Improvement of Robusta Coffee Aroma Quality

By

Chujiao Liu, BSc

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ABSTRACT

In this thesis, two main novel pre-treatment approaches were developed, with the aim to change the chemical reaction pathways during thermal treatment (roasting) and thereby regulate aroma formation during coffee roasting. The objective for this study is to up-value Robusta coffee beans with overall aim of increasing aroma chemistry similarities between Robusta and Arabica coffees.

Green Robusta beans were subjected to pre-treatment with the aim of reducing the perceived aroma difference between Arabica and Robusta coffee. The first approach was a short soaking procedure before roasting with varying concentrations of acetic acid (up to 5%). Samples were subjected to thermal treatment (roasted) and ground to a standardised particle size distribution. Aroma compounds were evaluated by headspace analysis using Solid Phase Micro Extraction - Gas Chromatograph Mass Spectrometry. Acetic acid pre-treatment significantly affected aroma formation during roasting and resulted in a modified level of pyrazines, furanic compounds and sulphur-containing compounds ($p < 0.05$). Principal Component Analysis (PCA) illustrated that the aroma profile of the acetic acid treated Robusta coffee was closer to the target Arabica coffee after roasting. Sensory results confirmed that the aroma of the 2% acetic acid pre-treated Robusta brew was similar to Arabica and the maximum inclusion level of Robusta coffee in a blend could be increased from 20% to 80%.

A second approach was attempted to improve Robusta sensory properties based on modification of the beans chemical composition before roasting. Building on the first approach, which modified bean pH through acid pre-treatment, sugar solutions (glucose, fructose and sucrose) were used to pre-treat Robusta coffee beans with

the aim to modify the concentration/availability of these aroma precursors. The treatment significantly affected aroma generation during roasting and lead to an altered level of pyrazines, furans, ketones, organic acid and heterocyclic nitrogen-containing compounds ($p < 0.05$). The optimum treatment was 15 % fructose. 80% treated Robusta could be blended with Arabica in coffee brew without significant aroma differences being perceived when compared to 100% Arabica brew.

The stability of volatile compounds found in Arabica and Robusta coffee beans were also investigated after roasting over six weeks of accelerated shelf-life storage (ASL) (5˚C, 25˚C and 35˚C, up to 6 weeks). As previously demonstrated, coffee bean pre-treatment technology enhances Robusta aroma by pre-soaking the beans in either acetic acid (2%) or 15 % fructose prior to roasting and the impact on aroma loss over shelf-life was also evaluated for these samples. Results indicated that Arabica coffee lost the greatest amount of volatile compounds during storage. Surprisingly, Robusta coffee treated with 2% acetic acid was the most stable over storage. Although the 15% fructose treated Robusta showed less aroma stability than non-treated Robusta during 6 weeks storage, the aroma of 15% fructose treated Robusta was still more stable compared with Arabica.

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ABBREVIATIONS:

ºC	Degrees Celsius
∆E	Total Colour Difference
3D	Three-Dimension
A	Current
ANOVA	Analysis of Variance
ASL	Accelerated Shelf Life
BCA	Bicinchoninic Acid
BSA	Bovin Serum Albumin
CGA	Chlorogenic Acid
cm	Centimetre
CO_2	Carbon Dioxide
CV	Coefficient of Variance
d	Day
FAME	Fatty Acid Methyl Esters
G	Relative Centrifugal Force
g	Gram
GC-MS	Gas Chromatography-Mass Spectometry
h	Hour
H_2O	Water
He	Helium
HPLC	High Performance Liquid Chromatography
ICO	International Coffee Organization
IS	Internal Standard
ISO	International Organization for Standardization
L	Litre
L*	Lightness
LC-MS	Liquid Chromatography-Mass Spectrometry
LRI	Linear Retention Index
m/z	Mass-to-Charge Ratio
MeOH	Methanol
mg	Milligram

MHz	Megahertz
min	Minute
mL	Millilitre
mm	Millimetre
mol	Mole
N_2	Nitrogen
Na_2CO_3	Sodium Carbonate
nm	Nanometre
O_2	Oxygen
PC	Principal Component
PCA	Principal Component Analysis
ppm	Part Per Million
psi	Pounds Per Square Inch
R & G	Roasted and Ground Coffee
RF	Radiofrequency
rpm	Revolutions Per Minute
s	Second
SDS	Sodium Dodecyl Sulphate
SPME	Solid-Phase Microextraction
V	Voltage
W1	Weight of Coffee Samples
W2	Dry Weight of Coffee Samples
Wd3	Weight of Glass Vial Containing the Dried Lipid Extract
Wg2	Weight of Glass Vial
Wp1	Weight of Coffee Powders
µCT	Micro-Computed Tomography
µL	Microliter
µm	Micrometre

PUBLICATIONS

Five papers have been published:

1. LIU C., YANG Q., LINFORTH R., IAN D. F., YANG N., 2018. Modifying Robusta Coffee Aroma by Green Bean Chemical Pre-Treatment. Food Chemistry, 272, 251-257.
2. DINU V., LIU C., ALI J., AYED C., GERSHKOVICH P., ADAMS G., HARDING S., FISK I. 2018. Analytical ultracentrifugation in saliva research: Impact of green tea astringency and its significance on the in vivo aroma release. Scientific Reports.
3. GIACALONE D., KREUZFELDT D. T., YANG N., LIU C., FISK I., & MÜNCHOW M., 2018. Common roasting defects in coffee: Aroma composition, sensory characterization and consumer perception. Food Quality and Preference.
4. ZHANG Y., YANG N., FRAY G.R., FISK I., LIU C., LI H., HAN Y., 2018. Characterization of volatile aroma compounds after in-vial cooking of foxtail millet porridge with gas chromatography-mass spectrometry. Journal of Cereal Science, 82, 8-15.
5. YANG N., LIU C., LIU X., KREUZFELDT D. T., MUNCHOW M. and FISK I., 2016. Determination of volatile marker compounds of common coffee roast defects. Food Chemistry, 211, 206–214.

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1. LIU C., YANG N., LINFORTH R., FISK D. I., 2018. Impact of Water-soluble Precursors Leaching from Green Beans on Aroma Generation

during Coffee Roasting In: Flavour Science: Proceedings of the XV Weurman Flavour Research Symposium Context Products Ltd. 119-122. (ISNB 978-3851255935)

2. YANG N., LIU C., ZAIDAN U., HUI G. and FISK D. I., 2015. Correlation of analytic techniques (GC-MS and APCI-MS) with chemometrics to differentiate roast coffee aroma profiles. In: Flavour Science: Proceedings of the XIV Weurman Flavour Research Symposium Context Products Ltd. 567-570.(ISBN 978-1899043705)

Another two papers are being prepared for publication:

1. The Progression of Volatiles Compounds and Change in Microstructure of the Arabica, Robusta and Treated Robusta Coffee Beans during the Accelerated Shelf Life Storage. Aimed at Food Research International (manuscript in preparation).
2. Enhancing Robusta Coffee aroma by modifying Maillard chemistry precursors in the green coffee bean (Manuscript submitted to Food Chemistry).

Chapter 1 Introduction

Coffee is one of the most popular beverages worldwide. It is consumed by millions of people every day and there is an increasing demand for high quality coffee and speciality coffees (Dulaney, 2018). Global coffee output for the year 2017/18 is estimated at 158.78 million bags (60 kg/bag), which is approximately 0.7% higher than that in 2016/17 (ICO, 2018). In the twelve months ending in January 2018, exports of Arabica totalled 77.37 million bags compared to 74 million bags last year; whereas Robusta exports amounted from 45.51 million bags compared to 45.52 million bags (ICO, 2018).

The Arabica plant has a lower yield and is more sensitive to insects compared with the Robusta plant. However, the pure Arabica beans are considered of finer flavour and more pronounced, therefore more expensive (Dulaney, 2018). Robusta coffee is primarily used for instant coffee. Some Robusta coffees are of high quality and valued especially in espressos for their deep flavour and good crema (Sherri, 2016). However, the majority of Robusta coffee flavours are described as neutral, harsh, earthy, rubbery, grainy, plain and bitter (Kalschne, Biasuz, De Conti, Viegas, Corso, & Benassi, 2018). These are all negative characters with regards to coffee quality. Although Robusta coffee beans are often blended with Arabica beans to reduce cost or create specific flavour profiles, the portion of Robusta is always limited due to the loss of flavour quality (Banks, McFadden, & Atkinson, 2000; Dias, Valderrama, Março, dos Santos Scholz, Edelmann, Yeretzian, 2018).

Currently, Robusta green beans are about half the price of Arabica green beans in the market (Dulaney, 2018). Therefore, there is significant market expectation to

up-value and improve the quality of Robusta coffee beans with the goal of making Robusta coffee flavour quality more like Arabica and further to reduce the coffee price.

Previous studies have attempted to modify the Robusta coffee quality by steaming or soaking, treating coffee beans with enzyme-containing solution under pressure, or created a flavour enhanced green beans by green extract heat treatment (Becker, Schlabs, & Weisemann, 1991; Dar, Bruckmann, & Kelly, 1985; Izumitani & Yajima, 1992; Penson, Brooks, Wragg, Ikenberry, Bradbury, Ozaki, *et al.*, 2008; Varsányi, Krajczár, Runtág, & Dörnyei, 1988). Different treatment has been applied to coffee to reduce unwanted smell/taste components in Robusta, mainly by increasing the pressure, temperature and moisture content during treatment. Early research in this area includes mainly patents. However, there is lack of research on the effect of other treatment (such as treated with low temperature, low pressure) on the Robusta beans and the aroma stability on the Robusta beans after they are treated and roasted. The subject needs further investigation, and therefore has become the focus in this study.

The aim of this study was to explore several novel approaches for improving the flavour quality of Robusta coffee. This study attempted to improve Robusta sensory properties based on modification of the beans chemical composition. Approaches used were either by removing/adding particular precursors or modifying the green bean local microchemistry (pH). The selected approach aimed to increase the sensory quality of Robusta to a level close to that of a higher quality species such as Arabica. Improved flavour characteristics and increased shelf life will therefore improve the commercial viability of Robusta beans.

1.1 Literature Review

1.1.1 Coffee Varieties and Production

The two main cultivated species of coffee are Arabica (*Coffeea Arabica* L.) and Robusta (*Coffeea canephora* P.) (Illy & Viani, 2005). In 2018, Arabica represents about 62% of the world production, while Robusta almost 37% (ICO, 2018). Due to the sensitivity to Fusarium xylarioides, the third most cultivated species, *Coffeea Liberica* only represented 1% of coffee production worldwide (Herrera & Lambot, 2017; Illy & Viani, 2005). The first wild coffee plant, *Coffea Arabica* was discovered in Ethiopia in the twelfth century (Herrera & Lambot, 2017). Conversely Robusta is a more recent discovery, having been found in the equatorial lowland forests of Guinea and Uganda during the nineteenth century (Illy & Viani, 2005).

The world coffee production grow enlarged significantly from 112 million bags in 2000/01 to approximately 159 million bags in 2017/18 (ICO, 2018; Illy & Viani, 2005). According to the International Coffee Organization 2018, the leading coffee producer and exporter was Brazil, which accounts for 32% of total production worldwide. Followed by Vietnam (18%) and Colombia (9%) (ICO, 2018).

1.1.2 Green Coffee Bean Processing

After harvesting, the coffee fruits are separated from the pulp by dry, pulped natural, honey or wet processing (Clarke & Macrae, 1985; Illy & Viani, 2005; Sanz-Uribe, Yusianto, Menon, Peñuela, Oliveros, Husson, *et al.,* 2017). Processing needs to be carried out on the same day as the harvest to reduce the risk of mould contamination and avoid unexpected fermentation during the storage period (Illy & Viani, 2005).

Dry processing is the process of drying the whole cherries under the sun in the open air or in mechanical dryers, followed by the separation of the hull (dried pulp and parchment) mechanically to yield the green beans (Farah, 2012). Harvesting must begin when the coffee cherries are ripe, especially in the regions which have been categorised as having a dry harvest season (Illy & Viani, 2005). It is a simple and inexpensive process which is mainly applied to Robusta coffee (Flament, 2002). The vast majority of Brazilian coffee beans are still processed via the dry method since Brazil is one of the few countries in the world that has the appropriate weather to do so successfully (Farah, 2012).

On the contrary, using a wet process requires greater investment and more care, but results in a superior coffee quality (Flament, 2002). The wet process is mainly applied to Arabica coffee (Clarke & Vitzthum, 2001). During the wet process, the pulp of the coffee cherries (exocarp) is removed mechanically, but the parchment remains attached to the beans (Clarke & Macrae, 1985). Then a natural fermentation step break down mucilage adhering to the parchment by enzymatic action, this can then be washed away (Clarke & Macrae, 1985). After drying, either under the sun or in a dryer, the parchment is removed to produce the green coffee beans.

The pulped natural process is an intermediate process between the dried and washed process. Pulped natural is a common processing method in Brazil. In the pulped natural process, bean cherries are mechanically pulped and then the beans still in the parchment are dried whilst surrounded by mucilage (Illy & Viani, 2005). A fermentation step to break down mucilage is not involved in this type of process.

In Honey processed coffee, coffee beans are dried with some or all of the mucilage remaining. Dependent upon the honey type, different percentages of mucilage are

removed. Yellow Honey production methods remove more than 50% mucilage, Red Honey production removed around 20% - 50% and Black Honey production retaining 100% of the mucilage. In humid environments a slower process is utilised where coffee cherries are picked, sorted, de-pulped, and then moved to drying patios or beds (Sanz-Uribe, *et al.*, 2017). A minor amount of fermentation occurs in the short amount of time it takes for the mucilage to dry for coffees processed in this manner. In Figure 1.1 provides the general information on coffee post-harvest processes steps (Clarke & Macrae, 1985; Flament, 2002; Illy & Viani, 2005; Sanz-Uribe, *et al.*, 2017).

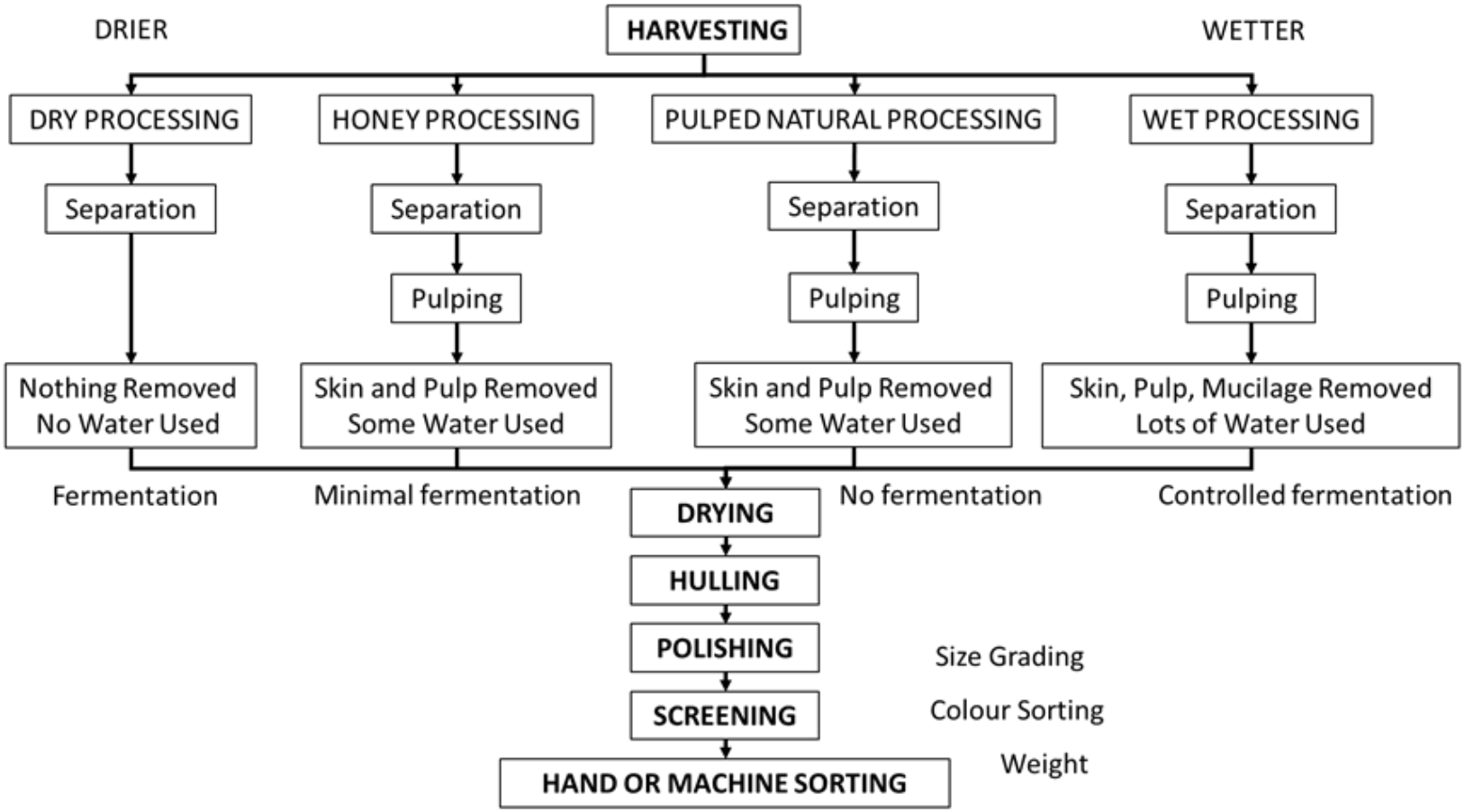


Figure 1.1 Flow chart of coffee post-harvest processes (dry, honey, pulped natural and wet processes).

Due to the nature of the processing, green bean chemical composition will vary between different types of postharvest processing (Oliveira Petkowicz, 2015). The contents of water-soluble polysaccharides were significantly higher in the wet processed green beans (2.79%) compared with unprocessed (1.95%), dry processed (1.98%) and puled natural processed (2.23%) (Oliveira Petkowicz, 2015). Duarte et al. reported that the coffee beans processed by the wet method were significantly higher in total chlorogenic acid (CGA) content ($p < 0.05$) than those processed by the puled natural method. No significant difference between wet processed and puled natural processed green beans was observed regarding to the caffeine content. The trigonelline content in green beans significantly increased in wet processed samples 0.9 g/100g compared with puled natural processed samples 0.81 g/100g ($p < 0.0001$) (Duarte, Pereira, & Farah, 2010).

1.1.3 Green Bean Composition (Arabica & Robusta)

Due to the difference in coffee species (Illy & Viani, 2005), geographical origins (Mehari, Redi-Abshiro, Chandravanshi, Combrinck, Atlabachew, & McCrindle, 2016), growth conditions (Schnabel, de Melo Virginio Filho, Xu, Fisk, Roupsard, & Haggar, 2017), and methods of processing and drying (Ameyu, 2017; Duarte, Pereira, & Farah, 2010; Oliveira Petkowicz, 2015), the chemical composition will differ within and between Arabica and Robusta green beans. The chemical composition of green Arabica and Robusta coffee beans is summarised in Table 1.2 below (Farah, 2012; Illy & Viani, 1995).

Table 1.2 Chemical composition of dry weight green Arabica and Robusta coffee beans.

Component	**Concentration (g/100g)**	
	Arabica coffee	**Robusta coffee**
Sucrose	6 - 9	0.9 - 4.0
Reducing sugars	0.1	0.4
other sugars	1.0	2.0
Polysaccharides	49.8	54.4
Lipids	15 - 17	7 - 10
Proteins	10 - 11	11 - 15
Free amino acids	0.5	0.8
Aliphatic acids	1.1	1.2
Quinic acid	0.4	0.4
Chlorogenic acid (CGA)	6.5	10.0
Caffeine	0.9 – 1.3	1.5 – 2.5
Trigonelline	0.6 – 1.3	0.3 – 0.9
Minerals (as oxide ash)	4.2	4.4
Water	8-12	8-12
Volatiles	traces	traces

Quoted from Illy and Viani (1995); Farah (2012).

As shown in Table 1.1, polysaccharides are the major constituents of green coffee, with 49.8 g/100g in Arabica and 54.4 g/100g in Robusta, which are an important factor in coffee roasting contributing to monosaccharides through hydrolysis, and formation of pigments. Sucrose is a low molecular weight carbohydrate, 342.3 g mol^{-1}, and is the main free sugar present in green beans. Sucrose accounts for 6 - 9% of dry weight in Arabica, whilst Robusta coffee sucrose content is around 0.9 - 4%.

There are a number of non-volatile compounds that are relevant for coffee overall flavour, these includes trigonelline, caffeine, CGA, lipids and minerals play a crucial role in coffee quality (Buffo & Cardelli-Freire, 2004). Some of these

compounds provide characteristic bitter, astringent or sour notes, which are key indicators of coffee quality. Caffeine and trigonelline are nitrogen-containing molecules that are characterized by a bitter note (Flament, 2002).

Nitrogenous compounds (protein, free amino acid, caffeine, trigonelline) are generally present at concentrations of approximately10 - 20%, while lipids are also present at similar concentrations. Green coffee beans have been reported to possess significantly different lipid contents between Arabica (15 - 17%) and Robusta (7 - 10%) (Illy & Viani, 2005). CGA and other compounds are also found at lower concentrations. In particular, trigonelline is about 0.6 - 1.3% in Arabica and 0.3 – 0.9% in Robusta, while caffeine is reported to be 0.9 - 1.3% in Arabica and 1.5-2.5% in Robusta coffee (Farah, 2012).

More than 180 volatile substances have been identified in green beans, which include aldehydes, alcohols, pyrazines, phenolic compounds, esters, furans, ketones, acids, and heterocyclic nitrogen-containing compounds (Boosfeld & Vitzthum, 1995; Cantergiani, Brevard, Krebs, Feria-Morales, Amadò, & Yeretzian, 2001). It was been reported that most volatile compounds are similar between Arabica and Robusta, whereas Arabica has a higher content of terpenes and is lower in aromatic compounds (Illy & Viani, 2005). However, most characteristic volatile compounds are formed during roasting (Gloess, Vietri, Wieland, Smrke, Schönbächler, López, *et al.*, 2014).

1.1.4 Coffee Roasting

Roasting is based on continuous heating of the green beans, causing a series of complex physical and chemical reactions. The following phenomena takes place during roasting of coffee beans (Illy & Viani, 2005):

- Bean surface: transferring heat via conduction, convection and radiation;
- Inside of the beans: temperature rise, endothermic water vaporization, exothermic reactions, volume enhancement, dry mass loss, temperature dependent transport, change of material properties and transport of water vapour, CO_2 and volatiles;

In the beginning, the coffee bean surface temperature increases and the thermal energy is transferred into the inside of the beans via conduction (Illy & Viani, 2005). Water evaporation will start during the first stage when the bean temperature reaches 50 ºC and the green coffee bean undergoes a moisture loss from the initial water content present in green beans of 8 - 12% in to about 5% in roasted coffee beans (Belitz & Grosch, 1999). During the first stage of roasting, the coffee bean walls are still relatively firm and strong. Although the water vapour has been generated inside of the beans, it cannot pass though the wall of the beans. Thus the pressure within the coffee bean increases, which makes the bean volume expand significantly. When the internal temperature of the beans reaches 100 ºC, the colour become slightly darker for about 20 - 60 s due to the endothermic reactions and vaporisation of water. When bean temperature reaches 140 - 160 ºC, Strecker degradation, Maillard and pyrolytic reactions start to take place, resulting in further darkening of the beans (Hernández, Heyd, Irles, Valdovinos, & Trystram, 2007). The build-up of water pressure, along with the large amount of gases generated causes the beans cell wall to crack, giving rise to the "first crack".

As heating continues at the roasting temperature of 160 - 190 ºC, the resulting coffee bean becomes darker and a second crack occurs as large volumes of carbon dioxide build-up and escape, which leaves the bean very porous (Wang & Lim,

2012). The reaction becomes exothermic, releasing heat/energy. It is necessary to build up sufficient pressure for the generation of coffee aroma (Illy & Viani, 2005). Finally, after roasting, the fresh roasted coffee beans need to be quickly cooled down to avoid over roasting (Yeretzian, Jordan, Badoud, & Lindinger, 2002). After the roast, the density of coffee bean is reduced from 550 g/L - 700 g/L (green beans) to around 400 g/L in (roasted beans) (Illy & Viani, 2005). The colour of the bean was changed from green to yellow at the first stage of roasting, it became darker with increasing roasting time and temperature.

Roast Profile is commonly use and record in the coffee roasting industry. A roast profile is a graphs that shows the temperature development during a roast cycle and preferably both bean and air temp is measured and logged. Previous study indicated that the changes of time temperature roasting profile could significantly influences the volatile organic compounds formation (Gloess, Vietri, Wieland, Smrke, Schönbächler, López, et al., 2014). An idealized profile is illustrated in Figure 1.3 below.

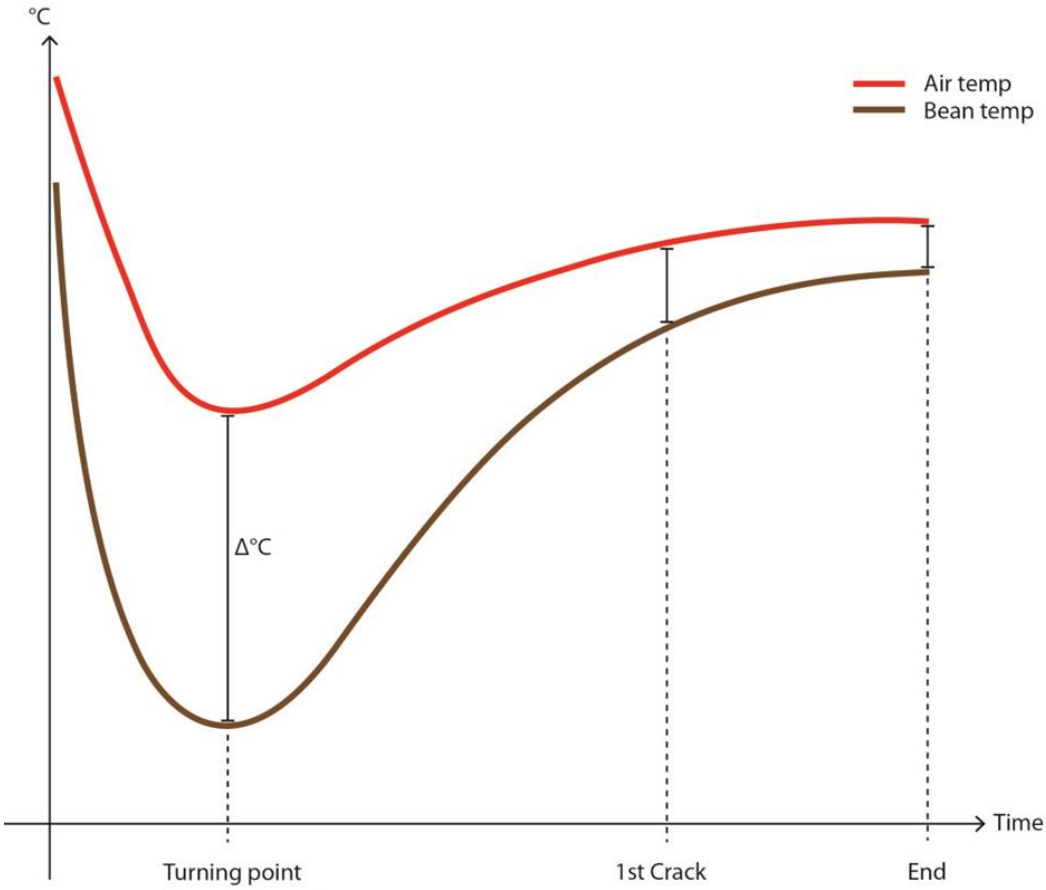


Figure 1.3 roasting profile for the ideal condition (quoted from CoffeeMind (2018))

Obtaining a homogeneous degree of roasting is extremely difficult, currently no universally accepted method to define roasting degree exists (Baggenstoss, Poisson, Kaegi, Perren, & Escher, 2008). However, the definition of roasting degree should be independent of variations in the raw material. There is agreement in the literature on the fact that colour assessment is not a reliable parameter for assessing coffee roasting, as different quality coffees can reach the same reflectance value, or coffees treated under the same time-temperature profile can present different colour values (Shan, Suzuki, Ogawa, & Kondo, 2015). Thus, visual assessment can give inaccurate determination as they depend on the subjectivity of the operator (Shan, Suzuki, Ogawa, & Kondo, 2015).

Typical roasting conditions using traditional industrial drum roasters vary from 180 to 240 ºC for a period of 7 - 20 min. Depending on the roasting intensity, roasted coffee has been largely categorized as light, medium or dark roasts. Light roast processing tends to give non-uniform bean colour with, grassy, sour, and underdeveloped flavour, while the medium roast process produces a balanced taste and aroma with citrus flavour (Lyman, Donald, Benck, Dell, Merle, & Murray, 2003). Medium roasted coffee is often reported as the best roasting degree to maximise the production of aroma compounds and flavour complexity of the final product. By contrast, dark roast process produces shiny black beans with an oily surface and a pronounced bitterness.

1.1.5 Coffee Flavour Generation

Many non-volatile constituents in green beans are the precursors of flavour compounds formed during the roasting process, particularly sucrose, proteins, and other nitrogen-containing compounds, as well as CGA (Figure 1.4).

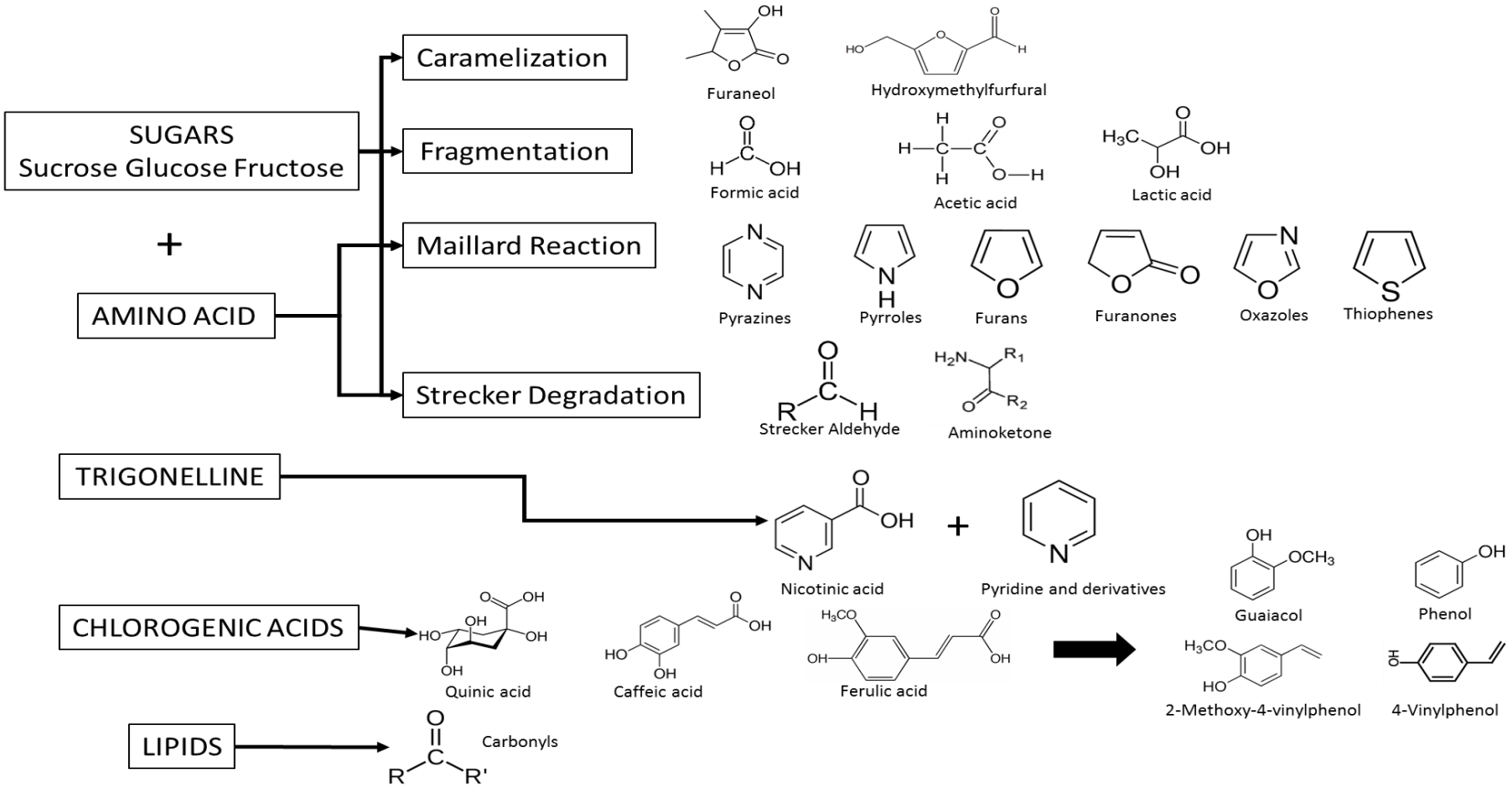


Figure 1.4 Relationship between green bean flavour precursors and the transformation into key aroma compounds (reproduced from Poisson, Blank, Dunkel, & Hofmann, 2017).

Compounds such as CGA contribute to a perception of astringency; sucrose degradation contributes to colour, volatile compounds, and acidic taste (Buffo & Cardelli-Freire, 2004). Overall, there is an increase in volatile acids, while the caffeine content remains almost unchanged (Buffo & Cardelli-Freire, 2004). The following reactions take place under the raised temperature/pressure during roasting leading to aroma compound generation.

- Maillard reaction between nitrogen-containing compounds and reducing sugars yielding pyridines, pyrazines, dicarbonyls (e.g. diacetyl), pyrroles and imidazoles, enolones (Bastos, 2012);
- Strecker degradation, between amino acids and dicarbonyl yielding aminoketones, that form nitrogen heterocyclic compounds or oxazoles (Berger, 2007);
- Sulphur amino acid degradation, which gives thiophenes and thiazoles (Uk Ahn, Joo Lee, Feng, Zhang, Lee, Jo, *et al.,* 2016);
- Caramelization and degradation of carbohydrates forming mainly volatile acids, furan derivatives and aldehydes (Rahn & Yeretzian, 2019; Yeretzian, Jordan, Badoud, & Lindinger, 2002);
- Trigonelline degradation, which forms alkyl-pyridines and pyrroles (Stadler, Varga, Hau, Vera, & Welti, 2002);
- Formation of melanoidins (brown pigments) via Maillard reaction or caramelization of carbohydrates following by polymerization (Illy & Viani, 2005);
- Chlorogenic acid degradation, which forms mainly phenols and

antioxidants (Kamiyama, Moon, Jang, & Shibamoto, 2015);

- Lipid oxidation, which forms aldehydes (Poisson, Blank, Dunkel, & Hofmann, 2017)

The Maillard reaction has been identified as the major pathway in the formation of volatile compounds during coffee roasting (Buffo & Cardelli-Freire, 2004). The Maillard reaction is a non-enzymatic browning reaction, which lead to the formation of higher molecular weight and lower molecular weight compounds. This includes melanoidins (brown coloured pigments) and flavour components providing earthy, caramel, roasted, nutty notes to the roasted coffee beans (Clarke & Vitzthum, 2001; Illy & Viani, 2005). During roasting, the reaction starts with the decomposition of sucrose into fructose and glucose which undergo Maillard reactions. During the Maillard reaction, reducing sugars such as glucose or fructose react with free amino acids to form N-substituted glycosylamine adducts (Schiff base), which are then rearranged to aminoketones and aminoaldoses by Amadori and Heyn's rearrangements (Poisson, Blank, Dunkel, & Hofmann, 2017). A complex reaction cascade of Amadori and Heyn's rearrangement products degrade to hydroxymethylfurfural or *C*-methyl reductones (Flament, 2002). The reaction is complex and results in the production of numerous volatile compounds and complex melanoidins. Due to the green bean chemical composition different, the composition of the roasted coffee Maillard reaction products can also vary. The flavour generation is influenced by many factors such as the type of amino acids and sugars involved (Kwak & Lim, 2004), reaction time/temperature (Ames, Guy, & Kipping, 2001; Bastos, 2012), pressure (Illy & Viani, 2005), pH (Ajandouz & Puigserver, 1999), and moisture content (Ames, Guy, & Kipping, 2001).

Research has revealed that sugar decomposition enhances the volatilisation and formation of formic acid, acetic acid and lactic acid in the initial stages of roasting (Yeretzian, Jordan, Badoud, & Lindinger, 2002). In the later stages, during roasting at high temperature, furaneol and hydroxymethylfurfural are generated via sugar caramelization. However, aroma formation is more likely through the Maillard route than caramelization due to lower activation energy in the presence of reactive nitrogen species (amino acids). Both caramelization and Maillard reaction are the main way to the formation of melanoidins (Illy & Viani, 2005).

Strecker degradation is partly involved in the Maillard reaction network. In Strecker degradation, free amino acids react with dicarbonyl to form mainly Strecker aldehydes and amino ketones. The amino ketones may react further to form a pyrazine or an oxazole (Clarke & Macrae, 1985). Strecker aldehyde may further form other volatiles as roasting continues via aldol condensation. Researchers have also reported that the formation of visual chromophors are also associated with Strecker degradation (Poisson, Blank, Dunkel, & Hofmann, 2017; Rizzi, 2008).

Sulphur-containing flavours can be generated during both the Maillard reaction and Strecker degradation via sulphur-containing amino acids such as cystine and methionine reacting with dicarbonyl compounds during roasting (Flament, 2002). Thiophenes and thiazoles can be formed by pyrolysis of cysteine. Thiazoles were reported to the formed via decarboxylation to the amine and ring closure to thiazolidine followed by oxidation (Clarke & Macrae, 1985).

CGA was first reported as a physiologically active substance by Robiquet and Boutron (Robiquet & Boutron, 1837). The CGA content is significantly reduced during roasting by a number of routes such as hydrolysis, oxidation, fragmentation,

polymerization and acyl migration (Clarke & Macrae, 1985). CGA was found to contribute to hydrolysis products such as guaiacol, 4-vinylguaiacol, and phenol in roasted coffee (Clifford, 1985).

Trigonelline is rapidly degraded during roasting and losses of between 50 - 80% occur. The degradation of trigonelline was mainly found to yield a range of volatiles such as pyrroles and pyridines (Viani & Horman, 1974). The residue of trigonelline can be demethylated to nicotinic acid, methyl ester and N-methylnicotinamide (Clarke & Macrae, 1985). The remaining trigonelline in the roasted beans will be completely extracted during the brewing process and can play an important role in the bitterness of coffee brew (Clarke & Macrae, 1985).

The lipids of coffee beans are composed of a coffee oil present in the endosperm, and coffee wax in the outer layers of the beans. There is a small change in the lipid fraction on roasting. Lipid oxidation of unsaturated fatty acids produces aldehydes such as nonenal, hexanal, other enals, and dienals, however, aldehydes may further react with other coffee compounds or undergo cyclization (Belitz, Grosch, & Schieberle, 2009).

1.1.6 Coffee "Staling"

As mentioned in 1.1.4, the formation of volatiles and CO_2 during roasting in combination with the high temperatures of the environment causes the expansion of the beans and the formation of pores (Pittia, Sacchetti, Mancini, Voltolini, Sodini, Tromba, *et al.,* 2011). The beans become very brittle and progressively lose their ability to entrap and retain volatiles. Thus, volatiles and CO_2 are easily released into the vapour phase due to diffusion mechanisms favoured by the pressure gradient

between the internal bean pores and the atmosphere (Manzocco, Calligaris, Anese, & Nicoli, 2016).

