acids itself. Another example is for *bitterness* in coffee which is thought to be influenced by many other factors, not only by trigonelline which is cited as a bitter taste-contributing alkaloid (Flament, 2002). Thus, confirming the study by Kerler et al. (Kerler et al., 2014), it is highly likely that there are other more important bitter-related compounds not currently being evaluated in this study such as Maillard reaction products.

It should also be noted that volatile compounds currently being investigated are generally present at above their reported sensory detection threshold for each coffee sample (with the exception of (E)-2-nonenal and geraniol that could not be detected or quantified in certain samples). For this reason, relationships observed could indeed be considered cause and effect. In other words, all volatiles measured were present in the coffee at an 'aroma-active' concentration which the sensory panel could perceive. Importantly, it is likely that other important compound shave not been measured, thus the absence of concentration data for certain volatiles may result in inadequate explanation of certain sensory properties.

After thoroughly visualising and exploring the sensory and physicochemical components of coffee by PCA, and developing a reduced dataset with less redundancy, the data were ready for modelling using Partial Least Squares regression to determine if the compositional data was sufficient to build predictions of the sensory attribute scores.

6.3.2 Prediction of coffee sensory properties using compositional data

Partial least squares regression was performed to identify physicochemical variables (volatile and non-volatile compounds and physical properties) that might be responsible for specific sensory characters in the coffees. In the PLS regression, the models were validated using full cross validation (Wakeling and Morris, 1993, Wold et al., 2001) and the optimal number of component (or factors) were chosen (C_{opt}). Partial least square 2 was performed to investigate the overall relationships between 22 physicochemical attributes (X) and 13 sensory attributes (Y) of 26 coffee samples (shown in the Appendix M, p.189). However, while PLS2 is beneficial for interpretation purposes, PLS1 offers better prediction of each individual sensory attributes (Naes, 2002). Therefore, PLS1 was

performed using each sensory attribute individually, to build an optimised predictive equation of a given sensory property based on the minimum number of physical and chemical variables possible. This approach tries to isolate the key variables that may cause a specific aroma or taste in coffee.

Partial least square 1 was calculated as per the Marten uncertainty test that involves cross validation, jack-knifing (JK) and stability plots, until optimum models for each of sensory attributes (Y) are achieved (CAMO, 2006). Thorough PLS1 exploration for prediction of Y was performed to the dataset in order to find more robust models. Initially, multivariate models for Y was created using the full dataset including the India Robusta coffee (n=26) which had been detected as a potential outlier due to high leverage and residual. Subsequently, the models without the India Robusta coffee (n=25) were also created for comparison.

Table 6.2 provides calibration statistics including coefficient of determination for calibration (Rc^2) and for validation (Rv^2), root mean square error of calibration (RMSEC), root mean square error of cross validation (RMSECV), and optimum number of components used (c_{opt}), and number of iterations or recalculation based on Marten's uncertainty test for each of sensory attribute (Y).

An example of the use of Marten's uncertainty test to predict sourness (Y) using 22 physicochemical attributes as predictors (X) was given in Figure 6.5 and Figure 6.6. Figure 6.5 shows the cumulative importance of X variables for certain Y sensory responses based on the size of regression coefficients. The blue highlighted bars indicate unimportant or unstable variables to the models which were removed in a step-wise fashion.

Further recalculation or iteration with the selected variables using Marten uncertainty test involves elimination of unimportant variables with correlation coefficient close to 0. The best possible predictive model achieved for sourness was indicated by higher Rv^2 and lower RMSECV by using fewer significant variables (Figure 6.6).

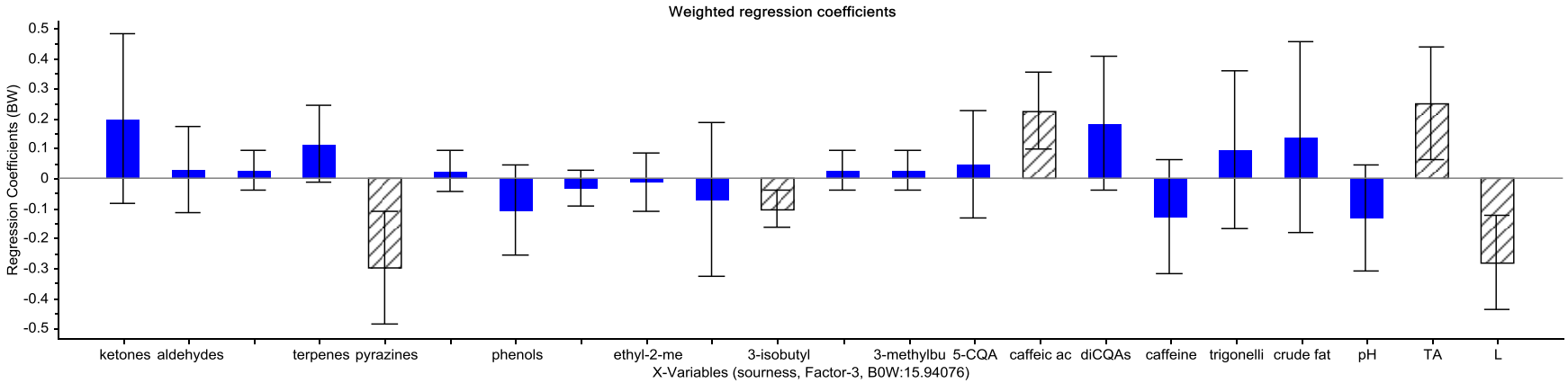

Figure 6.5 Physicohemical variables (X) important for sourness (Y) amongst 22 variables evaluated, except for the blue highlighted variables

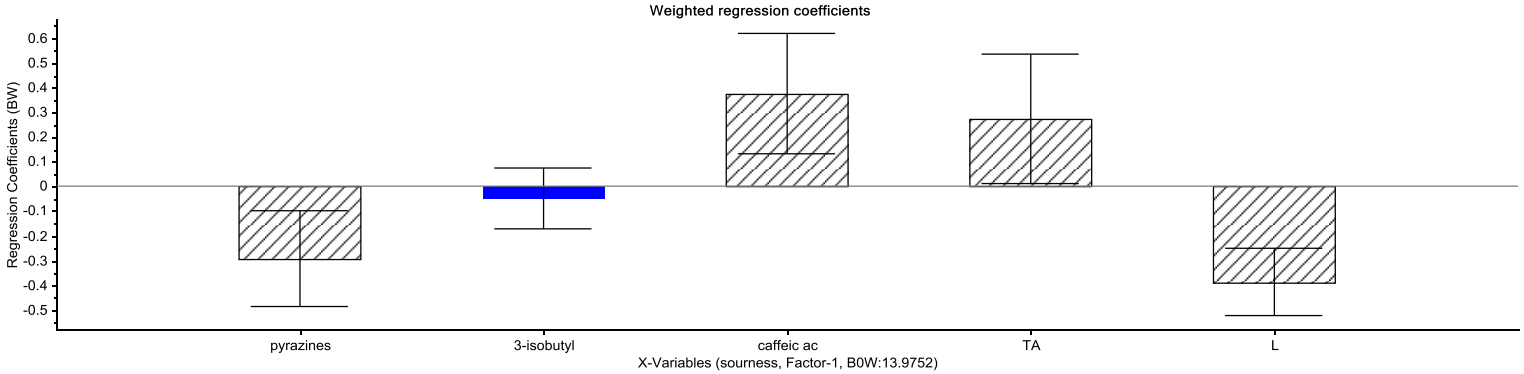

Figure 6.6 Physicohemical variables (X) important for sourness (Y) amongst 22 variables evaluated, re-calculated with marked attributes based on Martens uncertainty test (variable highlighted in blue is unimportant)

There is no single unit presented for level of error (RMSEC and RMSECV) since the X-variables were measured in different unit such as µg/kg and no unit for L variable (lightness). Data were also explored by adding other factors or components. However, in some cases the Rc^2 improved but could not be validated as indicated by lower Rv^2 and larger RMSECV showing that the model is over-fitted. Thus, only data from optimum component is presented in the Table 6.2. Calculation using data with the inclusion and exclusion of the India Robusta coffee is presented for comparison.

Based on Table 6.2, three out of 13 sensory attributes, including *citrus, earthy,* and *sourness,* could be adequately predicted with the inclusion of the India Robusta coffee, while seven out of 13 sensory attributes, including *aroma intensity, citrus*, *earthy*, *sourness*, *bitterness*, *flavour intensity* and *residual,* could be validated ($Rv^2 \geq 0.4$, RMSECV≤0.8) when the India Robusta coffee was excluded. This result confirms that the data from the one Robusta sample is influencing the development of models due to the Robusta being particularly different to the remaining 25 Arabica samples. For example, while a 'good' model could be created for *bitterness* as shown by $Rc^2=0.53$ with a dataset including the Robusta sample, it had low predictive power ($Rv^2=0.07$). Eliminating Robusta resulted in both a better prediction and therefore a more robust model for *bitterness* ($Rv^2=0.39$) with reduction of error or lower error of cross validation (RMSECV) even though the $Rc^2=0.49$ is not as strong as when the data from Robusta is included. Further, the Y variable *residual* showed a RMSECV of more than 0.8 even though predictive power was shown to be sufficient $R^2 \geq 0.4$. Removal of the Robusta data again offered a better prediction and lower error for this sensory attribute.

Important variables based on optimum PLS1 models for each y sensory attribute calculated with and without data from the India Robusta coffee are given in Table 6.3. However, only those Y variables that could be predicted ($Rv^2 \geq 0.4$) will be discussed further. As an example, *sourness* could be mathematically predicted by the measurement of four X-variables, namely: phenols, caffeine, and L*value (lightness) when the Robusta sample was included in the dataset. More robust models were created, however, without the inclusion of the Robusta sample. In this case, the ability to predict *sourness* relied on different variables including caffeic acid, titratable acidity, pyrazines and L*value with higher Rc^2 and Rv^2. Prediction for *citrus* relied on ketones and aldehydes mainly on the data set calculated without the Robusta coffee. Since the India Robusta is clearly

influencing some of the models for some parameters, further explanation will be based on the results from the calculation without the India Robusta coffee.

Sulfur-containing compounds that consisted of 2-furfurylthiol, methional, 3-mercapto-3-methylbutylformate were found to best predict *flavour intensity* positively. This suggests that when the concentration of these compounds was higher, coffee will be rated higher for *flavour intensity*. The compounds pyrazines (2,5-dimethylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-3,6-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine), *β*-damascenone, 5-CQA, trigonelline were found to be good predictors of *residual* and *bitterness* with a negative correlation meaning that when coffee contains lower concentration of those compounds, the *residual and bitterness* will be perceived as more intense. Interestingly, it appears the compounds responsible for *bitterness* in these coffees may have not been measured. Caffeic acid and titratable acidity were seen as a positive predictors of coffee *sourness* while L*value and the pyrazines (2,5-dimethylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-3,6-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine) were also important for the model.

It is interesting to note that titratable acidity was found to be more important to coffee *sourness* than pH, confirming an earlier study reporting the potential relationship between titratable acidity and acid taste (Bähre and Maier, 1999, Balzer, 2001). The variable pH was correlated negatively and responsible only for *aroma intensity* meaning that the aroma might be more intense when the pH is lower.

Table 6.2 Optimum PLS1 calibration (Rc^2, RMSEC) and validation statistics (Rv^2, RMSECV), number of optimum component (c_{opt}), and iteration for prediction of each Y sensory attributes

Predicted sensory attributes (y)	With the India Robusta coffee (n=26)						Without the India Robusta coffee (n=25)					
	Calibration		Validation		c_{opt}	Iteration	Calibration		Validation		c_{opt}	Iteration
	Rc^2	RMSEC	Rv^2	RMSECV			Rc^2	RMSEC	Rv^2	RMSECV		
aroma attributes												
aroma intensity	0.52	0.5	0.06	0.71	1	1	0.83	0.29	**0.49**	0.58	5	1
citrus	0.5	0.45	0.38	0.51	1	2	0.67	0.36	**0.36**	0.51	2	1
fruity	0.35	0.75	0.32	0.81	1	2	0.74	0.47	0.2	0.89	5	1
boiled vegetables	0.23	0.72	na.	0.89	1	1	0.31	0.68	0.03	0.84	1	1
aromatic spice	0.33	0.6	0.08	0.75	1	1	0.35	0.61	na.	0.8	1	1
earthy	0.92	0.19	0.64	0.4	5	1	0.71	0.27	**0.63**	0.31	3	2
nutty	0.34	0.56	0.19	0.68	1	1	0.25	0.56	na.	0.73	1	1
smoky	0.43	0.49	0.1	0.65	1	1	0.04	0.46	0.19	0.52	1	1
in-mouth flavour attributes												
sourness	0.67	0.52	0.5	0.62	2	1	0.72	0.44	**0.64**	0.5	3	2
bitterness	0.53	0.67	0.07	0.98	1	1	0.49	0.67	**0.39**	0.74	1	1
astringency	0.45	0.64	0.22	0.81	1	1	0.49	0.61	0.21	0.75	1	1
flavour intensity	0.28	0.73	0.26	0.81	1	2	0.55	0.58	**0.44**	0.7	1	1
residual	0.77	0.48	0.41	0.86	4	1	0.55	0.67	**0.52**	0.76	2	2

Acceptable models (R≥0.4) are presented in bold.

Table 6.3 Important variables based on optimum PLS1 models for each Y sensory attributes, comparison result between the inclusion and exclusion of the India Robusta coffee in the models

Predicted sensory attributes (Y)	With the India Robusta coffee (n=26)			Without the India Robusta coffee (n=25)		
	X-variable[a]	(+) loaded X-variable[b]	(-) loaded X-variable[c]	X-variable[a]	(+) loaded X-variable[b]	(-) loaded X-variable[c]
aroma attributes						
aroma intensity	5	furan aldehydes, sulfur-containing compounds, acetaldehyde, 3-methylbutyric acid, caffeic acid	-	2*	-	*β*-damascenone, pH
citrus	3*	ketones, aldehydes, caffeic acid	-	2*	ketones, aldehydes	-
fruity	2	crude fat	L*value,	0	-	-
boiled vegetables	1	-	caffeic acid	1	-	caffeic acid
aromatic spice	1	caffeic acid	-	2	sulfur-containing compounds, caffeic acid	
earthy	2*	Ketones	crude fat	4*	ketones, (E) -2-nonenal	caffeic acid, crude fat
nutty	4	ketones, furans aldehydes, sulfur-containing compounds, 3-methylbutyric acid		0	-	-

Predicted sensory attributes (Y)	With the India Robusta coffee (n=26)			Without the India Robusta coffee (n=25)		
	X-variable[a]	(+) loaded X-variable[b]	(-) loaded X-variable[c]	X-variable[a]	(+) loaded X-variable[b]	(-) loaded X-variable[c]
smoky	1	-	terpenes	2	-	pyrazines, *β*-damascenone
in-mouth flavour attributes						
sourness	3*	-	phenols, caffeine, L*value,	4*	caffeic acid, titratable acidity	pyrazines, L*value
bitterness	3	-	pyrazines, *β*-damascenone, 5-CQA	4*	-	pyrazines, *β*-damascenone, 5-CQA, trigonelline
astringency	5	furan aldehydes, sulfur-containing compounds, acetaldehyde, 3-methylbutyric acid, caffeic acid	-	5	furan aldehydes, sulfur-containing compounds, acetaldehyde, 3-methylbutyric acid, caffeic acid	-
flavour intensity	2	-	5-CQA, trigonelline	4*	sulfur-containing compounds	*β*-damascenone, 5-CQA, trigonelline
residual	3	-	pyrazines, 3-isobutyl-2-methoxypyrazine, 5-CQA	4*	-	pyrazines, *β*-damascenone, 5-CQA, trigonelline

[a]The number of X-variables used for optimum prediction

[b]X-variable that shows a positive correlation for the prediction of individual Y sensory attribute

[c]X-variable that shows a negative correlation for the prediction of individual Y sensory attribute

*showed significant prediction ($Rv^2 \geq 0.4$) of Y sensory attribute using a number of X-variables.

Some correlations found between X and Y attributes were counterintuitive to that of individual response to certain physicochemical attributes reported such as the case of *bitterness*. The attribute *earthy* could be predicted by the presence of ketones, (E)-2-nonenal and the absence of caffeic acid, crude fat (negatively loaded variables). While it could be suggested that the presence of higher caffeic acid and crude fat might enhance the perceived pleasant aromas such as *fruity*, thereby suppressing the *earthy* aroma, the explanation for ketones and (E)-2-nonenal vs-*earthy* relationship is challenging. This is counterintuitive with the individual aroma of those compounds since 2,3-butanedione and 2,3-pentanedione were reported to give *buttery-oily* aroma (Akiyama et al., 2007) and the (E)-2-nonenal with *green cucumber* aroma, rather than being perceived as *earthy*. Nevertheless, it is well known that a compounds odour contribution in a matrix may be quite different to its perceived odour as a neat chemical. The pyrazines, especially the ethenyl or alkylpyrazines (Oestreich-Janzen, 2010, Czerny et al., 1996), have often been mentioned as potential contributors to the *earthy* aroma in coffee but these were not found to be significant predictors of *earthy* in this study, showing that correlations found may not necessarily prove causality.

Ketones (2,3-butanedione and 2,3-pentanedione) and aldehydes (methylpropanal, 2-methylbutanal, and 3-methylbutanal) were found to be a positive predictors of *citrus* which is also at variance with the aroma previously described for these group of compounds. Earlier studies report that these compounds belong to *sweetish/caramel* group (Grosch, 2001a) even though it was also mentioned as having a *fruity green* aroma (Luebke, 2012). None of the terpenes (linalool, D-limonene and geraniol) and norisoprenoid (*β*-damascenone) expected to be important for *citrus* and/or *fruity* due to closer relationship observed in the PCA plot (Figure 6.1 and Figure 6.4), were found to be significant predictors of these sensory attributes. Given that certain volatile compounds are known to exhibit masking or synergistic effects with other volatile compounds, there is a possibility that the s*weet buttery malty caramel* group such as ketones and aldehydes may potentially enhance the *citrus* aroma perceived or may indeed be perceived as *citrus* when present at certain concentration levels and in a certain matrix.

It is clear that some of the sensory attributes could be predicted by certain X-variables in PLS models with an acceptable Rv^2 and RSMECV. There are between two and four grouped variables which were found to be of importance to the models of at least a third of

the sensory attributes. In other words, a few compositional variables were able to explain a great deal of the sensory information collected. The remaining attributes, for which adequate models were not developed, might be better predicted by other compositional variables which were not targeted and measured in this study. Further, coffee is an extremely complex beverage for which the flavour may not be adequately modelled using the approach of a limited number of targeted compositional variables as used in this study. More comprehensive compositional data together with addition of more samples would certainly be useful in a future study. Further analytical method development would be required to make this possible.

One limitation of the analytical method applied for the analysis was that the method conditions were not optimised for individual components specifically, rather it was a method that compromised across a diverse number of components to find the best method to measure a broad number of compounds in a single step (providing efficiency for high throughput). The downside of this approach is that many important coffee components and certain groups of compounds, including certain terpenes, aldehydes, ketones, pyrazines and certain sulfur-containing compounds, were not able to be adequately identified and/or quantified in a one-step method in such a complex coffee matrix. To measure these components, it may be necessary to develop discrete analytical approaches for each of these components individually, optimising for SPME headspace extraction, the type of fibre used, the GC column used or the amount of sample or pre-extraction steps etc. The discussion herein is therefore restricted by the analytical method and approach taken.

Another challenge that may affect the performance of the predictive models, may be the assumptions made while grouping and pre-treating the data which may have limited the information that could be extracted. Further, the quality of the sensory information may indeed be a limiting factor. The many challenges of coffee sensory evaluation involving human panellists have been previously explained in Chapter 3 (p.30), including the fatiguing nature of the beverage, and the limitations in sample preparation and presentation. These limitations may also include potential sensory adaptation due to frequent exposure to certain aroma and taste (Lawless and Heymann, 2010) and differences in sensitivity of the panellists to certain sensory attributes (Lawless and

Heymann, 2010, Lawless, 1999) that is supported by a complexity of flavour perception mechanism (Laing, 1994, Jinks and Laing, 2001, Engen, 1982).

6.4 Conclusion

Multivariate models between the physicochemical (volatile and non-volatile) attributes (X) and the sensory attributes (Y) could be validated for seven sensory attributes including *aroma intensity, citrus*, *earthy*, *sourness*, *bitterness*, *flavour intensity* and *residual*. As an example, *sourness* could be well predicted by the presence of caffeic acid and high titratable acidity (TA) together with low L (*lightness*) value as well as the absence of pyrazines (2,5-dimethylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-3,6-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine). *Citrus* aroma in coffee could be well predicted using concentration data for ketones (2,3-butanedione and 2,3-pentanedione) and aldehydes (methylpropanal, 2-methylbutanal and 3-methylbutanal) showing that those chemical components could indeed be playing a role in the *citrus* notes of coffee. Terpenes did not appear to show any importance to scoring of the *citrus* even though individually few terpenes were perceived as *citrus* or *fruity.*

Considering that coffee is very complex with 1000+ volatile compounds known to be present, it is highly likely that in this study not all the important components have been measured, thus not all predictive models performed well. However, this is the first study that involves a broad range of commercially available single-origin coffees and has a potential to provide the basis towards better understanding of coffee flavour.

Chapter 7 The evolution of chlorogenic acids in commercial single-origin coffees and their contribution to flavour

This chapter details an additional study with particular interest on chlorogenic acids of coffee. This chapter studied the evolution of chlorogenic acids from the green coffee beans to the coffee brews. The potential role of chlorogenic acids to flavour was also being investigated. The results offers more knowledge on chlorogenic acids in coffee while also providing a new insight on certain 'specialty' coffees.

7.1 Introduction

Coffee has been reported as one of the richest sources of chlorogenic acids in the human diet especially when compared to other beverages (Clifford, 1985b, Clifford, 2000). These compounds have been reported to offer many health benefits, most notably as an anti-bacterial agent together with anti-viral properties (Wang et al., 2009, Renouf et al., 2012). Chlorogenic acids also acts as a neuroprotective agent that inhibits key enzymes linked to Alzheimer's disease (Oboh et al., 2013) while also may act as a potential anti-obesity agent for humans (Williams et al., 2013). Other possible benefits that chlorogenic acids offer are for diabetic Type 2 treatment (van Dam and Feskens, 2002), the prevention of blindness in diabetic retinopathy patients (Shin et al., 2013) suffering from retinal damage due to diabetic complications, as well as supporting wound healing (Bagdas et al., 2014).

Chlorogenic acids comprise a family of esters formed between certain *trans*-cinnamic acids (phenolic acids most commonly caffeic, ferulic and *p*-coumaric acids in order of abundance) and quinic acid (Clifford, 1985b, Clifford, 1999). Major classes found in coffee are caffeoylquinic acids (CQA), dicaffeoylquinic acids (diCQA), feruloylquinic acids (FQA), *p*-coumaroylqunic acids (*p*-CoQA) and caffeoylferuloylquinic acids (CFQA) (Clifford et al., 2003). The compound 5-CQA had been reported as the main isomer of chlorogenic acids in coffee (Clifford, 1989), accounting for 80% of the total chlorogenic acids pool (Farah, 2012). Therefore 5-CQA often represents and refers to coffee chlorogenic acids.

In the past five decades, numerous studies have investigated chlorogenic acids in coffee. The most prominent studies were performed by Clifford's research group which identified

at least 45 isomers of chlorogenic acids in coffee (Clifford, 1986, Clifford, 1979, Clifford and Jarvis, 1988, Johnston et al., 2003, Clifford, 2006, Clifford et al., 2008). Current research has focussed on the health implications and absorption mechanisms or bioavailability of chlorogenic acids and its derivatives (Clifford, 2000, Olthof et al., 2001, Nardini et al., 2002, Monteiro et al., 2007, Farah et al., 2008). Several other studies examined chlorogenic acids relationship to genetics (Ky et al., 2001, Ky et al., 2013), environmental factors (Bertrand et al., 2006, Monteiro and Farah, 2012), the importance of processing (Duarte et al., 2010, Tfouni et al., 2014) as well as proposing the breakdown mechanism of these acids (Clifford, 2000, Dorfner et al., 2003, Coghe et al., 2004, Jaiswal et al., 2012).

There is increasing interest in premium coffees, such as single-origin, in the last decade due to their unique profile which makes them highly profitable. Previous research has identified chlorogenic acids from several single-origin coffees (Clifford and Jarvis, 1988, Ky et al., 2001, Alonso-Salces et al., 2009) but only a few coffees were studied. Therefore more information regarding the chlorogenic acids content of commercial single-origin roasted coffee beans and the corresponding brew would greatly assist in understanding the role of this important family of coffee bioactives.

The association of chlorogenic acids and coffee quality has been previously reported (Farah et al., 2006, Gichimu, 2014) with findings suggesting high levels of chlorogenic acids gives poor coffee quality. However, coffee quality is very complex so that the extent of the chlorogenic acids contribution to flavour requires further elucidation. More quantitative evidence is needed to fully understand the role of chlorogenic acids to coffee flavour (or quality). The current measurements are aimed at providing much-needed information which will contribute to a better understanding of coffee chlorogenic acids. The focus of this research was to observe the changes of chlorogenic acids from the green coffee beans, through roasting to cup (brew) and to assess any relationship between chlorogenic acids and certain volatile phenols important for coffee quality such as guaiacol, 4-ethylguaiacol and 4-vinylguaiacol. To the best of the authors' knowledge, this the first study that identifies and quantifies chlorogenic acids in Australian coffees and speciality coffees such as Monsooned and Luwak coffee.

7.2 Methodology

7.2.1 Analysis of chlorogenic acids in coffee

Since data for green and roasted coffee beans were extracted from previous Chapter 5 (p.89), only analytical procedures for the brew are explained in this section.

7.2.1.1 Materials

Samples of 26 commercial single-origin, medium-roasted, specialty coffees (Arabica and Robusta) and corresponding green coffee beans were obtained from coffee roasters and companies in Australia and Indonesia (see Chapter 3, p.39 for details).

Standard compounds and chemical reagents used involved 5-caffeoylquinic acid (5-CQA), 3,4-dicaffeoylquinic acid (3,4-diCQA), 3,5-dicaffeoylquinic acid (3,5-diCQA), and 4,5-dicaffeoylquinic acid (4,5-diCQA), acetonitrile and 2-propanol. Details provided in Chapter 5 (p.90).

7.2.1.2 Sample preparation

A 10 g of roasted coffee beans were taken out from -20°C and allowed to stand for 30 min before grinding using a CLIMAX Coffee Grinder (Climax Engineering, Melbourne, Australia).

7.2.1.3 Extraction

Extraction of chlorogenic acids from coffee brew was modified from Mills et al. (2013). Ground coffee (±1 g) was extracted using 45 mL boiled distilled water in a 50 mL falcon tube. The brews were allowed to stand for 1 min before vortex mixing for 10 s and then cooled immediately in an ice bath. A 4 mL of the subsequent brew was immediately taken and treated with Carrez reagents I & II (each of 0.1 mL) and 0.8 mL 100% methanol. The remainder of the brew was stored at 5°C until further analysis. The mixtures were then vortexed for 5 s and allowed to stand for 10 min before centrifugation at 4000 rpm for 5 min. The extract were filtered into HPLC vials (0.45 µm PVDF syringe filter) and stored at -80°C for HPLC analysis. Analyses were conducted in triplicates.

7.2.1.4 Instrumental conditions

Analysis was performed using a Shimadzu HPLC system with similar conditions detailed in Section 5.2.2.1, Chapter 5 (p.91).

7.2.2 Analysis of volatile phenols in coffee

As described in Chapter 4 (p.53), volatiles guaiacol, 4-vinylguaiacol and 4-ethylguaiacol were measured in beans of the roasted coffee samples. Concentration measurements were collected.

7.2.3 Statistical analysis

Data of chlorogenic acids and volatile phenols extracted from previous Chapters (Chapter 4, p.53 and Chapter 5, p.89), were tabulated together in Excel with current data for coffee brew. Further analysis of variance (ANOVA) and further Tukey-Kramer HSD at 95% confidence interval were performed using XLSTAT version 2015 (Addinsoft, New York, USA). Pearson's correlation matrix was constructed using XLSTAT version 2015 (Addinsoft, New York, USA) to know relationship between attributes.

7.3 Results and discussion

The objectives of this chapter were to profile and study the changes of the major coffee chlorogenic acids from the green coffee beans to the brew. The study targeted the CQA isomers namely 5-CQA, 3,4-diCQA, 3,5-diCQA and 4,5-diCQA (Figure 7.1) with an understanding that CQAs were responsible for approximately 80% of the total chlorogenic acids in coffee (Narita and Inouye, 2015, Farah, 2012). Relationships between chlorogenic acids and certain important volatile phenols such as guaiacol, 4-ethylguaiacol and 4-vinylguaiacol was also explored to determine if there is a relationship between chlorogenic acids content and resulting aroma notes of coffee.

7.3.1 Chlorogenic acids profiles

The compounds 5-CQA, 3,4-diCQA, 3,5-diCQA and 4,5-diCQA) were investigated across 26 single-origin commercial coffee samples to represent chlorogenic acids in coffee. An example of chromatogram obtained from analysis of green coffee beans that highlights

base-line resolution for the peaks of interest with a total running time of 11 minutes was depicted in Figure 7.2. This Figure 7.2 also visually shows the predominance of 5-CQA in green coffee beans as indicated by highest peak on the chromatogram. This is not surprising since previous studies reported similar trends with more than 50% of the total chlorogenic acids content being contributed by 5-CQA (Narita and Inouye, 2015, Farah, 2012).

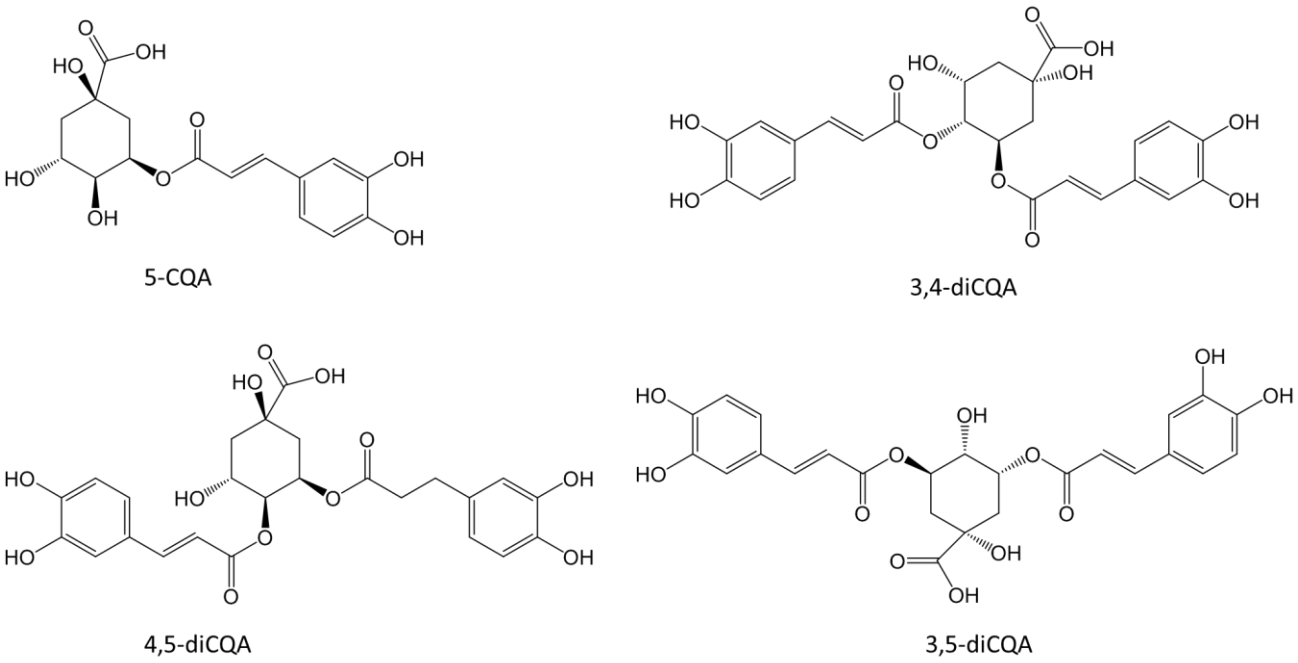

Figure 7.1 Chemical structures of 5-CQA, 3,4-diCQA, 3,5-diCQA and 4,5-diCQA

Table 7.1 presents a summary of chlorogenic acids measured with minimum, maximum, mean, standard deviation (SD), coefficient of variation (CV %) and significant difference (α=0.05), calculated from raw data across all coffee samples (26 samples x three replicates) for each of the green coffee beans, roasted coffees and the corresponding brews. Mean data of chlorogenic acid isomers evaluated for each coffee sample can be viewed in the Appendix N (p.190).