Many of the important coffee aroma compounds are known to be highly unstable and volatile, and are lost by volatilisation soon after roasting process (Akiyama, Murakami, Ohtani, Iwatsuki, Sotoyama, Wada, *et al.*, 2003; Yeretzian, Blank, & Wyser, 2017). The loss of freshness after roasting (coffee "staling") is due to the loss of their highly volatile compounds and a number of intrinsic chemical reactions such as thiol-binding, pH increase and oxidation. Many volatile compounds may react upon storage such as hexanal and acetic acid (Pérez-Martínez, Sopelana, de Peña, & Cid, 2008; Yeretzian, Blank, & Wyser, 2017). Elevated temperature (Cardelli & Labuza, 2001; Nicoli, Calligaris, & Manzocco, 2009), oxygen concentration (Illy & Viani, 2005; Ross, Pecka, & Weller, 2006) and relative humidity (Anese, Manzocco, & Nicoli, 2006; Cappuccio, Full, Lonzarich, & Savonitti, 2001) are the major factors that further accelerate the coffee staling.

Arabica coffee contains more than 15% lipid and Robusta green beans contain up to 10% lipid, consequently, volatiles associated with lipid oxidation by-products have been an early focus in studies on degradation markers for the coffee. A number of studies have reported the increase in n-hexanal over shelf life storage as a marker of lipid oxidation and correlated with coffee staling (Krešimir Marin, 2008). However, it has been reported other compounds associated with oxidative degradation of unsaturated fatty acids in roasted coffee do not play a significant role in the flavour development of roasted coffee. While hexanal seems to be the only notable exception, its formation cannot fully explain the loss of freshness, because a certain loss of odour intensity (freshness) and a significant loss of cup quality is noticeable much earlier during storage. Hence, the increase of n-hexanal

concentration occurs only at a quite advanced degree of staleness and is less relevant to address the freshness of speciality coffee (Krešimir Marin, 2008).

1.1.7 Aroma Differences between Roasted Arabica and Robusta

More than 1000 volatile compounds have been identified in both roasted and ground Arabica and Robusta coffee, the most common classes of compounds reported in the headspace include volatile acids, alcohols, aldehydes, furans, pyrroles, phenolic compounds, pyrazines, pyridines, and sulphur-containing compounds (Illy & Viani, 2005; Schenker, Heinemann, Huber, Pompizzi, Perren, & Escher, 2002).

Previous research has revealed that the major aroma profile differences between Arabica and Robusta coffee are mainly due to the predominance of sotolon, furaneol, 3,4-dimethylcyclopentenol-1-one in Arabica beans and a predominance of 2,3-diethyl-5-methylpyrazine, 3,5-dimethyl-2-ethylpyrazine, 4-vinylguaiacol and 4-ethylguaiacol in Robusta beans (Blank, Sen, & Grosch, 1991). Preparation of coffee brews enhance the flavour difference, as the concentration levels of water-soluble odorants (furaneol and sotolon) responsible for the sweet-caramel, sweet-roasty and nutty flavour increased more in the Arabica than Robusta coffee (Blank, Sen, & Grosch, 1991). Moreover, the guaiacols and alkylpyrazines have been shown to be responsible for the "harsh-earthy and spicy" aroma of Robusta coffee (Blank, Sen, & Grosch, 1991).

Later, in 1995, researchers found that the concentration of 2-methyl-3-furanthiol and 2, 3-diethyl-5-methyl-pyrazine was around 2 times higher in Robusta than Arabica (Semmelroch, Laskawy, Blank, & Grosch, 1995). In 2007, Nebesny confirmed that 2-furfurythiol and 4-vinylguaiacol were dominant in Robusta coffee,

as well as β-damascenone, methanethiol, alkylpyrazines, furanones, acetaldehyde, propanal, methyl-propanal, 2-and 3-methylbutanal (Nebesny, Budryn, Kula, & Majda, 2007).

1.1.8 Green Coffee Modification

In the literature, several approaches have been used to modify coffee for an overall quality improvement in the final products:

- Breaking down macromolecules to components that are more readily available for aroma formation reactions, such as protein to amino acids, polysaccharides to mono/disaccharides by modifying the fermentation process and enzyme treatment (Hashimoto, 1969; Kwak, Jeong, & Kim, 2018; Lee, Cheong, Curran, Yu, & Liu, 2016; Small & Asquith, 1990).
- Steaming or soaking pre-treatment, removal of specific precursors that cause undesired flavour in the final product (Becker, Schlabs, & Weisemann, 1991; Dar, Bruckmann, & Kelly, 1985; Lendrich, 1930; Maki Yoshiaki 1994).
- Adding flavour extracts into green beans to provide flavour-enhanced coffee beans (Penson, Brooks, Wragg, Ikenberry, Bradbury, Ozaki, et al., 2008;).

Fermentation in raw coffee refers to the microbial reaction of bacteria or yeasts breaking down the sugars and proteins in mucilage, which plays an important role in the formation of the coffee aroma profile (Lee, Cheong, Curran, Yu, & Liu, 2016). Optimized fermentation can impart desirable attributes to the corresponding coffee aroma while uncontrolled fermentation inevitably leads to off-flavours. A modified

fermentation process or enzyme treatment to improve the coffee bean flavour has been reported from several studies.

The Earliest study on the enzyme pre-treatment of coffee beans was done by Hashimoto and Fukumoto (1969) and suggested that mannan, manno-oligosaccharides and arabinogalactan in coffee can be hydrolysed by purified mannanase, mannosidase and galactanase. A later study (Small & Asquith, 1990) for treating coffee beans with enzyme-containing solutions under pressure, demonstrated that treated coffee had improved flavour and reduced bitterness. In this approach, a solution containing cell-wall-digesting, cell-storage-component-digesting or phenol oxidase enzymes under a pressure of at least 250 psi could enhance the level of desirable coffee aroma and flavour components.

Lee et al. (2016) illustrated that modulating coffee aroma via the fermentation of green coffee beans with a food-grade fungus Rhizopus oligosporus showed a positive impact on both aroma precursor content in the green beans and aroma generation in the roasted coffee. Proteolysis during fermentation resulted in a 1.5 fold increase in the concentrations of aspartic acid and proline which demonstrated high Maillard reactivity.

A recent study done by Kwak et al. (2018) demonstrated the relationship between yeast fermentation of green beans and consumer acceptability. The results indicated that the yeast fermentation was effective in fortifying the functionality of coffee by significantly increasing antioxidant activity. The yeast fermentation of green coffee beans did not generate a negative flavour and can be attractive with high antioxidant activity.

Regarding the steam treatment, the earliest experiments by Professor Karl Lendrich in the 1930's, showed that it helped remove unwanted substances in coffee. This approach involved heating with moisture under a pressure of 1 - 3 psi to remove unwanted substances such as carboxylic acids from the bean (Lendrich, 1930). A similar process was developed to reduce unwanted taste components in Robusta coffees, mainly by increasing the pressure in the vessel (Dar, Bruckmann, & Kelly, 1985). This approach means that more Robusta coffee can be added in the blends, with approximately the equivalent soluble solids in the pure Arabica coffee brew.

A study by Becker et al. (1991) suggested a method to improve the flavour profile by removal of 2-methylisoborneol, which is responsible for the musty and earthy flavours in the roasted coffee beans. The process involved increasing the moisture content in the green beans to about 30 - 45% with relatively high temperature steam (135 - 140 ºC) at a pressure of 3 - 4 bar for 60 - 120 min. Studies on the process for roasting coffee beans with steam by Maki and Haruyama (1994) utilised high temperature (251 - 400 ºC) and pressure (6.5 - 20.0 bar) steam during the roasting process which achieved a significant reduction in the undesired flavour (cereal flavour) of Robusta coffee beans with improved roasting efficiency. Floral and nutty aroma in the treated Arabica coffee beans was also enhanced and the sour flavour of the treated coffee was significantly reduced.

Penson et al. (2008) demonstrated that the flavour of roasted Robusta and Arabica coffee quality can be modified by green bean treatment. In this approach, the water-soluble precursors from the green coffee bean were extracted, then the water extract separated from the green coffee beans. Followed by modifying the water-soluble flavour precursors to obtain modified water-soluble flavour precursors in the

aqueous extract. Then the modified aqueous extract was added back to the extracted green coffee beans to create flavour enhanced green coffee beans.

1.2 Technical Hypothesis

Overarching hypothesis: Robusta coffee bean pre-treatment could affect aroma generation during roasting and may further increase aroma chemistry similarities between Robusta and Arabica coffees.

Previously studies have worked on elucidating the effects of bean variety, roast degree, and different time-temperature conditions, on the physical and chemical properties of coffee.

- **Hypothesis 1**: If the physical and chemical properties of coffee is related to the roasting time-temperature profiles, then modifying roasting condition will bring the Robusta coffee closer to Arabica.

Several approaches have been used to modify coffee for an overall quality improvement in final coffee products, most of the studies used water as a treatment carrier to remove the unwanted substance in green beans. However, during this process important water-soluble precursors such as sucrose, protein, CGA, caffeine and trigonelline are leached into water, hence compromising the flavour generation potential of the roasted coffee.

- **Hypothesis 2**: If water soluble precursors could be leached out into water during pre-treatment process, pre-treatment time and temperature could be optimized to minimize the difference between species and result in improved quality of Robusta coffee.

Cold plasma treatment can act as a surface treatment for food materials and can be used to control surface chemistry (detail explained in chapter 4), however, it has not be used to control coffee flavour.

- **Hypothesis 3**: If phenolic compounds content and sucrose content can be modified by cold plasma treatment, then green beans treated with cold plasma could have an enhanced flavour profile.

Previous researchers have been reported that pH can significantly affect the reaction rate of the Maillard-Type Reactions. Many steps in the Maillard cascade are affected by the pH. No comprehensive review of pH modification on green beans aroma generation is currently available.

- **Hypothesis 4**: Modifying the green bean pH could modify the aroma formation in Robusta coffee, which may bring the Robusta coffee aroma closer to Arabica.

Sugars are considered to be the main precursors in the aroma generation and colour formation during coffee roasting. Arabica contains almost twice the concentration of sugar than Robusta. There is no evidence currently available that shows the effectiveness of sugar modification on increasing the flavour quality of Robusta.

Hypothesis 5: Modifying the sugar content in green bean could modify the aroma formation in Robusta coffee, which may bring the Robusta coffee aroma closer to Arabica.

1.3 Aim and Objective

The overall aim of this PhD research was to improve the aroma properties of Robusta coffee using several novel processes to modify the chemical composition of the green bean with a relatively low processing cost. To achieve this research aim, specific objectives are detailed below and the chapters in which they discussed:

- Study the impact of roasting time-temperature profiles on aroma generation within Robusta coffee beans to explain the underlying working mechanisms behind the aroma formation. (Chapter 3)
- Explore the effect of cold plasma pre-treatment on the aroma generation within Robusta coffee beans. (Chapter 4)
- Investigate the loss of water-soluble precursors during pre-soaking of green coffee beans and the impact on aroma generation during coffee roasting. Optimize pre-soaking time and temperature to modify the aroma profile of Robusta coffee. (Chapter 4)
- Analyse the impact of green bean pH on aroma generation within Robusta coffee beans to bring the aroma of the final product closer to the target Arabica coffee. (Chapter 5).
- Evaluate the impact of green bean sugar content on aroma generation within Robusta coffee beans to bring the aroma of the final products closer to the target Arabica coffee. (Chapter 6).

Chapter 2 Material and Methods

2.1 Coffee Samples and Chemicals

Coffee beans were either single-origin wet processed Robusta green beans from Vietnam or high grade Arabica green beans (Type AA: cupping 93/100) from Aberdares, Mount Kenya. They were both supplied by Edgehill coffee UK. Reference chemical compounds for the volatiles and non-volatiles analysed were obtained from Sigma-Aldrich (Steinheim, Germany) (detail presented in appendix A1).

2.2 Physical and Chemical Analysis Methodologies of Green and Roasted Coffee Beans

2.2.1 Coffee Roasting

Coffee samples were roasted in the same batch by 10-tray convection oven (Mono Equipment, Swansea, UK) with varying roasting time and temperature profiles. Coffee beans were weighed before and after roasting. The roasting loss was measured by weight for green beans minus weight of roasted beans. Roasted samples were ground using an electronic coffee grinder (ground to fine powder approximately 1 min) (KG 49, Delonghi, Australia) then filtered by a metal sieve 710 μm (Endecotts, Essex, UK). The samples were ground to a uniform size, producing a specific particle size distribution and stored in a sealed aluminium bag in a freezer at -80 ºC until analysis.

2.2.2 Coffee Samples for Storage Test

After roasting, all coffee samples were cool down to 50 ± 2 ºC then stored in non-permeable aluminium bags with nitrogen gas protection under normal atmospheric

conditions. The use of this type of packaging was to minimise the moisture intake from the environment and control the photo autoxidation reaction induced by light.

Accelerated shelf life storage (ASL) test was carried out in various conditions that may be encountered in the product distribution chain and in the domestic storage environment. Samples (25 g) for ASL test were stored in the amber glass jars (G. Baldwin & Co., London, UK) without lid, open to the atmosphere. In this study, three storage temperatures were selected as 5, 25 and 35 ºC. Four replicates were prepared for each storage temperature. In order to determine the end point of the ASL test, coffee samples were stored previously at 35 ºC for 3 and 6 weeks, it was noticed that the product property changed significantly after 6 weeks storage from the fresh samples due to loss of the volatile compounds. Therefore, 6-week storage was determined as the end point of the ASL test.

Control samples were stored at -80 ºC with four replicates and remaining samples with four replicates were stored at temperatures 5, 25, and 35 ºC in a laboratory oven (Sanyo, Loughborough, UK). Samples were removed every two weeks from each storage condition and stored in sealed non-permeable aluminium bags with nitrogen gas protection at -80 ºC. All samples were tested together at the end of the storage trial in a randomised order for instrumental analysis.

2.2.3 Measurement of Moisture Content

All coffee samples were weighed using a balance (PEB 1200-2, KERN Ltd.) and their weight was recorded W1 (g). All samples were placed into a drying oven (GenLab Ltd.) at 105 ºC for 24 h and their weight recorded W2 (g). The moisture content (%) was calculated by $\frac{(W1-W2)}{W1} \times 100$

2.2.4 Measurement of Colour

Colour was determined for four replicates using a Hunter Lab (ColourQuest XE, HunterLab, US) by identifying lightness (L), a value, and b value. Positive a and b represent red and yellow, negative a and b represent green and blue respectively (Hunter, 1987). The conditions of the experiment were as follow: standard illumination: D65, colorimetric normal observer angle: 10°, ASTM E308 RSIN Mode, LAV, 1.00 Port, UV Nominal. The readings were made by CIELAB system. The Hunter Lab was preheated for 2 h and standardized by using the light trap standard (serial no. CQX2614) and diagnostic tile (serial no. CQX2614). 1 g of the coffee powder was put into cuvettes (SARSTEDT AG & Co. D-51588) and directly placed to the measurement aperture to test L, a and b value with three positions randomly. The total colour difference ∆E also can be calculated by equation:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

The ∆E is a single value which covers about the differences between L*, a and b of the sample and control sample (Hunter, 1987).

2.2.5 Measurement of pH

Green/roasted coffee bean powder (2 g) was extracted with 15 ml of boiling water in a 30 ml tube which was placed on a roller overnight (room temperature). The extracts were then filtered with No. 4 Whatman® filter paper before pH analysis. The pH of the extracts was measured using a pH meter (Inolab®).

2.2.6 Measurement of Total Lipid Content

The Bligh and Dyer's method was used to extract lipids from coffee (Bligh & Dyer, 1959). 0.1g of coffee powders were prepared and the weight was recorded Wp1 (g)

using an analytical balance (Sartorius 1712, Sartorius Ltd., Epsom, UK). An empty glass vial was weighed and the weight recorded Wg2 (g). 0.1 g of coffee powders was placed into a 15 mL centrifuge tube. 2 mL of chloroform: methanol (2:1) was added and vortexed for 1 min and then the samples were centrifuged at 1600 G for 10 min at 4 ºC. After the centrifugation, there were three phases in the samples; the bottom phase was the lipid extract, removed and placed into a new glass vial. The above processes were repeated three times to extract lipids completely. For each sample, the bottom phases of three extractions were combined into the glass vial and dried using N_2 gas. The weight of the glass vial containing the dried lipid extract was recorded Wd3 (g). The total lipid content by dry mass was calculated using the following formula:

$$\text{The total lipid content (g/kg)} = \frac{Wd3(g) - Wg2(g)}{Wp1(g)} \times 1000$$

2.2.7 Measurement of Total Protein Content

A bicinchoninic acid (BCA) assay kit (23225/23227, Thermo Scientific) was used to measure the total protein content. 0.1 g of coffee powder was weighed and 2 mL of chloroform was used to extract lipid with three replicates. After lipid extraction, the solid phase was dried at 60 ºC for 30 min and placed in a 15 mL centrifuge tube. After that, 1 mL of 2% sodium dodecyl sulphate (SDS) solution was added into the tube to eliminate interference from lipids and the tube was heated at 60 ºC in a water bath for 30 min. The sample was then vortexed for 1 min and centrifuged at 13,000 g for 3 min. Samples were then filtered and diluted 100 times using 2% SDS.

0.1 mL of each standard and sample replicate was added into a test tube. 2.0 mL of the working reagent containing 50 parts of BCA Reagent A (sodium carbonate,

sodium bicarbonate, bicinchoninic acid and sodium tartrate in 0.1 M sodium hydroxide) and 1 part of BCA Reagent B (4% cupric sulphate) was added to each tube and mixed well. All tubes were covered and incubated at 37 ºC for 30 min, after incubation, tubes were cooled to room temperature. Spectrophotometry was used to measure the absorbance of the samples (Genesys 10S Vis, Thermo Scientific). The absorbance of the samples was measured at wavelength 562 nm with subtracting of the average 562 nm absorbance measurement of the blank standard replicates. A standard curve was prepared by plotting the average blank-corrected 562 nm measurements for each bovin serum albumin (BSA) standard versus its concentration in µg/mL. This was used to determine the protein concentration of each sample.

2.2.8 Measurement of Sugar Content by LC-MS

0.1 g of coffee powder was placed in a 30 mL centrifuge tube with 20 mL of boiling water and vortexed for 5 min. Samples then were centrifuged at 1600 g for 10 min at room temperature. After centrifugation, the liquid phase was transferred into a new glass vial. The above processes were repeated three times for extracting sucrose, glucose and fructose completely. The mixture was cooled to room temperature and then filtered using a syringe filter (0.45 µm, 40 hydrophilic nylon syringe filter, Millipore Corporation). The final extract was diluted with methanol (MeOH) (1:1) prior to Liquid chromatography-mass spectrometry (LC/MS) analysis (the method was modified from Ky *et al.*, 2000,McCusker *et al.*,2003 and Perrone *et al.*, 2008).

The LC equipment (1100 Series, Agilent) consisted of a degasser (G1322A, Agilent), a pump (G1312A, Agilent), an auto-sampler (G1313A, Agilent). This LC

system was interfaced with a Quattro Ultima mass spectrometry (Micromass, UK Ltd.) fitted with an electrospray ion source. The Luna 5u NH2 100A (C18) column (250 ×3.20 mm, 5 µm, Phenomenex) was used to separate sucrose, glucose and fructose and the column was maintained at room temperature. Chromatographic separation was undertaken with an isocratic elution mobile phase of 80% acetonitrile. The flow rate was set at 0.7 mL/min, the volume injected was 5µl.

Peaks were identified by comparing retention times to those of sugar standards. Standard curves were made of fructose, glucose, and sucrose standards (Sigma Aldrich®). Standards were made at five levels of concentration: 1, 2.5, 5, 7.5, and 10 mg/mL. All dissolved in 50: 50 MeOH: H2O. The respective peak areas were used for the quantification.

2.2.9 Measurement of Chlorogenic Acid, Trigonelline and Caffeine Content by HPLC

Chlorogenic Acid, trigonelline and caffeine analysis by High Performance Liquid Chromatography (HPLC) method was modified from literature sources (Casal, Oliveira, Alves, & Ferreira, 2000; Moon & Shibamoto, 2009).

1g of sample was mixed with 50 mL of 50: 50 MeOH: H_2O in a 100 mL centrifuge tube. The analytes contained in the coffee were then extracted under stirring on a roller overnight at room temperature. The extracts were further treated with Carrez reagents I and II to eliminate polymeric components. The extracts (4 mL) along with 0.1mL each of Carrez reagents I and II and 0.8 mL of MeOH were vortex-mixed in centrifuge tubes and allowed to stand for 10 min. The precipitate was separated by centrifugation at 4000 rpm for 10 min. Then the solutions were filtered

with Acrodisc syringes with 0.2 μM Sartorius stedim Minisart filter, the samples were stored at 4 ºC until HPLC analysis.

Quantitative analyses of CGA, trigonelline and caffeine were performed using an Agilent 1100 model HPLC system equipped with Phenomenex C-18, 5 μ column (250 mm x3 mm) and a variable wavelength detector (Spectro Monitor®3000). Mobile phase A was water (Purelite®) containing 0.1% formic acid and mobile phase B was acetonitrile containing 0.1% formic acid. The gradient mode was initially set at an A:B ratio of 95: 5 which was hold for 9 min, then linearly increased to 75: 25 at 9 - 12 min and then to 50:50 at 12 - 15 min. The flow rate was 0.8 mL/min. Detection was accomplished at 265 nm for both trigonelline and caffeine; 325 nm for CGA; injection volume was 10 μL.

Concentrations of CGA, trigonelline and caffeine were calculated based on the regression equation obtained from a standard curve made using CGA, trigonelline and caffeine standard solutions at 50, 100, 500, 1000 and 2500 ppm. All dissolved in Purelite® water. The respective peak areas were used for quantitative analysis.

2.2.10 Measurement of Volatile Compounds by GC-MS

The ground coffee (1.5 g) was transferred into amber glass vials (20 mL) with four replicates for GC-MS analysis. An internal standard (IS) was prepared by adding 10 μL 3-heptanone (Sigma, Saint Louis, USA) into 10 mL methanol (Laboratory reagent grade, Fisher Scientific, UK). 2 μL of ISTD was added into each coffee sample and kept for 1 h equilibrium prior to GC analysis. All analytical samples were randomised for GC-MS analysis.

A trace 1300 series Gas Chromatograph coupled with a Single-Quadrupole Mass Spectrometer (Thermo Fisher Scientific, Hemel Hemptead, UK) was used for

analysis of volatile aroma compounds. Samples were incubated at 40 ºC for 5 min with shaking. A 50/30 μm DVB/CAR/PDMS SPME Fibre (Supelco, Sigma Aldrich, UK) was used to extract volatile aroma compounds from the sample headspace (extraction for 5 min then desorption for 2 min). The injector temperature was set at 200 ºC in splitless mode (constant carrier pressure was at 18 psi).

Separation was carried out on a ZB-WAX Capillary GC Column (length 30 m, inner diameter 0.25 mm, film thickness 1 μm; Phenomenex Inc., Macclesfield, UK). Column temperature was held initially at 40 ºC for 5 min, increased by 3 ºC/min to 180 ºC, then 8 ºC/min to 240 ºC and held for 2 min. Full scan mode was used to detect the volatile compounds (mass range from m/z 20 to 300).

Volatile compounds were identified by comparison of each mass spectrum with either the spectra from authentic compounds or with spectra in reference libraries (NIST/EPA/NIH Mass Spectral Library, version 2.0, Faircom Corporation, U.S.). The relative concentration of volatiles collected from the headspace was calculated from GC peak areas, by comparison with the peak area of the internal standard (3-heptanone). The relative abundance of each volatile compound presented in the headspace of Robusta or acetic acid treated Robusta (average of 4) was calculated by dividing its peak area with its peak area in Arabica as 100. All samples were analysed in one run in a randomised order.

2.3 Green Bean Pre-Treatment Methodologies

2.3.1 Cold Plasma Pre-Treatment

All treatments with cold atmospheric plasma were performed by placing the coffee samples on a plate with the lid on under the plasma pen leaving a distance of 10 ± 1 mm between the end of the plasma pen and the lid (Figure 2.1a). The plasma was

on for between 5 min to 30 min with four replicates each. A hole with a diameter of about 8 mm was made in the centre of a Petri dish lid which was then used to cover the plates while they were treated. Digital images for the plasma plume and plasma pen are shown in Figure 2.1.a and 2.1.b.

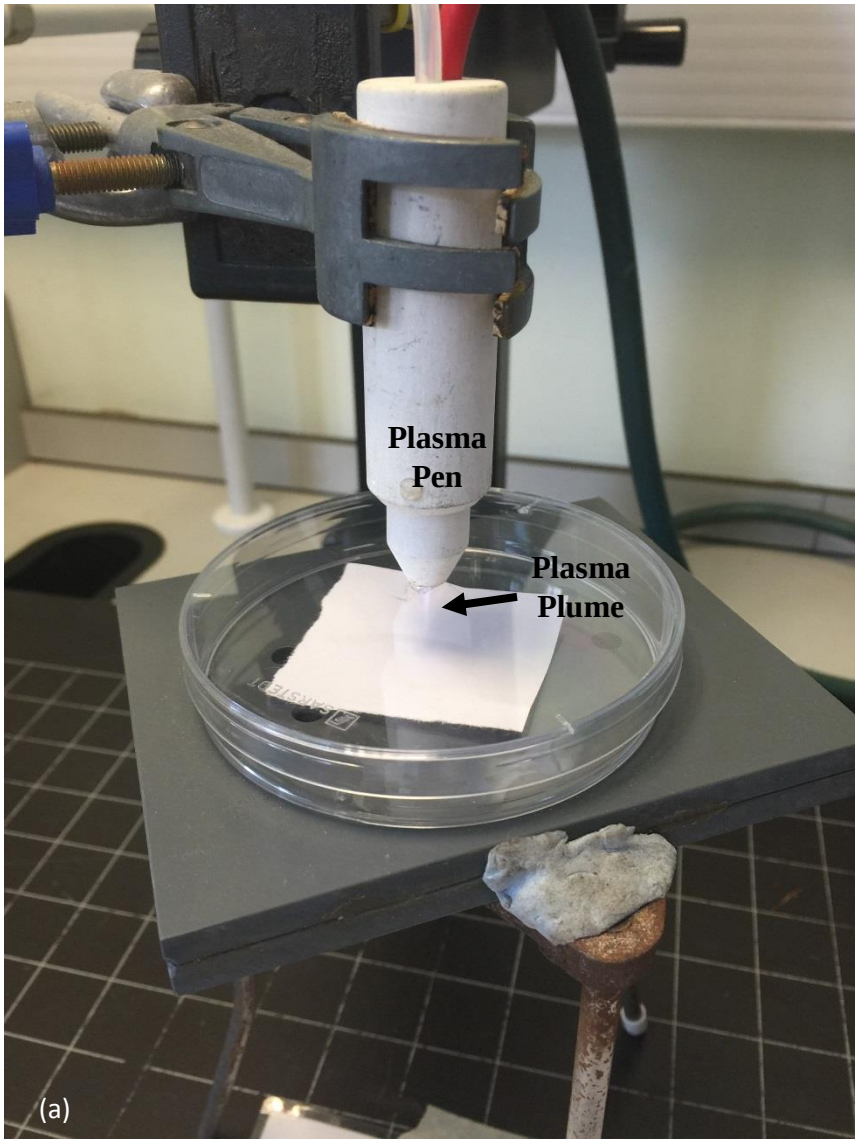


Figure 2.1(a) Digital images of the plasma pen.

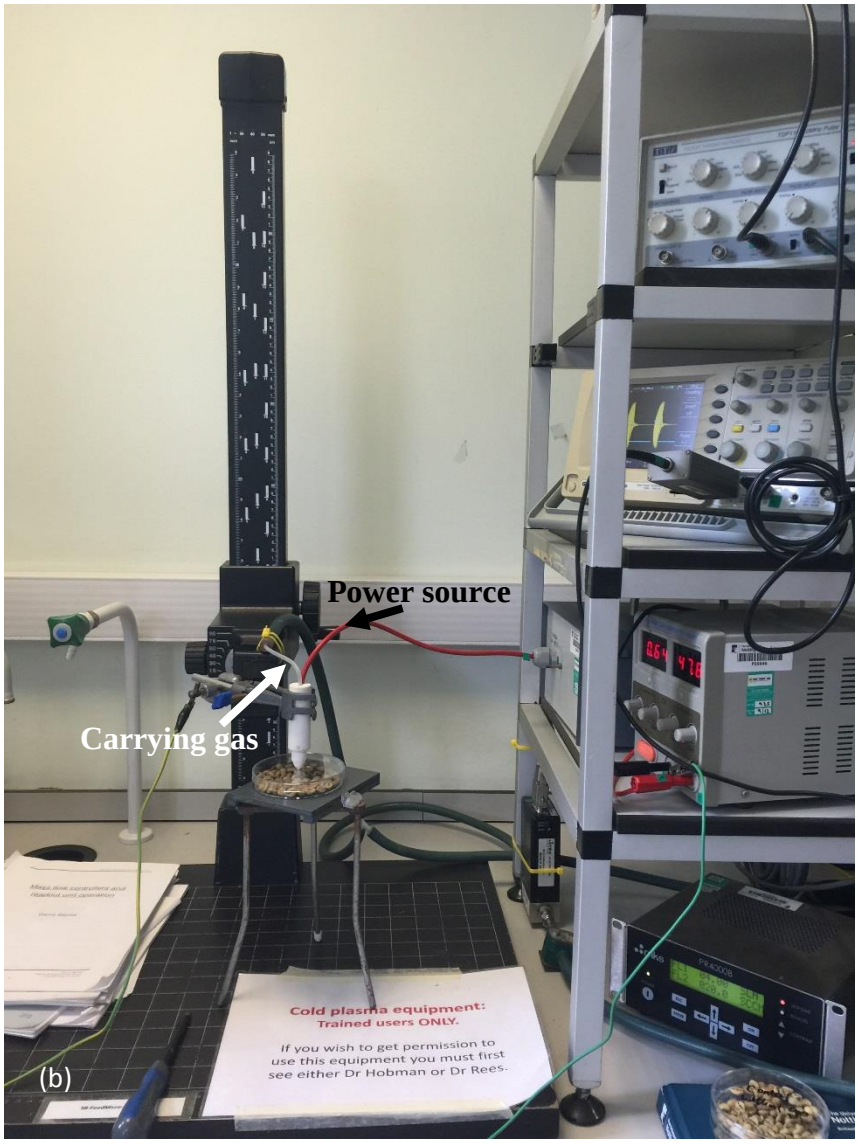


Figure 2.2 (b) Coffee was being treated.

Plasma treatments were carried out using the plasma rig at Sutton Bonington campus, University of Nottingham (made by Plasma and Pulsed Power Group at Loughborough University). The plasma was generated using a mixture of He and O_2 gases at flow rates of 4 L/min and 20 cm^3/min respectively with a radiofrequency (RF) = 4.7 MHz, and an input voltage 47.7 V. The temperature of the plasma plume was measured before any experiments using temperature indicator strips (20 to 50 ºC, reversible) (OMEGA, UK). It was always approximately 40 ºC.

2.3.2 Pre-Soaking Treatment with Varying Time and Temperature

Robusta green beans were soaked in water for different times (2, 4, 6, 8, 10 and 12 h) and temperatures (20, 40, 60, 80 and 100 ºC), three replicates each. Soaked Robusta green beans and non-treated Robusta green beans were placed into a desiccator with saturated sodium nitrate solution (relative humidity 65.5%) at room temperature (20 ± 2 ºC) for 20 d to control the moisture content (around 11.5%). Determination of the water changes during soaking and coffee roasting was carried out by weighing the coffee sample at every step.

2.3.3 Pre-Soaking Treatment with Varying Chemicals

Robusta green beans were soaked in 1% acetic acid (Sigma-Aldrich, Poole, UK), 10% sucrose (Sigma-Aldrich, Poole, UK), 5% lysine (Sigma-Aldrich, Poole, UK), or 1% sodium carbonate (Sigma-Aldrich, Poole, UK) water solution for 2 h at 20 ºC, four replicates each. Soaked Robusta green beans and non-treated Robusta green were placed into a desiccator with saturated sodium nitrate solution (relative humidity 65.5%) at room temperature (20 ± 2 ºC) for 20 d to control the moisture content (around 11.5%). Determination of the water changes during soaking and coffee roasting was carried out by weighting the coffee sample at every step.

2.3.4 Acetic Acid Pre-Treatment

25 g green Robusta coffee beans were placed into a Modulyo Freeze Dryer 1311-03/08 JM (Edwards, Crawley, UK) at −40 ºC for 72 h. Freeze dried Robusta green beans were soaked in different concentrations of acetic acid (Sigma-Aldrich, Poole, UK) 0, 1, 2, 3, 4, 5% for 2 h with four replicates each. 0% acetic acid (water only) treated sample was used as a control sample. Treated Robusta green beans, non-treated Robusta green beans and Arabica green beans were placed into a desiccator

with saturated sodium nitrate solution (relative humidity 65.5%) at room temperature (20 ± 2 ºC) for 20 d to control the moisture content (around 11.5%). Determination of the water changes during pre-treatment and coffee roasting was carried out by weighting the coffee samples at every step.

2.3.5 Sugar Pre-Treatment

25 g green Robusta coffee beans were placed into a Modulyo Freeze Dryer 1311-03/08 JM (Edwards, Crawley, UK) at −40 ºC for 72 h. Freeze dried Robusta green beans were soaked in different concentrations of glucose, fructose and sucrose (Sigma-Aldrich, Poole, UK) 0, 3, 6, 9, 12, and 15% using a steam retort for 30 min at 100 ºC with 2 bar pressure and a rotation of 1 rpm with four replicates each. 0% (water only) treated sample was used as a control sample. Treated Robusta green beans, non-treated Robusta green beans and Arabica green beans were placed into a desiccator with saturated sodium nitrate solution (relative humidity 65.5%) at room temperature (20 ± 2 ºC) for 20 d to control the moisture content (around 11.5%). Determination of the water changes during pre-treatment and coffee roasting was carried out by weighting the coffee sample at every step.

2.4 Sensory Analysis

2.4.1 Sensory Evaluation of Acetic Acid Pre-treated Samples

Two pre-treated Robusta coffee (2% and 4% acetic acid treated sample) were selected for sensory testing. The coffee samples for sensory testing were freshly brewed in cafetière in the morning of testing day to avoid flavour loss. Following manufacturers' instruction, 54 g of coffee were weighed and put in a cafetière (Argos, Stafford, UK) with 8-cup capacity. 860 mL boiling water was then poured into the cafetière, and stirred for 5 times. The coffee was then left to brew for 3 min.

Coffee was then poured out of the cafetière immediately into a glass beaker. 10 mL of coffee brew were prepared in amber vessel for each sample and served at ambient temperature (18 ± 2 ºC). In order to mask the colour differences, amber glass vessels were used in this study.

All sensory tests were carried out in individual sensory booth under northern hemisphere lighting at the Sensory Science Centre of the University of Nottingham. 84 volunteers were recruited from student and staff at the University of Nottingham to take part in this study. This study was approved by the School of Bioscience Ethic Committee (SBREC150118A) at the University of Nottingham, a small incentives was provided to the participants.

For each triangle test, volunteers were given three samples, and they were instructed to sniff the samples from left to right and identify which one was the different one. A 2 min break was given between triangles tests to allow nasal clearance. The presentation order between triangle tests and within each triangle test was randomised. All the data were collected using Compusense Cloud (Compusense, Ontario, Canada).

Volunteers were invited for two testing sessions on two separate days within a week. A total of 15 triangular tests were conducted over two sessions, each session lasted approximately 45 min. The first session was to determine how different/similar are the treated Robusta coffee brew (including Robusta, 2% acetic acid treated-Robusta, 4% acetic acid treated Robusta and non-treated Robusta blended with Arabica) to Arabica coffee brew. The second session was to determine how different/similar the Arabica was with Arabica blended with either 2% or 4% acid-treated Robusta (samples varied in blending ratio 20, 40, 60 and 80%).

2.4.2 Sensory Evaluation of Sugar Pre-treated Samples

Robusta samples treated by soaking in 15 g/100g fructose were chosen to be tested in the sensory study. The coffee brew was prepared and evaluated as described in 2.4.1. 98 volunteers were recruited from students and staff at the University of Nottingham to take part this study. This study was approved by the School of Bioscience Ethic Committee (SBREC160138A) at the University of Nottingham, a small incentives was provided to the participants.

2.5 Structure Analysis by X-Ray Computed Tomography

The microstructure of roasted Arabica coffee beans, roasted Robusta coffee beans and roasted and treated Robusta coffee beans (3 replicates each) was quantified using a Phoenix v|tome|x m 240 kV X-ray μCT system (GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany). Three replicate coffee beans were suspended in X-ray transparent polystyrene foam contained in a sample holder consisting of a plastic tube (dimensions 20 mm diameter x 50 mm height) attached to a plastic rod. The scan was conducted with an electron acceleration energy of 80 kV and a current of 120 μA. The distance between the X-ray source and the sample (FOD) and the X-ray source and the detector (FDD) was 40.93 mm and 818.69 mm, respectively, resulting in a geometric magnification of x19.9 and a spatial resolution of 10 μm. Each scan acquired 3598 project images over the 360° rotation, a detector exposure time of 250 ms, and no image averaging resulting in a total scan time of 10 min.

2D radiograph projections acquired during the scans were reconstructed into 3D volumes using DatosX|REC software (GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany) based on a filtered back-projection algorithm, and

then rendered, and exported as 16 bit grayscale Tiff images (XY axis) using VG StudioMAX v2.2 software (Volume Graphics GmbH, Germany).

To quantify the pores in the beans a multistage image analysis procedure was required to remove the surrounding background from the bean and the solid coffee bean matrix to leave only the pores. Firstly, image stacks were used to create a mask separating the beans from the background and any holding materials using segmentation tools in AvizoFire v6.0 (FEI, USA).

All images and masks were analysed using 'Image J' software version 1.49 (public domain Java analysis programme, developed by National Institute of Health, Maryland, US. http://imagej.nih.gov/ij) to obtain quantitative data of the coffee bean microstructure. Image stacks of the beans and the masks were imported and cropped to remove excess material from the images and reduce the file size of the data. The 'Original Bean' and 'Mask Bean' stacks were each threshold using the IsoData and minimum algorithms to classify the 'solid' material in each stack. The 'Image Calculator' function was then used to add the masked background to the threshold bean resulting in a new image stack containing only the pores of the coffee bean. The pores were then quantified using the 'Particle Analyser' function.