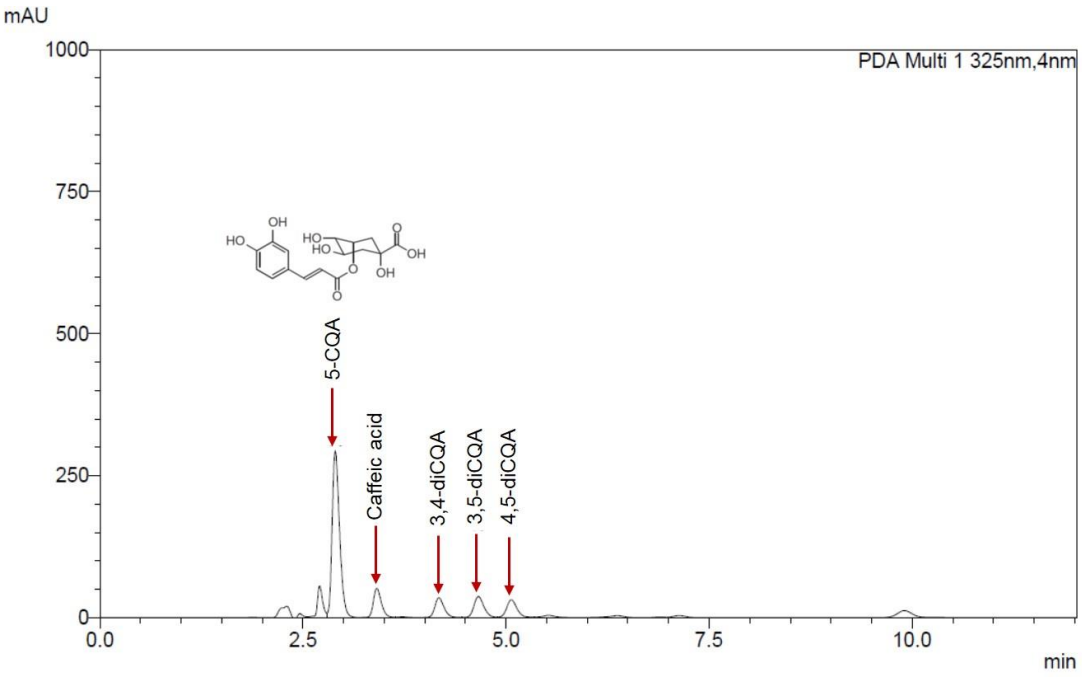

Figure 7.2 Chromatogram of chlorogenic acids and caffeic acid evaluated from one extract of green coffee bean

Table 7.1 Statistical summary of chlorogenic acid isomers investigated across coffee samples (26 samples x three replicates) for green coffee beans, roasted coffees and coffee brews

Attributes	Min	Max	Mean	SD	CV (%)
Green coffee beans					
5-CQA*	42	65	51	4.7	9.1
3,4-diCQA*	1.4	9.4	3.6	1.3	36
3,5-diCQA*	2.1	7.7	4.7	0.9	19
4,5-diCQA*	1.3	6.3	2.9	0.9	30
Roasted coffees					
5-CQA*	9.5	16	13	1.8	14
3,4-diCQA*	0.9	1.8	1.2	0.2	14
3,5-diCQA*	0.5	1.2	0.8	0.2	25
4,5-diCQA*	0.6	1.2	0.8	0.1	15
Coffee brews					
5-CQA*	79	136	107	12	11
3,4-diCQA*	4.3	8.4	5.4	0.8	15
3,5-diCQA*	3.3	6.4	5.1	0.7	15
4,5-diCQA*	1.8	4.1	3.3	0.4	13

Unit measurement for green coffee beans and roasted coffees were in mg/g dry matter, coffee brews were in mg/cup (200 mL). *Attributes are significantly different (α=0.05).

All of chlorogenic acid isomers such as 5-CQA, 3,4-diCQA, 3,4-diCQA, 4,5-diCQA were significantly different across the coffee samples assessed (α=0.05) in any processing stage i.e green beans, roasted beans, and the brews (Table 7.1). Generally, 5-CQA is the major chlorogenic acids in both the green and roasted bean and brew. However, the diCQAs showed the greatest variation amongst samples. This variation was found to be higher in green coffee beans as compared to roasted coffees and the brews (CV>15%) suggesting green coffee beans were quite diverse in diCQAs composition. This variation could be attributed due to many factors such as differences in variety, farming techniques as well as post-harvest treatments and processing. Subsequent processing such as roasting and brewing results in the reduction of CV (%) between samples.

Further comparison on chlorogenic acids profiles amongst 26 green coffee beans and the corresponding roasted samples were made. For this purpose, the individual diCQA isomers were summed as a 'diCQAs' group to simplify visualisation and to know contribution of these isomers to the total chlorogenic acids measured. Figure 7.2 shows comparison between the total chlorogenic acids and diCQAs in each of green coffee beans and roasted coffees while mean data for each coffee samples can be viewed in Appendix G-H (p.181-183). Total chlorogenic acids was a sum of all chlorogenic acid isomers measured (5-CQA, 3,4-diCQA, 3,4-diCQA, and 4,5-diCQA) while diCQAs is a sum of the diCQA isomers that includes 3,4-diCQA, 3,4-diCQA, and 4,5-diCQA.

The total chlorogenic acids of green India Robusta coffee beans was highest due to elevated concentrations of diCQA isomers (Figure 7.3). The discrepancy in chlorogenic acids content of green coffee beans can be attributed to the differences in genetic traits species (Ky et al., 2001, Ky et al., 2013) as well as post-harvest processing of coffee berries (Duarte et al., 2010). In the Arabica species, the Australian NSW coffee was found to have the highest total chlorogenic acids mostly due to elevated 5-CQA content while the coffees from north Queensland were found to possess lower chlorogenic acids. Besides the potential difference in cultivars grown in the New South Wales versus north Queensland, it is suggested that an increase in oxidative stress experienced by plants cultivated in the New South Wales could be the causative factor regarding chlorogenic acids concentration. This stress could be due to different weather conditions and geographical locations for coffee plant cultivation. The Australian north Queensland

coffees were cultivated under a different environment, one more mountainous and tropical with greater rainfall. Therefore, there might be less oxidative stress due to environmental factors as experienced by these coffee plants.

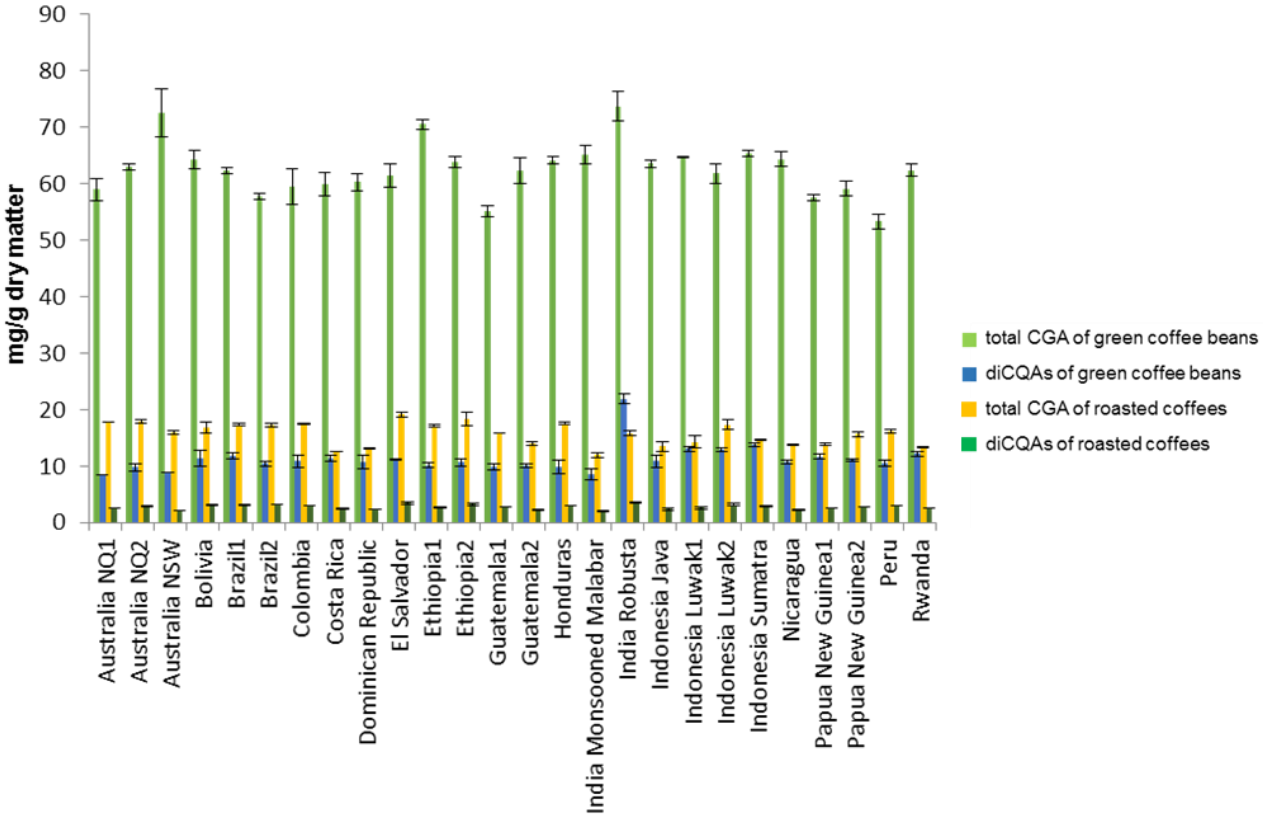

Figure 7.3 Total CGA (chlorogenic acids) and diCQAs calculated in green coffee beans and roasted coffees (n=3) for each 26 coffee samples.

The profiles of chlorogenic acids in green coffee beans were not necessarily similar to that of the corresponding roasted coffees due to different post-harvest treatment, physical properties and roasting profiles, for example, the India Robusta and the India Monsooned Malabar coffees. However, in the roasted coffee, the India Robusta still possessed the highest diCQAs concentration in comparison to other coffees (Figure 7.3). It was also clear that the diCQAs character were distinctive in the Robusta coffee species before and after roasting.

The roasted India Monsooned Malabar coffee had the lowest amount of chlorogenic acids content overall even though it was not the lowest in these compounds at the green bean

stage. This monsooned coffee was yellowish-green in colour and had a larger bean's size as compared to the other coffee samples. This can be explained as a result of exposure to high humidity monsoon conditions for weeks that allow green coffee beans to absorb moisture and sun-dried in cycles (Ahmad et al., 2003). Monsooning process was thought to increase susceptibility of chlorogenic acids degradation rate upon submission to roasting perhaps due to increasing porosity or surface area in contact with high temperature while roasting, and hydrolysis of chlorogenic acids.

The potential influence of different physical properties to the degradation rate of chlorogenic acids was also confirmed in the Indonesia Luwak coffees. The roasted Luwak 'Peaberry' (Indonesia Luwak 2) coffee has higher in total chlorogenic acids content than the non-peaberry (Indonesia Luwak 1), possibly because rounder-intact unsplit beans 'Peaberry' coffee beans has less surface area in contact to high roasting temperature which may have led to less chlorogenic acids degradation. There is no previous studies that could be used to compare the chlorogenic acids content of these specialty coffee types.

7.3.2 The evolution of chlorogenic acids in commercial coffee beans

It is understood that processing and preparation steps have a great influence on the levels of chlorogenic acids content of any given coffee. Therefore, the amount of chlorogenic acids consumed by coffee drinkers will depend very much on how much is lost during processing. Figure 7.4 presents the total chlorogenic acids levels in 26 commercial single-origin green coffee beans, roasted coffees and the brews under examination in the current study as well as the percent loss from green-to-brew. The percentage loss was presented as an average loss of each chlorogenic acid isomers (5-CQA, 3,4-diCQA, 3,5-diCQA, 4,5-diCQA) across coffee samples (26 samples x three replicates). Percent loss from roast to brew was calculated based on the concentration of chlorogenic acids on a fresh weight basis as per the domestic brewing procedure (55 g fresh ground coffee per 1 litre of water). An assumption of 200 mL of coffee per cup (11g of coffee/200 mL) was used.

As can be seen from Figure 7.4, this study found a significant decrease in chlorogenic acids content after roasting and brewing with the most recognisable reduction experienced

after roasting due to high temperature at approximately 180°C to 240°C, changing the composition and structure of coffee. The 5-CQA loss from green-to-roast processing was 74.7% while losses of lower magnitude were observed for the three isomers except for the 3,5-diCQA. In contrast, brewing was found to have a higher impact on the diCQAs when compared to the 5-CQA. Approximately 26% of chlorogenic acids remains in the roasted beans while only 13% left in the brew that we drink. A cup of 200 mL coffee contains 91.6-150.6 mg of chlorogenic acids, which is in a range of previously reported level in Arabica coffee brews (between 70-200 mg/200 mL) and in Robusta coffee brews (between 70-350 mg/200 mL) (Clifford (1999).

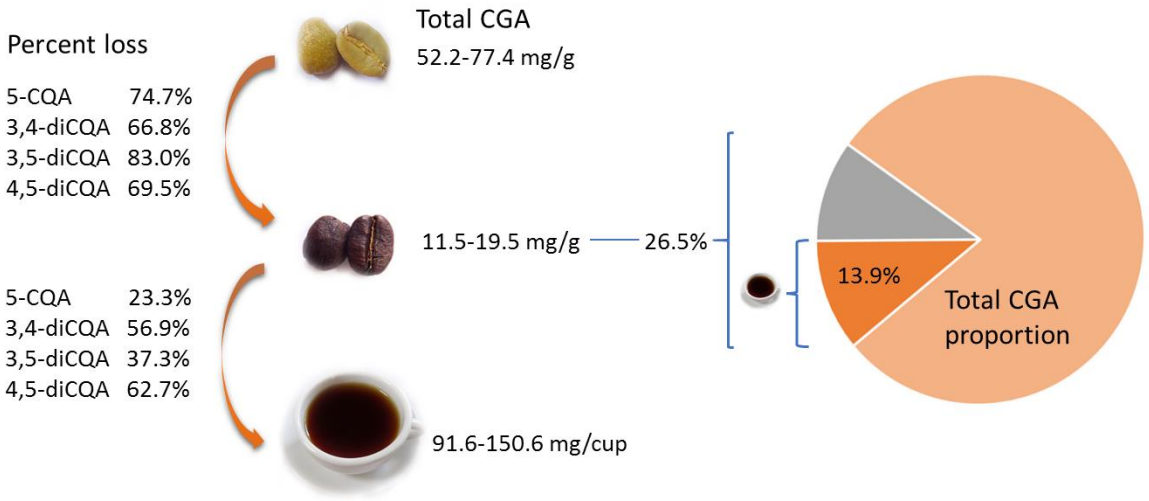

Figure 7.4 Total CGA (chlorogenic acids) levels and percent loss of chlorogenic acids measured from the green coffee beans to the coffee brews (26 samples x three replicates)

The reduction rate of chlorogenic acids in the processing depends on a number of factors, but it is mainly affected by the roasting parameters. A previous study reported that CQA levels of light roast coffee were higher than dark roast (Tfouni et al., 2014). Not only the roasting degree, the roasting time and speed to achieve certain colour level (or roasting level) may vary and thus affect compositional and quality characteristics of the end product (Toci et al., 2009). This suggests the same coffee roasted to the same degree such as in medium roasting level may differ in chemical composition if subjected to different roasting

conditions. Unfortunately, this roasting parameter couldn't be controlled in the current study since the coffees were obtained from commercial roasters and coffee producers. However, we can draw a line that all coffees observed are in the medium roasted level despite potential variation of this roasting level due to different roasting conditions. Similar to ground roasted coffee, the content of chlorogenic acids (including lactones) in brewed coffee has been reported to vary depending on blend, roasting temperature, coffee grind size, coffee-to-water proportions, brewing method, water temperature and length of time the coffee is in contact with water (Farah, 2012).

Chlorogenic acids is the major polyphenol found on the coffee seed's surface (Clifford and Kazi, 1987) synthesised as a defensive mechanism against environmental oxidative stress (Haard and Chism, 1996) and is potentially important for seed germination and cell growth (Clifford, 1985b). These compounds are susceptible to enzymatic and non-enzymatic oxidation (Rendón et al., 2014) as well as thermal degradation (Tfouni et al., 2014) along the cultivation, post-harvest and processing chain. Variability of chlorogenic acids could be contributed to by genetic variability, such as differing species (Ky et al., 2001, Ky et al., 2013), climate and growing elevation (Bertrand et al., 2012, Bertrand et al., 2006), cropping season (Monteiro and Farah, 2012) and different farming practices such as fertiliser application (Malta et al., 2003) as well as being subject to oxidative stress (Haard and Chism, 1996). Additionally, the fruit maturation stage has been reported to influence chlorogenic acids levels (Clifford and Kazi, 1987, Montavon et al., 2003) where CQA level decreased during maturation (Montavon et al., 2003), probably due to oxidation and hydrolysis of diCQAs into monoesters (Farah and Donangelo, 2006).

Discrimination of single-origin coffees based on phenolic content has also been reported (Mehari et al., 2016, Alonso-Salces et al., 2009). However, commercial single-origin coffees available in the market could be a mixture of coffee from different farms but still from the same country/area. These coffee beans may have been collected from fruit at different maturity levels and therefore a large variation of chlorogenic acids and distribution of isomers could be expected. The variability of chlorogenic acids concentration, along with other chemical components, contributes to the complexity of the coffee matrix and thus different reduction or degradation rates will be observed.

Structurally, the loss rate of chlorogenic acids in coffee depends on the binding of these compounds with other components in coffee and the strength of that bond. Chlorogenic acid is associated with cuticular wax, as well as the cytoplasm adjacent to the cell walls of the endosperm parenchyma (Clifford et al., 1987). The liberation will involve the breakdown of cell walls that could be caused by microbial infection, mechanical disruption, compositional changes in coffee fruit during maturation and/or other environmentally induced factors.

The evolution of chlorogenic acids during coffee processing involves the degradation of chlorogenic acids into smaller molecules as well as the binding of the chlorogenic acids and its derivatives to other compounds in the coffee matrix. Biochemical reactions responsible for the decrease of chlorogenic acids in roasting includes acyl migration, isomerisation, lactonisation, epimerisation, hydrolysis, degradation into low molecular weight compounds and polymerisation (Clifford, 1989, Jaiswal et al., 2012, Farah, 2012).

The isomerisation of chlorogenic acids takes place in the beginning of roasting process (Trugo and Macrae, 1984, Leloup et al., 1995). The diCQAs could be hydrolysed into CQA and caffeic acid that will again follow the hydrolysis and decarboxylation cycle. Prolonged roasting time will degrade the phenolic and quinic moiety and yield diverse phenolic compounds (Leloup et al., 1995). Another compound, chlorogenic lactones (CGL) could be generated during the roasting process. It has been reported that less than 8% of chlorogenic acids in green Arabica and Robusta coffee beans were transformed into 1,5-ɣ-quinolactones (Farah et al., 2005a, Farah et al., 2005b) through the loss of a single water molecule from the quinic acid moiety and the formation of an intramolecular ester bond (Farah, 2012). Besides the role in lactone formation, chlorogenic acids could be incorporated into melanoidins (Bekedam et al., 2008) as well as being partly bound to caffeine (Ky et al., 2013).

The aforementioned evolution mechanism of chlorogenic acids leads to the variability of coffee composition and thus could influence the sensory or cup quality. For example, the produced lactones have been reported to contribute to coffee bitterness (Farah et al., 2005a, Farah and Donangelo, 2006). The incorporation of chlorogenic acids in the complex polymers such as melanoidins indicates the involvement of chlorogenic acids to flavour since melanoidins are known to influence colour and flavour of coffee (Bartel et al.,

2015, Hofmann et al., 2001, Moreira et al., 2012). The production of certain volatile phenols from chlorogenic acids degradation could be important to coffee quality (Toci and Farah, 2014).

7.3.3 Impact of chlorogenic acids on coffee volatile phenols

In this study, three important volatile phenols (guaiacol, 4-ethylguaiacol and 4-vinylguaiacol), exhibiting phenolic, harsh medicinal or spicy aroma of the roasted coffee were investigated using a stable isotope dilution analysis/gas chromatography-mass spectrometry (SIDA/GC-MS) method (reported in Chapter 4, p.53). The structures of guaiacol, 4-ethylguaiacol and 4-vinylguaiacol are displayed in Figure 7.5.

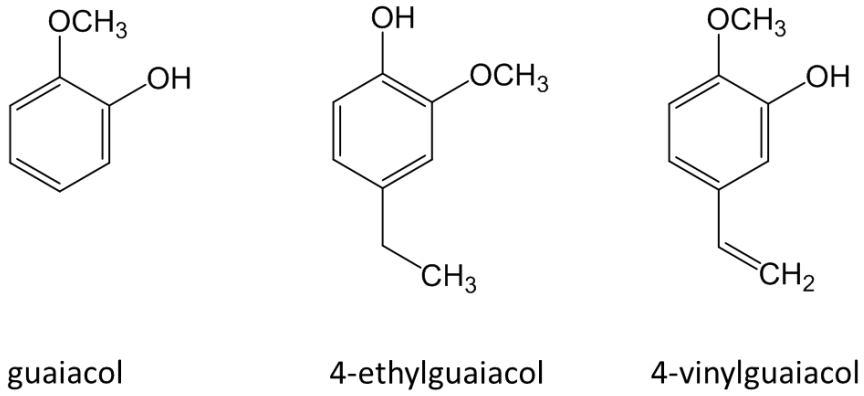

Figure 7.5 Chemical structures of guaiacol, 4-ethylguaiacol and 4-vinylguaiacol

Correlation matrix between chlorogenic acids isomers (5-CQA, 3,4-diCQA, 3,5-diCQA, 4,5-diCQA) and the volatile phenols (guaiacol, 4-ethylguaiacol and 4-vinylguaiacol) concentrations performed using mean data (26 samples x three replicates) of the compounds present in the green coffee beans, roasted coffees and coffee brews was presented in Table 7.2. While all of the volatile phenols were correlated ($r>0.5$), there was no correlation ($r<0.5$) between volatile phenols and chlorogenic acids except for that between diCQAs and 4-ethylguaiacol, particularly in the green bean form. Interestingly 3,4-diCQA is correlated positively to 4-ethylguaiacol in all forms of coffee: green, roasted & brew ($r>0.5$), suggesting this compound is more stable than the other chlorogenic acids isomers during processing and potential as a marker of 4-ethylguaiacol. The explanation could be related to the chemical structure of 3,4-diCQA that has the second strongest bond after the 1,3-diCQA and is therefore less susceptible to isomerisation or acyl

migration (Clifford, 1989). Thermal degradation was thought to firstly occur on the other weaker bonded diCQA isomers.

Certain phenols such as guaiacol, 4-ethylguaiacol and another non-phenolic group compounds were suggested as indicators of low quality coffee (Toci and Farah, 2014) formed such as via degradation of chlorogenic acids. However, there is no correlation found between percentage of chlorogenic acids loss from green to roasted coffee and the volatile phenols (Appendix O, p.191).

Certainly chlorogenic acids plays an important role in the volatile phenols synthesis pathway mainly through degradation of the quinic acid moiety. Its involvement in the production of lactones, incorporation in Maillard products and caffeine, or degradation into other lower molecular compounds via acyl migration, isomerisation, lactonisation, epimerisation, hydrolysis, and polymerisation has been recognised. The evidence gathered from this study indicates that the chlorogenic acids isomers generally has little impact on the poor aroma quality of coffee except for 3,4-diCQA that could be related to the spicy aroma of 4-ethylguaiacol. Since 4-ethylguaiacol has a lower odour activity value (OAV) than guaiacol and 4-vinylguaiacol, this compound was considered as less important to overall coffee aroma. Therefore, contribution of 5-CQA and diCQA to overall flavour might not be apparent except via indirect reactions or being the precursors of other compounds that may be associated with coffee quality. Potentially the feruloylquinic acid (FQA) could be more important to those volatile phenols via the ferulic acid degradation pathway. This isomer might show a greater association to 4-vinylguaiacol and guaiacol since these compounds were formed through the ferulic acid degradation pathway (Dorfner et al., 2003). Therefore, even though the FQA proportions to the total chlorogenic acids in roasted Arabica and Robusta coffee is lower than that of CQA and diCQA (Narita and Inouye, 2015), inclusion of this compound in future studies could offer more benefits.

Table 7.2 Pearson's correlation matrix between each chemical attributes analysed in green coffee beans, roasted coffees, and coffee brews (26 samples x three replicates)

Variables	guaiacol	4-ethylguaiacol	4-vinylguaiacol	5-CQA g	3,4-diCQA g	3,5-diCQA g	4,5-diCQA g	5-CQA r	3,4-diCQA r	3,5-diCQA r	4,5-diCQA r	5-CQA b	3,4 diCQA b	3,5 diCQA b	4,5 diCQA b
guaiacol	**1**														
4-ethylguaiacol	**0.63**	**1**													
4-vinylguaiacol	**0.74**	**0.70**	**1**												
5-CQA g	0.01	-0.06	-0.05	**1**											
3,4-diCQA g	0.10	**0.68***	0.29	-0.07	**1**										
3,5-diCQA g	0.46	**0.50**	0.40	-0.04	**0.53**	**1**									
4,5-diCQA g	0.14	**0.59**	0.21	-0.12	**0.87**	0.42	**1**								
5-CQA r	-0.15	-0.19	0.19	0.05	-0.17	0.00	-0.32	**1**							
3,4-diCQA r	0.22	**0.57***	0.48	-0.26	**0.64**	**0.50**	**0.52**	0.43	**1**						
3,5-diCQA r	-0.02	-0.05	0.23	-0.30	0.11	0.17	0.07	**0.65**	**0.57**	**1**					
4,5-diCQA r	0.17	0.37	0.44	-0.39	0.44	0.45	0.37	**0.58**	**0.93**	**0.72**	**1**				
5-CQA b	-0.27	-0.24	0.04	0.35	-0.06	-0.09	-0.14	**0.61**	0.14	0.35	0.24	**1**			
3,4 diCQA b	0.01	**0.53***	0.18	0.14	**0.81**	0.30	**0.76**	-0.13	**0.53**	0.05	0.32	0.24	**1**		
3,5 diCQA b	0.25	0.30	0.21	-0.19	0.39	0.34	0.48	-0.37	0.24	-0.07	0.20	-0.01	0.49	**1**	
4,5 diCQA b	0.06	-0.05	0.14	-0.33	0.05	0.22	0.14	0.19	0.26	0.37	0.40	0.23	0.21	0.47	**1**

Letter g, r, b means data obtained from green coffee beans, roasted coffees, and coffee brews, respectively. Bold typeface indicated significant correlation (α=0.05) with r>0.5, *indicated consistent significant correlation in green coffee beans, roasted coffees, and coffee brews .

Further exploration on the data was performed by calculating ratio between each of the chlorogenic acids isomers and the diCQAs measured. Interestingly, since 3,4-diCQA/diCQAs ratio was found to be quite stable during processing from green beans to brew, 3,4-diCQA by itself or the ratio of 3,4-diCQA/diCQAs has potential as discrimination markers of coffee origins, confirming research by Alonso-Salces et al. (Alonso-Salces et al., 2009) reporting 3,4-diCQA as a discriminating compound for African and American green coffee beans. Furthermore, it was revealed that the 5-CQA/diCQAs ratio of green coffee beans and roasted coffees were correlated as shown in Table 7.3 and Figure 7.6, while there was no correlation of 5-CQA/diCQAs ratio observed between green coffee beans or roasted coffees with the coffee brews. This indicates that discrimination of coffee origins using certain chlorogenic acids isomers or its ratio with total chlorogenic acids will only be applicable on green coffee beans and roasted coffees. Once hot water was introduced to the coffee through brewing process, this ratio and compositional balance will be changed significantly. Another investigation will be required to confirm this finding since this is out of the current research scope.

Table 7.3 Pearson's correlation matrix between 5CQA/diCQAs ratio calculated in green coffee beans, roasted coffees, and coffee brews (26 samples x three replicates)

Variables	5-CQA/ diCQAs g	5-CQA/diCQAs r	5-CQA/diCQAs b
5-CQA/diCQAs g	**1**	**0.87**	0.07
5-CQA/diCQAs r	**0.87**	**1**	-0.01
5-CQA/diCQAs b	0.07	-0.01	**1**

Letter g, r, b means data obtained from green coffee beans, roasted coffees, and coffee brews, respectively. Bold typeface indicated significant correlation (α=0.05).

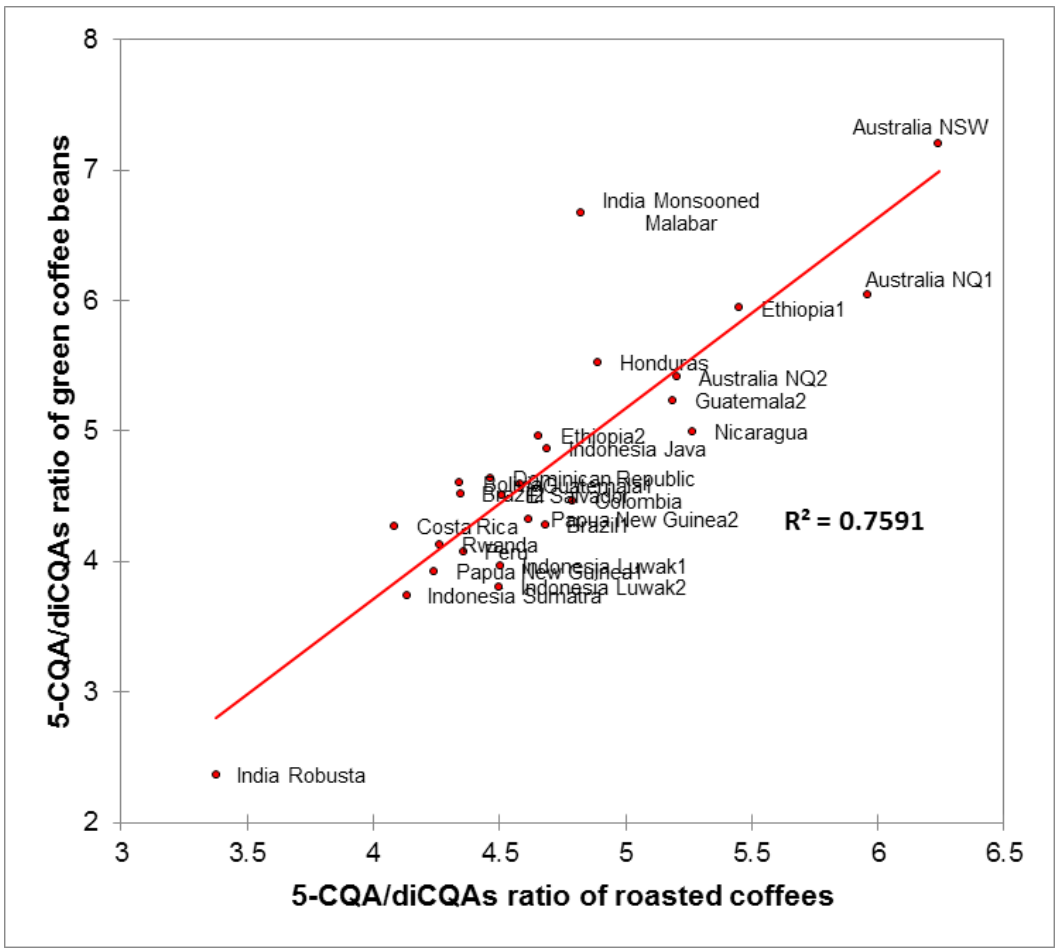

Figure 7.6 Scatterplot matrix between 5-CQA/diCQAs ratio of green coffee beans and roasted coffees (mean data, n=26)

7.4 Conclusions

It is clear that a difference in coffee species influenced the chlorogenic acids profiles of the green and roasted coffee beans. Additionally, the environmental factors and/or growing regions were believed to have an impact on the chlorogenic acids concentration in coffee. However these acids showed different non-linear behaviour after roasting and brewing due to further variables being involved with the greatest influencing factor being the roasting process.

Generally, roasting of the green beans to a medium level significantly reduces total chlorogenic acids concentration up to three quarter of its concentration in the green coffee

beans. Major degradation was observed on the 5-CQA as compared to the diCQA isomers. Reduction rates of coffee chlorogenic acids in the processing relied mainly on roasting parameters such as temperature, time and speed, as well as internal characteristics (composition) of its coffee matrix including the chemical bond of chlorogenic acids and availability of other compounds for further biochemical reactions. The brewing process halved the total chlorogenic acids levels in the roasted coffee beans. Nevertheless, chlorogenic acids intake from coffee brews through oral consumption is still considerably high as compared to other plant resources.

Despite providing useful information and evidence in this study, further information on whether or not the chlorogenic acids plays a prominent role in influencing coffee quality is still required. In order to better address this issue, further studies should be conducted that includes other chlorogenic acid isomers and/or other chlorogenic acids-related compounds, in conjunction with thorough sensory evaluation.

Chapter 8 General conclusions and recommendations for further research

8.1 General conclusions

The goal of this research was to understand how different components in coffee influence flavour perception, focusing on what could potentially become markers of coffee flavour. The exploration involves sensory and physicochemical profiling of sensorily diverse commercial single-origin 'specialty' coffees.

Initially, a thorough review of the scientific literature on coffee flavour was performed with a main focus to develop a list of target compounds that are likely to be important to coffee flavour (Chapter 2, p.5). Secondly, a sensory screening of a broad range of medium-roasted single-origin specialty coffees from around the world (n=59) was conducted to identify and select a set of coffees (n=26) that represented the sensory diversity of coffee flavour available commercially. Quantitative descriptive techniques, involving a trained sensory panellists, was applied to further evaluate sensory profiles of the selected coffees (Chapter 3, p.30). Thirdly, analytical chemistry methods were developed to measure a range of important volatile compounds in coffee, which were most likely to have an influence on coffee flavour. The analytical methods were applied to the selected coffees (n=26) and the data were analysed (Chapter 4, p.53). The selected sensorily diverse coffees (n=26) were also analysed for various non-volatile components and physical properties which were also thought to be of importance to coffee flavour (Chapter 5, p.89). Finally, multivariate data analysis techniques were used to predict sensory scoring of attributes using the compositional data. Thus the relationships between sensory properties of coffee and coffee components were explored (Chapter 6, p.104). Aside of the main project objective, an additional study was conducted whereby the evolution of a range of important chlorogenic acids from the green coffee beans to the coffee brews and the potential role to coffee flavour were also explored (Chapter 7, p.125).

There are very limited good quality sensory studies of coffee where sensory properties are linked to the compositional profiles of coffee. Much research has been conducted over the last century on coffee with most studies being dedicated to understanding the health

benefits of coffee and/or improving quality. Innumerable studies have been conducted reporting profiles of coffee volatiles, however, as efficient method is lacking that can simply and accurately identify as well as quantify a range of important coffee volatile compounds. Therefore, analytical approaches were developed and validated for this purpose involving headspace-solid phase micro extraction/stable isotope dilution analysis/gas chromatography-mass spectrometry (HS-SPME/SIDA/GC-MS), steam distillation/HS-SPME/SIDA/GC-MS, and steam distillation/liquid injection/ SIDA/GC-MS. Collectively, these methods provided the measurement of 27 important volatile compounds efficiently. There were some complications and challenges, however, mostly in relation to the complexity of coffee matrix as well as instability of certain compounds and instrumental limitation. Nevertheless, the developed method was proven to be applicable and provides an overall benefit in measuring many important compounds comprehensively in single run. This will give an advantage for application in research on coffee flavour or for industrial application where high throughput and rapid screening of many samples are required.

It is interesting to know that based on the results, certain volatile compounds could have potential as a discrimination marker of origins such as terpenes, ketones and aldehydes that specifically characterised Ethiopian coffees. This is also the first study that is successfully quantify geraniol in Ethiopian coffee and that is reporting volatile profiles of some more unique and lesser studied samples including Australian coffee, Indonesia Luwak (Arabica) and Indian Monsooned Malabar.