2.6 Statistical Analysis

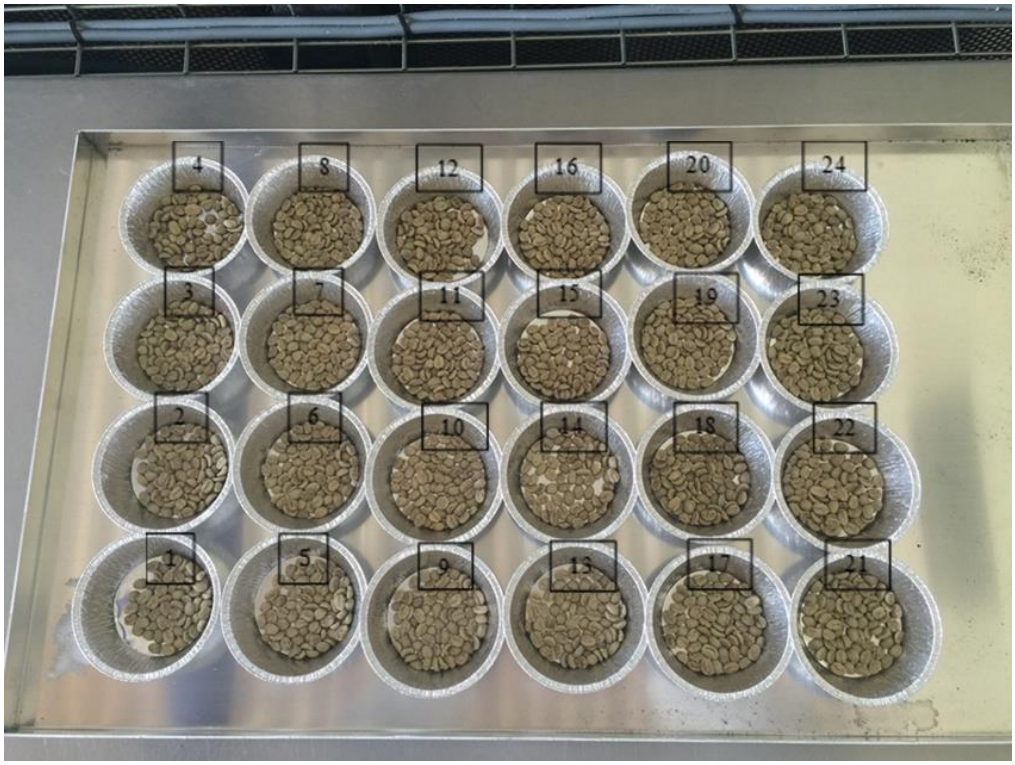
The relative abundance of each aroma compound was analysed by ANOVA to identify significant differences ($p < 0.05$) present for each compound between Arabica samples, non-treated Robusta samples and treated Robusta samples. All results were analysed by IBM® SPSS® Statistics version 21.0.0 and Microsoft excel XLSTAT 2013 using samples as the fixed effect in a Tukey's HSD post-hoc test. Results were also analysed by Design-Expert version 7.0.0 (Stat-Ease, Inc.,

Minneapolis, USA). All sensory data were collected and analysed using Compusense Cloud. Number of responses was compared to critical table in BS EN ISO 4120: 2007 (ISO4120:2007, 2007), α=0.05 was selected for difference testing, α=0.2, β=0.05, pD=30% were selected for similarity testing.

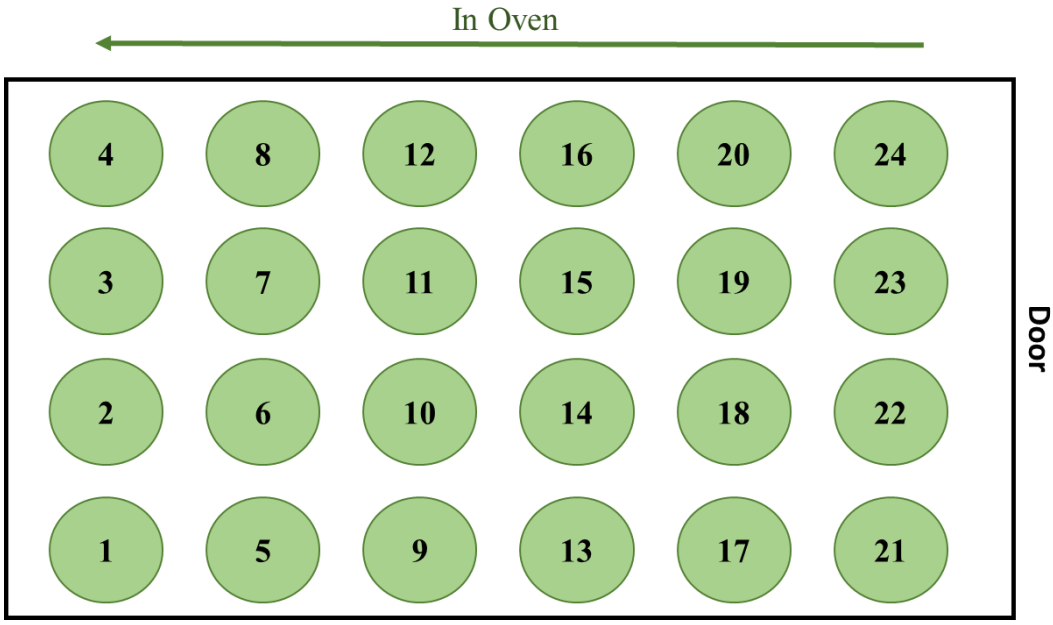
Chapter 3 Optimization of Roasting Conditions for Robusta Coffee

3.1 Preliminary Study on Roasting Variation

To evaluate the impact of roasting process on aroma generation within Robusta coffee, green beans were placed at different locations on a baking tray (Figure 3.1). The effect of potential variation on aroma generation and colour formation were evaluated. All the samples shown in the Figure 3.1 are single-origin washed Robusta green beans and from the same batch. These were roasted at 220 ºC for 15 min.



(a)



(b)

Figure 3.1 Locations of green coffee bean samples (1 - 24) on a baking tray (a) photo taken before roasting; (b) schematic diagram.

3.1.2 Results of Aroma Measurement

Fifty-seven volatile aroma compounds were identified within the coffee samples (Table 3.2), with an indication of the CAS number, Log P, molecular weight, linear retention index (LRI), and sensory description from the literature. Volatile compounds included 3 phenolic compounds, 6 furans, 3 organic acids, 9 heterocyclic compounds (N containing), 6 sulphur-containing compounds, 2 ethers, 4 aldehydes, 2 alcohols, 8 ketones and 13 pyrazines. In terms of chemical classes, the most represented groups were pyrazines, with 13 compounds. All the compounds have been previously reported in food materials and especially in coffee (please refer to the Table caption for the relevant literature).

Table 3.2 CAS number, Log P, molecular weight, LRI and odour description for 58 key aroma compounds (include internal standard) with different functional groups.

n.	Aroma compounds	CAS	Log P	Mol. WT	LRI	Literature LRI[b]	IM	Odour description [a]	Functional group
1	2-Methylfuran	000534-22-5	1.91	82.1	888	817-866	MS, L	Pungent, fruity	Furan
2	2-Methylbutanal	000096-17-3	1.23	86.13	930	903-984	MS, L	Malty	Aldehyde
3	2,5-Dimethylfuran	000625-86-5	2.46	96.13	975	938-951	MS, L, ST	Ethereal	Furan
4	2,3-Butanedione	000431-03-8	-1.34	86.09	1001	955	MS, L, ST	Buttery	Ketone
5	3-Hexanone	000589-38-8	1.24	100.16	1073	1046	MS, L	Fruity	Ketone
6	2,3,5-Trimethylfuran	010504-04-8	2.2	110.16	1076		MS	Ethereal	Furan
7	2,3-Pentanedione	000600-14-6	-0.85	100.12	1080	1053-1056	MS, L, ST	Oily buttery	Ketone
8	Dimethyl disulphide	000624-92-0	1.87	94.19	1097	1042-1054	MS, L	Onion	Sulphur
9	2-Vinylfuran	001487-18-9	2.26	94.11	1102	1064	MS, L	Ethereal, rum, cocoa note	Furan
10	Hexanal	000066-25-1	1.8	100.16	1106	1024-1087	MS, L, ST	Grassy, green oily	Aldehyde
11	2-Methylthiophene	000554-14-3	2.36	98.16	1113	1089-1095	MS, L, ST	Ash	Sulphur
12	3-Methylthiophene	000616-44-4	2.36	98.16	1144	1117	MS, L	Ash	Sulphur
13	3-Penten-2-one	000625-33-2	0.82	84.12	1153	1121-1137	MS, L, ST	Sweet, fruity pungent	Ketone
14	2,3-Hexanedione	003848-24-6	-0.35	114.15	1149	1125-1133	MS, L	cream	Ketone
15	1H-pyrrole,1-methyl	000096-54-8	1.43	81.12	1168	1123	MS, L	Smoky, woody, herbal	Heterocyclic N
16	IS_3-Heptanone	000106-35-4	1.73	114.19	1176	1162-1185	MS, L, ST	Fruit, green, fatty, sweet	Ketone
17	Pyridine	000110-86-1	0.8	79.1	1201	1183-1195	MS, L	Bitter, burnt	Heterocyclic N

18	1_ethylpyrrole	000617-92-5	1.92	95.15	1211		MS	Smoky, woody	Heterocyclic N
19	trimethyloxazole	020662-84-4	1.2	111.14	1220		MS	Ash	Sulphur
20	Pyrazine	000290-37-9	-0.06	80.09	1226	1192-1214	MS, L	Nutty	Pyrazine
21	Furfuryl methyl ether	013679-46-4	1.009	112.13	1227	1223	MS, L	Nutty, coffee grounds	Ether
22	Methylpyrazine	001333-41-1	1.35	93.13	1261	1260-1288	MS, L, ST	Nutty, roasted, chocolate	Pyrazine
23	Acetoin	000513-86-0	-0.36	88.11	1281	1265	MS, L	Sweet, buttery, creamy	Ketone
24	4-Methylthiazole	000693-95-8	1.54	99.15	1298	1282	MS, L	Ash	Sulphur
25	1-hydroxy,2-propanone	007339-87-9	1.19	136.15	1305	1294, 1308	MS, L	Sweet, caramellic	Ketone
26	2,5-Dimethylpyrazine	000123-32-0	1.03	108.14	1355	1316	MS, L, ST	Nutty, roasted, grassy	Pyrazine
27	2,6-Dimethylpyrazine	000108-50-9	1.03	108.14	1361	1319	MS, L	Nutty, sweet, fried	Pyrazine
28	Ethylpyrazine	013925-00-3	0.7	108.14	1368	1323-1325	MS, L	Nutty, roasted	Pyrazine
29	2,3-Dimethylpyrazine	005910-89-4	1.03	108.14	1381	1335	MS, L, ST	Nutty, roasted, green	Pyrazine
30	3-Ethylpyridine	000536-78-7	1.84	107.16	1395	1376-1397	MS, L, ST	Caramel, hazelnut	Heterocyclic N
31	2-Ethyl-6-methylpyrazine	013925-03-6	1.53	122.17	1418	1363-1381	MS, L	Roasted, hazelnut-like	Pyrazine
32	2-Ethyl-5-methylpyrazine	013360-64-0	1.53	122.17	1426	1383	MS, L, ST	Coffee-like	Pyrazine
33	Trimethylpyrazine	014667-55-1	1.58	122.17	1437	1387-1412	MS, L	Nutty, roasted	Pyrazine
34	Propylpyrazine	018138-03-9	1.47	122.17	1461		MS	Nutty	Pyrazine
35	Pyrazine,_2,6-diethyl-	013067-27-1	2.02	136.2	1470		MS	Nutty	Pyrazine
36	2,3-Dimethyl-6-ethylpyrazine	015707-34-3	1.5	136.19	1474	1449-1453	MS, L	Nutty	Pyrazine
37	2,5-Dimethyl-3-ethylpyrazine	013925-07-0	1.5	136.19	1482	1466-1469	MS, L	Earthy, roasted	Pyrazine

38	Acetic acid	000064-19-7	0.09	60.05	1484	1435-1459	MS, L, ST	Sour	Organic acid
39	Furfural	000098-01-1	0.83	96.09	1504	1447-1466	MS, L, ST	Bread, almond, sweet	Aldehyde
40	Acetol acetate	000592-20-1	-0.19	116.11	1512	1454	MS, L	Fruity, Buttery, Nutty	Acetate
41	Furfuryl methyl sulphide	001438-91-1	2	128.19	1524	1476-1480	MS, L	Coffee-like	Sulphur
42	2-Acetyfuran	001192-62-7	0.5	110.11	1546	1483-1542	MS, L	Balsamic-sweet	Furan
43	Pyrrole	000109-97-7	0.88	67.09	1553	1487-1504	MS, L, ST	Nutty, herbaceous	Heterocyclic N
44	2-Furanmethanol, acetate	000623-17-6	1.45	140.14	1568	1514	MS, L	Ethereal-floral	Acetate
45	Propanoic acid	000079-09-4	0.58	74.08	1569	1487-1574	MS, L	Pungent, acidic, cheesy	Organic acid
46	5-Methylfurfural	000620-02-0	1.38	110.11	1619	1551-1616	MS, L, ST	Spice, caramel, maple	Aldehyde
47	2,5-Dimethylpyrrole	000625-84-3	1.97	95.15	1631		MS, ST	Smoky	Heterocyclic N
48	2-Furfurylfuran	001197-40-6	2.99	148.16	1647	1586	MS, L	Caramellic, earthy	Furan
49	1_methyl_2_formylpyrrole	001192-58-1	1.14	109.13	1664	1610-1626	MS, L	Roasted, nutty	Heterocyclic N
50	Ethanone,_1-(1-methyl-1H-pyrrol-2-yl)-	000932-16-1	1.11	123.16	1675	1606	MS, L	Smoky, spicy	Heterocyclic N
51	2-Furanmethanol	000098-00-0	0.45	98.1	1690	1573-1682	MS, L, ST	Caramellic, burnt, smoky	Alcohol
52	3-Methylbutanoic acid	000503-74-2	1.49	102.13	1699	1670-1683	MS, L	Cheesy, fermented	Organic acid
53	Guaiacol	000090-05-1	1.34	124.14	1886	1850-1859	MS, L, ST	Phenolic, burnt, smoky	Phenolic
54	Maltol	000118-71-8	-0.19	126.11	2004	1968-2030	MS, L, ST	Caramel	Alcohol
55	2-Acetylpyrrole	001072-83-9	0.56	109.13	2029	1952-2001	MS, L	Earthy, roasted	Heterocyclic N

56	Phenol	000108-95-2	1.51	94.11	2054	1996-2051	MS, L, ST	Smoky	Phenolic
57	4-Ethylguaiacol	002785-89-9	2.38	152.19	2086	2020-2036	MS, L, ST	Smoky, spicy	Phenolic
58	4-Vinylguaiacol	007786-61-0	2.4	150.17	2262	2151-2210	MS, L	Smoky	Phenolic

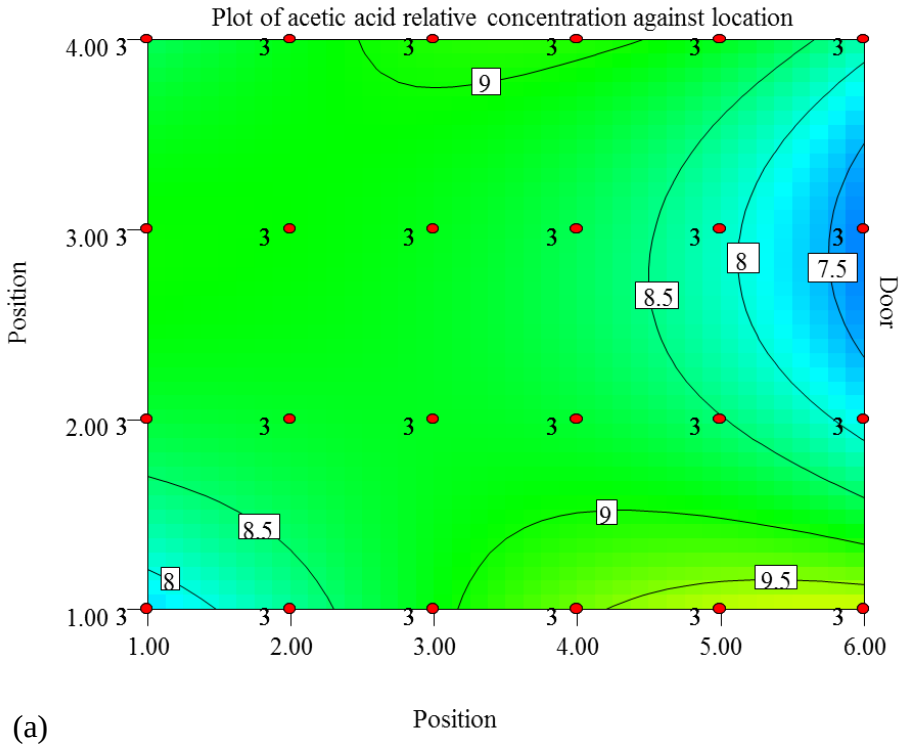
[a & b] *Quoted from (Akiyama, Murakami, Ikeda, Iwatsuki, Wada, Tokuno, et al., 2007; Boothroyd, Linforth, Jack, & Cook, 2014; Caporaso, Whitworth, Cui, & Fisk, 2018; Flament, 2002; Maeztu, Sanz, Andueza, Paz De Peña, Bello, & Cid, 2001; Piccino, Boulanger, Descroix, & Shum Cheong Sing, 2014; Robert; Semmelroch & Grosch, 1996) IM = identification method (MS = experimental mass spectra compared to database, or L = literature linear retention index; ST = standard compound, i.e. pure reference compound).*

Fifty-seven volatile compounds were found across the 24 coffee samples, there were 14 volatile compounds (4-ethylguaiacol, phenol, 4-vinylguaiacol, 2,5-dimethylfuran, dimethyl disulphide, 1H-pyrrole,1-methyl, pyrrole, 2-vinylfuran, 2-methylfuran, 3-methylpyridine, pyridine, 3-ethylpyridine, 2-furfurylfuran, and acetic acid,) which showed significant differences across sample location following ANOVA testing ($p < 0.05$). Samples located at 4, 8, 17 and 21 had significantly higher relative concentrations of dimethyl disulphide (7%), 5 heterocyclic N containing compounds (3-methylpyridine (16%), pyridine (12%), 3-ethylpyridine (11%), 1-methyl 1H-pyrrole (7%), pyrrole (15%)), 3 phenolic compounds (4-ethylguaiacol (11%), phenol (9%), 4-vinylguaiacol (12%)) and 2 furans (2-vinylfuran (8%), 2-furfurylfuran (11%)) when compared with the other coffee samples.

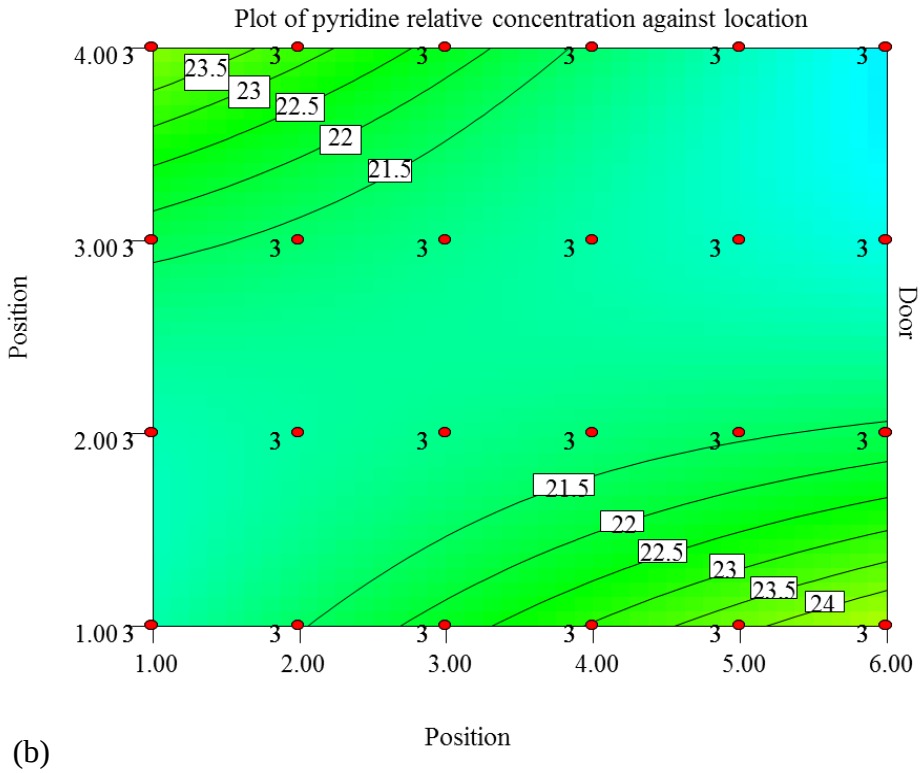
There was a significant decrease (6% - 13%) in relative concentration of acetic acid appearing in samples 1, 5, 19, 22, 23 and 24 which were at the edge of the tray (ANOVA, $p < 0.05$). Results for acetic acid relative concentration were plotted against sample location of on the tray (Figure 3.3(a)); the red dot indicates where the coffee samples was located. Symbol “3” on the plot which is near the red dot indicates that three replicates were evaluated in this experiment. Samples located at position 13, 17 and 21 indicated a significant higher (5% - 12%) in acetic acid content compare with other samples. There was no significant difference between the rests of the coffee samples. The results of volatile compounds analysis showed that samples roasted at the edge of the tray were significantly different with the samples roasted elsewhere on the tray.

Results for pyridine relative concentration were plotted against sample location on the tray in Figure 3.3 (b). There was a significant increase (9% - 12%) in relative

concentration of pyridine appearing in samples 3, 4, 8, 13, 17 and 21 which were at the edge of the tray (ANOVA, $p < 0.05$). Other aroma compounds showed the same trend as pyridine, correlation matrix from the Pearson distribution indicated a high correlation (≥ 0.72) between pyridine and the other 12 volatile compounds above (excluded acetic acid). Only acetic acid and pyridine plot has been presented which can give a visual impact on roasting variation. Coffee beans at the edge of the baking tray had different aroma profiles when compared with aroma profiles for beans roasted elsewhere on the tray according to ANOVA ($P < 0.05$). To get a consistent aroma profile, samples position in 1, 3, 4, 5, 8, 13, 17, 19, 21, 22, 23 and 24 were therefore discard in future experiments.



(a)



(b)

Figure 3.2 (a) Plot showing the acetic acid relative concentration (relative to the internal standard) across the sample location of the tray in the middle layer of the fan oven (same orientation as Figure 3.1); (b) Plot showing the pyridine relative concentration (relative to the internal standard) across the sample location of the tray in the middle layer of the fan oven (same orientation as Figure 3.1).

3.1.3 Results of Colour Measurement

Colour measurement (L, a, b) against the location of the tray (horizontal and vertical) was determined and plotted (Figure 3.4). Analysis using ANOVA revealed that horizontal placement on the tray had a significant impact on L ($p < 0.05$); while vertical position had a significantly effect on colour a and b. From the colour parameters, coffee samples roasted at the edge of the tray showed a significant difference when compared with the rest of the coffee samples. Therefore, the samples placed at the edge should be discarded from subsequent evaluation.

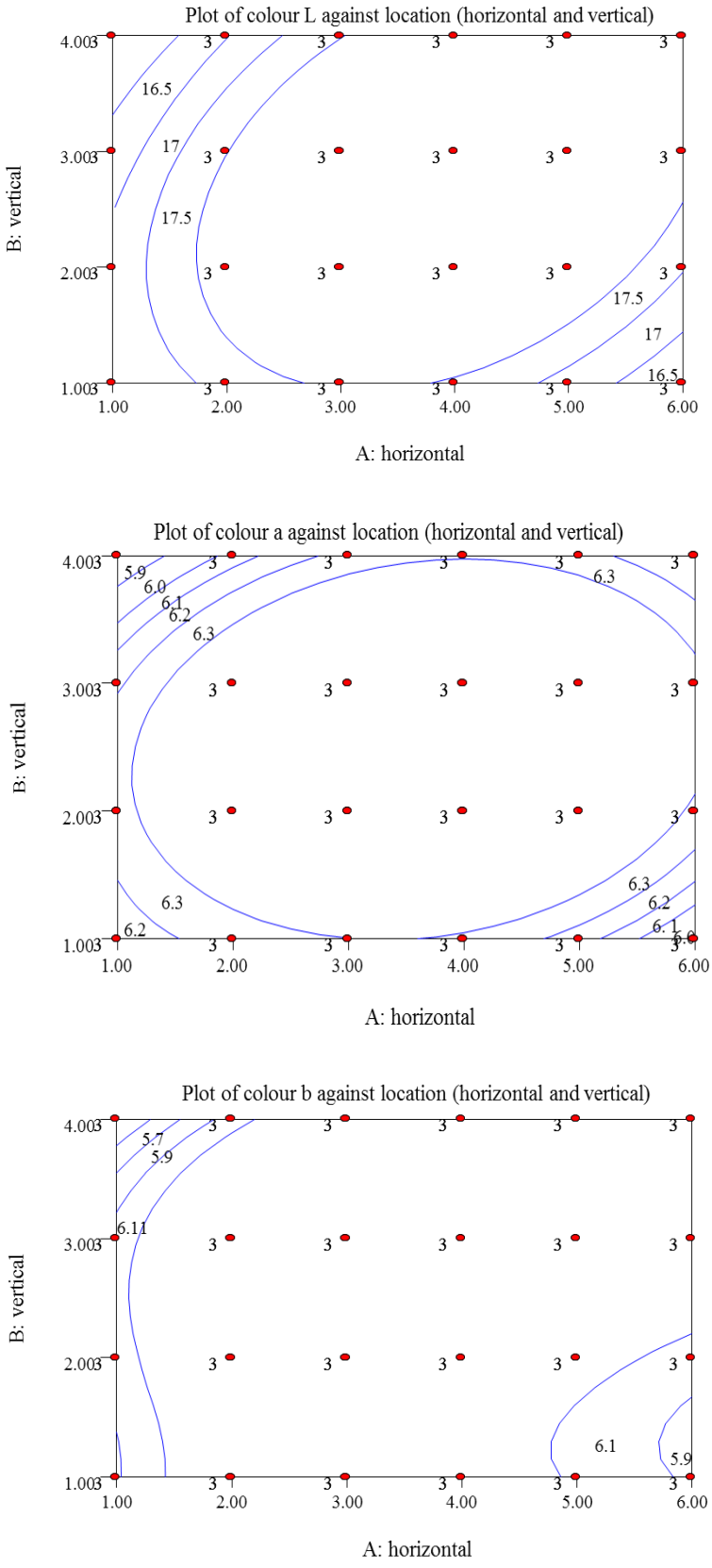


Figure 3.3 Plot of colour measurement (L, a, b) and location of coffee beans on the tray (horizontal and vertical).

To determine a region for optimal roasting with consistent colour and aroma, the contour maps of the 5 responses in a single contour plot (overlay plot) are superimposed on Figure 3.5. The optimum region obtained by superimposing the 5 contour maps is depicted in the middle region of Figure 3.5. This optimum region provides the coordinates of possible optimal levels of roasting consistency, therefore the only sample positions used for future analysis were the middle region (Figure 3.5), all edge samples were discarded.

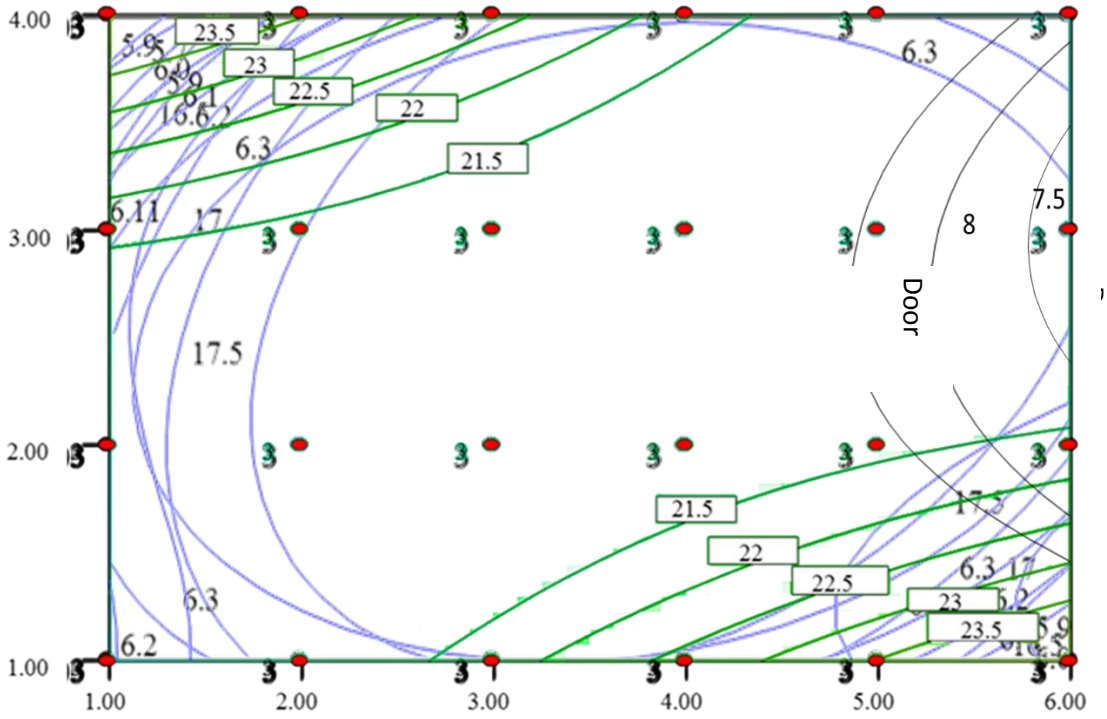


Figure 3.4 Superimposed contour plots of colour (L, a, and b), pyridine and acetic acid relative concentration of roasted coffee.

3.2 Difference between Arabica and Robusta

As mentioned earlier in chapter 1, the two coffee varieties, Arabica and Robusta, are known to differ in their organoleptic properties. Arabica is regarded to have smooth, mild, and rich flavour, while, Robusta possessed a flatter flavour, lacking in taste, and a muddy odour (Farah, 2012). An indispensable part in this thesis is to

identify the difference between Arabica and Robusta on both green bean precursors and roasted coffee volatile compounds.

3.2.1 Analysis of Green Bean Flavour Precursors in both Arabica and Robusta Coffee

Table 3.6 summarises the contents of coffee aroma precursors in the green coffee for both Arabica and Robusta. Highlighted numbers were either significantly higher in Arabica or Robusta green beans (t-test, $p < 0.05$). Robusta green beans showed higher protein, chlorogenic acid, and caffeine content, while Arabica was higher in trigonelline, sucrose and lipid content, which is concordant with Farah's 2012 and Illy & Viani's 2005 results in Table 1.2.

Table 3.6 Precursors of coffee aroma compounds found in green coffee beans

Precursor compounds	Arabica	Robusta
Protein	6.25% ± 0.12%	8.70% ± 0.29%
Chlorogenic acid	2.30% ± 0.14%	4.50% ± 0.04%
Trigonelline	1.21% ± 0.15%	0.90% ± 0.21%
Caffeine	1.45% ± 0.09%	3% ± 0.02%
Sucrose	6.20% ± 0.62%	3.20% ± 0.30%
Lipids	12.90% ± 1.0%	6.60% ± 0.53%

Mean ± Standard deviation

3.2.2 Results of Volatile Compounds on both Arabica and Robusta Coffee

Both Arabica and Robusta green beans were roasted to light, medium and dark roast. Light roast means the beans that has not been roasted beyond the first crack. Medium roast is between the end of the first crack and just before the beginning of the second crack. Dark roast is the bean roasted to the middle of the second crack (Baggenstoss, Poisson, Kaegi, Perren, & Escher, 2008; Illy & Viani, 2005; Kim, Ko, Kang, & Park, 2018).

The relative percentage (GC peak area (%) of the total peak area) of volatile compounds in the samples are shown in Table 3.7, and Principal component analysis results are shown in Figure 3.8. The two coffee species have distinctive aroma profiles mostly due to predominance of pyrazines, phenolic compounds and sulphur-containing compounds in Robusta and of furans, ketones and acids in Arabica (Table 3.7).

Pyrazines provide the nutty, baked, roasted and hazelnut or coffee like odour to coffee; out of all volatile compounds present pyrazines make up14.8% to 41.8%, which agreed with previous research done by Flament, 2002. Arabica coffee sample showed the lowest total pyrazine percentage when compare with Robusta samples across all roasting levels ($p < 0.001$) (Table 3.7).

The relative percentage for the total nitrogen containing heterocycle compounds is between 7.4% to 26.5% of the overall volatile content in both Arabica and Robusta coffee samples across all roasting levels. Light roasted Arabica sample showed the lowest total percentage of nitrogen containing heterocycle compounds when compared with Robusta samples ($p < 0.05$). The total nitrogen containing heterocycle compounds significantly increased (7.4% to 26.5%) with an increase in the roasting intensity ($p \leq 0.001$) in both Arabica and Robusta.

The total furans percentage was significantly higher in the Arabica sample compared to Robusta samples at all roasting levels, meanwhile total furans significantly increased with an increase in the roasting intensity in both Arabica and Robusta samples ($p \leq 0.001$). The Robusta coffee sample showed the lowest total aldehydes percentage (9.6%) in dark roasted Robusta sample when compared with the dark roasted Arabica (12.1%) ($p < 0.05$).

The results in Table 3.7 showed the percentage of each volatile compounds can be affected by the different coffee varieties and roasting intensity. The proportion of furans, sulphur-containing compounds, pyrazines, phenolic compounds and nitrogen-containing heterocycle compounds in the coffee samples can be increased by increasing roasting intensity. Whereas the proportion of compounds like aldehydes, ketones and acids in the volatile profile decreased with increasing roasting intensity.

Table 3.7 Relative percentage of the volatile compounds in both Arabica and Robusta roasted coffee beans (roasting intensity light, medium and dark with four replicates each). Each volatile compound was expressed as relative percentage of the GC peak area (%) of the total peak area for each coffee.

Compounds	Arabica Light	Robusta Light	Arabica Medium	Robusta Medium	Arabica Dark	Robusta Dark
2-Methylfuran	6.45	3.54	11.81	7.75	14.27	11.74
2,3,5-Trimethylfuran	0.04	0.02	0.09	0.06	0.11	0.08
2-Vinylfuran	0.71	0.32	0.81	0.47	0.72	0.45
2,5-Dimethylfuran	0.42	0.17	1.04	0.59	1.26	0.82
2-Furfurylfuran	0.02	0.01	0.05	0.03	0.06	0.05
2-Acetyfuran	0.34	0.08	0.38	0.18	0.22	0.13
Total Furans	**7.97**	**4.14**	**14.19**	**9.09**	**16.65**	**13.26**
Hexanal	0.12	0.71	0.05	0.26	0.05	0.18
2-Methylbutanal	8.86	11.27	6.73	9.13	6.26	8.40
5-Methylfurfural	3.28	0.89	1.67	0.89	0.71	0.41
Furfural	2.12	3.16	3.61	1.45	4.85	0.57
Total Aldehydes	**14.38**	**16.03**	**12.05**	**11.73**	**11.87**	**9.56**
3-Hexanone	0.53	1.33	0.39	0.56	0.37	0.52
2,3-Butanedione	2.87	1.35	1.87	1.31	1.59	1.23
2,3-Hexanedione	0.33	0.13	0.43	0.23	0.34	0.19
1-hydroxy,2-propanone	2.36	0.92	0.55	0.35	0.27	0.16
2,3-Pentanedione	11.56	10.70	9.11	6.23	12.63	3.61
3-Penten-2-one	0.21	0.08	0.27	0.13	0.20	0.08
Total Ketones	**17.86**	**14.51**	**12.62**	**8.81**	**15.39**	**5.79**
Dimethyl disufide	0.02	0.03	0.01	0.03	0.01	0.03
Furfuryl methyl sulfide	0.03	0.01	0.16	0.11	0.22	0.19
4-Methylthiazole	0.07	0.14	0.07	0.23	0.07	0.23
trimethyloxazole	0.03	0.08	0.06	0.12	0.07	0.14
2-Methylthiophene	0.08	0.10	0.14	0.24	0.17	0.28
3-Methylthiophene	0.00	0.01	0.01	0.02	0.01	0.02
Total Sulphur-containing compounds	**0.24**	**0.37**	**0.45**	**0.73**	**0.56**	**0.90**
1H-pyrrole,1-methyl	0.82	2.46	4.99	2.41	1.65	2.35
Pyrrole	0.37	2.41	0.62	1.62	0.66	1.27
2,5-Dimethylpyrrole	0.01	0.04	0.04	0.06	0.04	0.06
2-Acetylpyrrole	0.04	0.04	0.05	0.06	0.06	0.06
1_methyl_2_formylpyrrole	0.20	0.21	0.31	0.31	0.26	0.28
Ethanone,_1-(1-methyl-1H-pyrrol-2-yl)-	0.02	0.02	0.05	0.05	0.05	0.06
1_ethylpyrrole	0.05	1	0.12	0.26	0.16	0.29
3-Ethylpyridine	0.01	0.02	0.05	0.05	0.07	0.07
Pyridine	5.86	6.33	18.90	17.73	23.41	22.05
Total Heterocyclic N-containing compounds	**7.38**	**11.73**	**25.12**	**22.55**	**26.36**	**26.48**

Pyrazine	1.80	4.24	1.58	4.27	1.55	3.92
Methylpyrazine	8.97	18.33	7.30	15.31	7.01	16.17
2,5-Dimethylpyrazine	2.00	4.75	1.40	3.42	1.29	3.31
2,6-Dimethylpyrazine	2.50	5.64	2.04	4.90	1.93	4.83
Ethylpyrazine	1.16	3.07	1.00	2.81	0.95	2.65
2,3-Dimethylpyrazine	0.34	0.91	0.33	0.96	0.36	1.03
2-Ethyl-6-methylpyrazine	0.53	1.79	0.46	1.51	0.41	1.46
2-Ethyl-5-methylpyrazine	0.34	1.23	0.26	0.90	0.24	0.87
Pyrazine,_2,6-diethyl-	0.04	0.19	0.07	0.17	0.07	0.16
Trimethylpyrazine	0.29	1.07	0.24	0.89	0.23	0.88
Propylpyrazine	0.00	0.01	0.00	0.01	0.00	0.01
2,3-Dimethyl-6-ethylpyrazine	0.09	0.44	0.07	0.33	0.06	0.41
2,5-Dimethyl-3-ethylpyrazine	0.03	0.14	0.03	0.13	0.03	0.12
Total Pyrazines	**18.09**	**41.82**	**14.78**	**35.61**	**14.14**	**35.83**
Acetic acid	23.13	6.57	8.24	3.54	4.53	1.56
Propanoic acid	0.80	0.12	0.55	0.13	0.34	0.08
3-Methylbutanoic acid	0.11	0.15	0.05	0.07	0.03	0.04
Total Acids	**24.04**	**6.83**	**8.85**	**3.74**	**4.91**	**1.68**
Guaiacol	0.02	0.03	0.04	0.09	0.06	0.11
Phenol	0.02	0.02	0.05	0.05	0.07	0.07
4-Ethylguaiacol	0.00	0.00	0.00	0.01	0.00	0.01
4-Vinylguaiacol	0.02	0.05	0.01	0.04	0.01	0.03
Total Phenolic compounds	**0.05**	**0.11**	**0.10**	**0.19**	**0.14**	**0.23**
2-Furanmethanol, acetate	1.41	0.50	3.09	1.79	3.33	2.03
Acetol acetate	0.48	0.20	0.77	0.40	0.53	0.25
2-Furanmethanol	7.70	3.64	7.41	5.11	5.54	3.69
Maltol	0.03	0.02	0.07	0.03	0.08	0.03
Acetoin	0.19	0.06	0.12	0.07	0.07	0.04
Furfuryl methyl ether	0.17	0.04	0.38	0.16	0.42	0.23

The two principal components issued from principal component analysis (PCA) on the data matrix explain up to 88% of the total variance, being 46.2% explained by PC1 and 41.8% by PC2 (Figure 3.8). PCA clearly shows that the two species are fully separated by PC1, with Arabica on the left and Robusta on the right. Roasting intensity can clearly be separated by PC2 (from dark roast to light roast). Robusta coffee had more pyrazines and hexanal compared with Arabica coffee. Unlike Robusta, Arabica coffee tend to have more furans, acids, and ketones, which agreed with the previous studies (Blank, Sen, & Grosch, 1991). Although, both light roasted Arabica and Robusta samples indicated a relatively higher level in acids, ketones and aldehydes, the rest of volatile compounds indicated lower level in both light roasted samples.