Besides investigating volatile profiles of commercial single-origin coffees, this study also showed that these coffees were diverse in their non-volatiles physicochemical profiles. The India Robusta coffee was found to be the most distinctive mainly due to its double caffeine content as compared to the Arabica coffees. This non volatiles data is meaningful but will be more useful when used together with previous sensory and volatiles data to understand the role of the composition to sensory properties.

In attempt to determine the compositional basis of coffee flavour, all the data collected in this study for the 26 sensorily diverse coffees were brought together and explored using multivariate analysis tools. The coffee sensory, volatile, non-volatile and certain physical measurements were analysed through partial least squares modelling to understand potential relationships between compositional and sensory variables. The ultimate goal

was to achieve the best robust predictive models for each sensory attribute. Exploration included simplification of data using “grouped variables” to reduce redundancy, removal of non-significant attributes, inclusion and exclusion of the sample having highest leverage and residual (the India Robusta coffee). As many as seven out of 13 sensory attributes including *aroma intensity, citrus, earthy, sourness, bitterness, flavour intensity* and *residual* could be well validated in the model. Some of the results were in line with previous coffee studies even though some relationship appeared to be inconsistent. The reason could be because not all the important components have been measured, thus not all predictive models performed well. Some examples of the missing compounds includes all furanones group such as sotolon and abhexone that may contribute to *sweet, caramel, seasoning-like* notes could be associated to *cereal* or *chocolate*, a *sweet fruity* raspberry ketone compound such as 4-(4-hydroxyphenyl)-2-butanone, the *earthy* ethenylpyrazines, the *smoky-roasted* furans and some other pyrazines that might contribute to *nutty, toasted, dark chocolate* aromas. However, this the first scientific report of a comprehensive sensory-compositional study for such a diverse range of commercial single-origin coffees. Therefore, it has a potential to provide a strong framework to build on towards better understanding coffee flavour and diversity.

Aside from this project, this thesis also has particular interest on studying the evolution of chlorogenic acids from the green coffee beans to the coffee brews and to know the potential role to flavour. It is interesting to know that less than a fifth of chlorogenic acids out of the concentration in green coffee beans remains in a cup of 200 ml coffee that people drink, while three quarters of chlorogenic acids destroyed due to coffee roasting. It was understood that the chlorogenic acids role in coffee flavour might involve certain mechanisms such as the volatile phenols synthesis pathway through degradation of the quinic acid moiety, lactonisation into chlorogenic lactones, incorporation to other compounds, etc. However, no relationship was found between these phenols and the chlorogenic acids level even though it seemed that 3,4-diCQA might be associated with 4-ethylguaiacol (*spicy* aroma). Additionally, 5-CQA/diCQAs ratio could have a potential for discrimination of coffee origins. Further justification and evidence pertaining to whether or not the chlorogenic acids plays a prominent role in influencing coffee quality will be beneficial for further research.

8.2 Recommendations for further research

Since coffee is a very complex and challenging sample to evaluate, it is advised that more work need to be done to develop a carefully controlled method for different coffees and diverse sensory properties. The improvement could involve the choice and/or the development of sensory evaluation techniques and analytical methods.

It is also recommended to use or to combine this descriptive technique with other sensory evaluation methods such as time intensity (TI) and temporal dominance sensations (TDS) considering the flavour of coffee is dynamically changing during assessment. It is also potential to conduct real time analysis such as a combination between dynamic sensory and nosespace analysis (Charles et al., 2015) or a common gas chromatography-olfactometry (GC-O), where a direct comparison between sensory and chemistry could be performed. Certainly these analysis will be limited mainly to sensory versus volatiles (aroma) and therefore the non-volatiles and physical profiles investigation should be performed separately.

It is also suggested that the assessment of roasted coffee ground for aroma evaluation could sufficiently be beneficial such as for the discrimination of coffee from different origins or different processing since coffee ground aroma could represent aroma of the brews. Surely, different methods will have dissimilar advantages and drawbacks and therefore the choice should always consider the purpose of the study.

Coffee exhibit broad diversity that is likely to create equally diverse chemical compositional profiles. Consequently, the matrix effect add complications and challenges during development and application of analytical methods for coffee volatile analysis, which need to be addressed in the future work. For example, matrix influence on the volatile compounds analysis could be eliminated such as through solvent extraction or distillation procedures. However, since those techniques are typically time-consuming, and require a lot of solvents and are limited to a few compounds only. The simultaneous direct HS-SPME method described in this thesis can be applied for rapid screening of many coffee samples. Re-validation and improvement could be performed with the inclusion of more target volatile compounds should the standards be available. It should be noted that coffee volatiles are inherently unstable and therefore, sample extract should be prepared

carefully. The analysis of each compound should also be carried out against appropriate internal standard. The challenge to find a coffee-like model matrix for the calibration development could be addressed by formulating a solution resembling the coffee matrix in chemical composition without having a strong odour like that of coffee. In this way, better calibration functions for quantification could be achieved.

The inclusion of more targeted compounds, volatiles and non-volatiles in a further method improvement could improve the performance of multivariate models for a better predictions. Certainly the models needs more work to prove mathematical relationship that could be demonstrated by using some extra experiments such as reconstitution of the aroma models, omission and spiking, such that of previously performed by Grosch (2001b) and Czerny et al. (1999).

The potential of certain chemical properties such as chlorogenic acid isomers, volatile terpenes, aldehydes and ketones for discrimination of coffee origin will open up opportunities for further research that may include non-destructive methods such as near-infrared (NIR) and mid-infrared (MIR) spectroscopic techniques.

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APPENDICES

Appendix A 1. Evidence of UQ Ethical Clearance for the Research

THE UNIVERSITY OF QUEENSLAND

Institutional Human Research Ethics Approval

Project Title:	The Compositional Basis Of Coffee Flavour - 18/11/2013 - AMENDMENT
Chief Investigator:	Ms Wenny Bekti Sunarharum
Supervisor:	Dr Heather Smyth, Prof Mike Gidley, Dr David Williams, Dr Glen Fox
Co-Investigator(s):	Dr Heather Smyth
School(s):	Queensland Alliance for Agriculture and Food Innovation (QAAFI)
Approval Number:	2013000052
Granting Agency/Degree:	Endeavour Awards, Department of Industry, Innovation Science, Research and Tertiary Education
Duration:	30th June 2016

Comments/Conditions:

Note: if this approval is for amendments to an already approved protocol for which a UQ Clinical Trials Protection/Insurance Form was originally submitted, then the researchers must directly notify the UQ Insurance Office of any changes to that Form and Participant Information Sheets & Consent Forms as a result of the amendments, before action.

Name of responsible Committee:
Behavioural & Social Sciences Ethical Review Committee
This project complies with the provisions contained in the *National Statement on Ethical Conduct in Human Research* and complies with the regulations governing experimentation on humans.

Name of Ethics Committee representative:
Associate Professor John McLean
Chairperson
Behavioural & Social Sciences Ethical Review Committee

Signature JPMc Date 19/11/2013

Appendix A.2. Example of sensory worksheet

Name/ID:_____ Date: __ /__ /____

Booth #	Sample

Aroma **Scales***

	none	*high*
aroma intensity		
citrus		
fruity		
boiled vegetables		
aromatic spice		
woody		
earthy		
nutty		
cereal		
toasted		
smoky		
dark chocolate		
'other' aroma		

'other' aroma description :……

Taste/aftertaste	*low*	*high*
sourness		
bitterness		
astringency		
flavour intensity		
'other' flavour		

'other' flavour description ……

	short	*long*
residual		

Comments : ……
……

*15 cm line scale was adjusted to fit paper

Appendix B. Mean scores for each sensory attribute assessed for each coffee samples (14 panellists X three replicates), significance and multiple comparison of sample means at 95% confidence interval

Samples	*aroma intensity**	*citrus**	*fruity**	*boiled vegetables** #	*aromatic spice**	*woody*	*earthy**	*nutty** #	*cereal*	*toasted*	*smoky**	*dark chocolate*	*sourness**	*bitterness**	*astringency**	*flavour intensity**	*residual**
Australia NQ1	7.1 abc	1.5 ab	1.9 abc	3.4 abc	1.7 b	4.4	1.0 b	2.7 abcd	2.9	3.8	2.1 ab	2.7	6.3 abcd	6.0 abc	3.4 ab	6.9 ab	5.7 abc
Australia NSW	8.7 ab	1.8 ab	3.4 abc	3.2 abc	2.6 ab	4.1	0.7 b	4.2 abcd	4.0	3.1	1.4 ab	2.9	7.5 abc	5.8 abc	4.2 ab	8.0 ab	6.5 abc
Australia NQ2	6.3 c	1.1 ab	0.8 c	3.6 abcd	2.0 ab	5.0	2.6 ab	3.1 a	3.1	2.5	1.1 ab	2.0	5.6 abcd	5.0 c	3.3 ab	6.1 b	4.7 c
Bolivia	7.7 abc	1.7 ab	2.9 abc	2.1 cd	2.5 ab	4.6	1.4 ab	2.7 abcd	3.4	2.9	1.7 ab	3.4	6.8 abcd	6.2 abc	4.7 ab	7.2 ab	6.7 abc
Brazil1	8.2 abc	2.2 ab	3.6 abc	4.5 a	3.0 ab	5.0	1.2 ab	2.0 cd	3.0	2.6	1.1 ab	3.2	6.6 abcd	5.4 bc	4.1 ab	7.5 ab	6.4 abc
Brazil2	7.6 abc	1.3 ab	2.0 abc	3.0 abcd	2.8 ab	4.9	1.5 ab	3.0 abcd	3.7	3.0	1.1 ab	2.8	6.4 abcd	6.5 abc	4.7 ab	8.0 ab	7.1 abc
Colombia	7.7 abc	1.9 ab	2.3 abc	2.7 abcd	3.0 ab	5.0	2.2 ab	1.8 d	2.9	2.8	1.0 ab	2.5	7.2 abcd	6.1 abc	5.1 ab	8.1 ab	6.0 abc
Costa Rica	8.2 abc	1.0 ab	2.0 abc	4.2 ab	3.5 ab	4.8	2.2 ab	3.4 abcd	2.9	3.0	2.6 ab	2.2	6.7 abcd	8.5 ab	5.7 ab	9.3 a	8.4a
Dominican Republic	7.7 abc	0.9 ab	2.4 abc	3.9 abc	3.0 ab	5.5	1.2 ab	2.2 cd	2.8	2.9	1.5 ab	2.9	6.7 abcd	6.3 abc	4.3 ab	7.1 ab	6.1 abc
El Salvador	6.7 bc	1.1 ab	1.8 bc	2.0 cd	2.0 ab	4.1	1.8 ab	4.1 ab	3.5	3.3	1.8 ab	2.1	6.8 abcd	4.8 c	3.8 ab	7.1 ab	6.5 abc
Ethiopia1	8.1 abc	3.2 a	3.3 abc	1.5 d	3.5 ab	4.4	0.5 b	2.4 bcd	3.4	3.0	0.6 b	2.4	7.7 a	7.2 abc	5.3 ab	8.1 ab	7.5 abc
Ethiopia2	9.4 a	3.0 ab	5.2 a	2.4 bcd	5. 0 a	4.8	1.7 ab	2.1 cd	3.5	2.5	1.9 ab	2.8	7.6 ab	6.3 abc	4.3 ab	8.4 ab	7.7 abc
Guatemala1	9.0 ab	1.7 ab	3.4 abc	2.5 bcd	3.8 ab	3.6	0.9 b	2.8 abcd	3.6	3.4	1.9 ab	3.0	7.5 abc	7.2 abc	5.4 ab	8.6 ab	7.9 abc
Guatemala2	7.4 abc	2.0 ab	2.8 abc	2.7 abcd	2.0 ab	4.2	1.1 b	2.6 abcd	3.1	3.6	1.2 ab	3.0	6.9 abcd	6.2 abc	4.4 ab	7.7 ab	6.0 abc
Honduras	7.1 abc	0.7 b	1.8 bc	2.0 cd	2.3 ab	3.6	1.0 b	2.5 abcd	3.0	3.0	1.0 ab	4.0	4.9 cd	5.7 abc	2.8 b	6.0 b	4.8 bc
India Monsooned Malabar	8.4 abc	0.9 ab	1.7 bc	3.8 abc	3.5 ab	5.0	2.1 ab	3.8 abc	3.8	3.5	2.5 ab	2.4	5.0 bcd	7.6 abc	5.1 ab	8.3 ab	7.5 abc
India Robusta	9.0 ab	0.8 b	1.4 bc	3.9 abc	2.9 ab	6.7	3.8 a	4.0 ab	4.4	3.1	3.1 a	2.5	4.8 d	8.5 ab	5.7 ab	8.6 ab	8.1 ab
Indonesia Luwak1	8.0 abc	1.1 ab	2.8 abc	2.1 abcd	2.9 ab	3.1	1.2 ab	2.5 abcd	3.6	2.8	2.7 ab	3.2	7.5 abc	6.6 abc	4.5 ab	7.8 ab	7.5 abc

Samples	*aroma intensity**	*citrus**	*fruity**	*boiled vegetables** [#]	*aromatic spice**	*woody*	*earthy**	*nutty** [#]	*cereal*	*toasted*	*smoky**	*dark chocolate*	*sourness**	*bitterness**	*astringency**	*flavour intensity**	*residual**
Indonesia Luwak2	8.0 abc	1.4 ab	3.1 abc	2.0 cd	4.1 ab	4.2	1.0 b	3.3 abcd	4.1	3.2	2.5 ab	2.7	8.1 a	7.2 abc	5.6 ab	8.9 ab	7.6 abc
Indonesia Java	8.6 abc	1.7 ab	4.3 ab	2.8 cd	4.0 ab	4.8	1.3 ab	2. 5 abcd	3.8	3.1	2.6 ab	2.5	7.2 abcd	8.7 a	6.4 a	9.6 a	8.5 a
Indonesia Sumatra	8.7 ab	2.5 ab	3.3 abc	3.3 abcd	2.9 ab	5.2	1.4 ab	2.6 abcd	2.9	2.6	1.5 ab	3.5	8.2 a	7.2 abc	5.4 ab	8.4 ab	8.5 a
Nicaragua	7.6 abc	1.8 ab	1.7 bc	2.7 abcd	3.7 ab	5.5	2.0 ab	1.8 d	2.8	2.7	2.1 ab	2.8	6.6 abcd	7.4 abc	4.3 ab	8.6 ab	7.1 abc
Peru	7.7 abc	1.6 ab	2.5 abc	3.7 ab	2.8 ab	5.5	2.1 ab	2.0 cd	2.6	2.8	1.6 ab	2.2	7.4 abcd	6.3 abc	4.8 ab	8.7 ab	7.5 abc
Papua New Guinea1	8.7 ab	1.3 ab	2.5 abc	4.4 abc	3.4 ab	4.4	1.4 ab	2.2 abcd	3.4	2.8	1.7 ab	2.7	7.6 ab	7.5 abc	6.3 a	8.7 ab	7.6 abc
Papua New Guinea2	8.5 abc	1.3 ab	2.3 abc	3. 8 abc	3.0 ab	4.9	1.8 ab	2.7 d	3.1	3.4	1.5 ab	2.3	7.7 a	7.0 abc	4.9 ab	8.4 ab	6.7 abc
Rwanda	7.9 abc	2.0 ab	3.1 abc	3.2 abcd	2.4 ab	3.7	1.1 b	3.5 abcd	4.4	2.6	1.0 ab	3.3	7.4 abc	6.8 abc	4.5 ab	7.7 ab	7.0 abc
Pr > F	0.001	0.009	0.003	0.008	0.038	0.33	0.017	0.027	0.44	0.99	0.002	0.77	0.000	0.000	0.005	0.003	0.001
Significant	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No	Yes	No	Yes	Yes	Yes	Yes	Yes

*Indicates attributes that are significantly different (α=0.05)

Coffee samples sharing similar letters means no difference based on further Tukey-Kramer HSD

[#]Duncan multiple comparison was used to differentiate groups instead of Tukey-Kramer HSD.

Appendix C. Pearson's correlation coefficient matrix of 17 coffee sensory attributes

Variables	*toasted*	*nutty*	*earthy*	*boiled vegetables*	*woody*	*smoky*	*cereal*	*bitterness*	*residual*	*astringency*	*flavour intensity*	*aroma intensity*	*aromatic spice*	*fruity*	*sourness*	*citrus*	*dark chocolate*
toasted	**1**																
nutty	0.34	**1**															
earthy	-0.21	0.22	**1**														
boiled vegetables	-0.2	0.08	0.46	**1**													
woody	-0.21	-0.07	**0.74**	0.53	**1**												
smoky	0.26	0.28	0.43	0.18	0.21	**1**											
cereal	0.11	**0.64**	0.06	-0.1	-0.12	0.26	**1**										
bitterness	0.02	0.01	0.27	0.26	0.35	0.58	0.25	**1**									
residual	-0.03	0	0.21	0.26	0.26	0.5	0.2	**0.83**	**1**								
astringency	0	-0.08	0.16	0.24	0.31	0.37	0.22	**0.8**	**0.91**	**1**							
flavour intensity	-0.02	-0.1	0.17	0.26	0.33	0.48	0.12	**0.8**	**0.91**	**0.86**	**1**						
aroma intensity	-0.13	0.01	0.04	0.23	0.18	0.36	0.37	**0.62**	**0.68**	**0.66**	**0.72**	**1**					
aromatic spice	-0.2	-0.33	0	-0.07	0.14	0.29	0.1	0.54	0.55	0.55	**0.68**	**0.72**	**1**				
fruity	-0.25	-0.32	-0.48	-0.15	-0.19	-0.07	0.13	0.13	0.25	0.3	0.38	**0.61**	0.56	**1**			
sourness	-0.12	-0.36	-0.52	-0.21	-0.31	-0.23	-0.14	0.01	0.36	0.36	0.39	0.3	0.33	**0.61**	**1**		
citrus	-0.29	-0.34	-0.4	-0.33	-0.08	-0.4	-0.07	-0.07	0.03	0.06	0.17	0.29	0.35	**0.64**	0.55	**1**	
dark chocolate	-0.11	-0.21	-0.5	-0.31	-0.44	-0.19	0.06	-0.14	-0.3	-0.24	-0.32	0.09	-0.08	0.28	0.05	0.13	**1**

Bold typeface indicates significant correlation (α=0.05), r ≤ -0.60 and r ≥ 0.60.