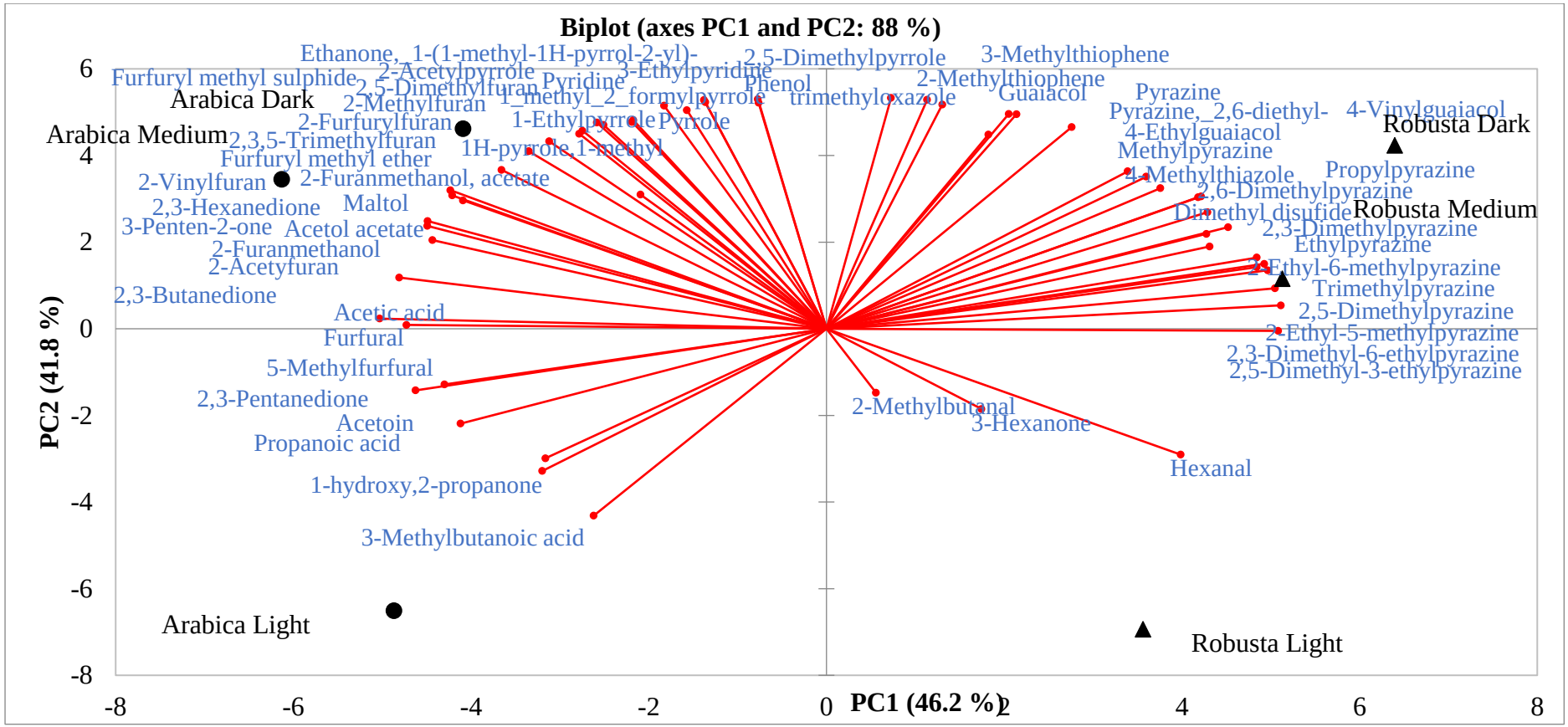


Figure .3.8 The biplot of PC1 and PC2 of aroma profile showing 88% variation among 6 coffee samples which include both Arabica (●) and Robusta (▲) coffee roasted at light, medium and dark level.

3.3 Effect of Roasting Condition

During coffee roasting a complex array of chemical reactions occurred, the products of which are responsible for generation of the much appreciated characteristic coffee aroma. Coffee aroma formation is dependent on many factors during roasting process, including the types of heat transfer such as conduction, radiation and convection (Fadai, Melrose, Please, Schulman, & Van Gorder, 2017), air flow (Kwak, Ji, & Jeong, 2017), roasting time and temperature (Madihah, Zaibunnisa, Norashikin, Rozita, & Misnawi, 2012). The unique aroma profiles of coffee are very closely related to the time-temperature profile used during the roasting process (Baggenstoss, Poisson, Kaegi, Perren, & Escher, 2008). Previous studies have attempted to modify the coffee roasting time and temperature with the aim to maximize aroma formation in roasted coffee (Franca, Oliveira, Oliveira, Agresti, & Augusti, 2009; Madihah, Zaibunnisa, Norashikin, Rozita, & Misnawi, 2012).

In general, different time-temperature profiles affect the reaction rate of dehydration and the chemical reactions that occur inside of the beans, which control gas formation, browning and flavour development (Flament, 2002). Depending on the extent of heat treatment, coffee can be crudely categorized as light, medium or dark roast (Lyman, Donald, Benck, Dell, Merle, & Murray, 2003). Light roast process tend to give non-uniform bean colour with sour, grassy, and underdeveloped flavour, while medium roast process produces a balanced taste and aroma with citrus flavour (Lyman, Donald, Benck, Dell, Merle, & Murray, 2003). In contrast, dark roast process produces coffee of low acidity and burnt notes sensory profiles (Lyman, Donald, Benck, Dell, Merle, & Murray, 2003). Which agreed with our results explained in 3.2.2. Researchers have reported the effects of time-temperature profile on coffee aroma properties (Schenker, Heinemann, Huber,

Pompizzi, Perren, & Escher, 2002). In general, high-temperature-short-time roasting produced less degradation of chlorogenic acid, more soluble solids, less burnt flavour, lower loss of volatiles, higher CO_2 desorption, and oil migration, as compared to low-temperature-long-time roasting process (Schenker, 2000). A consequence of this is that low-temperature-long-time roast processes often result in sour, grassy, woody, and underdeveloped flavour properties (Yang, Liu, Liu, Degn, Munchow, & Fisk, 2016). In comparison, high-temperature-short-time produced the higher quality coffee, more aroma volatiles and higher brew yield (Lyman, Donald, Benck, Dell, Merle, & Murray, 2003; Dell, Merle, & Murray, 2003).

According to the previous work described in 3.2.2, it is necessary to look deeper into the effect of different roasting time-temperature profiles on aroma generation within Robusta coffee. The overarching objective of this part was to roast Robusta coffee to minimise the differences between Arabica and Robusta. The basic physicochemical properties and volatile aroma compounds present in green coffee roasted to different time-temperature profile are evaluated and shown in 3.3.3.

3.3.1 Modelling of Robusta Coffee Aroma Formation at Different Roasting Time Temperature Profile

Building on the optimisation of the roasting process, the first step was to attempt to match the Robusta coffee aroma with Arabica coffee aroma by roasting Robusta coffee beans using different time–temperature settings. A D-optimal design was chosen as a tool for multifactor response surface analysis to minimise the number of experiments while allowing simultaneous assessments of variations of all the experimented factors studied (Aguiar, Bourguignon, Khots, Massart, & Phan-Than-Luu, 1995). The arrangement of D-optimal design for independent variables and the adequacy of the model was determined by using model analysis, lack of fit test and coefficient of determination (R^2). All responses were used based on quadratic model with backward alpha of 0.05.

3.3.2 Results of D-Optimal Design

Typical roasting conditions using traditional industrial drum roasters vary from 160 to 250 ºC for a period of 7 - 20 min (Clarke, 2012; Folmer, 2016). The roasting time-temperature profiles used in this experiment are shown in Table 3.9. The effect of the two independent variables, A = Time (7 - 20 min) B = Temperature (135 - 250 ºC) on 57 volatile compounds is shown in Table 3.10. The levels (values) of each parameter are automatically selected and resulted in 15 treatments.

Table 3.9 Roasting conditions for 15 roasting profiles used in the experiment for Robusta beans.

No.	Roasting temperature (ºC)	Roasting time (min)
1	135	7
2	135	13.5
3	135	20
4	164	10.3
5	164	16.8
6	173	7
7	173	20
8	193	13.5
9	212	7
10	212	20
11	222	10.3
12	222	16.8
13	250	7
14	250	13.5
15	250	20

3.3.3 Impact on Aroma Generation within Different Roasting Time and Temperature

Table 3.10 summarizes the significant difference across different roasting time-temperature profiles, R^2, and types of model graph for 57 volatile compounds found in all roasted and ground coffee samples. 19 volatile compounds (include 1 phenolic compound, 2 aldehydes, 5 ketones, 1 furan, 1 ether, 1 acetate, 1 sulphur-containing compound, 2 pyrazines, 4 pyrroles and 1 acid) showed a significant increase in their relative concentration with increase roasting temperature. There were no significant difference between roasting time and relative concentration for these volatile compounds. Other 38 volatile compounds indicated a significant difference in both roasting time and temperature ($p < 0.05$).

Table 3.10 Summary of significant difference across different time and temperature roasting profiles, R^2, and types of model graph (polynomial degree of the model) for 57 volatile compounds found in all roasted and ground coffee samples.

Aroma compounds	Sig Time	Sig Temperature	R^2	Model Graph Types
2-Methylbutanal	n	y	0.7822	-
2,3-Butanedione	n	y	0.7925	-
2,3-Pentanedione	n	y	0.8569	-
3-Penten-2-one	n	y	0.7637	-
2,3-Hexanedione	n	y	0.7201	-
2-Vinylfuran	n	y	0.8687	-
Furfuryl methyl ether	n	y	0.8106	-
2-Furanmethanol, acetate	n	y	0.4824	-
Dimethyl disufide	n	y	0.8647	-
Pyrazine	n	y	0.8044	-
2,3-Dimethylpyrazine	n	y	0.8644	-
Pyrrole	n	y	0.9134	-
1_methyl_2_formylpyrrole	n	y	0.5863	-
Ethanone,_1-(1-methyl-1H-pyrrol-2-yl)-	n	y	0.5967	-
2-Acetylpyrrole	n	y	0.7516	-
Acetoin	n	y	0.7341	-
Propanoic acid	n	y	0.6814	-
4-Vinylguaiacol	y	y	0.8695	-
Hexanal	y	y	0.8522	-
2,5-Dimethylfuran	y	y	0.9446	1
2-Acetyfuran	y	y	0.8348	1
2,3,5-Trimethylfuran	y	y	0.706	1
2-Furfurylfuran	y	y	0.7997	1
Furfuryl methyl sulfide	y	y	0.8106	1
2-Methylthiophene	y	y	0.9375	1
3-Methylthiophene	y	y	0.9467	1
trimethyloxazole	y	y	0.8774	1
4-Methylthiazole	y	y	0.8287	1
1H-pyrrole,1-methyl	y	y	0.9096	1
1_ethylpyrrole	y	y	0.9468	1
2,5-Dimethylpyrrole	y	y	0.92	1
Pyridine	y	y	0.9444	1
3-Ethylpyridine	y	y	0.9392	1
Maltol	y	y	0.6048	1
Guaiacol	y	y	0.9279	1
Phenol	y	y	0.9481	1
4-Ethylguaiacol	y	y	0.9241	1

Propylpyrazine	y	y	0.881	2
Methylpyrazine	y	y	0.7982	2
2,5-Dimethylpyrazine	y	y	0.8099	2
2,6-Dimethylpyrazine	y	y	0.7788	2
Ethylpyrazine	y	y	0.7956	2
2-Ethyl-6-methylpyrazine	y	y	0.8303	2
2-Ethyl-5-methylpyrazine	y	y	0.7888	2
Trimethylpyrazine	y	y	0.8331	2
Pyrazine,_2,6-diethyl-	y	y	0.7902	2
2,3-Dimethyl-6-ethylpyrazine	y	y	0.8714	2
2,5-Dimethyl-3-ethylpyrazine	y	y	0.8405	2
3-Hexanone	y	y	0.7005	2
1-hydroxy,2-propanone	y	y	0.7364	2
Furfural	y	y	0.6841	2
5-Methylfurfural	y	y	0.6116	2
2-Furanmethanol	y	y	0.6282	2
Acetic acid	y	y	0.7932	2
3-Methylbutanoic acid	y	y	0.771	2
Acetol acetate	y	y	0.6889	2

To determine the effect of relative concentration and roasting time temperature, different types of model graph are shown below. Due to some aroma compounds expressed the same trend, two types of model graph have been presented which can give a visual impression of roasting time-temperature profiles.

Figure 3.11 (a) shows the plot of pyridine relative concentration against roasting time temperature, which presented as type 1 model graph. Samples roasted at 250 ºC for 20 min has the highest pyridine content. There was only limited amount of pyridine generated in the samples roasted at temperatures below 190 ºC. High temperature was needed to initiate reactions leading to pyridine, and once formation began, pyridine concentration continuously increase during roasting, which is in accordance with the work done by Baggenstoss et al. (2008).

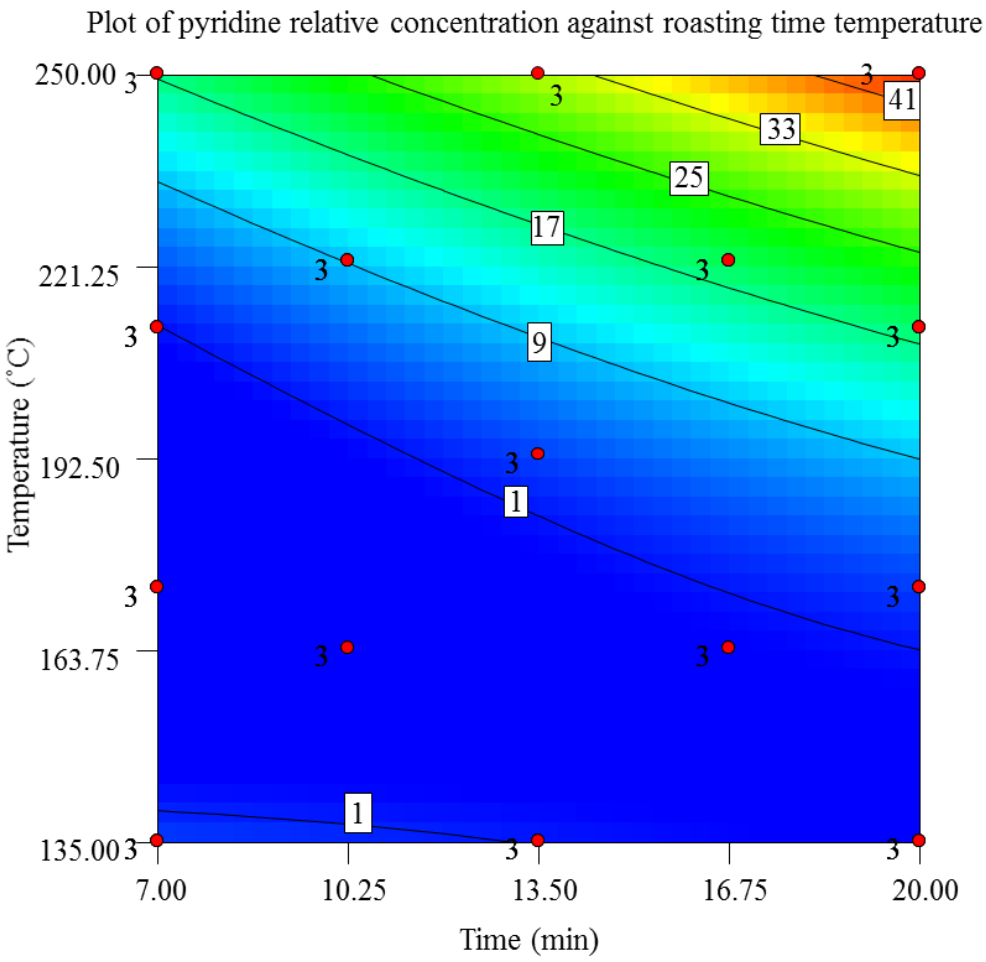


Figure 3.11 (a) Plot of pyridine relative concentration (relative to the internal standard) against time and temperature, presented as type 1 model graph

There were 19 volatile compounds (include 5 furans, 5 sulphur-containing compounds, 1 alcohol, 3 phenolic compounds and 5 Heterocyclic N-containing compounds in Table 3.10) showed the same trend with type 1 model graph (Figure 3.11 (a)). In this plot, the relative concentration increased with increasing time and temperature. Volatile compounds which showed the same trend as type 1 model graph obviously required high activation energy since formation started late in the roasting process.

The relative concentration of furan compounds increased continuously during the roasting process depending on temperature and time, and no decrease was observed,

which is in agreement with results from other authors (Gloess, Vietri, Wieland, Smrke, Schönbächler, López, et al., 2014; Heyns, Stute, & Paulsen, 1966; Mayer, Czerny, & Grosch, 1999; Schenker, 2000). Phenolic and Heterocycles nitrogen containing compounds are typical roasting products, resulting from the thermal decomposition of ferulic acid (guaiacols) and trigonelline degradation (pyridine, N-methylpyrrole). Evolution of phenolic compounds during roasting were highly dependent on time and temperature. In the endothermic roasting stage, formation of 4-vinylguaiacol is predominant because of low activation energy. Once exothermic roasting conditions are attained, 4-vinylguaiacol degrades yielding guaiacol, which then degrades to phenol (Gloess, Vietri, Wieland, Smrke, Schönbächler, López, et al., 2014).

Figure 3.11 (b) showed the plot of acetic acid relative concentration against roasting time temperature, which presented as type 2 model graph. There was only limited amount of acetic acid generated in the coffee roasting temperature below 160 ºC for around 15 min. Acetic acid increased significantly during the first stage of roasting with relative low temperature and time followed by decreasing concentration towards a high degrees of roast. Similar observations were made by Clarke (2001). Acetic acid contents in the medium and dark roast coffee consisted of 1.58% and 0.71% respectively (Clarke & Vitzthum, 2001).

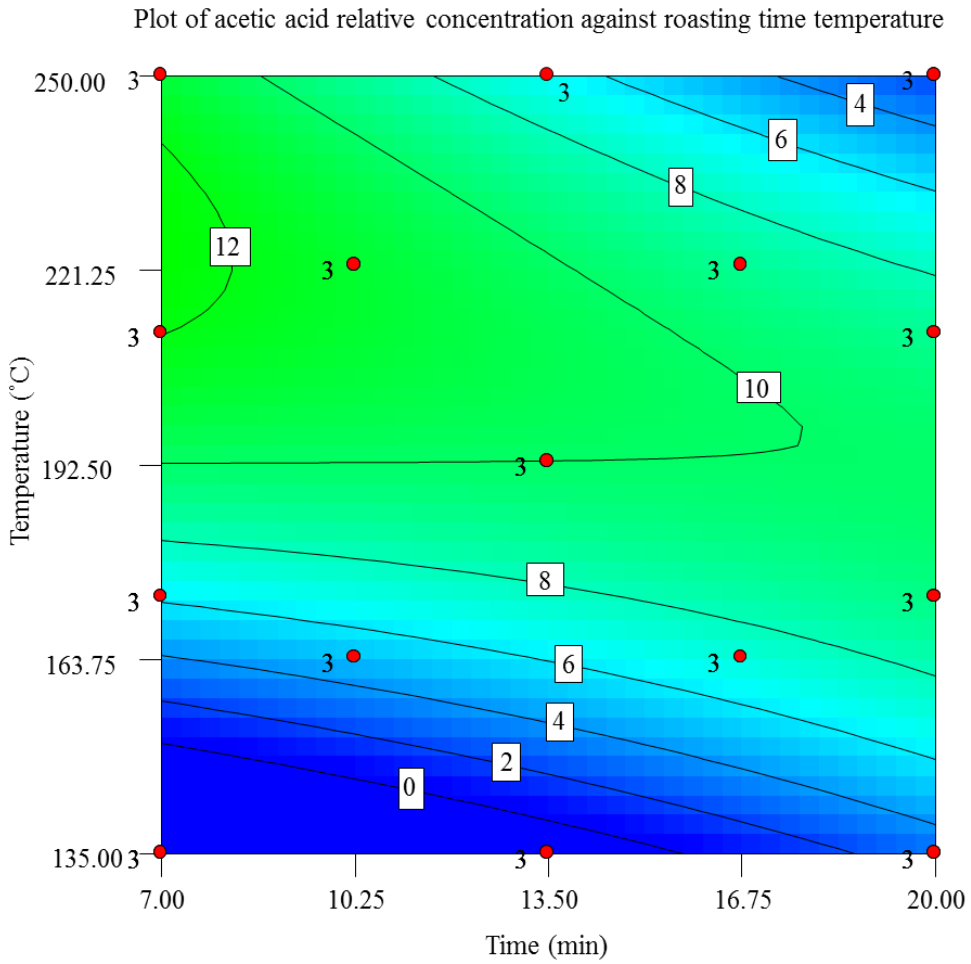


Figure 3.11 (b) Plot of acetic acid relative concentration (relative to the internal standard) against time and temperature, presented as type 2 model graph

Most pyrazines, aldehydes, ketones and acids had a similar behaviour to acetic acid. Pyrazines have been identified as the Maillard reaction products, which are highly dependent on the roasting time temperature. Previously studies has been reported that pyrazine formation increased highly in the early stage of roasting and degraded in the later stage of roasting (over-roasted) (Baggenstoss, Poisson, Kaegi, Perren, & Escher, 2008; Gretsch, Sarrazin, & Liardon, 2000). Formation of the Strecker aldehydes and ketones in the beginning of the roasting process seemed to be slightly favoured by high temperature, however the relative concentration decrease was observed when over roasted (Baggenstoss, Poisson, Kaegi, Perren, & Escher, 2008).

3.3.4 Results of Optimisation Solutions

The optimisation procedure was performed to obtain the optimal levels of two factors (A: time and B: temperature). In optimizing the roasting conditions, the goals for the concentration of the target aroma compounds were set as either medium roasted or dark roasted Arabica coffee. The predicted values of all selected marker compounds were calculated using the regression model and compared with the actual value. Six solutions were generated from the D-optimal design (Table 3.12).

Table 3.12 Optimized roasting conditions for Robusta using both dark roasted Arabica and medium roasted Arabica as references.

	Reference 1 Dark roasted Arabica		Reference 2 Medium roasted Arabica	
No.	Temperature (ºC)	Time (min)	Temperature (ºC)	Time (min)
1	228	11	237	9
2	200	20	200	20
3	196	20	196	14

3.3.5 Results of Optimizing Roasting Condition

The results of aroma compounds for roasted coffee samples were presented in the Figure 3.13 and 3.14. Principle component analysis was used to illustrate differences in aroma compounds across the 5 Robusta coffees with different roasting profiles (Table 3.12) and 2 Arabica samples shown in Figures 3.13. There were two identified principal components based on the main aroma compounds retrieved from the experiment. PC1 accounts for 55.1% of the variation across all samples, which mainly separate the coffee varieties from left to right (Robust to

Arabica) and is highly correlated with pyrazines, furans, ketones and acetic acid. PC2 accounts for 31% of the variation, which separate the samples according to their roasting intensity from top to bottom (dark to medium) and is highly correlated with phenolic compounds and sulphur-containing compounds.

When points are close to each other then have the most similar aroma profiles. In comparison with Arabica, none of the Robusta samples are located in the same quadrant, however following the second principal component, 200 ºC for 20 min Robusta samples are located closest to the dark roasted Arabica samples, which showed similarity in phenolic and sulphur containing compounds. However, all 5 Robusta coffee have more pyrazines and hexanal compare with Arabica coffee (Figure 3.13). Unlike Robusta, Arabica coffee tend to have more furans, acids, and ketones, which agreed with the previous studies done by Farah 2012 and Clarke 2012.

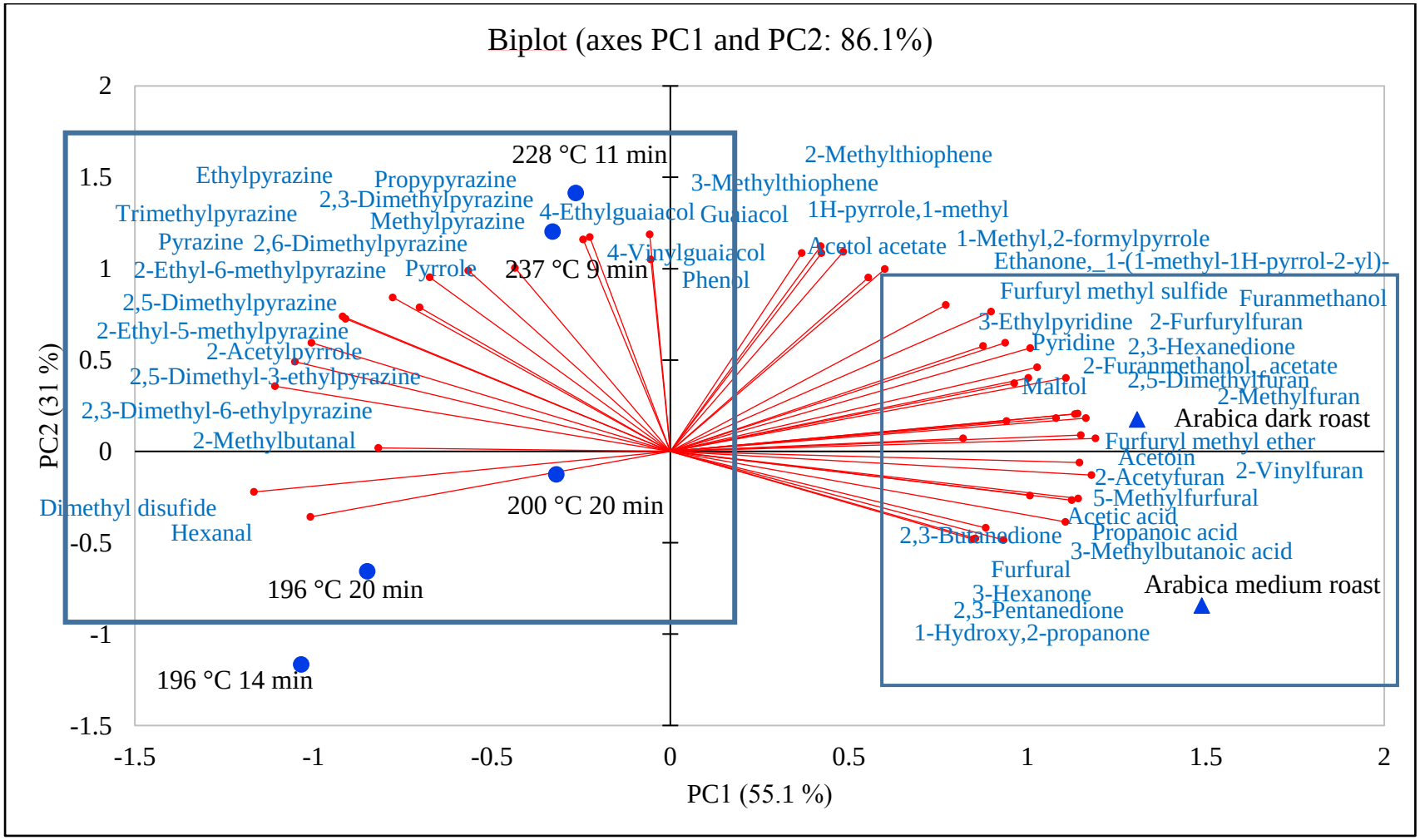


Figure 3.13 The biplot of PC1 and PC2 of aroma profile showing 86.16% variation among 5 Robusta (●) and 2 Arabica (▲) coffee samples

Rather than displayed all the replicates, the dispersion of each subcloud is summarized in Fig. 3.14 by its bootstrap hull. The convex hull can be defined as the vertices of the smallest convex polyhedron in variable space within or on which all the data points lie (Everitt, 2002). The hull is defined by the positions of the extreme points. In two dimensions the hull is a convex polygon. The analysis involves the initial definition of a convex hull bounding the extremities of a given area within the biplot.

The background of Fig. 3.13 is identical to the one of Fig. 3.14. The same words are shown with the same coordinates. In fact, in Figure 3.14 contains different information than Fig. 3.13, to show the shapes and size of the clusters, and the degree to which they overlap. The distribution of the seven samples in the biplot also gives a strong indication of the degree of variation among them. Whilst there was repeatable differences between Arabica and Robusta, careful section of roasting conditions could minimize these differences, in this chapter, a Robusta roasting temperature of 200 ºC and roasting time of 20 min are found to be the optimum conditions with the highest overlap with dark roasted Arabica coffee sample (Figure 3.14).

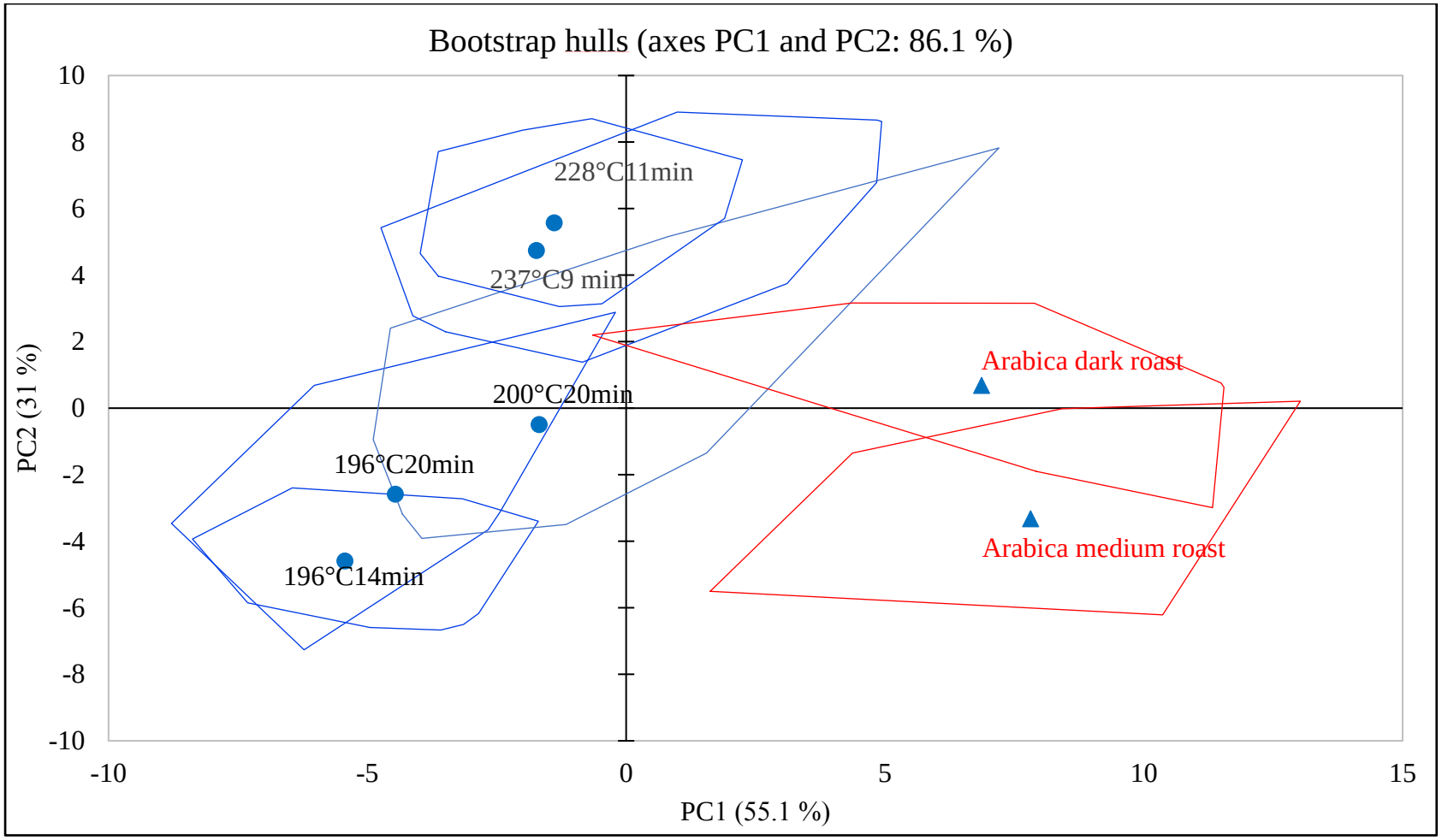


Figure 3.14 Bootstrap hull plot area of seven fields; Robusta (●) and Arabica (▲) coffee samples (4 replicates each).

3.4 Conclusion

In this chapter, a procedure for roasting Robusta coffee to reduce the differences between Arabica and Robusta was successfully generated. A roasting profile of 200 ºC with 20 min was selected as having the least aroma differences from dark roasted Arabica. These optimised roasting condition were applied in chapters 4, 5 and 6.

Chapter 4 Evaluation of the Impact of Different Pre-Treatment Method on Aroma Generation with Robusta Coffee Beans

In order to achieve the objective of this study, several potential green coffee bean pre-treatment methods were evaluated for their ability to reduce the differences in volatile aroma compounds between Arabica and Robusta. The methods are then further developed in the following chapters 5 and 6.

4.1 Cold Plasma Pre-Treatment

4.1.1 Introduction

Cold plasma is an emerging technology that is currently being evaluated for its use in the food industry. Due to the interactions of its energetically highly reactive plasma with the product surface, cold plasma has been widely trialled for toxin degradation (Bosch, Pfohl, Avramidis, Wieneke, Viöl, & Karlovsky, 2017), to enhance product shelf life (McClurkin-Moore, Ileleji, & Keener, 2017; Wang, Zhuang, Hinton, & Zhang, 2016) and food decontamination (Afshari & Hosseini, 2013; Niemira, 2012). Due to the interaction of highly reactive plasma with sensitive food components, cold plasma treatment has also been shown to impact food quality. Cold plasma has been shown to progress maturation in treated wheat flour by accelerating lipid oxidation (Bahrami, Bayliss, Chope, Penson, Perehinec, & Fisk, 2016). This study also concluded that although there was no significant difference in the total protein content, treated flour did produced a stronger dough due to the protein oxidation.

Previous researchers have reported that the cold plasma treatment can significantly increase free phenolic compound in fruit juice products (Garofulić, Jambrak, Milošević, Dragović-Uzelac, Zorić, & Herceg, 2015). Grzegorzewski observed that

after plasma treatment in lamb's lettuce the content of flavonoid increased significantly (Grzegorzewski, Ehlbeck, Schlüter, Kroh, & Rohn, 2011). Cold plasma treatment could also modify the sucrose content in the fruit juice (Almeida, Cavalcante, Cullen, Frias, Bourke, Fernandes, *et al.*, 2015; Rodríguez, Gomes, Rodrigues, & Fernandes, 2017). Almeida et al reported that the sucrose content in orange juice showed a significant increase after cold plasma treatment, which could be explained by degradation of oligosaccharides with high degree of polymerization in juice. (Almeida, *et al.*, 2015). Moreover, there were 23% of sucrose generated in the cashew apple juice after treatment with N_2 plasma flow rate of 30ml/min for 15 min, which agreed with the work done by Almerda (Rodríguez, Gomes, Rodrigues, & Fernandes, 2017). No work has previously been carried out to understand the effect of cold plasma pre-treatment on the aroma generation in Robusta coffee beans. In this study, a cold atmospheric plasma technology was applied, with the aim to modify aroma precursors and change the chemical reaction pathways during thermal treatment and thereby regulate aroma formation during the coffee roasting.

4.1.2 Results of Aroma Formation

Fifty-seven volatile aroma compounds were identified within all coffee samples (Table 3.2). Table 4.1 presents the volatile compounds that showed a significant difference between 30 min cold plasma treated Robusta coffee and non-treated Robusta coffee ($p \leq 0.05$). All 15 volatile compounds significantly increased (between 26.31% and 59.40%) after treatment with cold plasma (30 min) (Table

4.1). However, the rest of the 42 volatile compounds were not affected by cold plasma pre-treatment.

Table 4.1 p value of the effect of plasma (n = 4) (increase % between gas on and 30 min treated Robusta for 15 aroma compounds).

Aroma Compounds	p value	Increase (%)
Hexanal	0.00	+59.40
2-Furfurylfuran	0.00	+54.81
Dimethyl disulphide	0.01	+53.08
3-Methylthiophene	0.03	+51.72
Phenol	0.01	+46.44
2-Methylthiophene	0.04	+45.91
2,5-Dimethylfuran	0.00	+38.70
Furfuryl methyl ether	0.01	+38.06
2-Methylfuran	0.01	+37.73
3-Hexanone	0.01	+36.36
2-Methylbutanal	0.01	+33.85
2-Vinylfuran	0.05	+32.51
Furfuryl methyl sulphide	0.05	+31.16
3-Penten-2-one	0.05	+27.07
3-Methylbutanoic acid	0.02	+26.31

Hexanal has been reported as a marker for lipid oxidation in the staling of coffee. There was significant increase of hexanal content in cold plasma treated coffee. Cold plasma pre-treated coffee generated around 59% more hexanal after roasting. This may be due to cold plasma accelerating lipid oxidation during the treatment process, which is concordant with previous study done by Bahrami et al. (2016). Volatiles such as 2-methylfuran, 2, 5-dimethylfuran, 2-vinylfuran, 3-penten-2-one have been reported as sugar degradation products (Flament, 2002). Higher concentration of these volatiles may be due to the increased release of free sugars

during cold plasma treatment. These results are partly consistent with Almeida et al. (2015).

PCA was used to illustrate the variation between the 15 aroma compounds across the 2 cold plasma treated Robusta samples (including different treatment time) and 1 control Robusta sample and 1 sample with gas flashed only (control sample, which only treated with carrying gas, no plasma involved) (Figure 4.2). The PC1 accounted for 90 % of the variance in the dataset and showed separation between the control Robusta, process control Robusta (left) and cold plasma treated Robusta samples (right). The PC2 accounted for 9% of the variance and indicated minor differences between different volatile compounds. Samples treated with cold plasma showed more furans, ether, aldehydes, ketones, and acid and sulphur-containing compounds. Cold plasma treated beans have a significantly higher concentration of all these volatile compounds ($p < 0.05$) as compared to non-treated beans and gas flashed beans, there were no significant difference (ANOVA, $p > 0.05$) with time, indicating that only a small level of treatment is required.

Although there were significant difference in the cold plasma treated Robusta coffee and non-treated Robusta coffee, the modified aroma profile was not comparable to Arabica coffee samples (Figure 4.3). In summary, this study demonstrated the capability of cold plasma to alter the aroma profile of Robusta, however not to the extent that it showed similarity with the volatile aroma profile of Arabica. Further studies need to be completed in order to meet this aim. Alternative approaches like pre-soaking methods were explored in the following chapters.