Appendix D. Concentration of targeted volatile compounds: aldehydes, ketones, esters, pyrazines, phenols, terpenes and norisoprenoids measured in commercial single-origin roasted coffees

Samples	methylpropanal	3-methylbutanal	2-methylbutanal	(E)-2-nonenal	2,3-butanedione	2,3-pentanedione	ethyl-2-methylbutyrate	2,5-dimethylpyrazine	2,3-dimethylpyrazine	2-ethyl-3,5-dimethylpyrazine	2-ethyl-3,6-dimethylpyrazine	2,3-diethyl-5-methylpyrazine	3-isobutyl-2-methoxypyrazine	D-limonene	guaiacol	4-ethylguaiacol	4-vinylguaiacol	linalool	geraniol	β-damascenone
Australia NQ1	2224	3312	1309	*nd*	16759	14207	1	131291	20668	1757	9932	132	31	408	2444	292	23590	92	*nd*	6
Australia NSW	2832*	3311	1649	*nd*	20868	12141	1	104156*	16819	1543*	10003*	155	10	559	3167	295	25626	238	*nq*	3
Australia NQ2	1786	2611*	1124	1	15231	12679*	2	95557	14336*	1701	10333	170	21	494	2344	212	20793	191	*nq*	4
Bolivia	3699	5300	1906	*nd*	23896	13553	1	120139*	17903*	1786*	9335*	143	23	505	4002*	337	24552	205*	*nq*	4
Brazil1	3784	4722	1985	1	22754	14090	2	141488	22157	2460	16757*	259	21	488	2683	238	24059	235	*nq*	4
Brazil2	1374	2550	1000	1*	13292	10374*	1	126093*	15599	1952	14012*	197	23	563	2891	268	24641	230	*nq*	5
Colombia	2160*	3463	1315	31*	21961	13687	8	106516*	16016*	1282	6863	168*	115*	612	4356	546	28878	207*	*nq*	4
Costa Rica	2578	3816	1501	9	20484	14271	2	83870	13753	1077	6430	88	15	473	4357	320	21982	120	*nd*	2
Dominican Republic	2083	3167	1288	5	11709	9681	3	82244	12105	1205	7615	123	18	499	3575	319	23343	226	*nq*	3
El Salvador	2139	3513	1310	1	15945	14447	1	93085	14137	1524	10053	162	19	445	2126	178	19765	148	*nd*	3
Ethiopia1	2799	4231	1604	2	21605	16065	2	114128	17544	1832	11448	179	9	743	3894	250	21511	485	71	4
Ethiopia2	5497	6708	2696	2	35392	29488	5	126335	20859	1930	11602	153	6	619	4203	240	24852	453	65	3
Guatemala1	4338	6251	2136	1	28065	21211	2	125887	17901	1780	11716	164	11	485	3742	268	24197	190	*nq*	3
Guatemala2	3251	4100	1701	1	22255	17504	2	104565	13039	1273	9097*	135*	11	520	2887	236	19563	191	*nq*	3
Honduras	1984	2616	1152	1	6287	10051	1	142217	10122	2219	12297	201	24	472	3190	280	22964	195	*nq*	4

Samples	methylpropanal	3-methylbutanal	2-methylbutanal	(E)-2-nonenal	2,3-butanedione	2,3-pentanedione	ethyl-2-methylbutyrate	2,5-dimethylpyrazine	2,3-dimethylpyrazine	2-ethyl-3,5-dimethylpyrazine	2-ethyl-3,6-dimethylpyrazine	2,3-diethyl-5-methylpyrazine	3-isobutyl-2-methoxypyrazine	D-limonene	guaiacol	4-ethylguaiacol	4-vinylguaiacol	linalool	geraniol	β-damascenone
India Monsooned Malabar	2352	2759	1375	1	13708*	7449	1	71071	12715*	1499	8964	168	12	438	3199	356	21457	90	*nd*	1
India Robusta	2317	3091	1399	2	12946	4807	1	77216*	12238*	1961*	12167*	251*	1	391	4162	824	29075	44	*nd*	4
Indonesia Luwak1	1959	2628	1247	1	12888	9995	1	62618	7420	874	7265	117	15	547	2622	336	20762	118	*nd*	2
Indonesia Luwak2	1680	2277	965	1	15495	9437	1	68427	9050	951*	6498*	99	15	525	1984	222	21369	141	*nq*	2
Indonesia Java	2156	2856*	1079	2	16531	10526*	1	47923*	7139	637*	3521*	52*	17	477	2656	277	18307	111*	*nq*	2
Indonesia Sumatra	3015	4144	1789	2	20581	11624	2	63515	9105	947	6162	100	16	449	2316	295	20416	134	*nd*	2
Nicaragua	2546	3175	1324	1	18176	10801	1	48654	6972	764	4093	68	11	464	1731	182	14013	102	*nd*	2
Peru	3309	4598	1815	4	26835	14904	1*	73033	10964	1034	6172	100	16	483	2214	220	18202	126	*nd*	3
Papua New Guinea1	3062	4583	1875	1	27272	16741	1	84746	10194*	995	5917	83	11	431	3249	293	19235	167	*nq*	3
Papua New Guinea2	1008	1703	662	2	16426	5647	2	42145	6520	729	4089	73	10	494	2522	234	23714	206	*nq*	3
Rwanda	1161	1926	750	1	10096	5874	2	52029	7542	865	4838	80	12	546	2909	321	22052	193	*nq*	4

Mean concentration (µg/kg) in dry matter was calculated based on at least two assays (CV <20%, except those indicated in asterix *)

nd indicates compounds not detected

nq indicates compounds detected but could not quantified

All compounds were significantly different (α=0.05).

Appendix E. Concentration of targeted volatile compounds: acetaldehyde, acids, furans, and sulfur-containing compounds measured in commercial single-origin roasted coffees

Samples	acetaldehyde	furfural	3-methylbutyric acid	5-methylfurfural	2-furfurylthiol	methional	3-mercapto-3-methylbutylformate
Australia NQ1	48887	93210	72352	46279	2091*	851	338
Australia NSW	63486	128945	107235	55591	2803	1541	449
Australia NQ2	33818	84381	60085	43011	1274	933	240
Bolivia	59349	85726	49127	46160	3559	1454	439
Brazil1	55849	101842	51907	50921	3806	1281	316
Brazil2	68273	86414	37930	48154	3671*	1296	208
Colombia	67706	153818	67706	64091	3130	1879	512
Costa Rica	53718	90299	84696	48774	3750	1538	982
Dominican Republic	64794	99986	80581	49664	3764	1613	693
El Salvador	58595	89693	48447	49429	3211	1263	267
Ethiopia1	64846	135289	133972	61884	2174	1393	539
Ethiopia2	57732	105567	87753	49485	2577	1422	438
Guatemala1	70012	124321	72303	61506	4158	1747	420
Guatemala2	57836	107456	76238	55207	3246	1563	492
Honduras	53770	73563	42555	39586	3023	1025	251
India Monsooned Malabar	60196	53912	43658	31752	4691	1025	360
India Robusta	49054	40823	50700	26337	4337	794	233
Indonesia Luwak1	52467	90746	78866	47518	2061	1193	597
Indonesia Luwak2	54318	126084	72424	57610	1967*	1591	655
Indonesia Java	56750	91725	56750	46852	1265*	1272	501

Samples	acetaldehyde	furfural	3-methylbutyric acid	5-methylfurfural	2-furfurylthiol	methional	3-mercapto-3-methylbutylformate
Indonesia Sumatra	75752	110334	65871	55332	4362	1694	725*
Nicaragua	55730	70570	52762	40561	3051	1254	436
Peru	68950*	114147	61032	54764	2767	1383	498
Papua New Guinea1	54305	96432	72735	51343	1740	1366	645
Papua New Guinea2	68230	130197	75152	59001	2689	1823	535
Rwanda	48589	85940	86270	45945	2390	1260	375

Mean concentration (µg/kg) in dry matter was calculated based on at least two assays (CV <20%, except those indicated in asterix *)

All compounds were significantly different (α=0.05).

Appendix F. Pearson's correlation coefficient matrix of 27 coffee volatile compounds

Variables	2,3-butanedione	methylpropanal	3-methylbutanal	2-methylbutanal	(E)-2-nonenal	2,3-pentanedione	ethyl-2-methylbutyrate	D-limonene	guaiacol	4-ethylguaiacol	4-vinylguaiacol	linalool	geraniol	*β*-damascenone	2,5-dimethylpyrazine	2,3-dimethylpyrazine	2-ethyl-3,6-dimethylpyrazine	2-ethyl-3,5-dimethylpyrazine	2,3-diethyl-5-methylpyrazine	3-isobutyl-2-methoxypyrazine	acetaldehyde	2- furaldehyde	3-methylbutyric acid	5-methylfurfural	2-furfurylthiol	methional	3-mercapto-3-methylbutylformate
2,3-butanedione	**1**																									
methylpropanal	**0.85**	**1**																								
3-methylbutanal	**0.87**	**0.96**	**1**																							
2-methylbutanal	**0.85**	**0.98**	**0.96**	**1**																						
(E)-2-nonenal	0.13	-0.08	0.00	-0.05	**1**																					
2,3-pentanedione	**0.84**	**0.84**	**0.87**	**0.82**	0.05	**1**																				
ethyl-2-methylbutyrate	0.31	0.21	0.25	0.21	**0.87**	0.35	**1**																			
D-limonene	0.24	0.12	0.15	0.11	0.27	0.32	**0.41**	**1**																		
guaiacol	0.29	0.37	**0.42**	**0.39**	**0.41**	0.30	**0.54**	0.33	**1**																	
4-ethylguaiacol	-0.19	-0.10	-0.11	-0.05	0.38	-0.34	0.26	-0.16	**0.56**	**1**																
4-vinylguaiacol	0.01	0.06	0.08	0.09	0.37	-0.01	**0.47**	0.18	**0.67**	**0.65**	**1**															
linalool	**0.42**	**0.39**	**0.42**	0.39	0.02	**0.56**	0.34	**0.84**	0.38	-0.28	0.21	**1**														
geraniol	**0.43**	**0.44**	**0.43**	**0.43**	-0.05	**0.56**	0.24	**0.71**	0.37	-0.13	0.08	**0.85**	**1**													
β-damascenone	-0.03	-0.05	0.06	-0.02	0.07	0.04	0.11	0.18	0.18	0.21	**0.55**	0.29	0.13	**1**												
2,5-dimethylpyrazine	0.27	**0.46**	**0.51**	**0.48**	0.01	**0.54**	0.20	0.23	0.38	-0.05	**0.46**	**0.45**	0.27	**0.61**	**1**											
2,3-dimethylpyrazine	**0.48**	**0.58**	**0.62**	**0.60**	0.07	**0.60**	0.29	0.26	**0.44**	0.01	**0.51**	**0.47**	0.38	**0.58**	**0.85**	**1**										
2-ethyl-3,6-dimethylpyrazine	0.07	0.35	0.34	0.38	-0.20	0.29	-0.01	0.14	0.27	0.10	**0.49**	0.36	0.25	**0.54**	**0.84**	**0.76**	**1**									

Variables	2,3-butanedione	methylpropanal	3-methylbutanal	2-methylbutanal	(E)-2-nonenal	2,3-pentanedione	ethyl-2-methylbutyrate	D-limonene	guaiacol	4-ethylguaiacol	4-vinylguaiacol	linalool	geraniol	β-damascenone	2,5-dimethylpyrazine	2,3-dimethylpyrazine	2-ethyl-3,6-dimethylpyrazine	2-ethyl-3,5-dimethylpyrazine	2,3-diethyl-5-methylpyrazine	3-isobutyl-2-methoxypyrazine	acetaldehyde	2- furaldehyde	3-methylbutyric acid	5-methylfurfural	2-furfurylthiol	methional	3-mercapto-3-methylbutylformate
2-ethyl-3,5-dimethylpyrazine	0.08	0.37	0.37	0.39	-0.14	0.30	0.05	0.11	0.36	0.15	**0.54**	0.37	0.28	**0.61**	**0.88**	**0.78**	**0.96**	**1**									
2,3-diethyl-5-methylpyrazine	-0.06	0.20	0.19	0.24	0.02	0.08	0.11	0.09	0.34	0.39	**0.61**	0.22	0.15	**0.54**	**0.70**	**0.63**	**0.92**	**0.91**	**1**								
3-isobutyl-2-methoxypyrazine	0.00	-0.15	-0.07	-0.13	**0.89**	0.01	**0.76**	0.21	0.23	0.26	0.37	-0.02	-0.17	0.29	0.22	0.19	-0.05	0.02	0.12	**1**							
acetaldehyde	0.32	0.21	0.26	0.23	0.24	0.13	0.23	0.24	0.12	-0.08	0.10	0.21	0.10	-0.14	0.00	0.03	-0.07	-0.12	-0.08	0.15	**1**						
2- furaldehyde	0.31	0.21	0.25	0.23	0.24	0.12	0.23	0.24	0.12	-0.08	0.10	0.21	0.10	-0.15	-0.01	0.02	-0.08	-0.13	-0.09	0.15	**1.00**	**1**					
3-methylbutyric acid	0.31	0.21	0.25	0.23	0.24	0.12	0.23	0.25	0.12	-0.08	0.10	0.21	0.10	-0.16	-0.02	0.01	-0.08	-0.13	-0.09	0.15	**1.00**	**1.00**	**1**				
5-methylfurfural	0.30	0.20	0.24	0.22	0.24	0.11	0.23	0.25	0.12	-0.07	0.09	0.21	0.10	-0.17	-0.02	0.00	-0.09	-0.14	-0.10	0.14	**1.00**	**1.00**	**1.00**	**1**			
2-furfurylthiol	0.30	0.20	0.24	0.22	0.24	0.10	0.22	0.25	0.12	-0.07	0.09	0.21	0.10	-0.18	-0.03	-0.01	-0.10	-0.14	-0.10	0.14	**1.00**	**1.00**	**1.00**	**1.00**	**1**		
methional	0.29	0.20	0.23	0.21	0.24	0.09	0.22	0.26	0.12	-0.07	0.08	0.22	0.11	-0.19	-0.04	-0.02	-0.10	-0.15	-0.11	0.13	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**	**1**	
3-mercapto-3-methylbutylformate	0.29	0.19	0.22	0.21	0.23	0.09	0.22	0.26	0.12	-0.07	0.08	0.22	0.11	-0.20	-0.05	-0.03	-0.11	-0.15	-0.11	0.13	**0.99**	**0.99**	**1.00**	**1.00**	**1.00**	**1.00**	**1**

Bold typeface indicates significant correlation (α=0.05).

Appendix G. Mean data of physicochemical (non-volatiles) components measured in green coffee beans

Samples	5-CQA (mg/g)	3,4-diCQA (mg/g)	3,5-diCQA (mg/g)	4,5-diCQA (mg/g)	caffeic acid (mg/g)	caffeine (mg/g)	trigonelline (mg/g)	pH	L*value
Australia NQ1[1]	51±2.6 cdef	2.7±0.7 e	4.3± 0.3 cd	1.4±0.2 i	2.9±0.3 bc	11±0.01 hi	13±0.1 ab	6.2±0.03 ab	70±0.5 klm
Australia NSW[2]	64±0.9 a	2.7±0.1 e	4.4± 0.3 cd	1.7±0.6 hi	2.8±0.2 bc	9.5±0.1 j	10±0.1 i	6.2±0.02 a	72±0.4 efghij
Australia NQ2[2]	53±4.9 bcd	3.2±1.1 de	3.5± 0.7 de	3.1±0.1 bcdef	2.8±0.4 bc	11±0.0 4 ghi	12±0.04 abc	5.6±0.1 e	74±0.05 bcdef
Bolivia[1]	53±1.8 cde	3.4±0.2 cde	6.0± 0.6 ab	2.0±0.3 ghi	2.0±1.4 bc	13±1.0 bcde	11±0.3 fghi	5.9±0.1 abcde	74±0.3 cdefgh
Brazil1[1]	51±0.6 cdef	3.7±0.2 bcde	5.0± 0.3 bc	3.1±0.2 bcdef	2.7±0.1 bc	11±0.1 fghi	12±0.2 cdefg	5.7±0.1 de	73±0.1 defghij
Brazil2[3]	47±1.2 defg	3.6±0.9 bcde	4.2± 0.7 cd	2.7±0.4 cdefgh	2.5±0.3 bc	13±0.01 bcdef	12±0.2 cdefg	5.9±0.2 abcde	72±0.1 hijklm
Colombia[1]	49±4.7 defg	3.4±0.1 cde	4.9± 0.7 bcd	2.6±0.3 cdefgh	2.9±0.1 bc	11±0.1 fgh	11±0.04 defg	5.8±0.1 cde	70±1.4 lm
Costa Rica[1]	49±1.8 defg	2.7±0.9 e	5.3± 0.7 bc	3.3±0.5 bcd	2.8±0.3 bc	12±0.1 efgh	11±0.01 defg	5.8±0.2 cde	75±1.0 bcd
Dominican Republic[4]	50±2.6 cdefg	3.1±0.1 de	4.9± 0.1 bc	2.6±0.01 cdefgh	3.2±0.1 b	11±0.2 fghi	12±0.3 cdefg	5.7±0.1 de	69±0.5 m
El Salvador	50±4.3 cdef	3.4±0.6 cde	5.0± 0.1 bc	2.8±0.1 cdefg	2.6±0.1 bc	13.±0.0 3 b	11±0.1 fghi	5.9±0.01 bcde	72±0.4 fghijk
Ethiopia1[1]	60±1.7 ab	2.5±0.4 e	5.4± 0.4 bc	2.2±0.3 efghi	2.5±0.1 bc	12±0.2 defgh	13±0.2 a	5.7±0.1 cde	73±0.4 defghi
Ethiopia2[2]	53±1.1 cd	3.2±0.2 de	4.6± 0.4 bcd	3.0±0.3 cdefg	2.5±0.1 bc	11±0.1 hi	12±0.1 bcd	5.7±0.02 cde	73±1.0 defghi
Guatemala1[1]	45±1.6 fg	3.0±0.2 e	4.6± 0.3 bcd	2.2±0.1 efghi	2.5±0.1 bc	12±0.3 efgh	11±0.2 fgh	5.8±0.0 cde	74±0.9 bcdefg
Guatemala2[1]	52±2.1 cdef	2.5±0.9 e	5.4± 0.8 bc	2.2±0.4 fghi	2.6±0.3 bc	12±0.6 bcdefg	12±0.6 bcdef	5.8±0.1 cde	70±0.4 m
Honduras[1]	54±0.8 bcd	3.3±0.9 cde	4.2± 0.5 cd	2.3±0.4 defghi	2.3±0.5 bc	13±0.1 bc	11±0.1 cdefg	5.9±0.05 abcde	75±0.5 bcd
India Monsooned Malabar[5]	58±1.3 abc	3.1±0.7 de	2.4± 0.4 e	3.0±0.4 cdefg	1.9±0.3 bc	13±0.3 bcde	11±0.2 cdefg	6.0±0.01 abcde	78±0.5 a
India Robusta[2]	52±2.7 cdef	8.8±0.7 a	7.1± 0.6 a	5.9±0.7 a	4.8±0.4 a	24±0.04 a	8.6±0.02 j	5.9±0.1 abcde	73±0.6 defghi
Indonesia Luwak1[7]	52±0.5 cdef	4.9±0.01 bc	4.6± 0.4 bcd	3.5±0.2 bc	2.9±0.04 bc	12±0.1 efgh	11±0.03 fgh	6.0±0.01 abcde	72±0.1 fghijk
Indonesia Luwak2 peaberry[7]	49±3.4 defg	4.7±0.2 bcd	4.9± 0.4 bc	3.3±0.2 bcd	3.1±0.2 b	12±0.1 cdefgh	12±0.1 cdefg	6.1±0.02 abc	76±0.1 abc
Indonesia Java[6]	53±0.7 cde	3.8±0.2 bcde	4.2± 0.2 cd	2.8±0.3 cdefg	3.0±0.2 bc	12±0.3 defgh	11±0.3 ghi	6.0±0.1 abcd	76±0.3 ab
Indonesia Sumatra[6]	52±1.3 cdef	5.1±0.2 b	4.6± 0.2 bcd	4.1±0.1 b	3.0±0.03 bc	13±0.1 bcd	11±0.1 efg	5.8±0.1 cde	71±0.2 jklm
Nicaragua[1]	54±1.7 bcd	3.7±0.3 bcde	4.1± 0.3 cd	2.9±0.3 cdefg	2.4±0.2 bc	12±0.5 efgh	8.3±0.4 j	6.0±0.1 abcd	72±0.01 hijklm
Peru[1]	43±0.6 g	3.2±0.2 de	4.5± 0.2 cd	2.8±0.3 cdefg	2.6±0.1 bc	11.±0.4 hi	10±0.2 hi	5.9±0.1 abcde	72±0.2 ghijkl
Papua New Guinea1[1]	46±1.9 efg	3.7±0.3 bcde	5.1± 0.4 bc	2.9±0.1 cdefg	2.7±0.1 bc	11±0.1 fghi	10±0.1 hi	5.8±0.01 cde	71±0.7 ijklm

Samples	5-CQA (mg/g)	3,4-diCQA (mg/g)	3,5-diCQA (mg/g)	4,5-diCQA (mg/g)	caffeic acid (mg/g)	caffeine (mg/g)	trigonelline (mg/g)	pH	L*value
Papua New Guinea2[1]	48±1.5 defg	3.2±0.4 de	4.6± 0.2 bcd	3.2±0.2 bcde	1.8±1.0 c	12±0.1 defgh	8.9±0.1 j	5.7±0.0 4 de	74±0.4 bcde
Rwanda[1]	50±1.6 cdef	3.9±0.04 bcde	4.8± 0.2 bcd	3.5±0.1 bc	2.9±0.1 bc	10±0.04 ij	12±0.1 bcde	5.9±0.01 abcde	72±0.04 hijklm
n	3	3	3	3	3	2	2	2	2

*Green beans treatment details collected from suppliers: [1]washed, [2]natural, [3]semi-dry (pulped natural), [4]natural-washed mixture, [5]dry-monsooned, [6]semi-dry (wet-hulled), [7]Luwak fermentation

Concentrations of 5-CQA, 3,4-diCQA, 3,5-diCQA, 4,5-diCQA, caffeic acid, caffeine and trigonelline were presented in dry matter

Data for each attributes presented as mean ± standard deviation. All attributes were significantly different (α=0.05) based on ANOVA results.

Tukey HSD with confidence interval 95% was performed, data presented as letters following each mean value. Mean value sharing similar letters means no difference.

Appendix H. Mean data of physicochemical components (5-CQA, 3,4-diCQA, 3,5-diCQA, 4,5-diCQA, caffeic acid, caffeine, and trigonelline) measured in roasted coffees

Samples*	5-CQA (mg/g)	3,4-diCQA (mg/g)	3,5-diCQA (mg/g)	4,5-diCQA (mg/g)	caffeic acid (mg/g)	caffeine (mg/g)	trigonelline (mg/g)
Australia NQ1[1]	15± 0.5 ab	1.1± 0.02 bcdefg	0.7± 0.01 hij	0.8± 0.01 defgh	1.8± 0.01 bcde	11±0.2 kl	9.8±0.3 a
Australia NSW[2]	14± 0.5 abcdef	1.0± 0.1 fg	0.6± 0.1 ij	0.7± 0.1 hi	2.0± 0.1 abc	9.8±0.2 l	7.1±0.1 j
Australia NQ2[2]	15± 1.4 abc	1.1± 0.02 cdefg	0.9± 0.02 abcdef	0.9± 0.03 bcdefg	1.4± 0.03 g	12±0.3 jk	9.1±0.2 bc
Bolivia[1]	14± 0.2 abcdef	1.2± 0.03 bcd	1.0± 0.2 abcd	0.9± 0.02 bcde	2.0± 0.03 abcd	13±0.2 bcdef	7.5±0.1 hij
Brazil1[1]	14± 0.3 abcd	1.2± 0.1 bcdefg	1.0± 0.04 abc	0.9± 0.1 bcdefg	1.7± 0.1 efg	12±0.1 ghij	8.5±0.02 cdef
Brazil2[3]	14± 0.1 abcde	1.2± 0.04 bcde	1.1± 0.04 a	0.9± 0.03 bcde	1.8± 0.03 cde	13±0.2 bcde	8.5±0.1 cdef
Colombia[1]	14± 0.1 abcd	1.3± 0.02 bcd	0.8± 0.01 cdefghi	1.0± 0.02 abcd	2.1± 0.02 ab	12±0.1 hij	8.6±0.01 cdef
Costa Rica[1]	10± 0.03 ij	1.0± 0.03 defg	0.7± 0.01 hij	0.8± 0.02 defghi	1.7± 0.1 cdef	13±0.2 defghij	7.4±0.1 ij
Dominican Republic[4]	11± 0.6 hij	1.0± 0.1 defg	0.6± 0.1 hij	0.7± 0.1 efghi	1.7± 0.1 cdef	12±0.2 fghij	8.1±0.1 fg
El Salvador	16± 0.3 a	1.3± 0.02 bc	1.1± 0.03 a	1.0± 0.03 ab	1.7± 0.1 cdefg	14±0.1 bc	8.6±0.1 cdef
Ethiopia1[1]	14± 1.9 abcd	1.1± 0.1 bcdefg	0.7± 0.1 fghij	0.8± 0.1 bcdefgh	2.2± 0.2 a	13±0.1 defgh	9±0.02 c
Ethiopia2[2]	15± 0.05 abc	1.2± 0.01 bcd	1.1± 0.01 ab	0.9± 0.002 abcde	2.0± 0.01 abcd	12±0.1 hij	9.6±0.002 ab
Guatemala1[1]	13± 0.4 cdefg	1.2± 0.02 bcdef	0.8± 0.02 defghi	0.9± 0.02 bcdef	2.0± 0.1 abc	13±0.1 bcd	8.1±0.01 fg
Guatemala2[1]	12± 0.4 fghij	1.0± 0.02 fg	0.6± 0.02 hij	0.7± 0.01 ghi	1.7± 0.1 cdefg	13±0.3 bcdef	8.2±0.1 fg
Honduras[1]	15± 0.7 abc	1.2± 0.1 bcde	0.9± 0.2 abcdefg	0.8± 0.04 bcdefg	1.8± 0.1 cde	14±0.1 b	8.4±0.02 defg
India Monsooned Malabar[5]	9.9± 0.5 j	0.9± 0.1 g	0.5± 0.05 j	0.6± 0.1 i	1.5± 0.1 fg	13±0.1 cdefg	7.9±0.02 ghi
India Robusta[2]	12± 1.2 defghi	1.7± 0.2 a	0.9± 0.1 bcdefgh	1.1± 0.1 a	1.7± 0.2 defg	25±0.8 a	6.1±0.2 k
Indonesia Luwak1[7]	12± 1 fghij	1.1± 0.1 bcdefg	0.7± 0.1 ghij	0.8± 0.1 defgh	1.7± 0.03 cdefg	13±0.1 bcdef	8.2±0.02 efg
Indonesia Luwak2 peaberry[7]	14± 0.1 abcd	1.3± 0.03 b	0.8± 0.02 bcdefgh	1.0± 0.04 abc	1.9± 0.01 bcde	13±0.2 defgh	8.8±0.1 cde
Indonesia Java[6]	11± 1.5 ghij	1.0± 0.2 defg	0.6± 0.1 hij	0.7± 0.1 fghi	1.9± 0.2 bcde	12±0.1 ij	7.2±0.1 j
Indonesia Sumatra[6]	12± 0.2 efghij	1.2± 0.1 bcd	0.7± 0.01 efghij	0.9± 0.04 bcdefg	1.9± 0.03 abcde	13±0.2 defgh	8.0±0.1 fghi
Nicaragua[1]	12± 0.4 fghij	1.0± 0.004 efg	0.6± 0.01 ij	0.7± 0.01 hi	1.8± 0.1 cde	12±0.03 efghij	5.9±0.04 k
Peru[1]	13± 0.7 bcdefg	1.2± 0.03 bcdefg	1.0± 0.2 abcde	0.9± 0.03 bcde	1.8± 0.1 cdef	12±0.1 ij	7.9±0.1 ghi

Samples*	5-CQA (mg/g)		3,4-diCQA (mg/g)		3,5-diCQA (mg/g)		4,5-diCQA (mg/g)		caffeic acid (mg/g)		caffeine (mg/g)		trigonelline (mg/g)	
Papua New Guinea1[1]	11± 0.5	ghij	1.1± 0.1	bcdefg	0.7± 0.02	efghij	0.8± 0.1	cdefgh	1.8± 0.1	cde	12±0.1	fghij	7.3±0.2	j
Papua New Guinea2[1]	13± 0.1	cdefgh	1.2± 0.01	bcdefg	0.7± 0.01	efghij	0.9± 0.01	bcdefg	1.9± 0.01	abcde	13±0.2	defghi	8.0±0.4	fgh
Rwanda[1]	11± 0.1	ghij	1.1± 0.04	bcdefg	0.6± 0.01	hij	0.8± 0.03	defghi	1.7± 0.01	cdef	12±0.4	hij	8.9±0.3	cd
n	3		3		3		3		3		2		2	

*Green beans treatment details collected from suppliers: [1]washed, [2]natural, [3]semi-dry (pulped natural), [4]natural-washed mixture, [5]dry-monsooned, [6]semi-dry (wet-hulled), [7]Luwak fermentation

Concentrations of 5-CQA, 3,4-diCQA, 3,5-diCQA, 4,5-diCQA, caffeic acid, caffeine and trigonelline were presented in dry matter

Data for each attributes presented as mean ± standard deviation. All attributes are significantly different (α=0.05)
Tukey HSD with confidence interval 95% was performed, data presented as letters following each mean value. Mean value sharing similar letters means no difference.

Appendix I. Mean data of physicochemical components (crude fat, titratable acidity, pH, L*value) measured in roasted coffees

Samples*	crude fat (%)		titratable acidity (mL NaOH/g)		pH		L*value	
Australia NQ1[1]	15 ± 0.02	bcdefgh	1.5 ± 0.01	efgh	5.2 ± 0.01	bcdefg	40 ± 1.1	efg
Australia NSW[2]	15 ± 0.3	bcdefg	1.2 ± 0.04	i	5.2 ± 0.04	efg	38 ± 0.2	g
Australia NQ2[2]	12 ± 0.6	j	1.6 ± 0.2	cdefgh	5.1 ± 0.1	efg	42 ± 0.8	bcde
Bolivia[1]	14 ± 0.4	efghi	1.5 ± 0.04	fgh	5.3 ± 0.01	bcdefg	41 ± 0.2	bcdef
Brazil1[1]	14 ± 0.3	defghi	1.5 ± 0.01	efgh	5.2 ± 0.02	bcdefg	41 ± 1	cdefg
Brazil2[3]	14 ± 0.3	fghi	1.6 ± 0.04	abcdef	5.1 ± 0.05	fg	42 ± 1	bcde
Colombia[1]	15 ± 0.1	bcdef	1.8 ± 0.02	abc	5.1 ± 0.01	g	42 ± 1.2	bcde
Costa Rica[1]	15 ± 0.7	cdefghi	1.6 ± 0.02	abcdef	5.3 ± 0.05	bcdef	40 ± 0.2	efg
Dominican Republic[4]	16 ± 0.1	bcde	1.6 ± 0.004	bcdefg	5.2 ± 0.01	bcdefg	38 ± 0.1	g
El Salvador	13 ± 0.3	i	1.8 ± 0.03	abcd	5.5 ± 0.01	abc	43 ± 0.4	ab
Ethiopia1[1]	16 ± 0.1	bcd	1.7 ± 0.004	abcdef	5.4 ± 0.02	bcd	40 ± 0.9	efg
Ethiopia2[2]	14 ± 0.4	hi	1.6 ± 0.03	abcdef	5.3 ± 0.01	bcdefg	40 ± 0.3	cdefg
Guatemala1[1]	18 ± 0.5	a	1.5 ± 0.02	defgh	5.2 ± 0	efg	39 ± 0.4	efg
Guatemala2[1]	16 ± 0.5	bc	1.7 ± 0.1	abcdef	5.3 ± 0.05	bcdefg	39 ± 0.3	fg
Honduras[1]	14 ± 0.1	ghi	1.4 ± 0.2	fghi	5.3 ± 0.04	bcdefg	42 ± 0.4	bc
India Monsooned Malabar[5]	11 ± 0.5	j	1.3 ± 0.05	hi	5.5 ± 0.01	ab	42 ± 0.7	bcd
India Robusta[2]	6.8 ± 0.2	k	1.3 ± 0.01	ghi	5.7 ± 0.03	a	46 ± 0.2	a
Indonesia Luwak1[7]	14 ± 0.2	fghi	1.8 ± 0.02	a	5.1 ± 0.01	fg	39 ± 0.5	fg
Indonesia Luwak2 peaberry[7]	14 ± 0.01	i	1.6 ± 0.003	abcdef	5.2 ± 0.02	defg	40 ± 1.1	efg
Indonesia Java[6]	17 ± 0.2	b	1.5 ± 0.02	fgh	5.3 ± 0.01	bcdefg	39 ± 0.3	efg
Indonesia Sumatra[6]	14 ± 0.3	defghi	1.7 ± 0.01	abcde	5.4 ± 0.03	bcde	39 ± 0.2	fg
Nicaragua[1]	15 ± 0.1	cdefgh	1.6 ± 0.04	bcdefg	5.4 ± 0.04	bcde	40 ± 0.8	cdefg
Peru[1]	14 ± 0.2	fghi	1.7 ± 0.1	abcdef	5.4 ± 0.1	bcde	40 ± 0.04	defg
Papua New Guinea1[1]	16 ± 0.2	bcd	1.6 ± 0.1	abcdef	5.2 ± 0.3	cdefg	40 ± 0.4	cdefg
Papua New Guinea2[1]	15 ± 0.2	cdefghi	1.8 ± 0.1	ab	5.2 ± 0.02	cdefg	40 ± 0.5	efg
Rwanda[1]	15 ± 0.3	bcdef	1.5 ± 0.03	fgh	5.3 ± 0.03	bcdefg	40 ± 0.3	efg
n	2		2		2		2	

*Green beans treatment details collected from suppliers: [1]washed, [2]natural, [3]semi-dry (pulped natural), [4]natural-washed mixture, [5]dry-monsooned, [6]semi-dry (wet-hulled), [7]Luwak fermentation

Data for each attributes presented as mean ± standard deviation. All attributes are significantly different (α=0.05)

Tukey HSD with confidence interval 95% was performed, data presented as letters following each mean value. Mean value sharing similar letters means no difference.