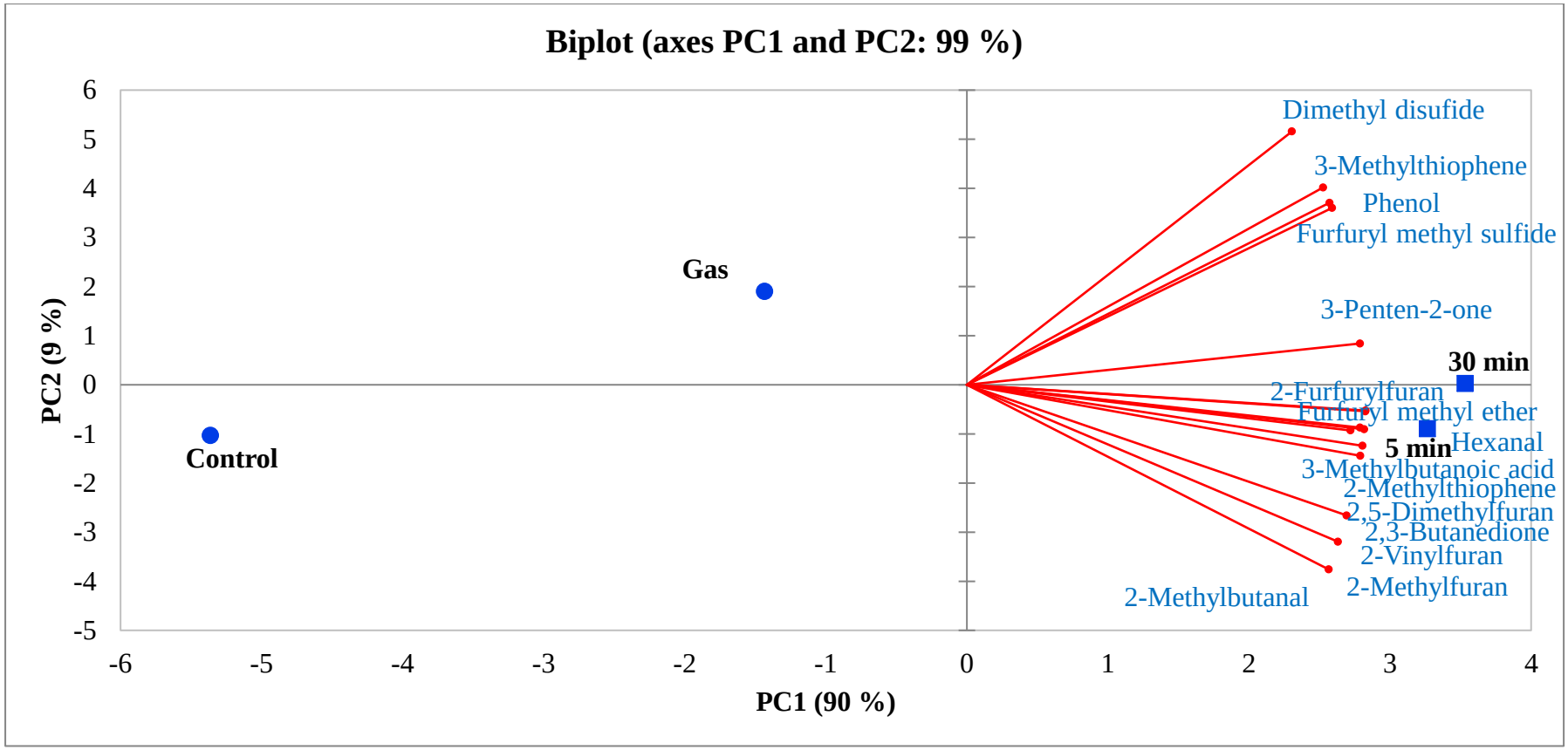


Figure 4.2 The biplot of PC1 and PC2 of aroma profile showing 99.08% variation among 4 coffee samples (n = 4) (● = control and gas flashed Robusta samples, ■ = cold plasma treated samples).

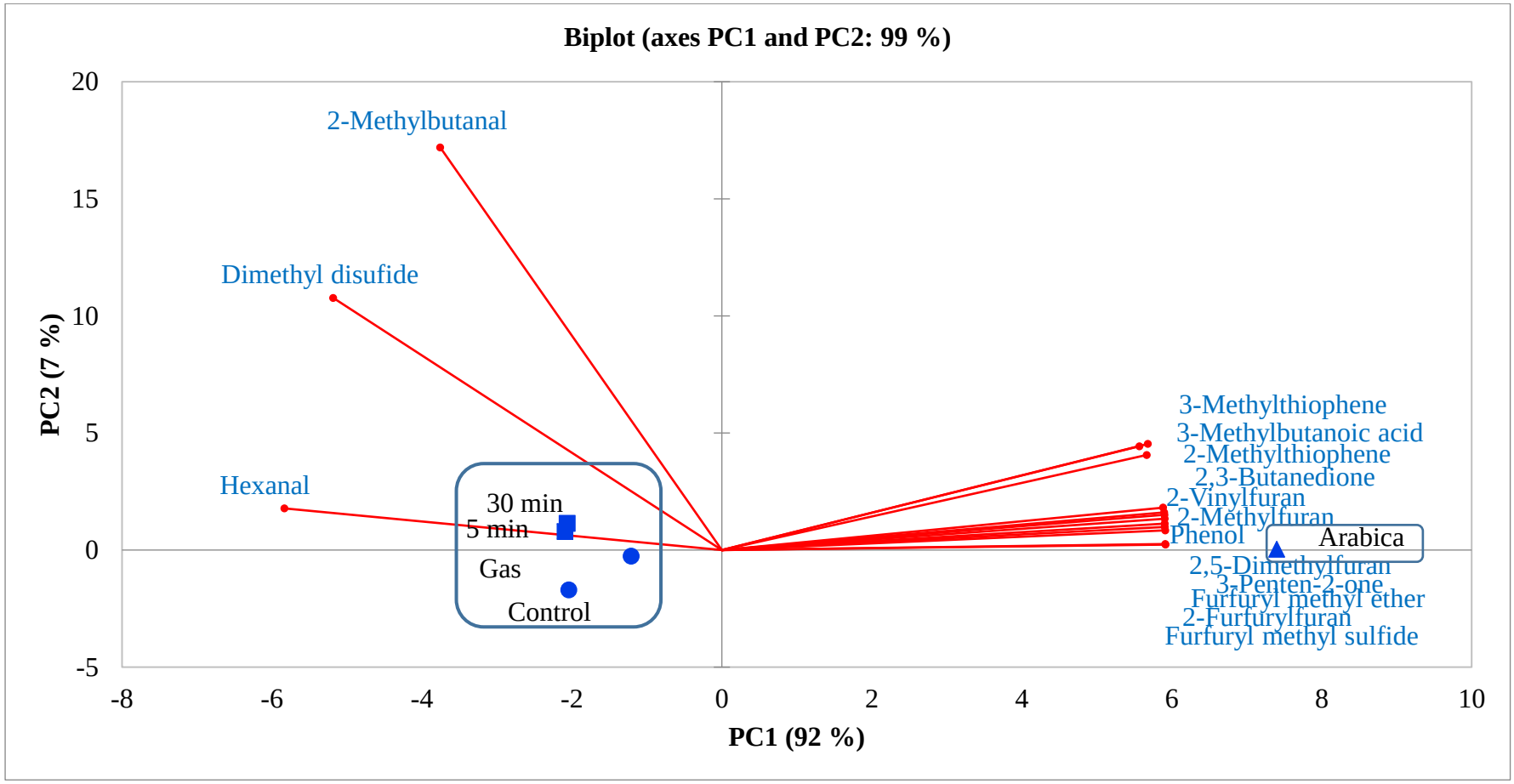


Figure 4.3 The biplot of PC1 and PC2 of aroma profile showing 98.51% variation among 5 coffee samples (n = 4) (● = control and gas flashed Robusta samples, ■ = cold plasma treated samples, ▲ = Arabica sample).

4.2 Pre-Soaking Treatment with Varying Time and Temperature

4.2.1 Introduction

As mentioned early in chapter 1, aroma formation in coffee is directly related to the chemical composition of the green coffee beans and typical coffee aroma compounds are developed during the roasting process due to complex reactions. A number of studies have improved the quality of Robusta coffee by passing the green Robusta beans through steam or soaking into the enzyme containing solution to remove substances such as 2-methylisoborneol, which is responsible for the muddy odour (Becker, Schlabs, & Weisemann, 1991; Small & Asquith, 1990).

However, during this process important water soluble precursors such as sucrose, protein, CGA, caffeine and trigonelline are leached into water, hence compromising the flavour generation potential of the roasted coffee. The amino acids, CGA, trigonelline and sugar are considered to be some of the main precursors in the aroma generation and colour formation during coffee roasting (Poisson, Blank, Dunkel, & Hofmann, 2017). Therefore, it is necessary to investigate how much water-soluble precursors are lost during pre-soaking of green coffee beans and its impact on aroma generation during coffee roasting. This study was conducted to identify an optimal pre-soaking time and temperature that would result in acceptable quality of Robusta coffee in terms of different volatile compounds. The proposed pre-soaking condition are applied in chapter 4 and 5.

4.2.2 Results of Moisture Content in Pre-Soaked Robusta Green Beans

The results for pre-soaked Robusta green bean moisture content across different soaking time temperature are shown in Figure 4.4. Both soaking time and soaking temperature play an important role on the green bean moisture content. Moisture

content for the non-soaked Robusta green bean was 12%. Green bean moisture content increased from 12% to 75% after soaking in 20 ºC for 2 h. Increased soaking temperature results in increased moisture content (from 75% to 130%) for the green beans soaked at 2 h; increased soaking time results in increased moisture content (from 75% to 135%) for the green bean soaked in 20 ºC. The highest moisture content for the soaked green bean is 156%, which soaked at 100 ºC for 10 h. There were no significant difference between green bean soaked at 100 ºC for 10 h and 12 h ($p \geq 0.05$).

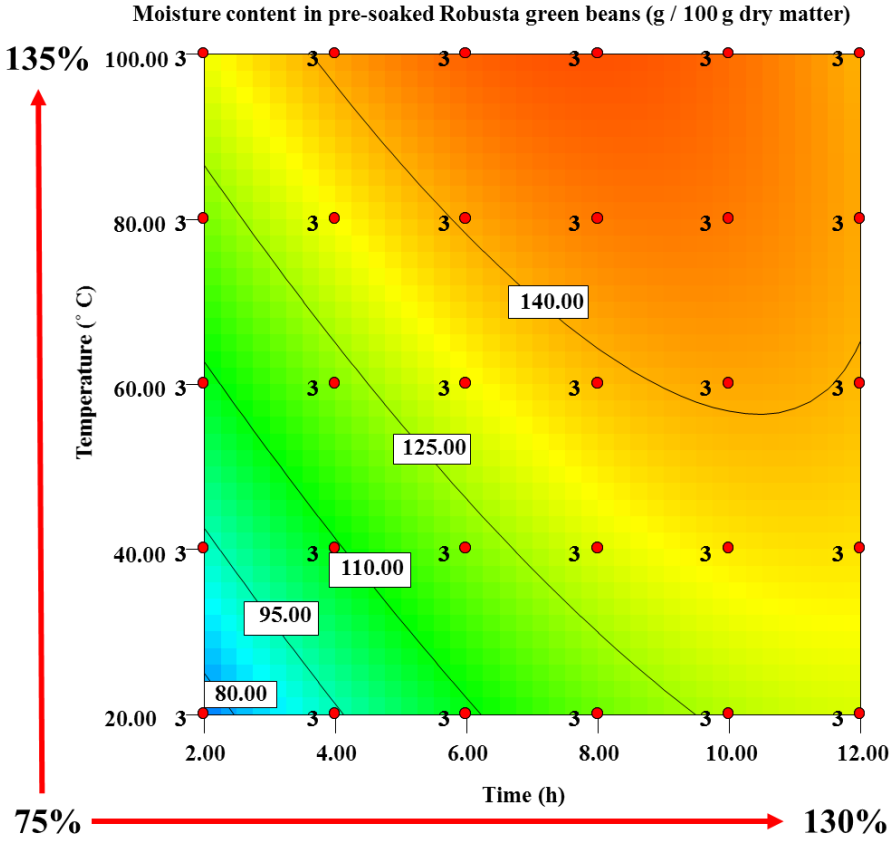
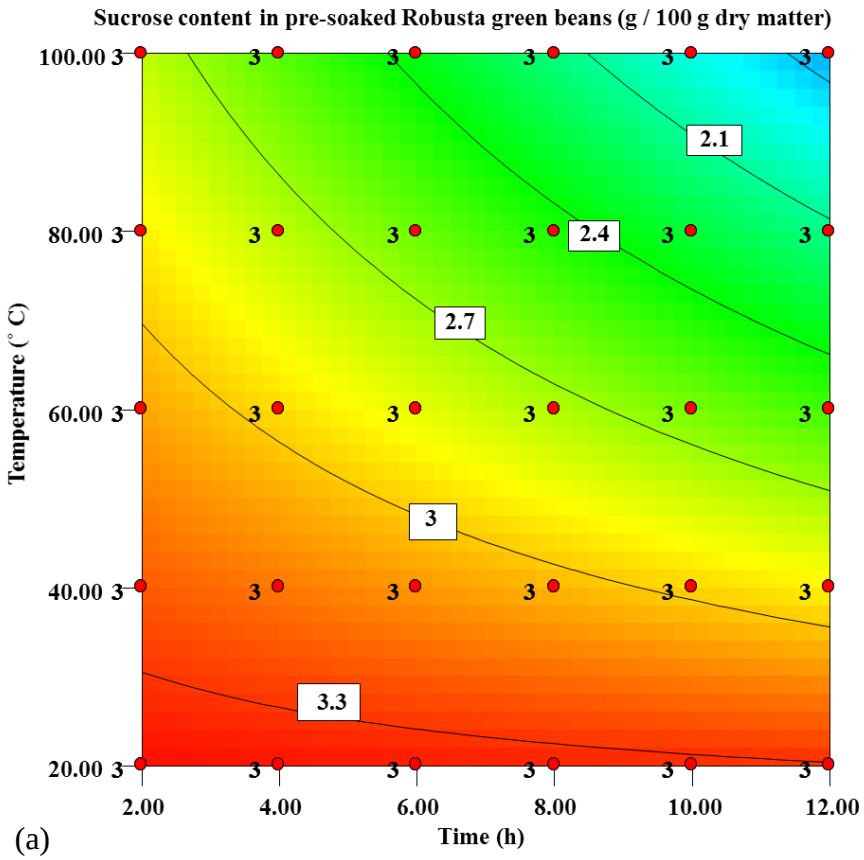


Figure 4.4 Plot showing the Moisture content for the Robusta soaked beans across different soaking time temperatures (Moisture content in the non-treated green Robusta bean is 12g / 100 g dry matter).

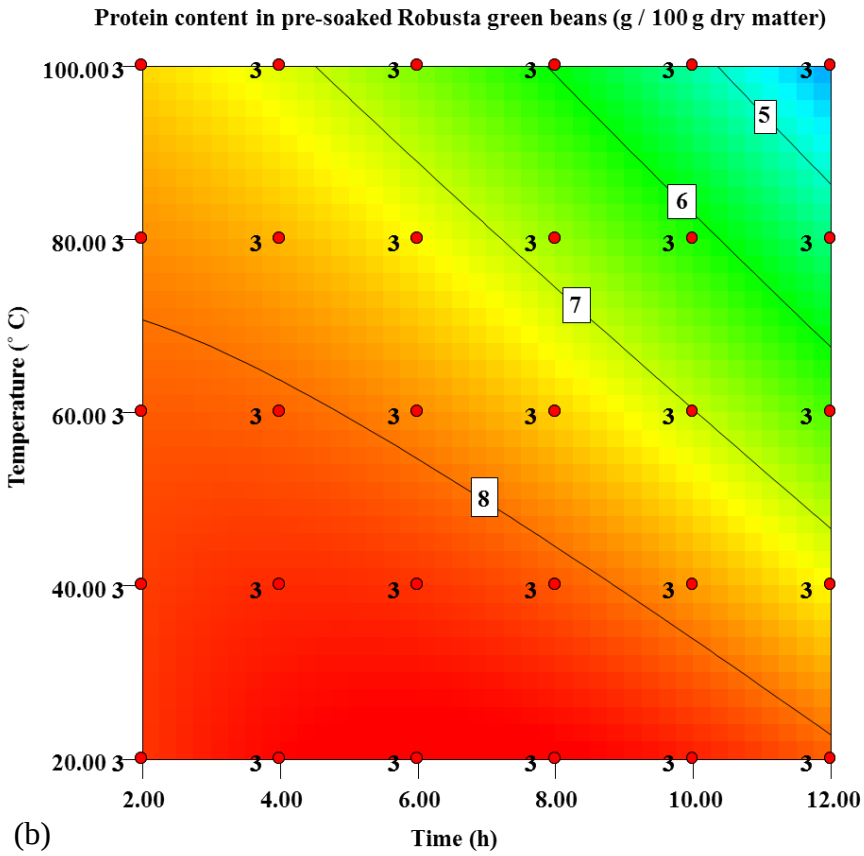
4.2.3 Results of Water-Soluble Precursors

The effect of two independent variables, time (2 - 12 h) and temperature (20 -100 ºC) on five response variables sucrose, protein, CGA, caffeine and trigonelline content were studied using response surface methodology. The value of R^2 for sugar, protein, CGA, caffeine and trigonelline were 0.83, 0.95, 0.84, 0.91 and 0.95 respectively which were obtained using quadratic model. In Figure 4.5, sucrose, protein, CGA, caffeine and trigonelline content showed a significant decrease with increased soaking time and temperature ($p < 0.001$). Increase soaking time and temperature resulted in a significant loss in sucrose (from 3.1 g/100g to 1.6 g/100g), protein (from 8.3 g/100g to 3.3 g/100g), CGA (from 44.4 mg/g to 17 mg/g), caffeine (from 29.5 mg/g to 4 mg/g) and trigonelline (from 8.13 mg/g to 2.46 mg/g) content (Figure 4.5).

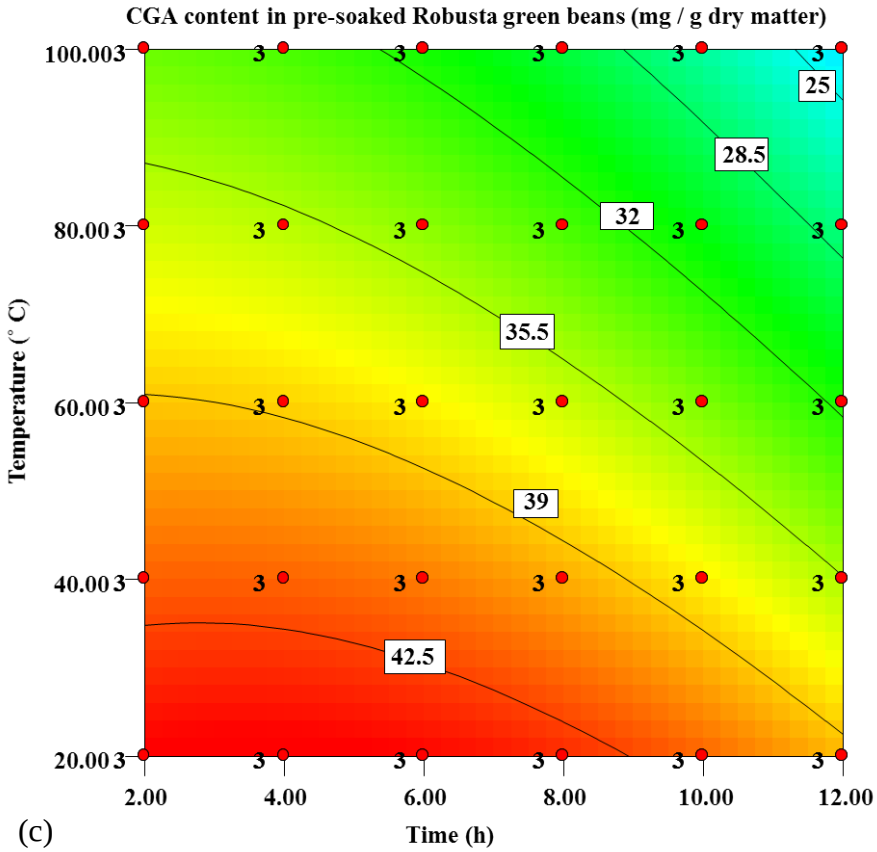
In summary, there was a significant impact of soaking temperature and time on the water soluble precursors ($p < 0.001$), this may significantly impact the aroma profile of roasted coffee beans, which were investigated in the following section.


Sucrose content in pre-soaked Robusta green beans (g / 100 g dry matter)
Temperature (° C)
Time (h)
100.00
80.00
60.00
40.00
20.00
2.00
4.00
6.00
8.00
10.00
12.00
2.1
2.4
2.7
3
3.3

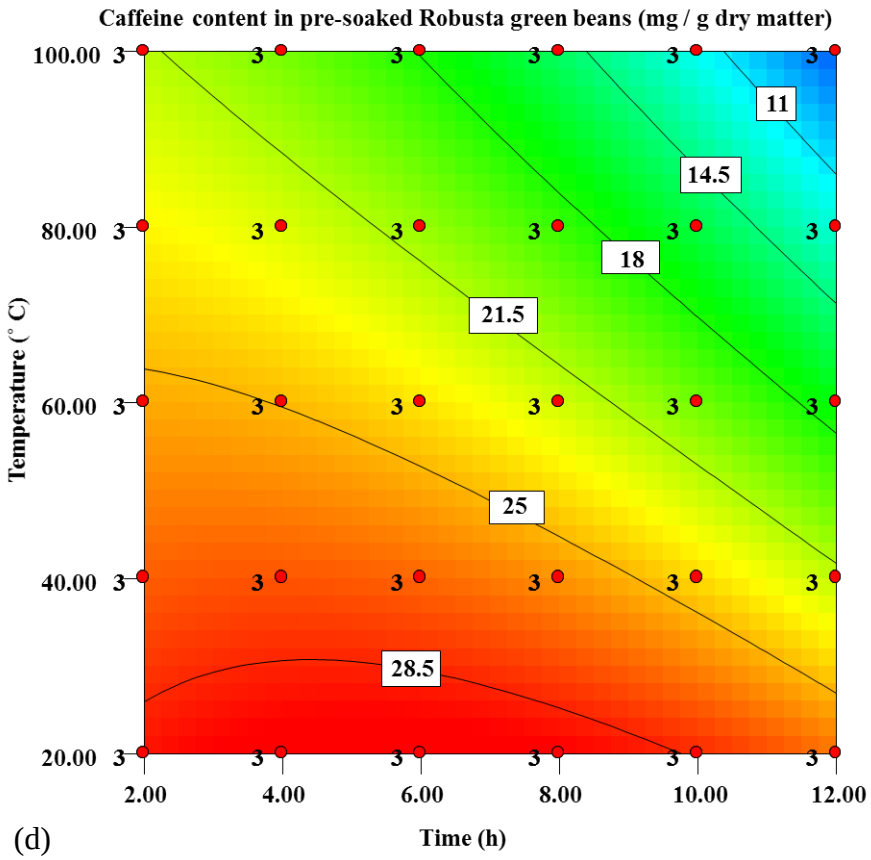
(a)


Protein content in pre-soaked Robusta green beans (g / 100 g dry matter)
Temperature (° C)
Time (h)
100.00
80.00
60.00
40.00
20.00
2.00
4.00
6.00
8.00
10.00
12.00
5
6
7
8

(b)


CGA content in pre-soaked Robusta green beans (mg / g dry matter)
Temperature (° C)
Time (h)
100.00
80.00
60.00
40.00
20.00
2.00
4.00
6.00
8.00
10.00
12.00
25
28.5
32
35.5
39
42.5

(c)


Caffeine content in pre-soaked Robusta green beans (mg / g dry matter)
Temperature (° C)
Time (h)
100.00
80.00
60.00
40.00
20.00
2.00
4.00
6.00
8.00
10.00
12.00
11
14.5
18
21.5
25
28.5

(d)

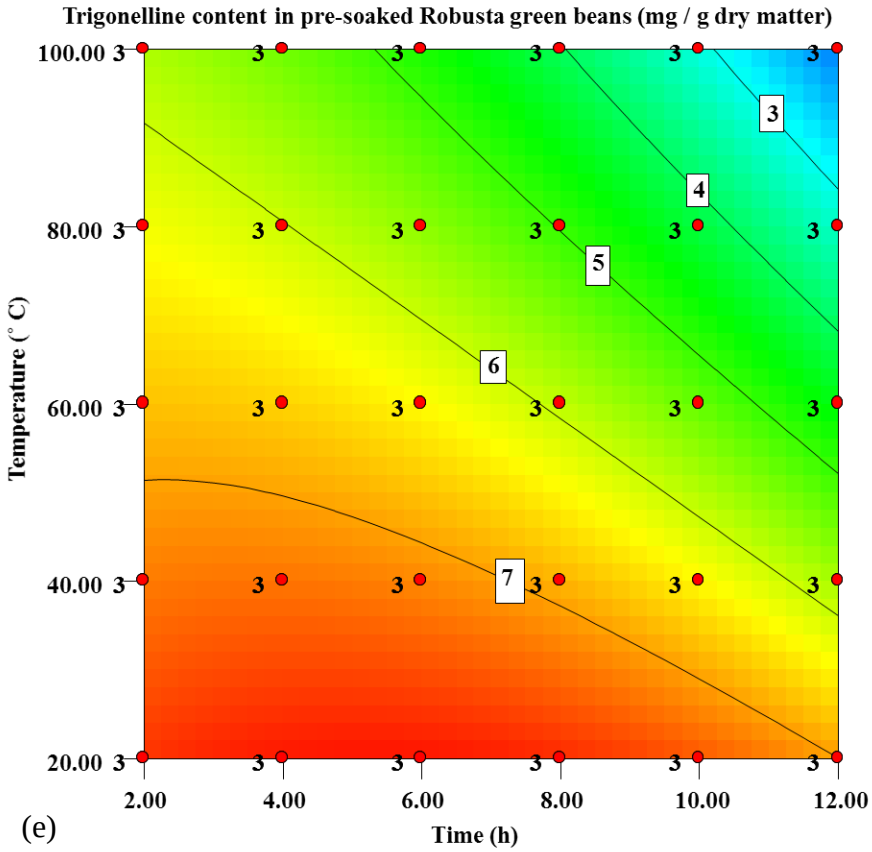


Figure 4.5:Contour plot for a) Sucrose content for the soaked green beans (sucrose content in the non-treated Robusta green bean is 3.2%); b) Protein content for the soaked green beans (protein content for the non-treated green Robusta bean is 8.7%); c) CGA content for the soaked green beans (CGA content in the non-treated green Robusta bean is 45 mg/g); d) Caffeine content for the soaked green beans (caffeine content in the non-treated green Robusta bean is 30 mg/g); e) Trigonelline content for the soaked green beans (trigonelline content in the non-treated green Robusta bean is 9.01 mg/g); in dry weight.

4.2.4 Results of Aroma Profile

Fifty-seven volatile aroma compounds were identified within all coffee samples (Table 3.2). Table 4.6 indicated 21 volatile compounds showed a significant decrease in their relative amount with increasing soaking time and temperature ($p < 0.05$). There was a significant decrease in the concentration of all volatile acids (acetic acid, 3-methylbutanoic acid and propanoic acid) in the pre-soaked samples (amount of lost is between 50.9% and 79.8%) when compared with the original Robusta samples. Lentner and Beatherage in (1959) pointed out that decomposition of sugar enhanced the formation of acetic acid, rearrangement of sugars and decarboxylation of CGA attributed to the decarboxylation of acids, led to change in acid content (Lentner & Deatherage, 1967). Significantly decrease in phenolic compounds appeared in the soaked Robusta coffee, which lost around 55% to 72% compared with non-soaked Robusta (Table 4.6). Phenol was reported to be formed from 5-caffeoylquinic acid degradation over roasting (Flament, 2002). Degradation of CGA causes the appearance of a series of neo-formation products (Kamiyama, Moon, Jang, & Shibamoto, 2015). Hence, a reduction in both sucrose content and CGA content in the green beans (Figure 4.5) could explain the reduction of acids (50% - 80%) and phenolic compounds formation (55% - 72%).

There was also a significant decrease in ketone compounds such as 3-penten-2-one (60%), 2, 3-hexanedione (54%), 2, 3-pentanedione (78%), 1-hydroxy-2-propanone (56%), 3-hexanone (65%) and 2, 3-butanedione (79%) in the soaked Robusta (Table 4.6). These were all been previous reported as carbohydrate pyrolysis products (Flament, 2002). Therefore, the ketone formation during roasting process significantly affected by the leaching out of sucrose (mentioned in Figure 4.5) in the pre-soaked green beans . The formation of pyridine can be identified by the

thermal degradation of Amadori intermediates (Charalambous, 2012). Pyridine also been reported as a trigonelline decomposition product during roasting of coffee (Poisson, Blank, Dunkel, & Hofmann, 2017). A reduction in the trigonelline and protein content in the soaked green beans (Figure 4.5) could significantly affect the heterocyclic N containing compounds formation during the roasting process.

Table 4.6 Summary of significant difference across different time and temperature soaking profiles on amount of lost (calculated by minimum soaking time-temperature (20 ºC for 2 h) minus maximum soaking time-temperature (100 ºC for 12 h) then divided by non-soaked Robusta multiply by 100) for 21 compounds found in all roasted and ground coffee samples n = 3.

No.	Aroma compounds	Sig time	Sig temperature	Amount of loss%
1	Acetic acid	y	y	50.89
2	Phenol	y	y	55
3	3-Methylbutanoic acid	y	y	79.75
4	2-Acetylpyrrole	y	y	68.51
5	3-Penten-2-one	y	y	60.45
6	3-Ethylpyridine	y	y	56.91
7	2,3-Hexanedione	y	y	54.32
8	2-Furfurylfuran	y	y	70.95
9	Guaiacol	y	y	71.59
10	5-Methylfurfural	y	y	67.31
11	1H-pyrrole,1-methyl	y	y	47.6
12	2,3-Pentanedione	y	y	78.19
13	Propanoic acid	y	y	68.41
14	1-Hydroxy-2-propanone	y	y	56.3
15	Furanmethanol	y	y	60.15
16	3-Hexanone	y	y	64.71
17	Maltol	y	y	53
18	Furfural	y	y	75.97
19	2,3-Butanedione	y	y	78.87
20	Ethanone,_1-(1-methyl-1H-pyrrol-2-yl)-	y	y	74.47
21	Pyridine	y	y	65.87

The optimisation procedure was performed to obtain the optimal levels of two factors (A: time and B: temperature) by Design-Expert version 7.0.0 (Stat-Ease, Inc., Minneapolis, USA). The optimisation condition which was based on the

maximum level of flavour compounds (Table 4.6) and moisture content (Figure 4.4) with minimum level of undesirable water-soluble precursors loss (Figure 4.5), predicted an optimum soaking time of 2 h and temperature for 20 ºC.

4.3 Pre-Soaking Treatment with Varied Chemicals

4.3.1 Introduction

As mentioned early in chapter 1, the flavour generation is influenced by many factors such as green bean flavour precursors, pH and moisture content. Previous studies have treated the coffee beans with a treatment solution containing an effective amount of enzymes, sugars and food grade bases for a period of time at a range of temperature and under a pressure of 250 psi to enhance the level of desirable coffee aroma and flavour components (Small & Asquith, 1990). In this chapter, instead of using high pressure, more gentle treatment (soaking time of 2 h and temperature for 20 ºC) was applied with the intention to retain more reaction precursors for the Maillard reaction to create similar volatile aroma compounds of Arabica during roasting of beans. Different chemicals were added in the soaking solution (1% Na_2CO_3, 10% sucrose, 1% acetic acid, and 5% lysine). Colour and aroma of these treated beans were analysed and discussed in the following section.

4.3.2 Results of Colour change in Pre-Soaked Robusta with Varied Chemicals

Lightness (L*) is often used as a measurement of the roasting intensity, which is related to the roasting temperature and time (Illy & Viani, 1995). Colour analysis was completed for all coffee bean samples. It was found that there were significant differences (ANOVA $p < 0.05$) between Arabica beans and non-treated Robusta. ΔE was used to determine the overall distance between two colours. According to the previous study, ΔE of 3.0 is the minimum colour difference that human eyes

can detect (depends on the hue) (Martínez-Cervera, Salvador, Muguerza, Moulay, & Fiszman, 2011). Therefore, a ΔE value of less than 3.0 is almost imperceptible.

Clear differences were perceived between sodium carbonate treated Robusta green bean and non-treated Robusta green bean with a total colour difference ΔE of 15.85. However, the colour difference after roast is almost imperceptible as ΔE is 1.01. The total colour differences were lower than 3 in all R&G coffee samples, indicating only minimum colour difference that human eyes can detect when compared with non-treated Robusta.

Table 4.7: Total colour difference (ΔE) in 5 treated Robusta compared with non-treated Robusta n = 4.

Coffee bean soaked in varying chemical solutions	ΔE for ground green coffee beans	ΔE for roasted and ground coffee beans
Na_2CO_3	15.85	1.01
water	3.96	1.26
Acetic acid	1.74	2.19
Sucrose	3.67	0.47
Lysine	6.89	0.54

4.3.3 Results of Volatile Compounds in Pre-Soaked Robusta with Varied Chemicals

PCA was used to illustrate the differences between the 57 aroma compounds across the 7 R & G samples (Figure 4.8). The PC1 accounted for 69% of the variance in the dataset and showed a trend with decreasing levels of pyrazines from left to right, increasing level of furans from left to right. Robusta (left) was clearly separated from Arabica (right) by PC1. The PC2 accounted for 17% of the variance and showed separation between different volatile compounds.

There were visible differences between Arabica and Robusta, characterised by these samples being located in different corners of the PCA plot. The lysine treated

Robusta sample had greater levels of pyrazines and phenolic compounds when compared with the other samples. The reason may due to more precursors has been infused into the Robusta green bean. During roasting amino acids, such as lysine, play a critical role in the development of flavour compound via the Maillard reaction and Strecker degradation. Lysine obviously was a key factor in promoting pyrazines and phenolic compounds formation (Figure 4.8). Results in concordant with the previous research done by He et al. 2018, lysine could effectively promote pyrazine and phenolic compound generation in Chinese Arabica coffee (He, Zhang, Wen, Hu, Wu, Zeng, *et al.*, 2018). As the results demonstrated in chapter 3 Table 3.7 (difference between Arabica and Robusta), although lysine treated Robusta can accelerate the formation of pyrazines and phenolic compounds, lysine cannot be used to bring Robusta coffee aroma closer to Arabica coffee aroma.

Sodium carbonate is the disodium salt of carbonic acid with alkalinizing property. When dissolved in water, sodium carbonate forms carbonic acid and sodium hydroxide. As sodium hydroxide is a strong alkali, sodium hydroxide neutralizes acid thereby acting as alkali. The pH for 1mM sodium carbonate is around 10.52. Green bean soaked in a 1% sodium carbonate aqueous solution could therefore modified green bean pH from 4.86 to 5.21. Sodium carbonate treated Robusta had a greater levels of pyrazines, pyridine, phenolic compounds and sulphur-containing compounds when compared with the other samples (Figure 4.8). This result is supported by Meynier & Mottram, (1995) who defined an alkali condition favours pyrazine formation in the Maillard reaction because increased reactivity of the amino group at high pH due to the deprotonation (Meynier & Mottram, 1995). The content of thiazoles such as 4-Methylthiazole trimethyloxazole increased with pH, which could be due to the increased availability of ammonia at higher pH (Mulders,

1973). However, sodium carbonate treated Robusta was not sufficient to make an aroma profile imitating that of the Arabica coffee. On the contrary, although there were clearly separation between all different chemical treated Robusta and Arabica, 1% acetic acid treated Robusta showed the most similarity to Arabica (Figure 4.7).

There was no discrimination between sucrose treated Robusta and process controlled Robusta (soaked in water only) which were located together in the centre of the plot. It may due to sucrose is a disaccharide, it is too big to infuse into the green beans during soaking process (20 ºC for 2 h).

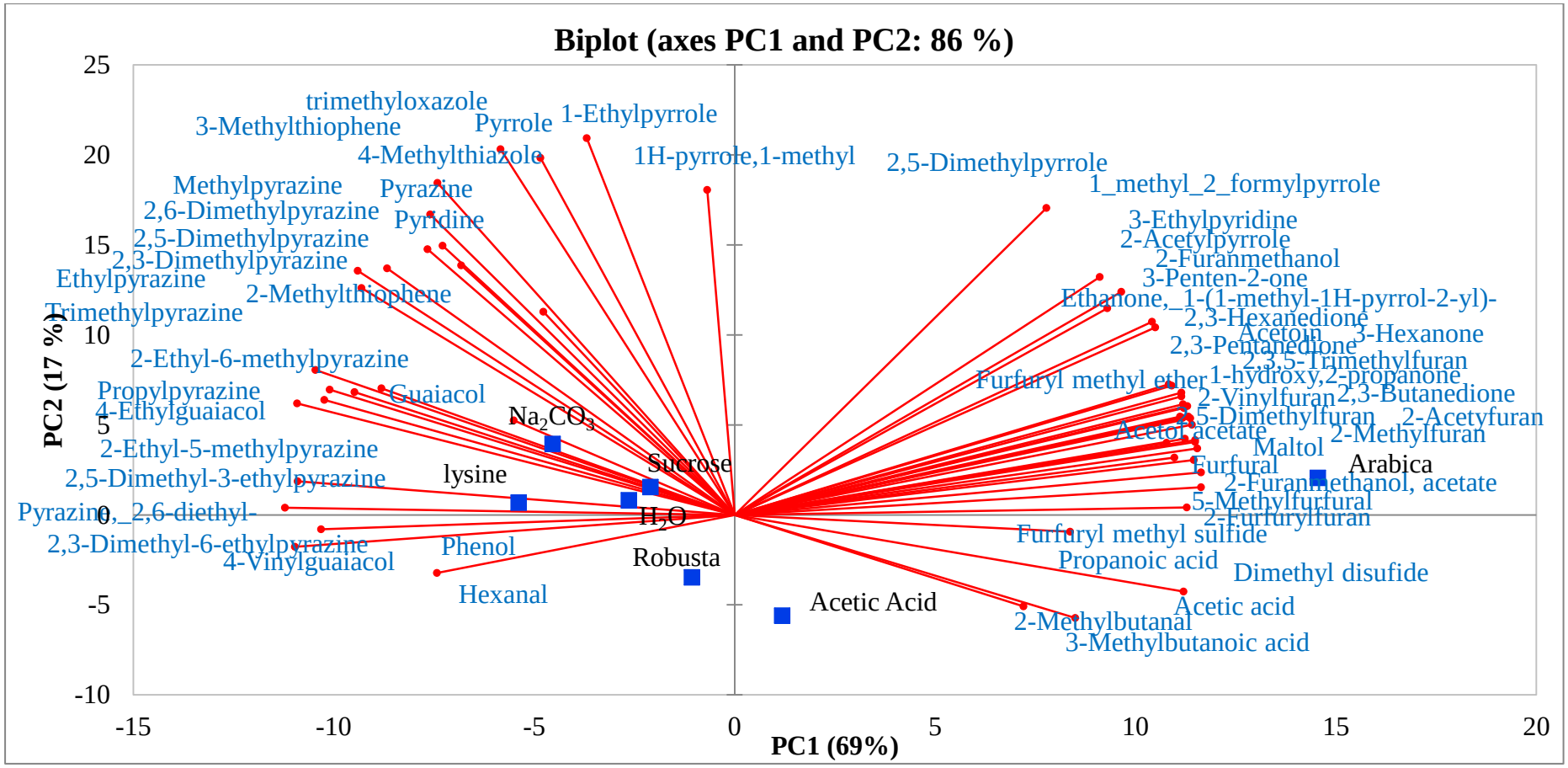


Figure 4.8 Principal component analysis (Bi-plot) of the aroma compliment of Arabica, Robusta, water treated Robusta, 1% acetic acid treated Robusta, 10% sucrose treated Robusta, 5% lysine treated Robusta and 1% sodium carbonate treated Robusta analysed by GC-MS (n = 4).