Appendix J. ANOVA results of physicochemical components measured in green beans and roasted coffees (26 samples x two replicates or three replicates)

Samples	5-CQA	3,4-diCQA	3,5-diCQA	4,5-diCQA	caffeic acid	caffeine^{ns}	trigonelline	pH	L*value
Green coffee beans	51 a	3.6 a	4.7 a	2.9 a	2.7 a	12 a	11 a	5.9 a	73 a
Roasted coffees	13 b	1.2 b	0.8 b	0.8 b	1.8 b	13 a	8.1 b	5.3 b	40 b

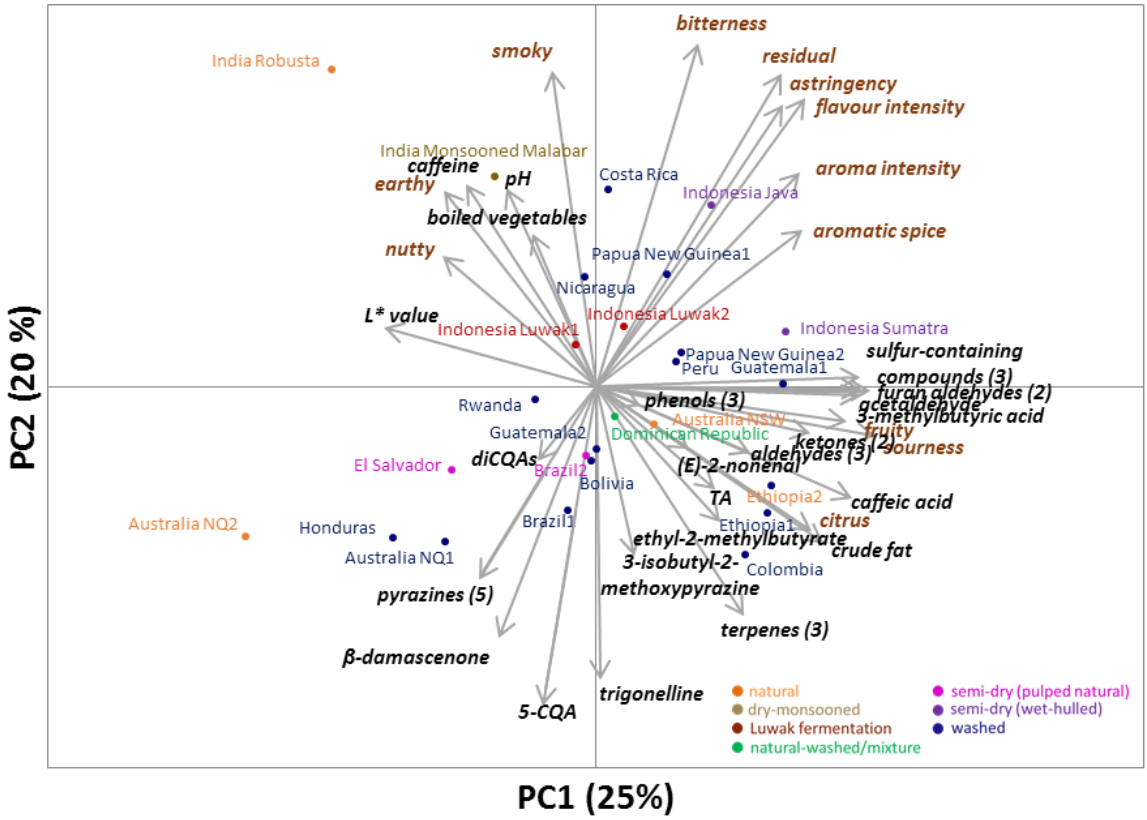
5-CQA, 3,4-diCQA, 3,5-diCQA, 4,5-diCQA, caffeic acid were assessed in three replicates while caffeine, trigonelline , pH and L*value were assessed in two replicates

Concentrations of 5-CQA, 3,4-diCQA, 3,5-diCQA, 4,5-diCQA, caffeic acid, caffeine and trigonelline were presented in dry matter

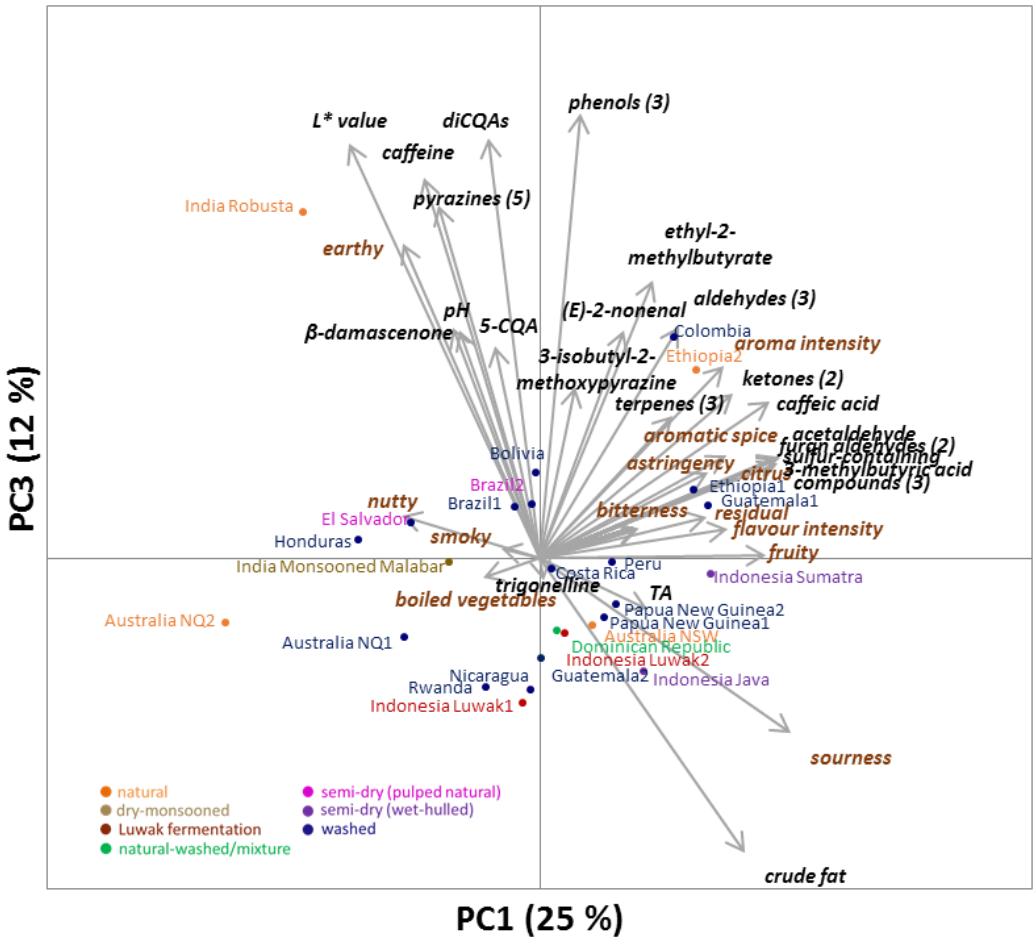
^{ns}indicates attribute that was not significantly different (α=0.05)

Tukey HSD with confidence interval 95% was performed, data presented as letters following each mean value. Mean value sharing similar letters means no difference.

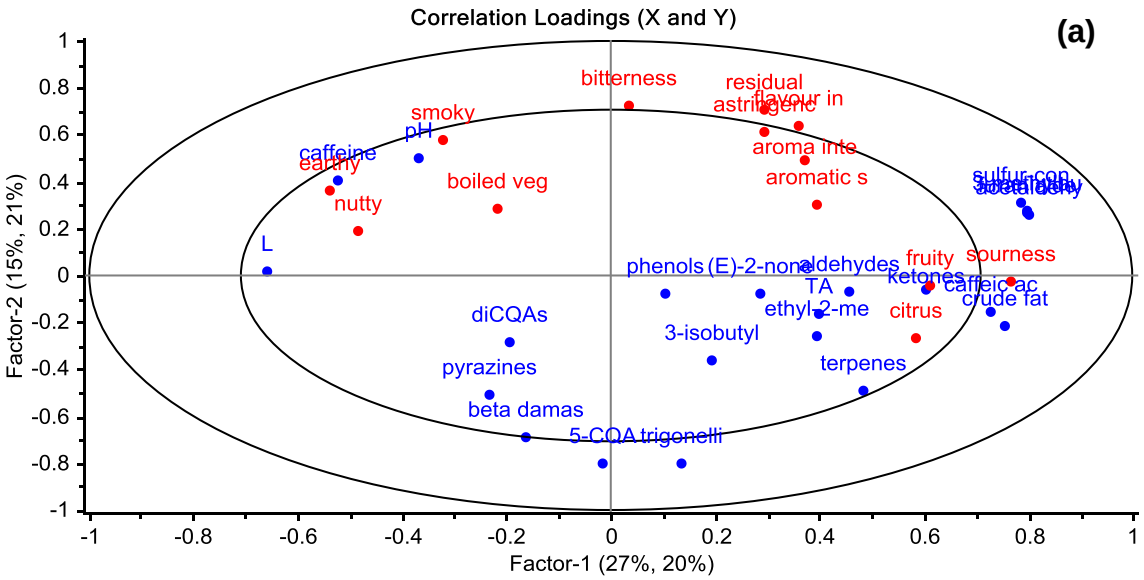
Appendix K. PCA bi-plot 26 coffee samples based on 35 variables, PC1 (25%) versus PC2 (20%)

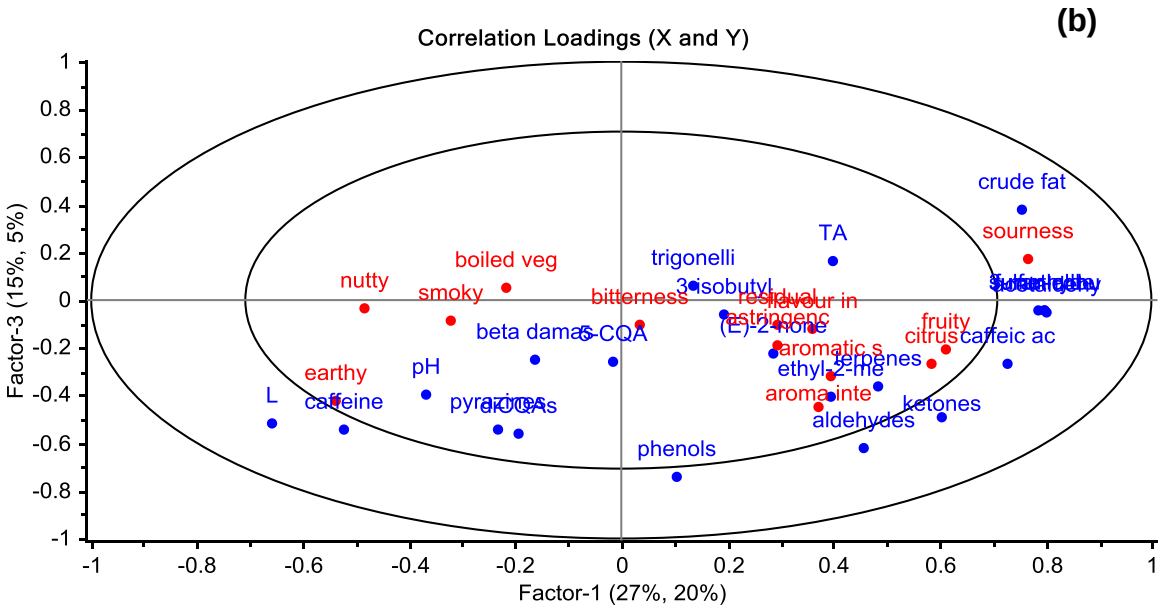


Appendix L. PCA bi-plot 26 coffee samples based on 35 variables, PC1 (25%) versus PC3 (12%)



Appendix M. PLS2 correlation loadings, PC1 versus PC2 (a) and PC1 versus PC3 (b) created using X=22 Y=13 of 26 coffee samples





Appendix N. Mean data of the chlorogenic acid isomers measured in coffee brews (26 samples x three replicates)

Samples	5-CQA	3,4-diCQA	3,5-diCQA	4,5-diCQA
Australia NQ1	110 ± 8.5	4.7 ± 0.3	3.4 ± 0.1	3.4 ± 0.2
Australia NSW	125 ± 8.4	5.3 ± 0.3	5.1 ± 0.3	2.8 ± 0.1
Australia NQ2	119 ± 16	5.1 ± 0.5	4.3 ± 0.4	3.5 ± 0.2
Bolivia	101 ± 10	4.7 ± 0.4	5.0 ± 0.5	3.5 ± 0.3
Brazil1	120 ± 5.7	5.4 ± 0.3	4.7 ± 0.3	3.5 ± 0.2
Brazil2	106 ± 12	5.2 ± 0.6	5.3 ± 0.4	3.7 ± 0.4
Colombia	109 ± 6.8	5.0 ± 0.5	5.4 ± 0.3	3.5 ± 0.3
Costa Rica	85 ± 6.0	4.7 ± 0.4	5.5 ± 0.2	3.5 ± 0.3
Dominican Republic	98 ± 5.1	5.1 ± 0.4	5.5 ± 0.3	3.2 ± 0.6
El Salvador	122 ± 12	5.6 ± 0.4	4.8 ± 0.4	3.7 ± 0.2
Ethiopia1	115 ± 7.8	5.5 ± 0.3	4.9 ± 0.2	3.5 ± 0.2
Ethiopia2	119 ± 6.4	5.5 ± 0.5	4.7 ± 0.4	3.6 ± 0.4
Guatemala1	104 ± 7.3	4.8 ± 0.3	5.4 ± 0.3	3.6 ± 0.3
Guatemala2	101 ± 6.8	4.6 ± 0.2	5.1 ± 0.1	3.3 ± 0.2
Honduras	115 ± 11	4.9 ± 0.4	4.1 ± 0.4	2.8 ± 0.6
India Monsooned Malabar	104 ± 1.4	5.9 ± 0.1	5.1 ± 0.1	3.1 ± 0.1
India Robusta	101 ± 3.6	8.2 ± 0.3	5.9 ± 0.1	3.3 ± 0.4
Indonesia Luwak1	109 ± 7.6	6.4 ± 0.5	6.0 ± 0.4	3.3 ± 0.3
Indonesia Luwak2	109 ± 3.8	5.8 ± 0.4	5.5 ± 0.3	3.2 ± 0.2
Indonesia Java	92 ± 0.8	5.2 ± 0.2	3.5 ± 0.1	2.5 ± 0.6
Indonesia Sumatra	112 ± 3.1	6.6 ± 0.2	6.2 ± 0.2	3.7 ± 0.4
Nicaragua	95 ± 8.3	5.2 ± 0.3	4.6 ± 0.3	2.8 ± 0.2
Peru	108 ± 8.1	5.1 ± 0.4	4.9 ± 0.3	3.5 ± 0.3
Papua New Guinea1	96 ± 4.5	4.9 ± 0.0	5.9 ± 0.2	3.5 ± 0.4
Papua New Guinea2	102 ± 2.7	4.8 ± 0.3	5.5 ± 0.5	3.6 ± 0.6
Rwanda	101 ± 6.8	5.8 ± 0.3	5.8 ± 0.2	3.6 ± 0.4

Data for each attributes presented as mean ± standard deviation

Concentration unit was mg/cup (200 mL).

Appendix O. Pearson's correlation matrix between percent loss of each chlorogenic acid isomers from green coffee beans to roasted coffees and the volatile phenols level in roasted coffees (26 samples x three replicates)

Variables	guaiacol	4-ethylguaiacol	4-vinylguaiacol	5-CQA	3,4-diCQA	3,5-diCQA	4,5-diCQA
guaiacol	**1**						
4-ethylguaiacol	**0.63**	**1**					
4-vinylguaiacol	**0.74**	**0.70**	**1**				
5-CQA	0.14	0.13	-0.19	**1**			
3,4-diCQA	-0.05	0.31	0.02	0.36	**1**		
3,5-diCQA	0.26	0.29	0.03	**0.58**	0.29	**1**	
4,5-diCQA	0.03	0.23	-0.12	**0.58**	**0.68**	0.20	**1**

Bold typeface indicates significant correlation (α=0.05).