4.3 Conclusion

Further building on the chapter 3, different pre-treatment methods were developed with the aim of reducing the differences between Arabica and Robusta roasted coffee aroma. Cold plasma pre-treatment altered the aroma profile of Robusta, however did not show similarity with Arabica. A pre-soaking method was developed as a tool and was optimised for pre-soaking time and temperature. The proposed pre-soaking condition (2 h for 20 ºC) was used in subsequent chapters with further modifications to reduce Arabica-Robusta aroma differences (chapter 5).

Different chemicals were used to pre-soak green Robusta coffee with the aim to reduce the difference between two coffee species. Results indicated that both lysine pre-treated Robusta and sodium carbonate treated Robusta drifted further away from Arabica. Acetic acid pre-soaked Robusta did alter the aroma formation during roasting process and showed the most similarity to Arabica. Therefore, chapter 5 further describes the work on the acetic acid treatment. As sucrose is too big to infuse into Robusta green bean by pre-soaking, there was no difference between sucrose treated Robusta and process controlled Robusta. Further research need to be done to achieve the aim (detail explained in chapter 6).

Chapter 5 Modifying Robusta Coffee Aroma by Green Bean acetic acid Pre-Treatment

5.1 Introduction

As illuminated in Chapter1, Maillard reactions have been identified to be one of the major pathways in the formation of volatile compounds during the coffee roasting process. While numerous factors can influence the generation of flavours by the Maillard reaction (Nursten, 2005) the pH at which the reaction is conducted influences the kinetics of certain reaction pathway of the volatiles formed and, hence, the flavour of the final product (Jousse, Jongen, Agterof, Russell, & Braat, 2002 Russell, & Braat, 2002).

The influence of pH on the Maillard reaction and caramelization in sugar-amino acid model system has been previously studied (Ajandouz, Desseaux, Tazi, & Puigserver, 2008; Petriella, Resnik, Lozano & Chirife, 1985; Ajandouz, Tchiakpe, Ore, Benajiba & Puigserver, 2001; Hodge, 1953; Nie, Huang, Hu, Zhang, Wang, Li, *et al.*, 2013; Buera, Chirife, Resnik, & Lozano, 1987). The earliest study observed the rate of D-glucose mutarotation can be accelerated by both hydroxyl ions and protons (Lowry, 1903). Wolfrom et al. reported that weak acids and their conjugate bases are capable of promoting enolization of the sugars. Which may accelerate the Maillard reaction and caramelization as the enolized sugar is more easily dehydrated and fragmented than the ring form (Wolfrom, Cavalieri, & Cavalieri, 1947). Six year later, Hodge (1953) evaluated further that sugar polymerization can be strongly enhanced with increasing pH. In the model system, Amadori compounds give rise preferentially to reductones and highly reactive dicarbonyls are formed essentially under alkaline conditions, while furfurals and

related derivatives under acidic conditions. Moreover, colour formation was also highly positive correlated with pH, which is related to the reactivity of the amino acid (Petriella, Resnik, Lozano & Chirife, 1985). Recent work done by Nie et al. (2013) showed that pH had a significant effect on thermally induced furan formation when the temperature was above120 ºC. At pH 4.18, significantly more furan was generated than at pH 7 or 9 in both glucose–glycine and sucrose–glycine models (Nie, *et al.*, 2013).

Robusta green bean contains more protein and less sugar than Arabica, more pyrazines and less furans were generated in the Robusta coffee after roasting (chapter 1 and 3). In this chapter, green beans were pre-soaked in an aqueous acetic acid solution to modify green bean pH and therefore modify aroma chemistry reaction pathways that occur during thermal treatment (roasting). The hypothesis tested in this chapter is that, green Robusta beans treated with acetic acid could influence the kinetics of certain reaction pathway of the volatiles formed in green Robusta beans during the roasting process (without significant reduction in the product shelf life) and, hence, the flavour of the final product will be closer to the target Arabica coffee. GC-MS with SPME was used to compare aroma compounds in the treated Robusta coffee, non-treated Robusta and target Arabica coffee. Sensory analysis was used to determine the maximum percentage of Robusta that can be blended with Arabica without any perceivable sensory difference.

As mentioned earlier in chapter 1, many of the important coffee aroma compounds are known to be highly unstable and volatile, and are lost directly after roasting is complete. In order to evaluate the impact of treatment process on the roasted coffee shelf life, the chemical stability of acetic acid treated Robusta, untreated Robusta and Arabica coffee beans was investigated over six weeks of accelerated shelf-life

storage (5 ºC, 25 ºC and 35 ºC, for up to 6 weeks).

Computed Tomography (CT) is a non-destructive technique for visualising solid interiors, and X-ray attenuation is a function of density of the material (Babin, Della Valle, Dendievel, Lourdin, & Salvo, 2007). By sample rotation, a series of 2-D X-ray images can be obtained, which can then be rendered into a 3-D image and allow accurate reconstruction of the internal microstructure (Kerckhofs, Schrooten, Van Cleynenbreugel, Lomov, & Wevers, 2008). To understand the accelerated shelf-life storage results and explore the changes in the structure of the coffee bean and the impact of treatment process on the structural porosity, the roasted coffee beans (Arabica, non-treated Robusta and treated Robusta) were imaged using 3D X-ray CT.

5.2 Results and Discussion

5.2.1 Evaluation of pH and Acetic Acid in Robusta, Treated Robusta and Arabica

Coffee brew acidity is an important sensory quality attribute (Clarke & Vitzthum, 2001) and the pH of the Robusta green bean and roasted coffee was significantly higher than Arabica (Table 5.1). The pH of the coffee bean extract increased with roasting. There was no significant difference between the non-treated Robusta and the process control (0%), however pH significantly reduced with increasing concentrations of acetic acid treatment ($p < 0.05$). There was no significant difference between the pH of green Arabica and treated green Robusta when 2 - 5% acid treatment levels were used. There was no significant difference between the pH of roasted Arabica and roasted Robusta when 4 - 5% acid treatment levels were used.

The relative abundance of acetic acid in non-treated Robusta, treated Robusta and Arabica sample were shown in Table 5.1 (using its level in Arabica as 100%). Non-treated Robusta (acetic acid 52 ± 8.4) and 0% acetic acid treated Robusta (acetic acid 65 ± 4.4) had significantly lower concentrations of acetic acid when compared with Arabica (acetic acid 100 ± 12.6) ($p < 0.05$). There were no significant difference in relative abundance of acetic acid for both 1% and 2% acetic acid treated Robusta when compared with Arabica sample. However, at higher treatment levels (3% - 5%) the treated coffee had significantly higher levels of acetic acid than Arabica.

Table 5.1 pH and relative abundance of acetic acid in Arabica, Robusta and acetic acid treated Robusta coffee (0, 1, 2, 3, 4, and 5% acetic acid).

	Green bean extract pH	Roasted bean extract pH	Relative Abundance for Acetic Acid
Arabica	4.42 ± 0.09^{ab}	5.05 ± 0.03^{a}	100 ± 12.6^{b}
Robusta	4.86 ± 0.05^{d}	5.55 ± 0.01^{d}	52 ± 8.4^{a}
0%	4.84 ± 0.04^{d}	5.51 ± 0.01^{d}	65 ± 4.4^{a}
1%	4.6 ± 0.02^{c}	5.34 ± 0.02^{c}	102 ± 16.1^{b}
2%	4.47 ± 0.06^{b}	5.23 ± 0.08^{bc}	170 ± 39.1^{b}
3%	4.45 ± 0.03^{b}	5.2 ± 0.03^{b}	316 ± 19.5^{c}
4%	4.42 ± 0.02^{ab}	5.13 ± 0.04^{ab}	326 ± 11.3^{c}
5%	4.39 ± 0.01^{a}	5.01 ± 0.03^{a}	391 ± 6.7^{d}

^{abcd} samples with the same letter code in any column are not significantly different ($p \geq 0.05$); Mean ± standard deviation (n = 4).

5.2.2. Determination of the Aroma Compounds in Robusta Beans with Different Pre-treatment Concentrations

The average (n = 4) relative abundance (relative to Arabica 100%) of each volatile compound present in the headspace of the eight coffee samples is shown in Table 5.2. A range of volatile compounds which showed a significant difference between acetic acid treated Robusta coffee and non-treated Robusta coffee ($p < 0.05$).

Table 5.2 Average relative abundance of each volatile compound (relative to Arabica) presented in the headspace was calculated in the eight coffee samples (exclude acetic acid) (n = 4).

	Aroma compound	Robusta	0%	1%	2%	3%	4%	5%	Arabica
1	2,6-Diethylpyrazine	498 ± 7.9^{e}	338 ± 35^{d}	278 ± 24^{cd}	246 ± 23^{bcd}	235 ± 20^{bc}	174 ± 35^{ab}	208 ± 22^{bc}	100 ± 17^{a}
2	3-Ethyl-2,5-dimethylpyrazine	435 ± 39^{d}	340 ± 49^{cd}	266 ± 22^{bc}	256 ± 20^{bc}	221 ± 8.6^{b}	176 ± 19^{ab}	226 ± 18^{b}	100 ± 18^{a}
3	2-Ethyl-6-methylpyrazine	327 ± 21^{d}	276 ± 27^{cd}	233 ± 14^{bc}	210 ± 18^{bc}	197 ± 16^{bc}	148 ± 25^{ab}	183 ± 38^{ab}	100 ± 26^{a}
4	Trimethyl pyrazine	314 ± 27^{d}	244 ± 23^{cd}	193 ± 26^{bc}	176 ± 17^{abc}	160 ± 19^{ab}	123 ± 12^{ab}	150 ± 29^{ab}	100 ± 14^{a}
5	2-Ethyl-5-methylpyrazine	302 ± 22^{d}	265 ± 17^{cd}	228 ± 24^{bcd}	207 ± 22^{bc}	197 ± 17^{bc}	153 ± 21^{ab}	187 ± 36^{abc}	100 ± 20^{a}
6	Ethyl pyrazine	230 ± 21^{c}	185 ± 16^{bc}	172 ± 34^{abc}	150 ± 26^{ab}	144 ± 19^{ab}	118 ± 19^{ab}	141 ± 27^{ab}	100 ± 16^{a}
7	2,5-Dimethylpyrazine	223 ± 13^{b}	165 ± 5.8^{b}	149 ± 23^{ab}	123 ± 16^{a}	122 ± 12^{a}	86 ± 11^{a}	104 ± 12^{a}	100 ± 13^{a}
8	2,6-Dimethylpyrazine	204 ± 21^{c}	181 ± 17^{bc}	154 ± 27^{abc}	134 ± 26^{ab}	130 ± 25^{ab}	98 ± 23^{a}	112 ± 18^{ab}	100 ± 11^{a}
9	Methyl pyrazine	169 ± 16^{b}	128 ± 15^{ab}	111 ± 4.6^{a}	91 ± 5.9^{a}	90 ± 9.8^{a}	76 ± 17^{a}	79 ± 10^{a}	100 ± 14^{a}
10	2,3-Dimethylpyrazine	178 ± 23^{c}	137 ± 12^{bc}	113 ± 6.2^{ab}	104 ± 9.2^{ab}	93 ± 6.5^{ab}	77 ± 8.3^{a}	92 ± 8.6^{ab}	100 ± 8.2^{ab}
11	Pyrazine	124 ± 3.1^{d}	102 ± 5.1^{c}	76 ± 2.6^{b}	68 ± 9.0^{ab}	62 ± 8.6^{ab}	60 ± 8.3^{ab}	54 ± 6.8^{a}	100 ± 3.4^{c}
12	Furfural	94 ± 8.2^{a}	108 ± 4.2^{ab}	149 ± 20^{abc}	152 ± 23^{abc}	201 ± 22^{c}	154 ± 19^{abc}	174 ± 12^{bc}	100 ± 4.1^{a}
13	2-Furanmethanol	34 ± 12^{ab}	29 ± 3.9^{a}	39 ± 6.9^{ab}	48 ± 7.1^{ab}	55 ± 6.9^{ab}	58 ± 6.3^{ab}	65 ± 12^{b}	100 ± 5.6^{c}
14	4-Methylthiazole	192 ± 2.3^{c}	133 ± 17^{b}	89 ± 2.2^{a}	77 ± 11^{a}	68 ± 15^{a}	63 ± 16^{a}	64 ± 12^{a}	100 ± 3.3^{ab}
15	Trimethylthiazole	107 ± 5.1^{c}	88 ± 6.2^{ab}	84 ± 1.9^{ab}	82 ± 9.3^{ab}	77 ± 2.0^{a}	73 ± 2.1^{a}	73 ± 3.5^{a}	100 ± 2.3^{bc}
16	3-Methylthiophene	96 ± 11^{bc}	82 ± 17^{abc}	78 ± 4.1^{ab}	71 ± 1.2^{a}	64 ± 3.1^{a}	73 ± 2.1^{a}	62 ± 2.3^{a}	100 ± 3.1^{c}
17	Propanoic acid	37 ± 6.2^{a}	45 ± 8.1^{ab}	59 ± 2.3^{bc}	66 ± 5.3^{c}	69 ± 4.7^{c}	66 ± 5.1^{c}	68 ± 1.1^{c}	100 ± 5.9^{d}

18	Hydroxyacetone	107 ± 5.9^{ab}	132 ± 14^{b}	103 ± 6.1^{ab}	97 ± 5.2^{ab}	108 ± 12^{ab}	89 ± 2.4^{a}	84 ± 5.1^{a}	100 ± 8.7^{ab}
19	Hexanal	710 ± 23^{c}	667 ± 36^{bc}	876 ± 20^{d}	475 ± 52^{b}	607 ± 47^{bc}	494 ± 5.2^{b}	520 ±55^{bc}	100 ± 15^{a}
20	1-Methyl-2-formylpyrrole	264 ± 6.9^{b}	131 ± 17^{a}	128 ± 5.0^{a}	99 ± 2.8^{a}	102 ± 1.7^{a}	93 ± 8.1^{a}	80 ± 12^{a}	100 ± 14^{a}
21	Pyrrole	247 ± 15^{e}	160 ± 7.2^{d}	106 ± 3.5^{c}	96 ± 10^{abc}	84 ± 5.2^{ab}	81 ± 7.9^{ab}	78 ± 3.2^{a}	100 ± 4.2^{bc}
22	1-Methylpyrrole	100 ± 1.6^{c}	55 ± 2.2^{b}	46 ± 5.6^{ab}	35 ± 7.0^{ab}	34 ± 1.1^{a}	33 ± 7.5^{a}	29 ± 2.6^{a}	100 ± 2.4^{c}
23	1-Ethylpyrrole	94 ± 5.9^{b}	52 ± 7.9^{a}	55 ± 8.7^{a}	44 ± 3.3^{a}	45 ± 4.2^{a}	42 ± 3.5^{a}	39 ± 9.1^{a}	100 ± 3.2^{b}

^{abcde} *Samples with the same letter code in any row are not significantly different ($p \geq 0.05$).*
Mean ± standard deviation

5.2.3 Summary of All Coffee Samples via Principal Component Analysis

PCA was used to illustrate the differences between the 23 aroma compounds (excluding acetic acid) across the 8 coffee samples (Figure 5.3). PC1 accounted for 68.6% of the variance in the dataset and showed a trend with increasing levels of pyrazines from left to right and Arabica (left) was clearly separated from Robusta (right). PC2 accounted for 24.7% of the variance and showed separation among the Arabica, treated Robusta and untreated Robusta samples. Pyrazines and hydroxyacetone were positively correlated with PC1 and furfural and propanoic acid were negatively correlated with PC1. There were visible differences between Arabica and Robusta, characterised by these samples being located in different corners of the PCA plot. The non-treated Robusta sample had greater levels of pyrazines when compared with the other samples. There were no discrimination among 2%, 3%, 4% and 5% acetic acid treated Robusta which were located together in the centre of the plot.

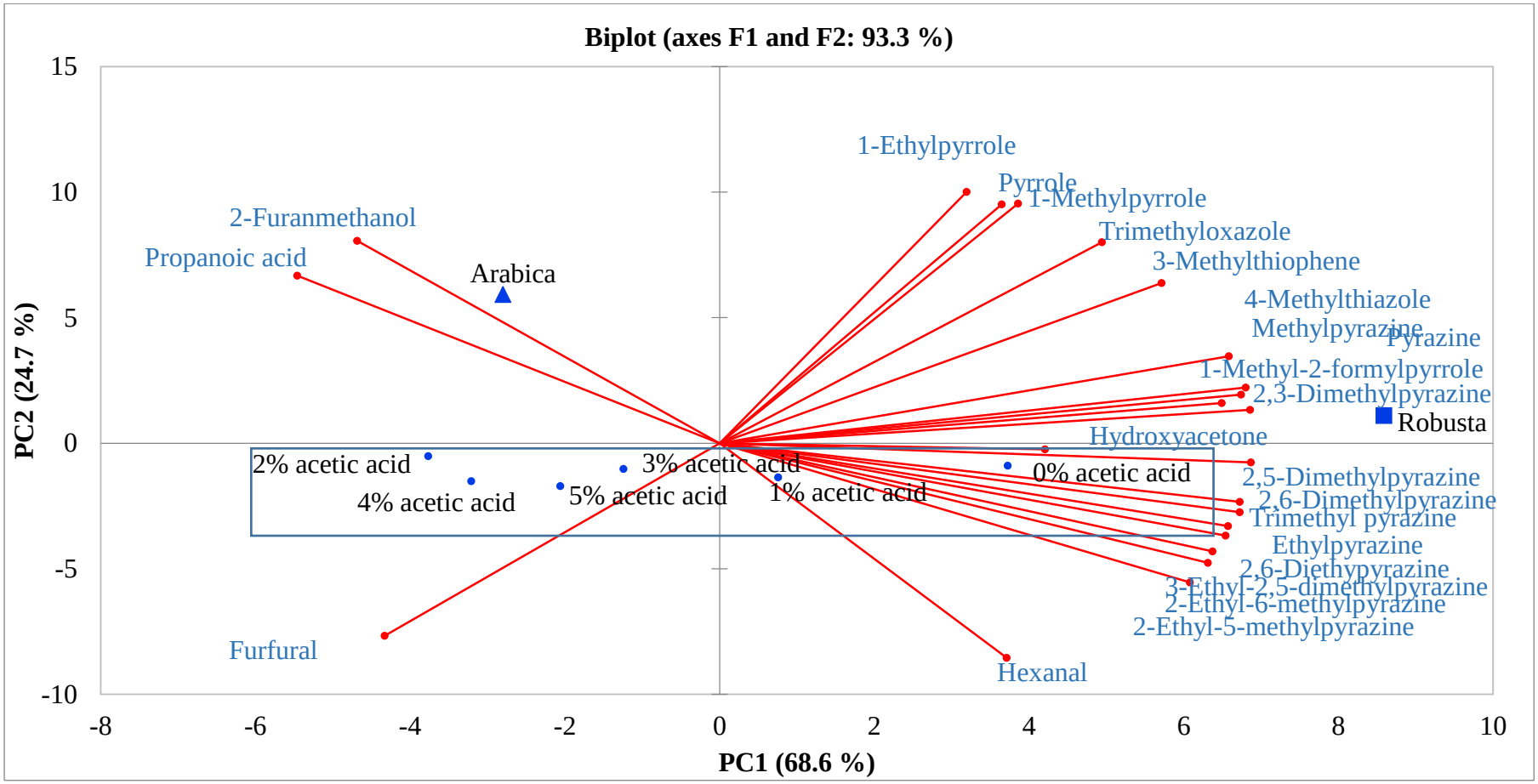


Figure 5.3 Principal component analysis (Bi-plot) of the aroma compliment of Arabica, Robusta and acetic acid treated Robusta coffee (0, 1, 2, 3, 4, and 5% acetic acid) analysed by GC-MS (n = 4).

5.2.4 Pyrazine Content in Acetic Acid Treated Robusta Samples

Pyrazines belong to a class of heterocyclic nitrogen-containing compounds which contain four carbon and two nitrogen atoms in a ring skeleton and are one of the most important roast aromas in a large number of thermal treated foods (Maga, 1992). According to Silwar and Lullmann (1993), the pyrazine formation in Arabica after roasting is lower than Robusta, this is due to the content of free amino acids being lower in green Arabica than in Robusta. Since pyrazines account for more than 14% of the total volatile compounds in roasted coffee (Flament, 2002), it is important in our study to understand pyrazine formation. A reduction in pyrazine concentration could make the aroma profile of Robusta more similar to that of Arabica.

A group of pyrazines were identified and their relative abundance is shown in Table 5.2. The relative abundance of all selected pyrazines were higher in Robusta than Arabica but were significantly reduced by acid pre-treatment (Table 5.2) ($p < 0.05$). For the four pyrazines, 2, 5-Dimethylpyrazine, 2, 6-Dimethylpyrazine, Ethylpyrazine and 2, 3-Dimethylpyrazine, there was no significant difference between the Arabica control and the 1% acetic acid treated Robusta. The relative abundance of total pyrazines was calculated and shown in Figure 5.4. The total pyrazines reduced significantly ($p < 0.05$) as the levels of acetic acid in the soaking solution increased (Figure 5.4). There was no significant difference between the Arabica and the 2%, 3%, 4% and 5% acetic acid treated Robusta samples ($p \geq 0.05$).

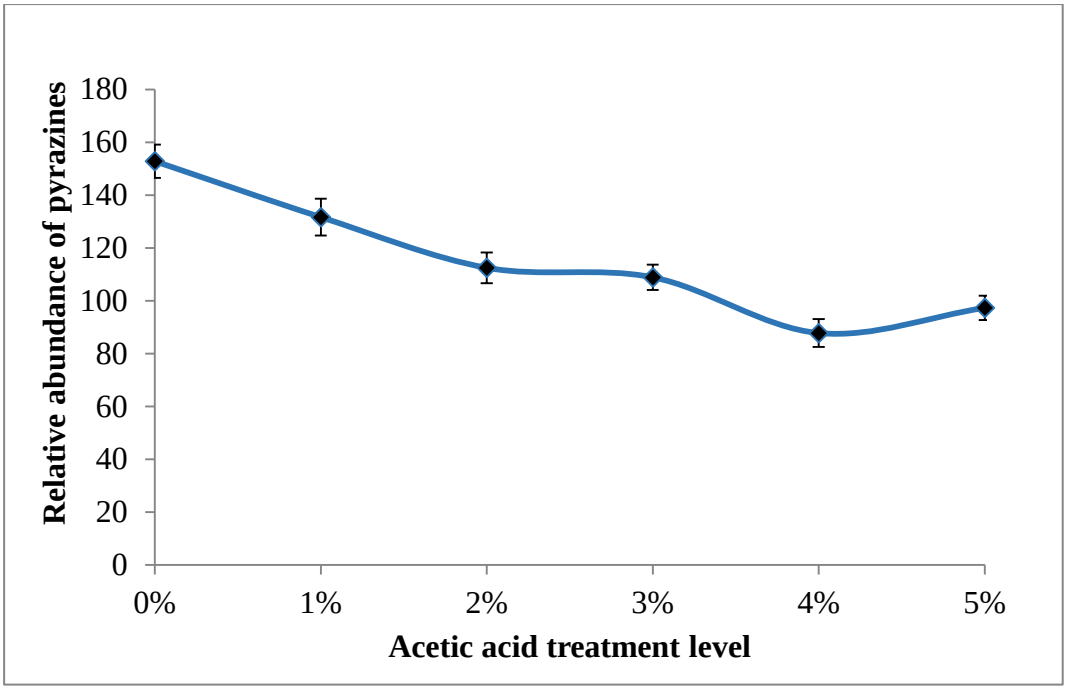


Figure 5.4 Relative abundance of pyrazines (relative to Arabica) for acetic acid treated Robusta (non-treated Robusta was 193.2 ± 1.8 (mean ± standard deviation)). Error bars represent the standard deviation (n = 4).

The first step of pyrazine generation is regarded as condensation of the carbonyl and amine groups to yield a Schiff base (Shahidi, Ho, & Van Chuyen, 2013). The Schiff base rearranges to Amadori product. The initial step formation of the Schiff base is one of the rate-limiting steps (Nursten, 2005). The reaction rate of the amine is highly dependent upon the pH of the sample, the maximum rate of most amine reactions occurs in an alkaline environment (Westheimer, 1939). This is due to the nitrogen becoming more nucleophilic at higher pH. Pre-soaking the green Robusta coffee beans with acetic acid decreased the green bean pH (from 4.86 ± 0.05 to 4.39 ± 0.01), at lower pH conditions, the rate of carbonyl-amine condensation reactions would be decreased significantly, because of the relatively lower reactivity of the protonated amine group (Wong & Bernhard, 1988). Nursten also reported a consequence of protonation and deprotonation of various moieties of the carbohydrates (Nursten, 2005). After loss of water from the amine and carbonyl

groups, fragmentation of the carbohydrate occurs through reverse aldol, enolization, and dehydration reaction (Shibamoto & Bernhard, 1977). According to the literature, reverse aldol, enolization and dehydration are all base catalysed (Shibamoto & Bernhard, 1977). These results are consistent with acidic conditions limiting the production of pyrazines by reducing the reactivity of the amino group (Koehler, 1970).

5.2.5 Furanic Compounds Content in Acetic Acid Treated Robusta Samples

Furfural and 2-furanmethanol were measured in all samples from coffee (Table 5.2). There was a significantly lower relative amount of furanic compounds in non-treated Robusta samples when compared with Arabica ($p < 0.05$). The relative abundance of furfural and 2-furanmethanol increased by about 2-fold in the 5% acetic acid treated Robusta when compared with the non-treated Robusta. The total relative abundance of furanic compounds significantly increased ($p < 0.05$) as the concentration of acetic acid in the soaking solution increased (Figure 5.5). There were no significant differences between Arabica and 3%, 4%, 5% acetic acid treated Robusta samples ($p \geq 0.05$). These results indicate that acidic conditions favoured the formation of furfural and 2-furanmethanol, which is concordant with Hodge's result. In a model system involving L-ascorbic acid and L-threonine/L-serine the total furanic compounds formation increased with increasing acidity (Yu & Zhang, 2010). Previous study done by Madruga and Mottram also indicated that the concentrations of furans and furfural increased significantly as the pH was reduced (Madruga & Mottram, 1998).

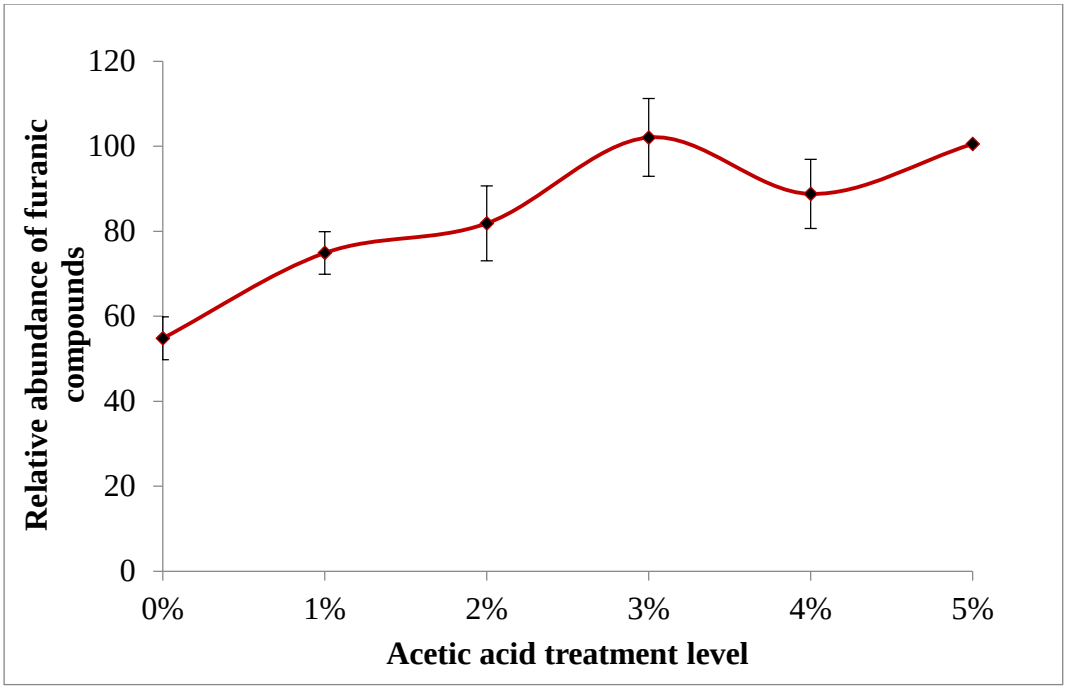


Figure 5.5 Relative abundance of furanic compounds (relative to Arabica) for acetic acid treated Robusta (non-treated Robusta was 53.2 ± 5.2 (mean ± standard deviation)). Error bars represent the standard deviation (n = 4).

Furanic compounds products are markers of sugar degradation (Heyns, Stute, & Paulsen, 1966; Nursten, 2005). According to Flamment (2002), furfural is formed by thermal degradation of glucose and was shown to be present in cysteine/glucose model systems. However, formation could also be from Amadori pentose compounds and an intermediate 3-deoxyosone. This compound provides sweet, caramel like but also cinnamon-almond like aroma.

Generally, roasted Arabica beans have higher levels of furfural and 2-furanmenthanol when compared with roasted Robusta beans, this is due to Arabica having a higher sucrose content. The increase in furfural and 2-furanmethanol by the pre-treatment method therefore reduces the difference between Arabica and Robusta beans.

5.2.6 Sulphur-Containing Compounds Content in Acetic Acid Treated Robusta Samples

Sulphur-containing compounds were identified in the samples and the relative abundance of 3-methylthiophene, trimethylthiazole and 4-methylthiazole was presented in Table 5.2. All sulphur-containing compounds were significantly reduced ($p < 0.05$) in the treated samples (Table 5.2). The relative abundance of 4-methylthiazole was significantly higher in non-treated Robusta when compared with Arabica. However, there were no significant difference between acetic acid treated Robusta and Arabica in the levels of 4-methylthiazole. Non-treated Robusta sample showed no significant difference when compared with the Arabica control in trimethylthiazole and 3-methylthiophene ($p \geq 0.05$), but acid treatment reduces its abundance. The total relative abundance of sulphur-containing compounds showed a decrease trend as the concentration of acetic acid in the soaking solution increased (Figure 5.6). However, the process control sample (0%) also indicated a significantly reduced ($p < 0.05$) level of sulphur-containing compounds compared with the Robusta control sample.

The formation of sulphur-containing volatile compounds is due to Maillard reactions and Strecker degradation involving sulphur containing amino acids. (Schenker, Heinemann, Huber, Pompizzi, Perren, & Escher, 2002). The main amino acids involved in the formation of sulphur-containing volatile compounds are cysteine and methionine (Flament, 2002; McGorrin, 2011). Since most of the main amino acid and sugar for the sulphur-containing volatile compounds are water-soluble, they could be lost during the green beans pre-treatment process, when green beans were soaked into water solution in this study.

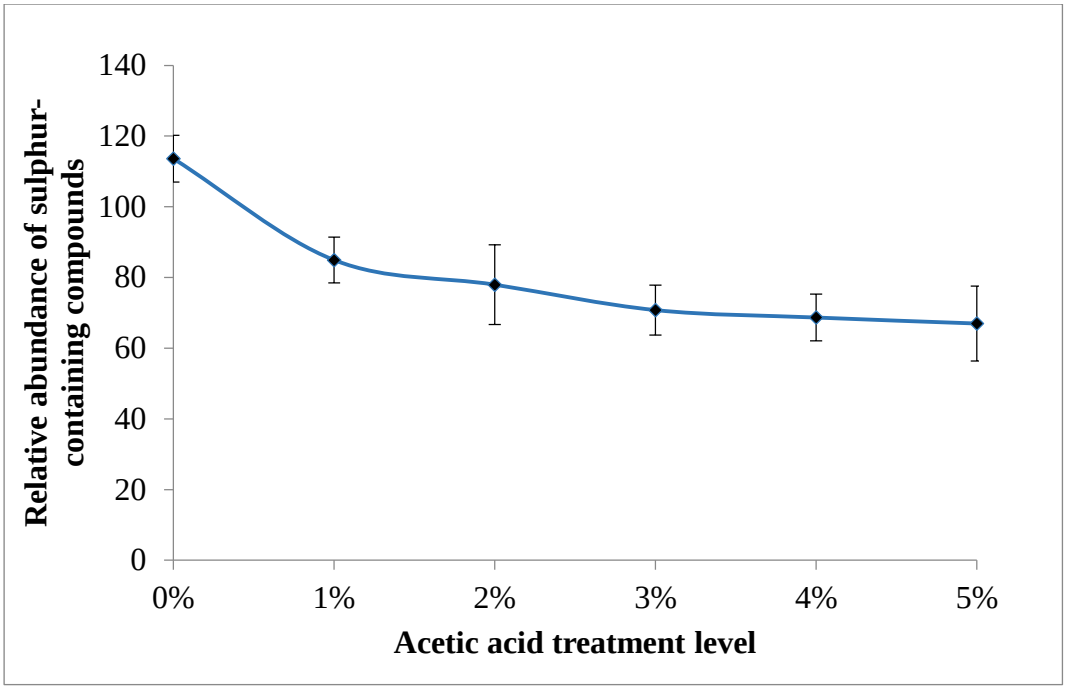


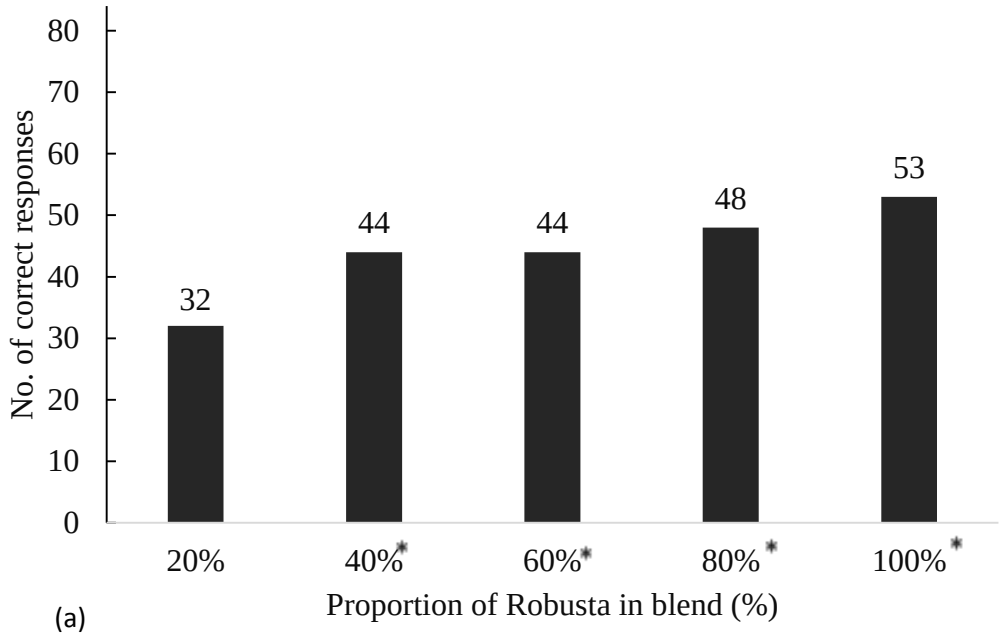
Figure 5.6: Relative abundance of sulphur-containing compounds (relative to Arabica) for acetic acid treated Robusta (non-treated Robusta sample was 142.9 ± 6.2 (mean ± standard deviation)). Error bars represent the standard deviation (n = 4).

5.2.7 Results of Sensory Analysis

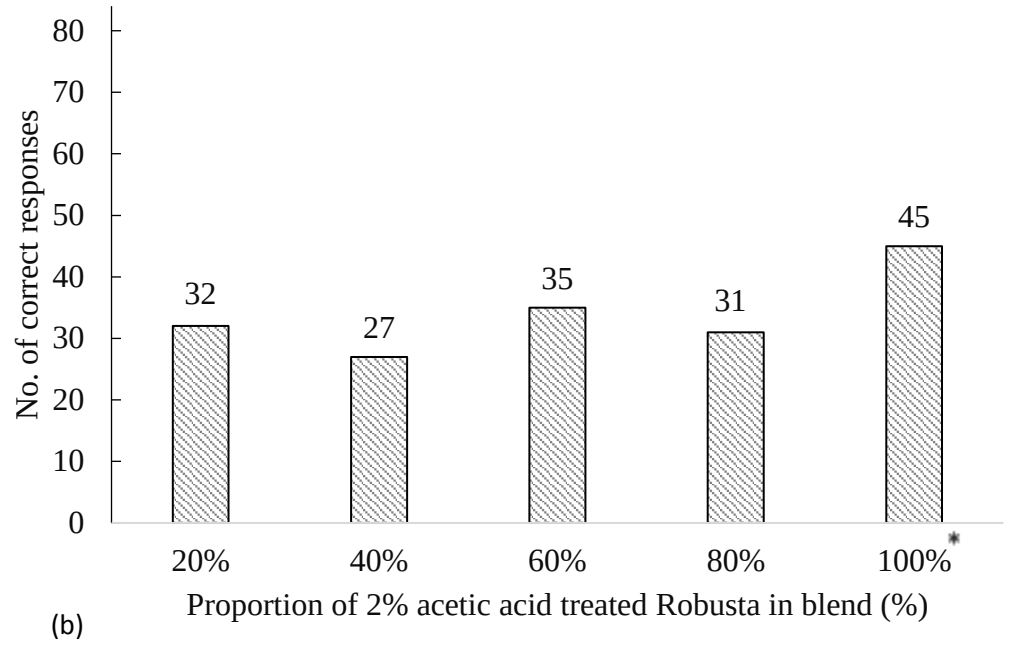
The aim of the sensory study was to determine the maximum percentage of Robusta that can be blended with Arabica without any perceived sensory difference. Triangle test has been used in this study. Robusta coffee, and 2%, 4% acetic acid treated Robusta coffee, were blended with up to 80% Arabica and compared with the Arabica control, the results for the numbers of correct responses in triangle test are shown in Figure 5.7. The Numbers of correct response indicates how many people chose the correct answer. Numbers of correct responses were compared to critical tables in ISO (ISO4120:2007); samples were classed as being similar to Arabica if the number of correct responses was less than 36 out of 84.

Sensory results of 2%, 4% acetic acid treated Robusta blend and non-treated Robusta blend were summarized in Figure 5.7. Participants could not discriminate

the aroma of Arabica and 20% Robusta blended with Arabica (number of correct responses was 32) (Figure 5.7 (a)). However, as the blending ratio of Robusta was increased to 40% and above, participants started to constantly perceive its aroma difference from 100% Arabica. When 2% acetic acid treated Robusta was used, participants could not discriminate any difference up to 80% Robusta inclusion when compared with Arabica (Figure 5.7 (b)). At 4% acetic acid treatment, 60% blending could be achieved with no significant aroma difference can be perceived from Arabica (Figure 5.7 (c)). However, when it increased to 80% and above, participants began to perceive a difference.



(a)



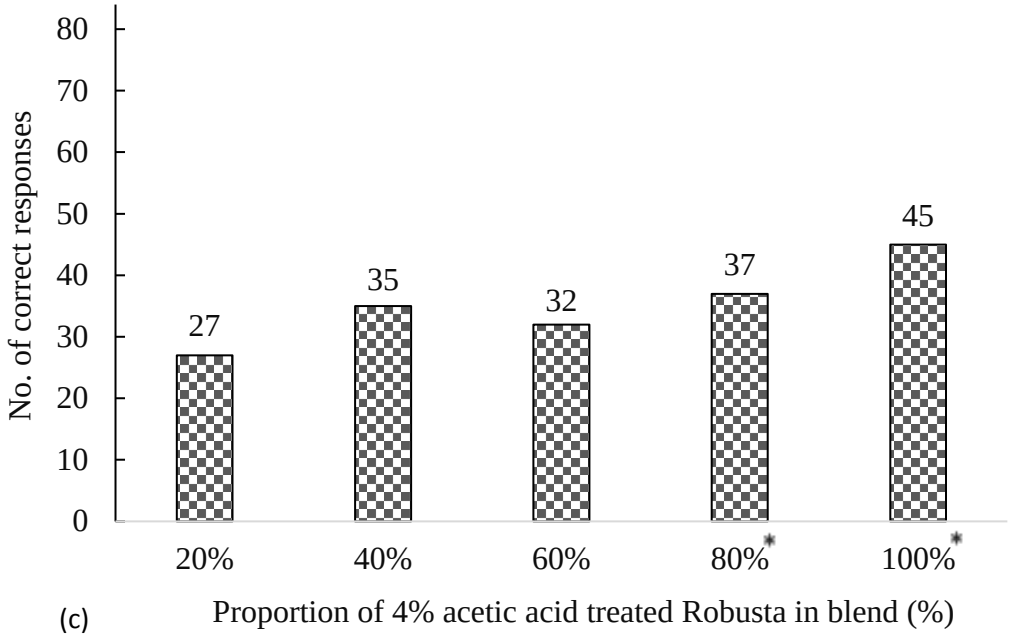


Figure 5.7: Sensory testing (triangle test) to identify similarity of 20%, 40%, 60% and 80% Robusta blends with the Arabica control (a) non-treated Robusta; (b) 2% acetic acid treated Robusta; (c) 4% acetic acid treated Robusta.

** indicates a significant difference compared with the Arabica control.*

In general, the sensory evaluation results provided meaningful evidence to conclude that both 2% and 4% acetic acid treated samples made the aroma profile more similar to Arabica, compared to the original Robusta aroma. The maximum proportion of Robusta that could be blended to Arabica increased from 20% for the non-treated coffee to 60% for the 4% acetic acid treated and 80% for the 2% acetic acid treated coffee. The treatment of Robusta with acetic acid before roasting clearly alters its aroma chemistry and allows it to be blended with Arabica in greater proportions.

5.2.8 Influence of Accelerated Shelf-life Storage on the Volatile Compounds

Thirty-four aroma compounds were identified and selected within all coffee samples (Table 5.8). The relative abundance of each volatile compounds present in the headspace of the three coffee samples stored for six weeks at 5 ºC, 25 ºC, and 35 ºC is shown in Table 5.8. All selected volatile compound showed a significant difference between the sample stored at -80 ºC for 6 weeks and 35 ºC stored for 6 weeks in Arabica coffee ($p < 0.05$). Each volatile compounds was normalised to (100%) as their original level (control -80 ºC) in each coffee. The use of relative abundance in Table 5.8 was aimed to avoid different starting points for Arabica, Robusta and treated Robusta coffee before storage as these two varieties contain different amounts of the volatile compounds after roasting. For Arabica, all compounds significantly decreased over the storage period by around 50% ($p < 0.05$). The only exception was acetic acid that increased three-fold over the six weeks' time.

Table 5.8 Average relative abundance (relative to control n = 4) of each volatile compounds presented in the headspace of the three coffee samples stored for six weeks at 5 ºC, 25 ºC, and 35 ºC. Each volatile compound relative concentration for each stored coffee was normalised to its original level in respective coffee stored at -80 ºC, which was regarded as 100%.

6 weeks	**Arabica**				**Robusta**				**Treated Robusta**			
Aroma compounds	**Control -80 ºC**	**5 ºC**	**25 ºC**	**35 ºC**	**Control -80 ºC**	**5 ºC**	**25 ºC**	**35 ºC**	**Control -80 ºC**	**5 ºC**	**25 ºC**	**35 ºC**
Pyrazine	100^{d}	83^{b}	79^{b}	53^{a}	100^{a}	93^{a}	83^{a}	78^{a}	100^{a}	107^{a}	114^{a}	102^{a}
Methylpyrazine	100^{c}	76^{b}	68^{b}	43^{a}	100^{a}	88^{a}	96^{a}	104^{a}	100^{a}	107^{a}	102^{a}	92^{a}
2,5-Dimethylpyrazine	100^{d}	74^{b}	65^{b}	41^{a}	100^{a}	88^{a}	81^{a}	79^{a}	100^{a}	104^{a}	96^{a}	99^{a}
2,6-Dimethylpyrazine	100^{c}	75^{b}	66^{b}	42^{a}	100^{a}	86^{a}	87^{a}	75^{a}	100^{a}	103^{a}	94^{a}	97^{a}
Ethylpyrazine	100^{c}	79^{b}	70^{b}	45^{a}	100^{b}	85^{a}	82^{a}	76^{a}	100^{a}	105^{a}	100^{a}	89^{a}
2,3-Dimethylpyrazine	100^{c}	78^{b}	69^{b}	44^{a}	100^{b}	79^{a}	82^{a}	87^{a}	100^{b}	100^{b}	93^{ab}	87^{a}
2-Ethyl-6-methylpyrazine	100^{c}	81^{b}	75^{b}	49^{a}	100^{b}	86^{a}	86^{a}	87^{a}	100^{b}	94^{ab}	96^{ab}	85^{a}
2-Ethyl-5-methylpyrazine	100^{c}	88^{bc}	78^{b}	52^{a}	100^{a}	85^{a}	91^{a}	71^{a}	100^{a}	99^{a}	103^{a}	88^{a}
Trimethylpyrazine	100^{c}	83^{bc}	77^{b}	53^{a}	100^{b}	79^{a}	81^{a}	84^{a}	100^{b}	86^{ab}	92^{ab}	82^{a}
2,6-Diethylpyrazine	100^{b}	87^{ab}	94^{b}	67^{a}	100^{b}	88^{a}	85^{a}	72^{a}	100^{a}	83^{a}	94^{a}	88^{a}
2,3-Dimethyl-6-ethylpyrazine	100^{b}	89^{ab}	94^{b}	67^{a}	100^{a}	87^{a}	85^{a}	72^{a}	100^{a}	90^{a}	94^{a}	87^{a}
2-Methylfuran	100^{c}	59^{b}	42^{a}	31^{a}	100^{b}	80^{a}	65^{a}	64^{a}	100^{b}	106^{b}	59^{a}	63^{a}
2,5-Dimethylfuran	100^{d}	62^{c}	44^{b}	29^{a}	100^{b}	73^{a}	64^{a}	59^{a}	100^{b}	105^{b}	64^{a}	65^{a}
2,3,5-Trimethylfuran	100^{d}	75^{c}	54^{b}	35^{a}	100^{b}	83^{ab}	76^{a}	67^{a}	100^{b}	106^{b}	73^{a}	74^{a}
2-Vinylfuran	100^{d}	64^{c}	42^{b}	24^{a}	100^{b}	82^{ab}	78^{ab}	67^{a}	$100^{\mathbf{b}}$	102^{b}	79^{a}	70^{a}

2-Butylfuran	100^{c}	82^{b}	69^{b}	47^{a}	100^{a}	98^{a}	101^{a}	82^{a}	100^{a}	101^{a}	103^{a}	106^{a}
2,3-Butanedione	100^{c}	58^{b}	43^{a}	34^{a}	100^{c}	80^{b}	65^{ab}	57^{a}	100^{b}	104^{b}	62^{a}	59^{a}
2,3-Pentanedione	100^{c}	66^{b}	53^{ab}	41^{a}	100^{b}	75^{a}	68^{a}	56^{a}	100^{b}	110^{b}	68^{a}	61^{a}
2,3-Hexanedione	100^{d}	65^{c}	50^{b}	35^{a}	100^{c}	73^{b}	76^{b}	58^{a}	100^{b}	101^{b}	77^{a}	72^{a}
1H-pyrrole,1-methyl-	100^{d}	70^{c}	53^{b}	36^{a}	100^{b}	76^{a}	70^{a}	76^{a}	100^{b}	103^{b}	67^{a}	62^{a}
Pyridine	100^{d}	80^{b}	77^{b}	52^{a}	100^{a}	93^{a}	89^{a}	80^{a}	100^{a}	109^{a}	113^{a}	95^{a}
1-Ethylpyrrole	100^{d}	67^{c}	55^{b}	32^{a}	100^{b}	81^{a}	72^{a}	70^{a}	100^{b}	99^{b}	77^{a}	72^{a}
3-Methylpyridine	100^{c}	35^{b}	32^{ab}	20^{a}	100^{b}	19^{a}	19^{a}	20^{a}	100^{b}	44^{a}	19^{a}	15^{a}
3-Ethylpyridine	100^{c}	73^{bc}	66^{b}	36^{a}	100^{a}	89^{a}	64^{a}	76^{a}	100^{a}	112^{a}	114^{a}	97^{a}
2,5-Dimethylpyrrole	100^{c}	72^{bc}	68^{b}	32^{a}	100^{b}	94^{b}	102^{b}	85^{a}	100^{b}	102^{b}	78^{a}	64^{a}
Acetic acid	100^{a}	148^{b}	293^{c}	299^{c}	100^{a}	157^{b}	232^{c}	225^{c}	100^{a}	127^{b}	184^{c}	198^{c}
Hexanal	100^{b}	73^{a}	105^{bc}	109^{c}	100^{a}	71^{a}	95^{a}	104^{a}	100^{a}	86^{a}	97^{a}	93^{a}
Furfural	100^{c}	84^{b}	79^{b}	62^{a}	100^{a}	113^{a}	118^{b}	106^{a}	100^{a}	117^{b}	116^{b}	111^{b}
5-Methylfurfural	100^{c}	80^{b}	75^{b}	52^{a}	100^{a}	109^{ab}	104^{a}	104^{a}	100^{a}	106^{a}	101^{a}	111^{b}
2-Methylbutanal	100^{c}	70^{b}	58^{ab}	50^{a}	100^{b}	72^{a}	68^{a}	61^{a}	100^{b}	96^{b}	72^{a}	62^{a}
2-Methylthiophene	100^{d}	68^{c}	49^{b}	32^{a}	100^{b}	73^{a}	69^{a}	70^{a}	100^{b}	99^{b}	74^{a}	73^{a}
2-Furanmethanol acetate	100^{c}	82^{b}	71^{b}	47^{a}	100^{a}	102^{a}	117^{b}	95^{a}	100^{a}	116^{c}	114^{b}	110^{a}
2-Furanmethanol	100^{b}	108^{bc}	124^{c}	84^{a}	100^{a}	133^{b}	190^{c}	146^{b}	100^{a}	125^{b}	184^{c}	126^{b}
furfuryl methyl ether	100^{d}	75^{c}	56^{b}	34^{a}	100^{a}	96^{a}	103^{a}	84^{a}	100^{a}	107^{a}	106^{a}	99^{a}

^{abcd} *Samples with the same letter code in any row are not significantly different*

All eleven pyrazines significantly reduced over the storage time and temperature for Arabica (Table 5.8) ($p < 0.05$). For the non-treated Robusta, Ethyl pyrazine, 2, 3-Dimethylpyrazine, 2-Ethyl-6-methylpyrazine, Trimethylpyrazine, 2, 6-Diethylpyrazine significantly decreased over the six weeks at all storage temperature ($p < 0.05$). However, the 2% acetic acid treated Robusta only showed a significant decrease at 35 ºC in the 2-Ethyl-6-methylpyrazine, Trimethylpyrazine and 2, 3-Dimethylpyrazine. The change in relative concentration for each functional group is shown in Figure 5.9 to Figure 5.14 for the 35 ºC. In Figure 5.9 the concentrations of total pyrazines reduced significantly as the storage time increased in Arabica, more than 50% was lost after 4 week storage. For the non-treated Robusta samples, pyrazine significant decreased by 15% after four weeks storage at 35 ºC. There was no significant reduction in total pyrazines in the treated Robusta sample.

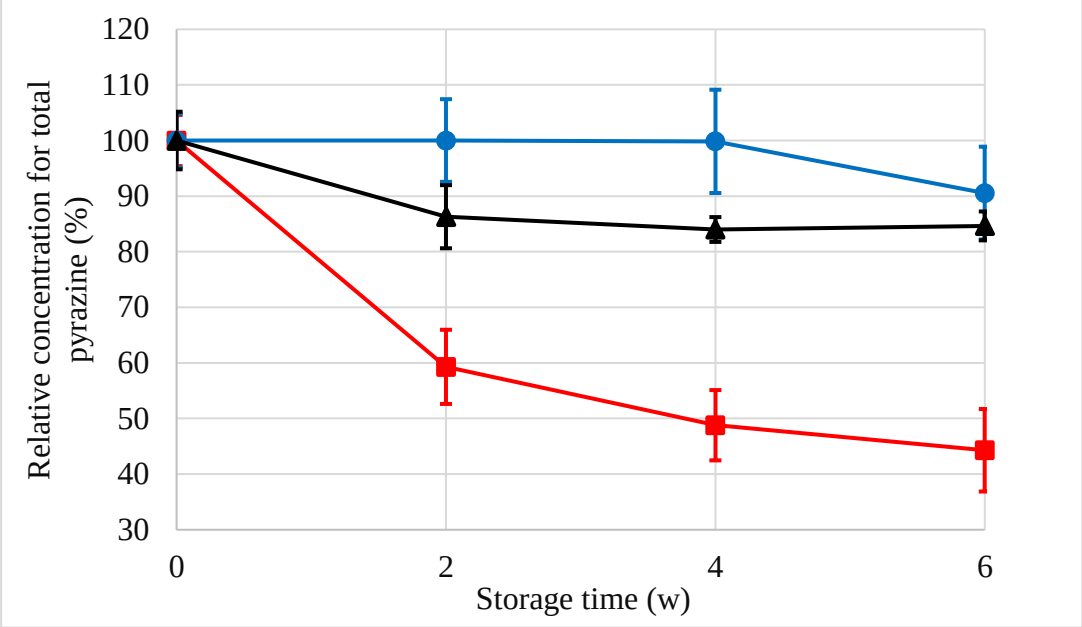


Figure 5.9 The effect of storage time (35 ºC) on the relative concentration of total pyrazines at week zero, two, four and six for three coffee samples; Arabica coffee beans (red square markers), Robusta coffee beans (black triangle markers) and treated Robusta coffee beans (blue circles). Error bar represents the standard deviation (n = 4).

All five furans significantly decreased with storage time and temperature increased in Arabica coffee by 53% - 76% over the six weeks period ($p < 0.05$). Non-treated Robusta coffee showed a significant decrease in 2-Methylfuran and 2, 5-Dimethylfuran at all storage temperature and 2-Vinylfuran only showed a significant decrease at 35 ºC storage temperature, which decreased by 33% over six weeks storage time ($p < 0.05$) (Table 2). For the treated Robusta, 2-methylfuran, 2-vinylfuran, 2, 5-dimethylfuran and 2, 3, 5-trimethylfuran only presented a significant decrease at 25 ºC and 35 ºC storage temperature during six week storage. There was no significant difference between the non-treated Robusta and treated Robusta for 2-butylfuran over the storage time and at different temperatures ($p > 0.05$).

Total furans significantly decreased (by around 50%) with storage time and temperature increased in Arabica coffee over the six week period ($p < 0.05$) (Figure 5.10). The relative concentration of total furans significantly decreased ($p < 0.05$) over 4 weeks storage in the non-treated Robusta at 35 ºC, however the treated Robusta coffee samples was more stable and only showed a significant decrease after six weeks of storage.

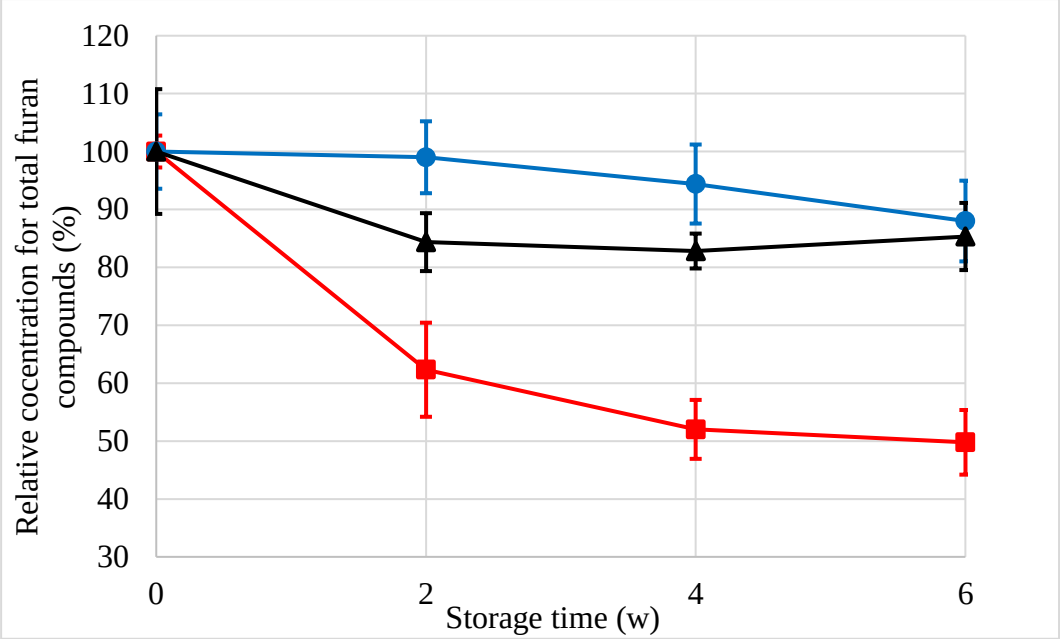


Figure 5.10 the effect of storage time (35 ºC) on the relative concentration of total furan compounds at week zero, two, four and six for three coffee samples; Arabica coffee beans (red square markers), Robusta coffee beans (black triangle markers) and treated Robusta coffee beans (blue circles).Error bar represents the standard deviation (n = 4) .

All three ketones (2, 3-butanedione, 2, 3-pentanedione and 2, 3-hexanedione) indicated a significant reduction in both Arabica and non-treated Robusta samples during 6 weeks storage across all storage temperature ($p < 0.05$). In the treated Robusta sample, no significant decrease can be observed during 6 weeks stored at 5 ºC. While, there was a significant reduction at 25 ºC and 35 ºC in all three ketones ($p < 0.05$).

In Arabica, non-treated Robusta and treated Robusta coffee samples, the relative concentrations of total ketone reduced significantly over storage time (Figure 5.11). However, the treated Robusta samples was more stable with no significant loss for the first two weeks at storage temperature 35 ºC ($p \geqslant 0.05$).

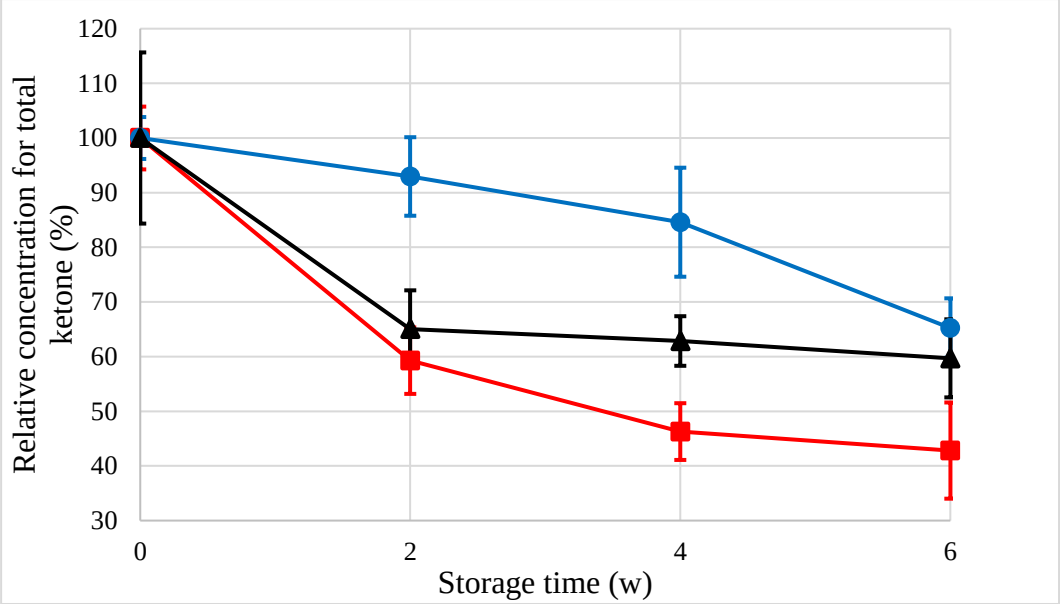


Figure 5.11 the effect of storage time (35 ºC) on the relative concentration of total ketones at week zero, two, four and six for three coffee samples; Arabica coffee beans (red square markers), Robusta coffee beans (black triangle markers) and treated Robusta coffee beans (blue circles). Error bar represents the standard deviation (n = 4).

All six heterocycle N-containing compounds reduced significantly in Arabica samples during 6 weeks storage across all storage temperature (Table 5.8) ($p < 0.05$). Non-treated Robusta showed a significant but smaller decrease in most of the heterocycle N-containing compounds (exclude pyridine) during the 6 week storage period ($p < 0.05$). The relative concentration of pyridine compounds in both non-treated Robusta and treated Robusta showed no significant changes after six weeks storage ($p > 0.05$). 1H-pyrrole, 1-methyl, 1-ethylpyrrole and 2,5-dimethylpyrrole only showed a significant decrease after six weeks stored at 25 ºC and 35 ºC ($p < 0.05$). No significant decease can be observed in treated Robusta sample after six week stored at 5 ºC ($p > 0.05$).

The total concentration of heterocycle N-containing compounds reduced significantly (50%) during storage at 35 ºC in the Arabica sample ($p< 0.05$) (Figure 5.12). Untreated Robusta had a significantly lower loss (15%) of N-containing

heterocycle compounds at week four and six as compared to control ($p < 0.05$). However, treated Robusta coffee samples were much more stable and only showed a decrease (~ 10%) after week six ($p < 0.05$).

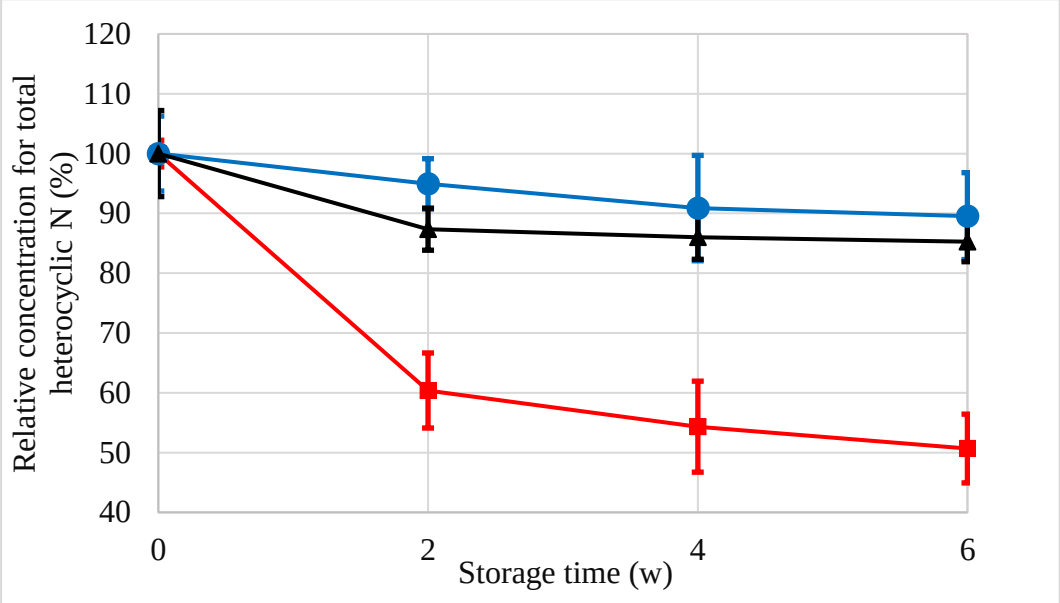


Figure 5.12 the effect of storage time (35 ºC) on the relative concentration of total heterocyclic N-containing compounds at week zero, two, four and six for three coffee samples; Arabica coffee beans (red square markers), Robusta coffee beans (black triangle markers) and treated Robusta coffee beans (blue circles).Error bar represents the standard deviation (n = 4).

The relative concentration of acetic acid significantly increased ($p < 0.05$) as the storage time increased in all coffee samples, the total concentration increased by two to three fold over the six week time course. Arabica coffee had the greatest increase in acetic acid content compared to the Robusta coffee and the treated Robusta coffee after storage (Figure 5.13). Previous studies on the staling and rancidity in coffee concluded that the volatile compounds (such as furfural, acetaldehyde) can be oxidised to the corresponding volatile acids during coffee storage period (Bengis, 1936; Elder, 1937). As Arabica coffee lost the greatest amount of furfural during storage compared with both treated Robusta and non-treated Robusta (Table 2). Therefore the highest volatile acids formation in Arabica

coffee during coffee storage could due to the oxidation of furfural. The following results suggested that oxidation of aroma constituents may be happening during storage.

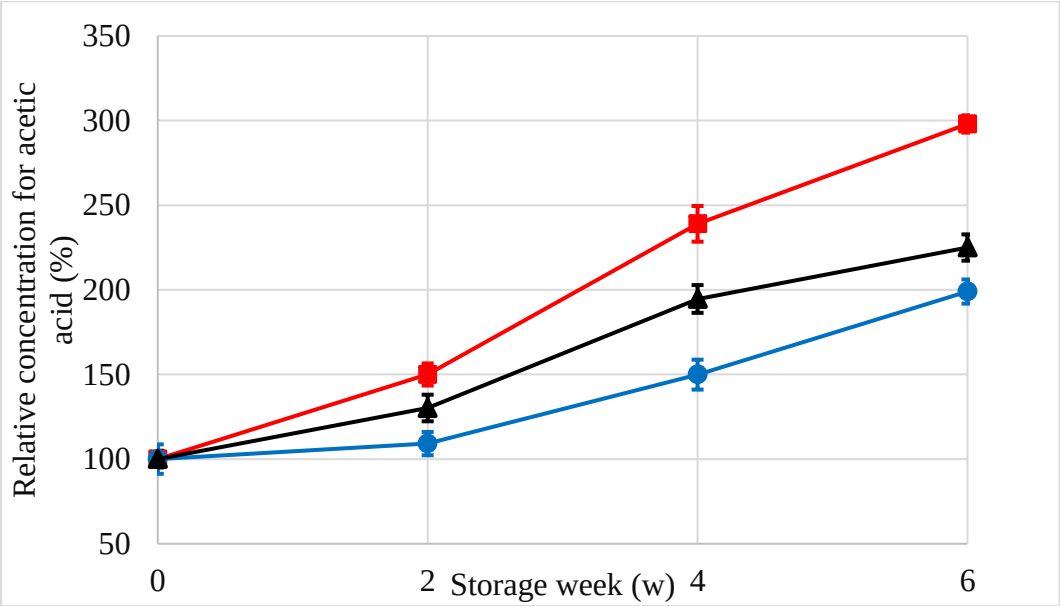


Figure 5.13 the effect of storage time (35 ºC) on the relative concentration of acetic acid at week zero, two, four and six for three coffee samples; Arabica coffee beans (red square markers), Robusta coffee beans (black triangle markers) and treated Robusta coffee beans (blue circles). Error bar represents the standard deviation (n = 4).

It is known a number of aldehydes are derived from the oxidation of polyunsaturated fatty acids, such as linoleic acid, which are abundant in coffee (Toledo, Pezza, Pezza, & Toci, 2016). Hexanal is a secondary marker for lipid oxidation, and therefore, changes in hexanal were used to monitor degradation of lipid in coffee beans (Buffo & Cardelli-Freire, 2004). Hexanal only showed a significant increase at 35 ºC stored for six weeks when compare with week zero for Arabica coffee samples ($p < 0.05$) (Table 5.8). However, it only increased by 8%. No significant difference was observed in both Robusta and treated Robusta coffee at the different storage time and temperature ($p > 0.05$). No change in hexanal

content was observed in all coffee samples when stored at 5 ºC and 25 ºC for six weeks.

The total aldehydes decreased over the 6 week storage trial for all coffee samples ($p < 0.05$). However, no significant difference was observed when comparing week zero and week two in the treated Robusta samples ($p \geq 0.05$). The non-treated Robusta and treated Robusta showed less of decrease than the Arabica and were similar to each other.

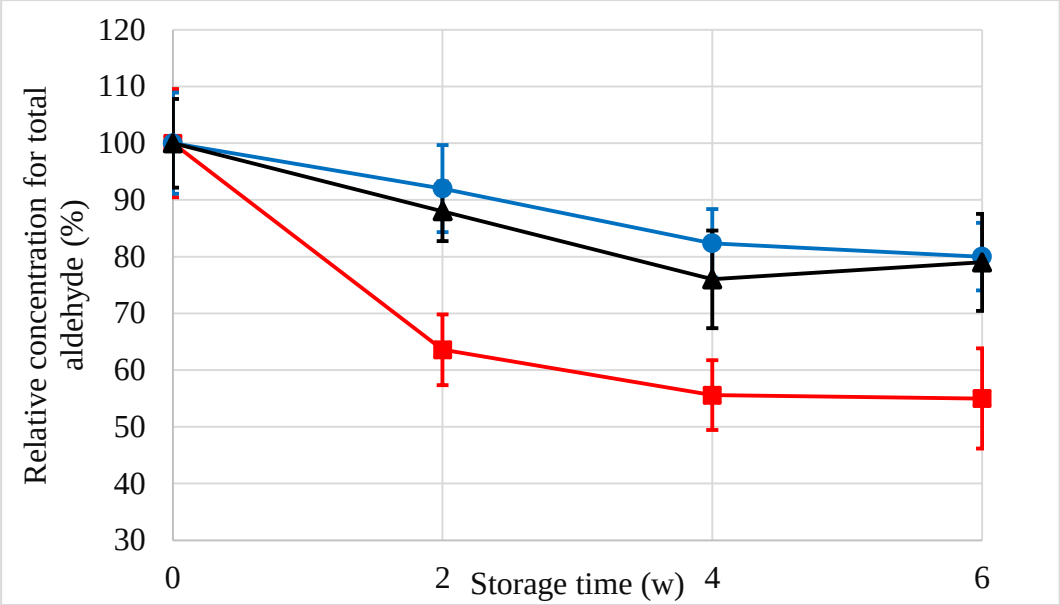


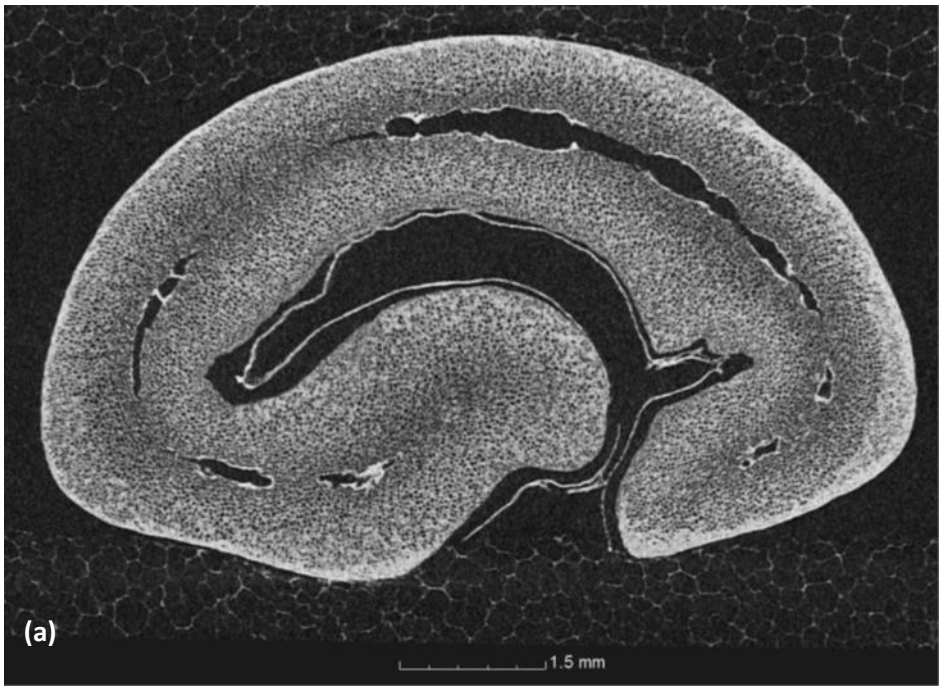
Figure 5.14 the effect of storage time (35 ºC) on the relative concentration of total aldehyde at week zero, two, four and six for three coffee samples; Arabica coffee beans (red square markers), Robusta coffee beans (black triangle markers) and treated Robusta coffee beans (blue circles). Error bar represents the standard deviation (n = 4).

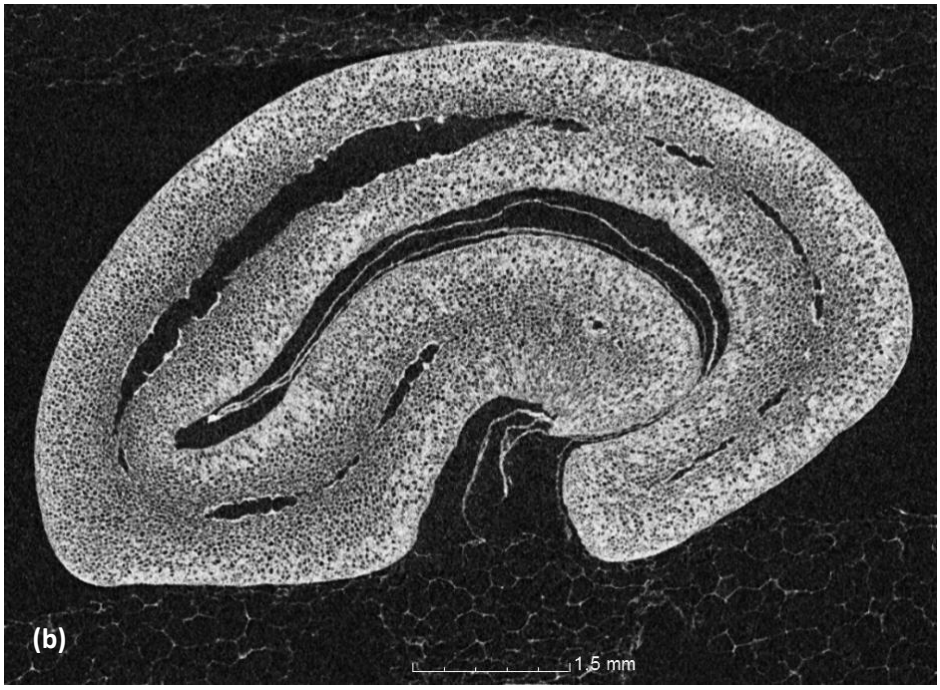
In summary, whilst a significant difference in volatiles compounds was associated with different varieties (i.e. Arabica and Robusta) and noticeable differences were shown by the treatment process. The concentrations of total pyrazine, furan, ketone, N-heterocycle compounds, and total aldehyde all decreased significantly during storage time for all coffee samples, apart from acetic acid that increased. Most of the volatiles in Arabica coffee showed a greater loss over storage when compared

to the non-treated Robusta and coffee bean pre-treatment with acetic acid enhanced aroma stability in the roasted coffee Robusta bean over the course of the shelf life stability test.

5.2.9 Results of X-ray Analysis

X-ray micro tomography imaging successfully allowed the quantification of coffee bean microstructure at high resolution. Without any physical destruction, a cross section view of the vertical slice for treated Robusta coffee, Robusta coffee and Arabica coffee was obtained from CT scan, as demonstrated in Figure 5.15. The brighter shades of grey shown are the higher density coffee bean matrix, and the darker areas inside the coffee beans indicated pores. These images were used to analyse the average size of the pores and the coffee bean porosity.





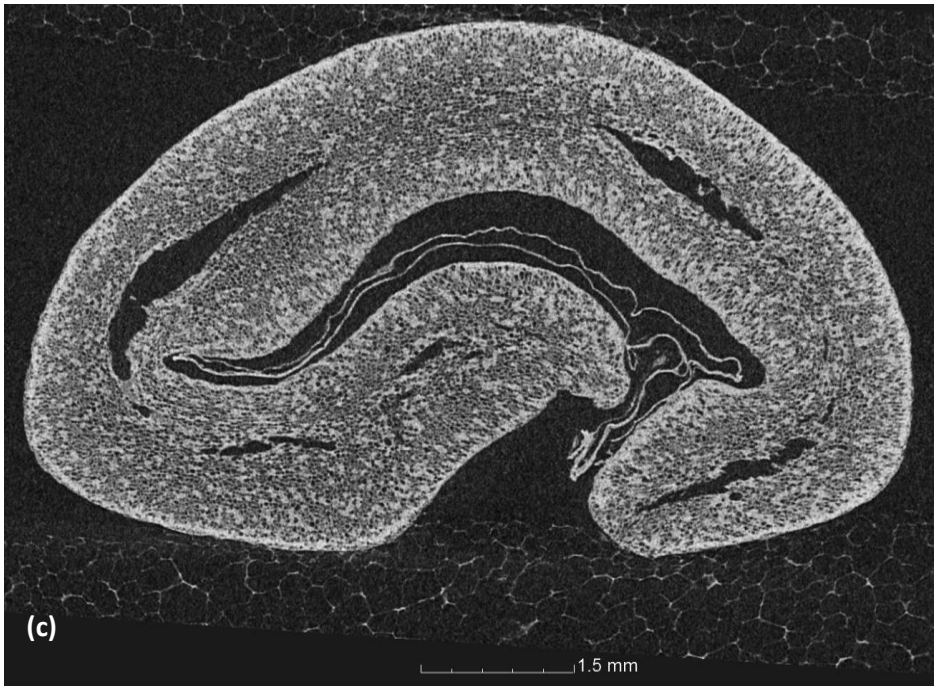


Figure 5.15 Image from a lateral layer slice of (a) acetic acid treated Robusta coffee (b) Robusta coffee and (c) Arabica coffee with 1.5 mm scale indicator

Based on the image analysis results obtained from an X-ray CT scan (Figure 5.15).Table 5.16 illustrated the total number of pores and the average pore size for

Arabica, Robusta and treated Robusta green beans. The average pore size for Arabica coffee (0.0018 ± 0.0006 mm^2) was significantly larger ($p < 0.001$ by ANOVA) than that of the treated Robusta (0.0012 ± 0.0004 mm^2) and Robusta (0.0008 ± 0.0004 mm^2). Arabica coffee beans were significantly more porous (6.69E+06) as compared to Robusta coffee beans (3.92E+06) and treated Robusta coffee beans (5.20E+06). That may explain why Arabica coffee lost more volatiles than Robusta during the ASL test.

Table 5.16 illustrated the total number of pores (average n = 3), average pore size and standard deviation for Arabica, non-treated robusta and treated robusta green beans

	Total Number of Pores	Average Pore Size mm^2	Standard Deviation
Arabica	6.69E+06	0.0018	0.0006
Robusta	3.92E+06	0.0008	0.0004
Treated Robusta	5.20E+06	0.0012	0.0004

Moreover, treated Robusta indicated a significantly larger average pore size ($p < 0.001$ by ANOVA) and significantly more porous ($p < 0.001$ by ANOVA) than that of non-treated Robusta. It may due to water uptake and loss during the pre-soaking treatment stage, which may have increased porosity and affected the structure of the Robusta green beans. However it should be noted that during the soaking process, green beans soaked in the acetic acid solution for 2 h at 20 ºC. The moisture content in green bean after soaking was increased from 12% to 75% (Chapter 4). There was 63% of water has been permeated into the green beans. After soaking, the whole green bean size has been enlarged. While after drying, it was back to the normal size. Furthermore, the treated beans had a similar bulk density compared with non-treated green beans, the internal structure of the beans may have changed, which agreed with previous study carried out by Davis & Gordon (1982). Figure

5.17 indicated the pore size distribution for Arabica, Robusta and treated Robusta coffee beans and showed the highest frequency pore size for all coffee beans is 0.0001mm^2.

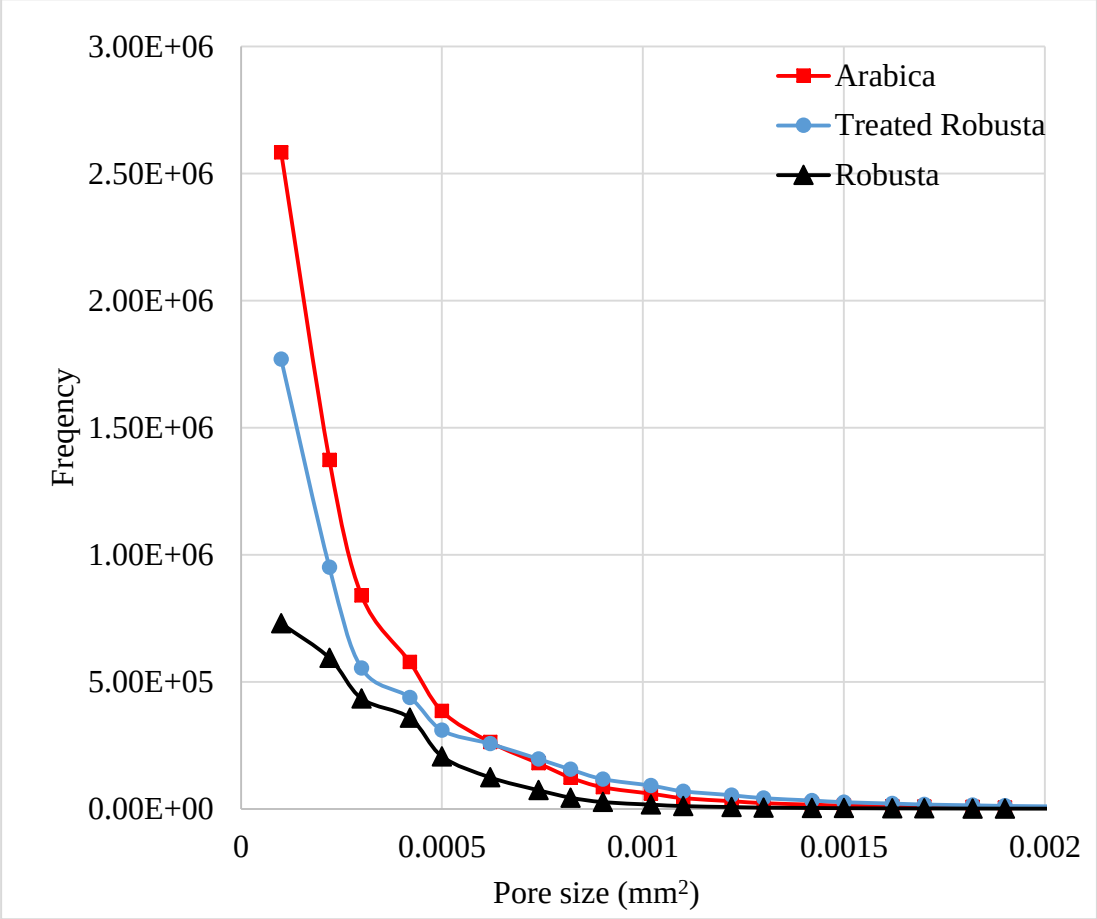


Figure 5.17 Scatter plot illustrated the Frequency against pore size (mm) of the Arabica coffee beans (square markers), Robusta coffee beans (triangle markers) and treated Robusta coffee beans (circles) (three replicates each).

The spatial distribution of porosity from the top to the bottom of the coffee is shown in Figure 5.18. It was evident from Figure 5.18, that there is a higher porosity in the centre (3 - 5 mm) of the coffee beans than the edges. In the centre of the beans (4mm), the treated Robusta coffee beans (39% ± 1.6%) and Arabica coffee beans (30% ± 2.3%) contained significant higher porosity ($p < 0.05$) than that of the non-treated Robusta coffee beans (23.4% ± 1.4%). However at the edges of the bean (both top and bottom), treated Robusta and non-treated Robusta contained

significant lower porosity ($p < 0.05$) than that of the Arabica coffee beans. Treated Robusta coffee beans had the lowest porosity at the edge of the bean (1, 2 and 7 mm) ($p < 0.05$), which may led to a lower loss of volatile compounds through volatilisation and further explain the increased aroma stability during storage.

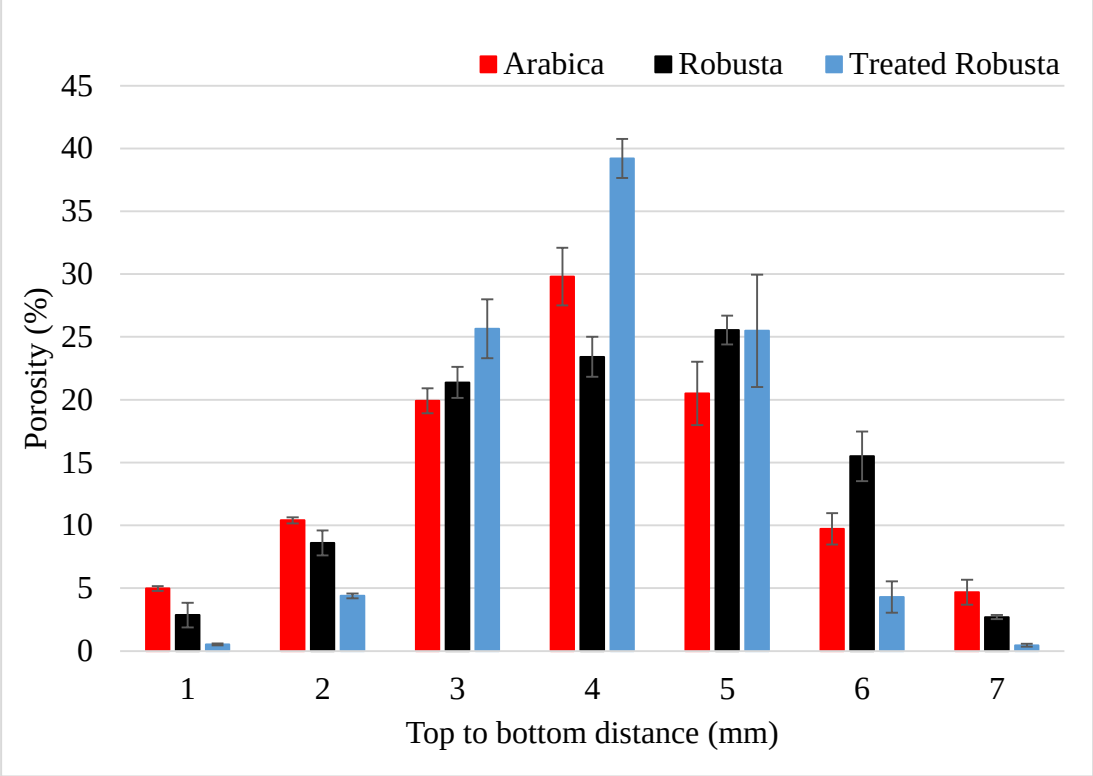


Figure 5.18 column chart of porosity (%) cutting from top to bottom of Arabica coffee beans, Robusta coffee beans and treated Robusta coffee beans (three replicates each).

5.3 Conclusion

In conclusion, this project has successfully developed a pre-treatment method for Robusta coffee with an improved aroma profile that is closer to Arabica's aroma. Principal component analysis based on analytical results showed the acetic acid treated Robusta coffee had a closer aroma profile to Arabica coffee. Sensory test results validate this finding, which showed that acid treated Robusta had a similar perceived aroma as Arabica. The maximum permissible blending proportion of

Robusta increased from 20% for the non-treated coffee to 60% for the 4% acetic acid and 80% for the 2% of acetic acid treated coffee. The acetic acid treatment before roasting evidently modifies its aroma profile and allows it to be blended with Arabica in larger proportions.

The concentrations of most aroma compounds (total pyrazine, furan, ketone, N-Heterocycle compounds, and total aldehydes) decreased significantly during storage for all coffee samples (acetic acid showed a significant increase). Most of the volatiles in Arabica coffee showed a greater loss over storage when compared to the Robusta coffee. The pre-treatment process that was applied to the Robusta coffee led to a significant increase in aroma stability over shelf life. This may be partially explained by changes in the internal pore structure. By using high resolution μCT imaging we successfully demonstrated that the treated Robusta coffee beans and Arabica coffee beans had significantly higher porosity compared to the non-treated Robusta coffee beans (in the centre). Spatial distribution of the coffee bean microstructure was also revealed, showing that treated Robusta coffee beans had a lower porosity at the edge of the bean which may have prevented volatiles being lost during storage and therefore enhance the aroma stability.

Chapter 6 Modifying Robusta Coffee Aroma by Green Bean Sugar Pre-Treatment

6.1 Introduction

The aroma of coffee is directly related to the chemical composition of the green coffee beans and typical coffee aromas are developed during the roasting process (detail explained in Chapter 1). Controlling sugars, amino acids and processing will therefore influence the aroma generation and the final flavour of the coffee (Wong, Aziz, & Mohamed, 2008). For example, a reaction comprising proline amino acid with reducing sugars is well known to generate roasty popcorn like aromas when thermally treated whereas a cysteine amino acid - glucose thermally treated reaction produces sulphuric roasted caramel like notes (Hofmann & Schieberle, 1997).

Sucrose is considered significant for the organoleptic qualities of coffee. Green Arabica coffee contains 6.2% sucrose (dry weight), whilst Robusta contains 3.2% (Chapter 3). However, while most sucrose is lost during roasting process, the higher sucrose content results in an enhanced aroma formation for Arabica (Farah, 2012). In Argentina, Spain and Singapore, there is a special type of roasted coffee called Torrefacto, it is produced by roasting whole beans with sucrose or glucose (maximum proportion is around 15% of added sugar during roasting process) (Wrigley, 1988). The sugar supplement in this treatment did not increase the sweetness of the coffee brew but it protects the beans from oxidation by forming a thin sugar film on the surface and speeds up the Maillard reaction (Wrigley, 1988). This procedure has also been demonstrated to mask the poor quality of low grade beans, especially Robusta (Wrigley, 1988).

In the research reported in this chapter, green beans were pre-soaked in three types of sugar solution (sucrose, glucose and fructose) at a range of concentrations (0 – 15%). The hypothesis of this chapter is that modify sugar content in green beans could modify the aroma formation in Robusta coffee, which may divert aroma chemistry reaction pathways that occur during thermal treatment (roasting) with the aim of increasing the aroma similarity between Arabica and Robusta coffee. Aroma analysis was done by GC-MS with SPME. Sensory analysis was performed to determine the maximum percentage of Robusta or treated Robusta that can be blended with Arabica without any perceived sensory difference. Accelerated shelf life test was also performed to define the impact of green bean pre-treatment on aroma stability during storage.

6.2 Results and Discussion

6.2.1. Levels of Sugar and Colour Compared Among Non-Treated Robusta, Sugar Treated Robusta and Arabica Coffee

The sugar content in the green coffee beans and the colour of the roasted coffee beans were presented in Table 6.1. Non-treated Robusta had significantly lower concentrations of sucrose when compared with Arabica (respectively: 3.20% ± 0.38; 6.20% ± 0.10) ($p < 0.05$). There was no significant difference in the glucose concentration between Arabica and non-treated Robusta ($p \geq 0.05$). However, the fructose concentration in the non-treated Robusta (0.76% ± 0.20) was significantly higher than Arabica (0.13% ± 0.06).

Table 6.1: average sugar content, Lightness (L), a, b and Total colour difference (E) in 16 treated Robusta, non-treated Robusta and Arabica coffee samples (0%: process control Robusta; S: sucrose treated Robusta; G: glucose treated Robusta; F: fructose treated Robusta) n = 4.

	Sugar content in green beans			Colour for roasted beans			
Coffee samples	Sucrose content (%)	Glucose content (%)	Fructose content (%)	Lightness (L)	a	b	ΔE
Arabica	6.20 ± 0.10	0.36 ± 0.12	0.13 ± 0.06	13.47 ± 0.05	4.32 ± 0.09	3.23 ± 0.09	-
Robusta	3.20 ± 0.38	0.39 ± 0.17	0.76 ± 0.20	19.08 ± 0.61	7.07 ± 0.26	7.34 ± 0.30	7.48
0%	0.72 ± 0.07	0.15 ± 0.06	0.14 ± 0.04	18.33 ± 0.12	6.76 ± 0.08	6.86 ± 0.12	6.53
3% S	1.66 ± 0.06	0.19 ± 0.02	0.15 ± 0.04	18.34 ± 0.98	6.40 ± 0.57	6.44 ± 0.48	6.18
6% S	1.90 ± 0.29	0.12 ± 0.02	0.12 ± 0.06	17.20 ± 0.33	6.11 ± 0.43	5.98 ± 0.44	4.96
9% S	3.08 ± 0.21	0.21 ±0.03	0.14 ± 0.02	17.08 ± 0.22	6.32 ± 0.19	6.12 ± 0.25	5.03
12% S	3.89 ± 0.19	0.16 ± 0.08	0.11 ± 0.07	15.47 ± 0.36	5.64 ± 0.13	5.11 ± 0.17	3.04
15% S	4.98 ± 0.18	0.13 ± 0.07	0.12 ± 0.05	15.04 ± 0.12	5.20 ± 0.13	4.60 ± 0.17	**2.26**
3% G	0.72 ± 0.09	2.74 ±0.42	0.13 ± 0.06	16.76 ± 0.35	6.27 ± 0.27	5.98 ± 0.32	4.70
6% G	0.72 ± 0.12	5.26 ± 0.45	0.16 ± 0.06	16.20 ± 0.48	5.85 ± 0.11	5.37 ± 0.13	3.79
9% G	0.80 ± 0.13	5.59 ± 0.29	0.16 ± 0.05	15.85 ± 0.51	5.73 ± 0.22	5.27 ± 0.27	3.44
12% G	0.62 ± 0.15	6.04 ± 0.46	0.17 ± 0.08	15.23 ± 0.18	5.48 ± 0.13	4.87 ± 0.09	**2.67**
15% G	0.62 ± 0.01	7.39 ± 0.13	0.12 ± 0.02	14.74 ± 0.27	5.17 ± 0.18	4.51 ± 0.14	**1.99**
3% F	0.69 ± 0.02	0.12 ± 0.03	2.06 ± 0.20	17.48 ± 0.71	6.56 ± 0.21	6.38 ± 0.28	5.56
6% F	0.75 ± 0.20	0.11 ± 0.04	3.82 ± 0.78	16.36 ± 0.37	5.91 ± 0.15	5.44 ± 0.17	3.96
9% F	0.72 ± 0.06	0.16 ± 0.05	5.15 ± 0.76	15.60 ± 0.34	5.46 ± 0.24	4.89 ± 0.19	**2.93**
12% F	0.78 ± 0.09	0.13 ± 0.00	5.74 ± 0.52	14.96 ± 0.15	5.31 ± 0.08	4.59 ± 0.06	**2.25**
15% F	0.78 ± 0.05	0.15 ± 0.02	7.35 ± 0.30	13.65 ± 0.42	4.57 ± 0.38	4.31 ± 0.34	**1.12**

Mean ± Standard deviation

To accelerate the diffusion of sucrose, glucose and fructose into the coffee beans, pre-soaking was carried out at 2 bar pressure. A rotation of 1 rpm was used to create even distribution of the treatment solution. The process control (water treated Robusta) was significantly lower in sucrose, glucose and fructose content when compared with the non-treated Robusta. This is due to the nature of the treatment process as, sucrose, glucose and fructose are water soluble and can be leached out into the process water during the treatment.

Increasing the sugar concentration in the treatment solution increased the sugar content in the treated green beans (Table 6.1). At the highest treatment level, Robusta samples were treated by soaking in 15% sugar (fructose, glucose, or sucrose), which are represented as 15% F, 15% G and 15% S accordingly. There was 4.98% sucrose in the 15% S treated green beans; 7.39% glucose in the 15% G treated green bean; 7.35% 15% F in the fructose treated green bean. At the highest sucrose treatment level the treated Robusta coffee still had a lower sucrose concentration (4.98%) than Arabica (6.20%). There was a significant increase in glucose and fructose concentrations between the glucose and fructose treated Robusta samples compare with the Arabica sample (Table 6.1). It should be noted that less sucrose was detected in the sucrose treated samples than glucose or fructose in their treated samples. Sucrose is a disaccharide and may penetrate the sample matrix less readily than monosaccharides like glucose and fructose.

Colour analysis of the coffee bean samples showed significant differences in L*, a, b ($p < 0.05$) between Arabica beans and the non-treated Robusta. ΔE was used to determine the overall distance between two colours. Clear differences were seen between Arabica and non-treated Robusta with a total colour difference ΔE of 7.48 (Table 6.1). This is the greatest colour difference between the Arabica and other

coffee samples. At 15% S, 12% G, 15% G, 9% F, 12% F and 15% F treatment, total colour differences were lower than 3, indicating minimised colour difference when compared with the Arabica coffee sample. As a result, it can be seen that sugar pre-treatment reduced the colour difference between Arabica and Robusta after roasting.

All coffee samples were roasted at 200 ºC for 20 min (known as a "dark roast"). Increasing sucrose, glucose and fructose levels in the Robusta beans did alter colour making the treated coffee more similar to that of the Arabica bean. The colour formation is mainly due to the Maillard reaction (Markowicz, Monaro, Siguemoto, & Mariana, 2012), and sugar caramelization processes, which can occur simultaneously, hence it is hard to separate the two reactions (Wong, Abdul Aziz, & Mohamed, 2008). Ganesan and Benjakul did a similar study on the basis of glucose treatment on pidan white (pickled duck eggs). They hypothesised and proved that glucose could improve brown colour development principally through accelerating the Maillard reaction (Ganesan, Benjakul, & Baharin, 2014), which is consistent with our result in Table 6.1.

6.2.2 Determination of the Aroma Compounds Presented in Coffee Derived from Robusta Beans with Different Treatment Methods

Thirty-four aroma compounds were selected within all the coffee samples, which all showed significant differences ($p < 0.05$) between Arabica and non-treated Robusta samples. All aroma compounds have previously been shown to be key aroma compounds and noted as having sensory significance in coffee. Volatile aroma compounds included 5 furans, 2 organic acids, 5 heterocyclic compounds (N containing), 4 sulphur-containing compounds, 2 aldehydes, 3 ketones and 9 pyrazines, 1 ether, 1 alcohol and 2 phenolic compounds.

6.2.3 Summary of All Coffee Samples via Aroma Chemistry

PCA was used to illustrate the variation between the 18 samples related to the level of 34 volatiles compounds formed during the roasting process (Figure 6.2). PC1 accounted for 64% of the variance in the whole dataset and showed more pyrazines and phenolic compounds on the left and more furans, ketones, aldehydes, ether, alcohol and acids on the right. PC2 accounted for 19% of the variance and indicated a positive correlation with pyrroles and negative correlation with sulphur-containing compounds. The non-treated Robusta sample had greater levels of pyrazines and phenolic compounds (left with triangle mark). While Arabica has a positive correlation with acids, furans, ketones and aldehydes (right with triangle mark). The main categories of compounds found higher proportion in Arabica were furans, acids, aldehydes and pyridines, which could give a more pronounced and finer aroma of roasted sweet caramel (Petisca, Pérez-Palacios, Farah, Pinho, & Ferreira, 2013). Robusta on the other hand is known to have a spicy burnt earthy odour due to higher concentrations of pyrazines and derivatives (Kerler, 2010),

which is concordant with our results in the Figure 6.2. Increasing the sugar treatment level moved the aroma profile from left to right, closer to Arabica. The 15% fructose treated coffees (square marked in the Figure 6.2) was the closest to the Arabica samples.

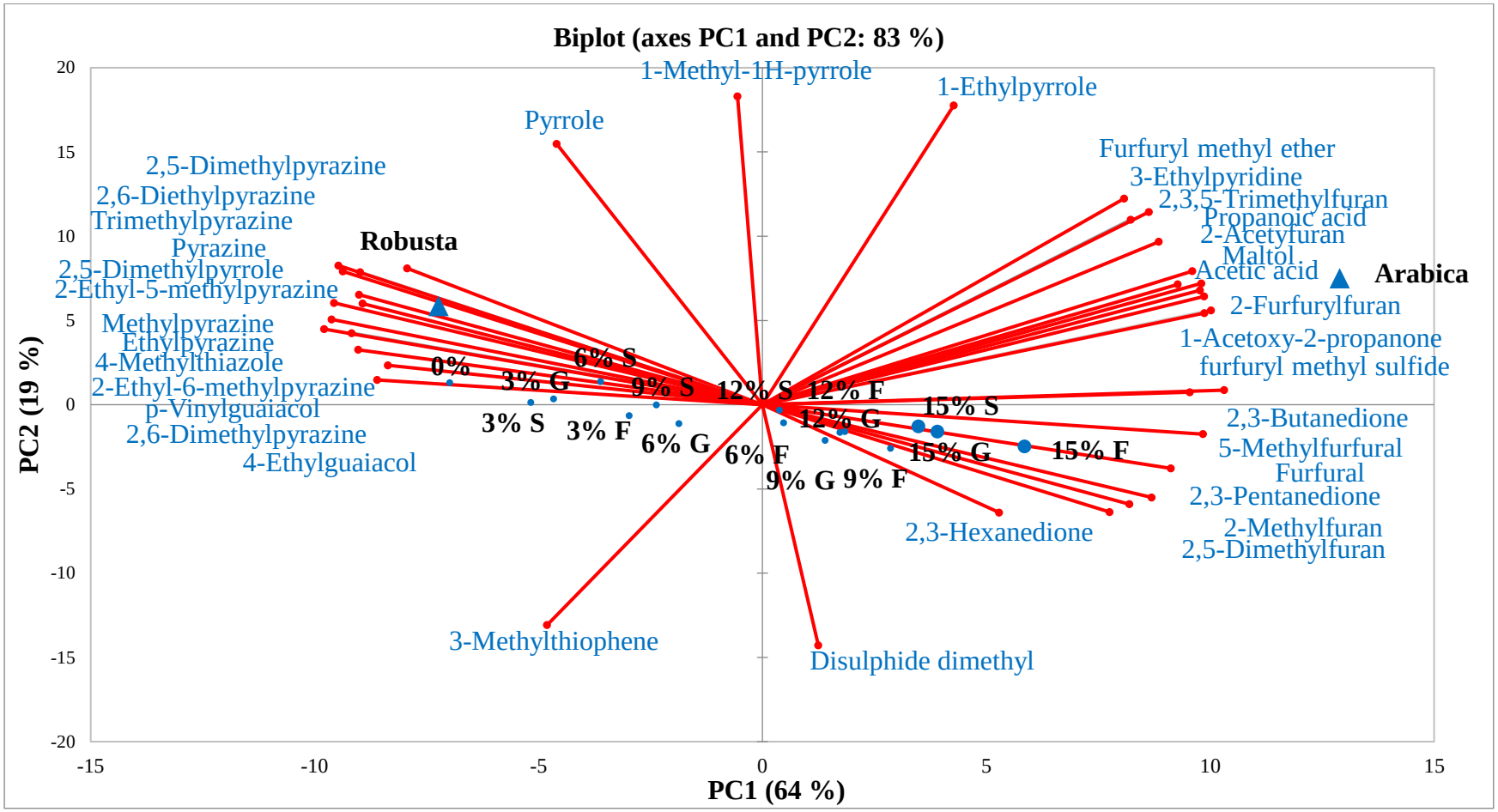


Figure 6.2 Principal component analysis (Bi-plot) of the aroma compliment of Arabica (▲), Robusta (▲) and sugar treated Robusta (●) coffee analysed by GC-MS (four replicates each).

6.2.4 Aroma Chemistry of Non-Treated Robusta, Sugar Treated Robusta and Arabica

The roasting aroma profile between Arabica, sugar treated and non-treated Robusta sample is illustrated in Figure 6.3 to Figure 6.7, where the level of 34 key volatile compounds in treated and non-treated Robusta coffee is presented and normalised by their respective results in Arabica coffee.

6.2.4.1 Non-treated Robusta

Significant differences were shown in all 34 key aroma compounds between Arabica and non-treated Robusta (Figure 6.3). Non-treated Robusta coffee had 2 to 4 times higher concentrations of all pyrazines, pyrroles, phenolic compounds and 4-methylthiazole when compare with Arabica coffee. However, for the rest of the volatile compounds such as furans, ketones, aldehydes, and acids, Arabica coffee had up to 8 times greater concentration than non-treated Robusta coffee.

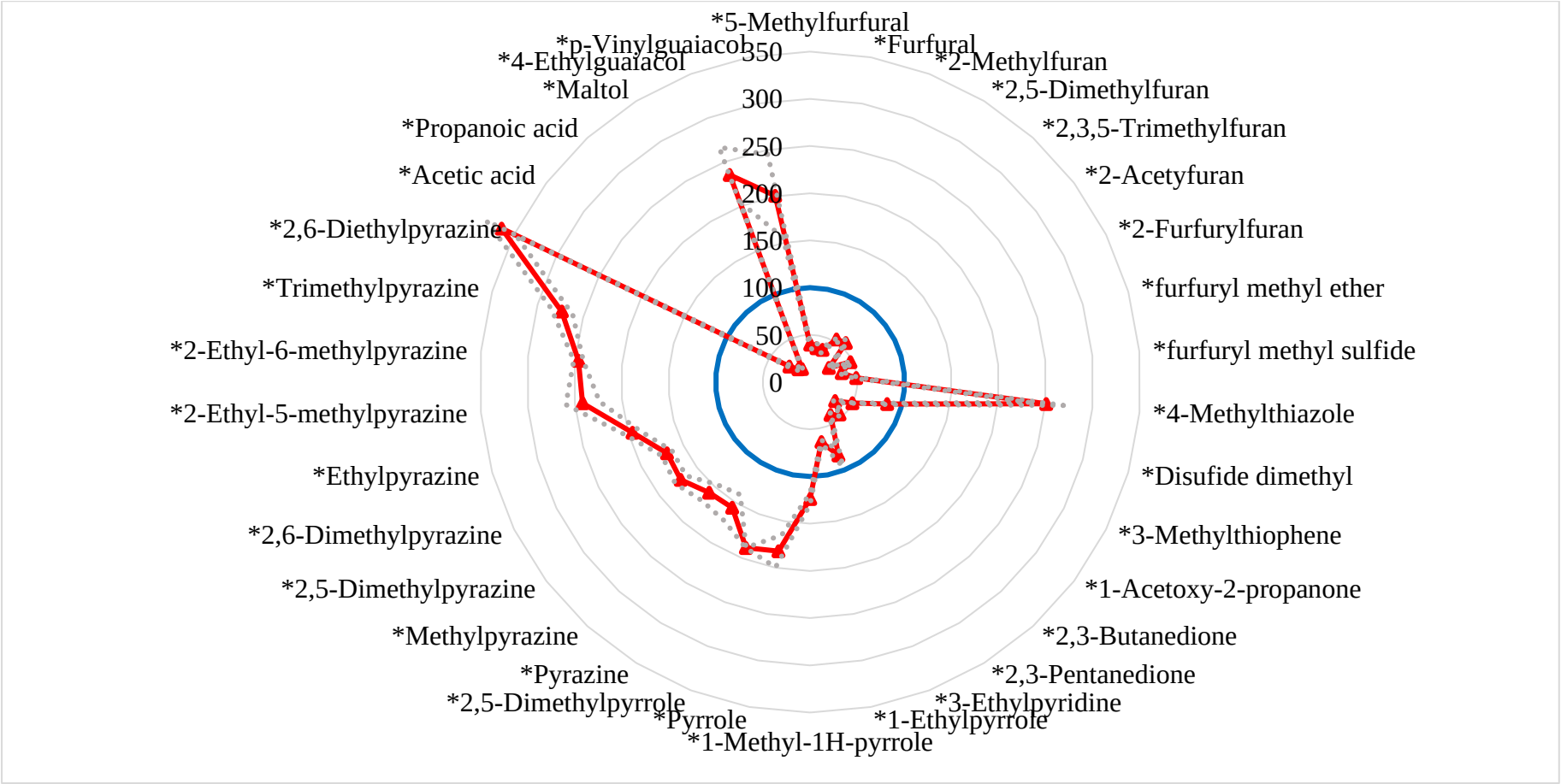


Figure 6.3 Aroma profile between Arabica (blue smooth circle) and non-treated Robusta (red spider diagram with triangle markers); graph with +/− standard deviation showed in grey dotted lines and compounds with ∗ indicated a significant difference between Robusta sample and Arabica sample (four replicates each) ($p < 0.05$).

6.2.4.2 Process Control Robusta

As shown in Figure 6.4, the aroma profile for the process control Robusta sample indicated significant differences ($p < 0.001$) in 32 volatile compounds compared to Arabica samples apart from pyrrole and disulphide dimethyl. These include a significantly greater level of pyrazines, phenolic compounds and 4-methylthiazole and lower levels of compounds such as furans, ketones, acids and aldehydes. . Similar to non-treated Robusta, the process control Robusta had a similar pattern but, the differences were smaller. These included a significant decreased levels of compounds such as pyrazines, furans, aldehydes, ketones and pyrroles. This change can be explained by the leaching of water soluble precursors during treatment as shown in Table 6.1. Volatiles such as furfural, 2-methylfuran have been reported as sugar degradation products (Flament, 2002).

In addition, an alteration to the bean structure could also alter the thermal reaction pathways during aroma formation. High density beans are more resistant to absorption of heat and takes a longer time to roast (Pittia, Dalla Rosa, & Lerici, 2001). Applying steam and pressure to the beans may open up bean pores and could alter the density of the green coffee beans. As a result, treated beans could have a lower density and less resistant to heat.

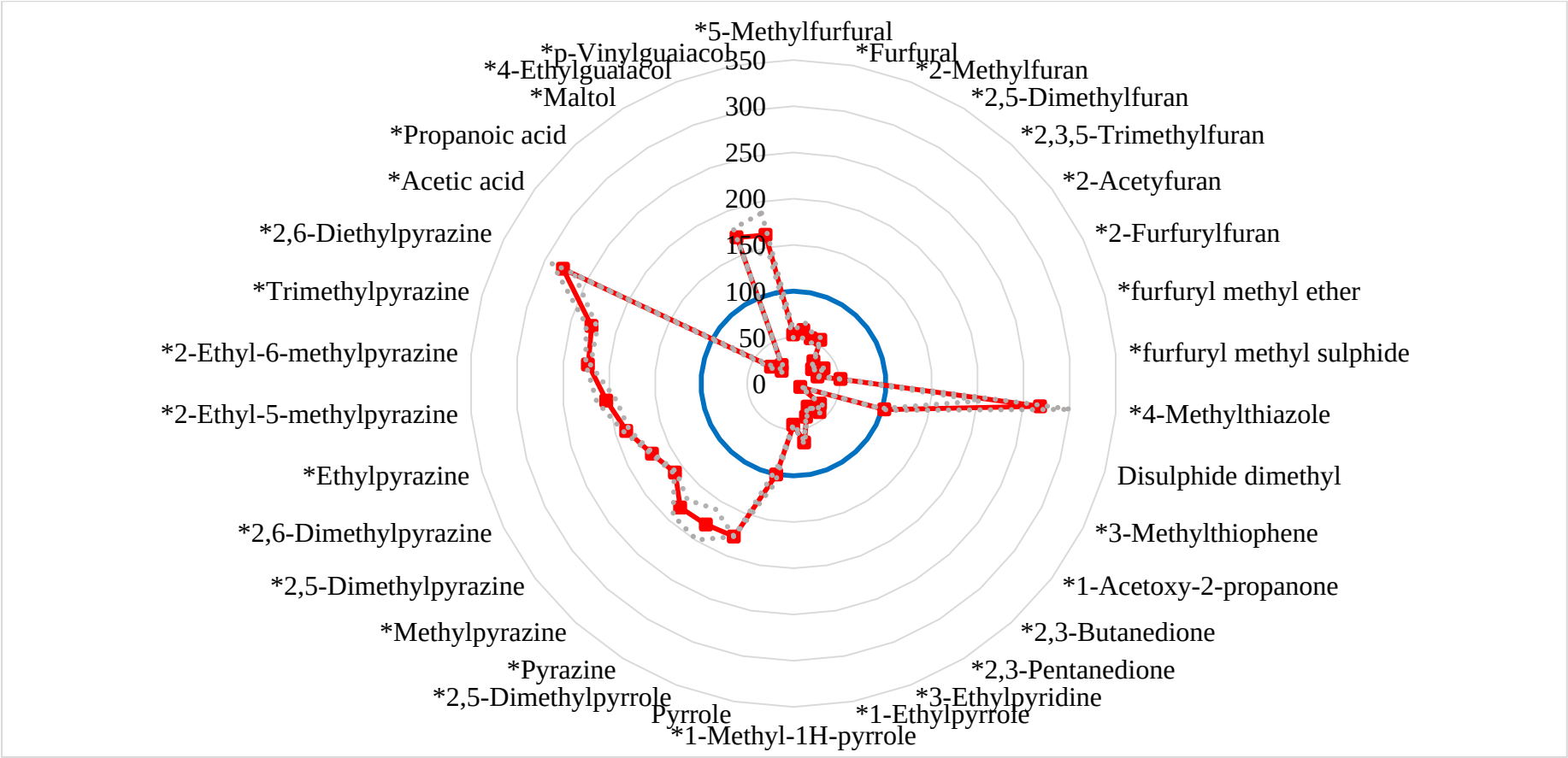


Figure 6.4 Aroma profile between Arabica (blue smooth circle) and processing controlled Robusta (0%) (red spider diagram with square markers) graph with +/− standard deviation showed in grey dotted lines and compounds with ∗ indicated a significant difference between process control Robusta sample and Arabica sample (four replicates each) ($p < 0.05$).

6.2.4.3 15% Fructose Treated Robusta

Figure 6.5 indicated the aroma profile between Arabica and 15 % fructose treated Robusta. There were no significant difference in the concentration of 16 compounds (including all pyrazines, aldehydes, 2, 5-dimethylfuran, 4-methylthiazole, 4-vinylguaiacol, 1-ethylpyrrole and 2, 5-dimethylpyrrole) between Arabica and 15% fructose treated Robusta. Although most furans, ketones and organic acids were still lower in the 15% fructose treated Robusta coffee compared with the Arabica, all furans, ketones and organic acids indicated a significant increase in 15% fructose treated Robusta (2-3 fold) when compare with non-treated Robusta and processing controlled Robusta, which made it closer to Arabica's profile.

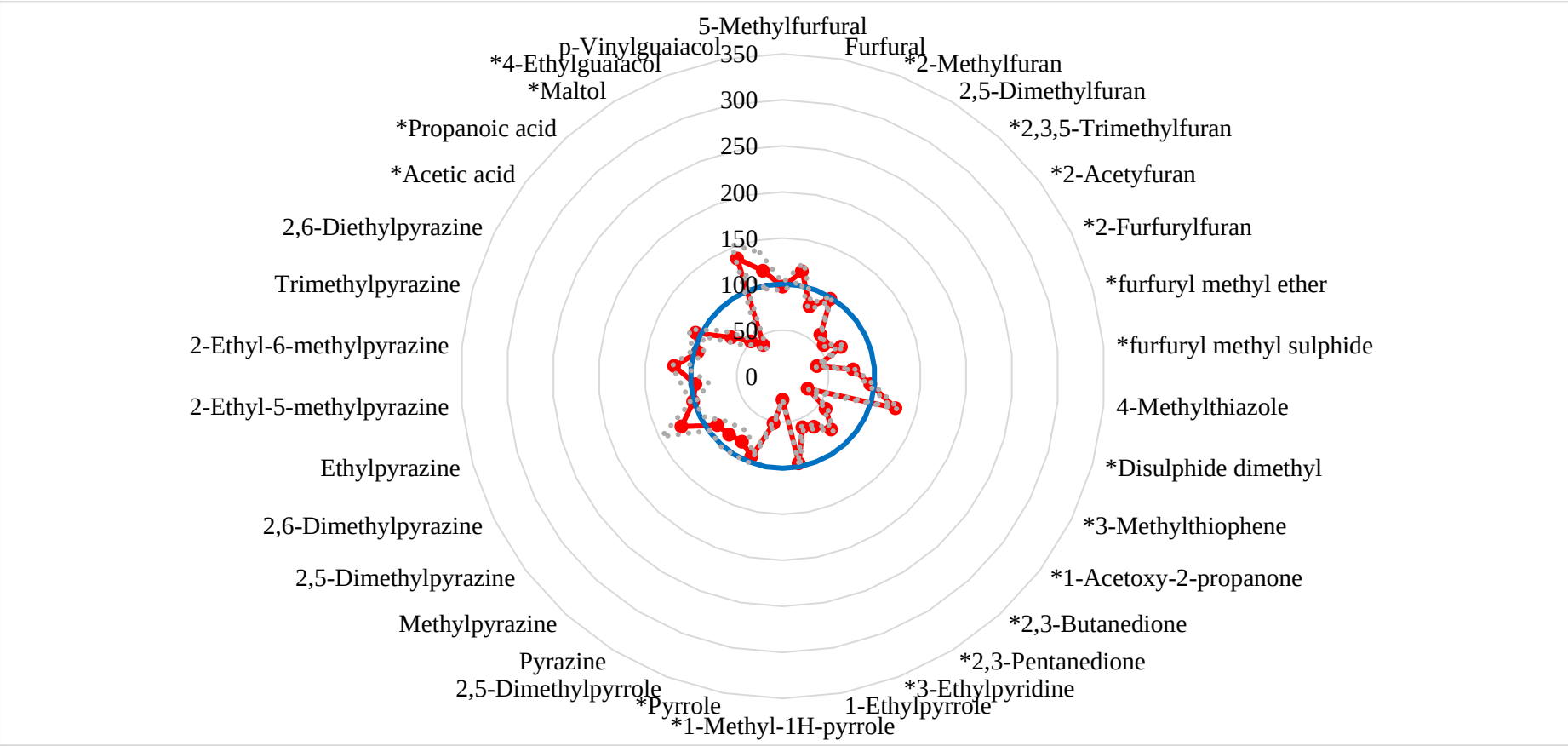


Figure 6.5 Aroma profile between Arabica (blue smooth circle) and 15 % fructose treated Robusta (red spider diagram with circle markers); graph with +/− standard deviation showed in grey dotted lines and compounds with ∗ indicated a significant difference between 15 % fructose treated Robusta sample and Arabica sample (four replicates each) ($p < 0.05$).

6.2.4.4 15% Glucose Treated Robusta

Figure 6.6 indicated the aroma profile between Arabica and 15 % glucose treated Robusta. There were no significant difference in 6 compounds (including 2, 5-dimethylpyrazine, 2-ethyl-5-methylpyrazine, methyl pyrazine, pyrazine, 1-ethylpyrrole, and 2, 5-dimethylfuran) between Arabica coffee and 15% glucose treated Robusta coffee. Some pyrazines like (2, 5-Dimethylpyrazine, 2-ethyl-5-methylpyrazine, methyl pyrazine, pyrazine) indicated a significant decrease in 15% glucose treated Robusta (60% - 100%) compare with non-treated Robusta sample (Figure 6.3). The concentration of 1-ethylpyrrole and 2, 5-dimetylfuran increased around 30% to 50% respectively in the 15% glucose treated Robusta when compare with non-treated one.

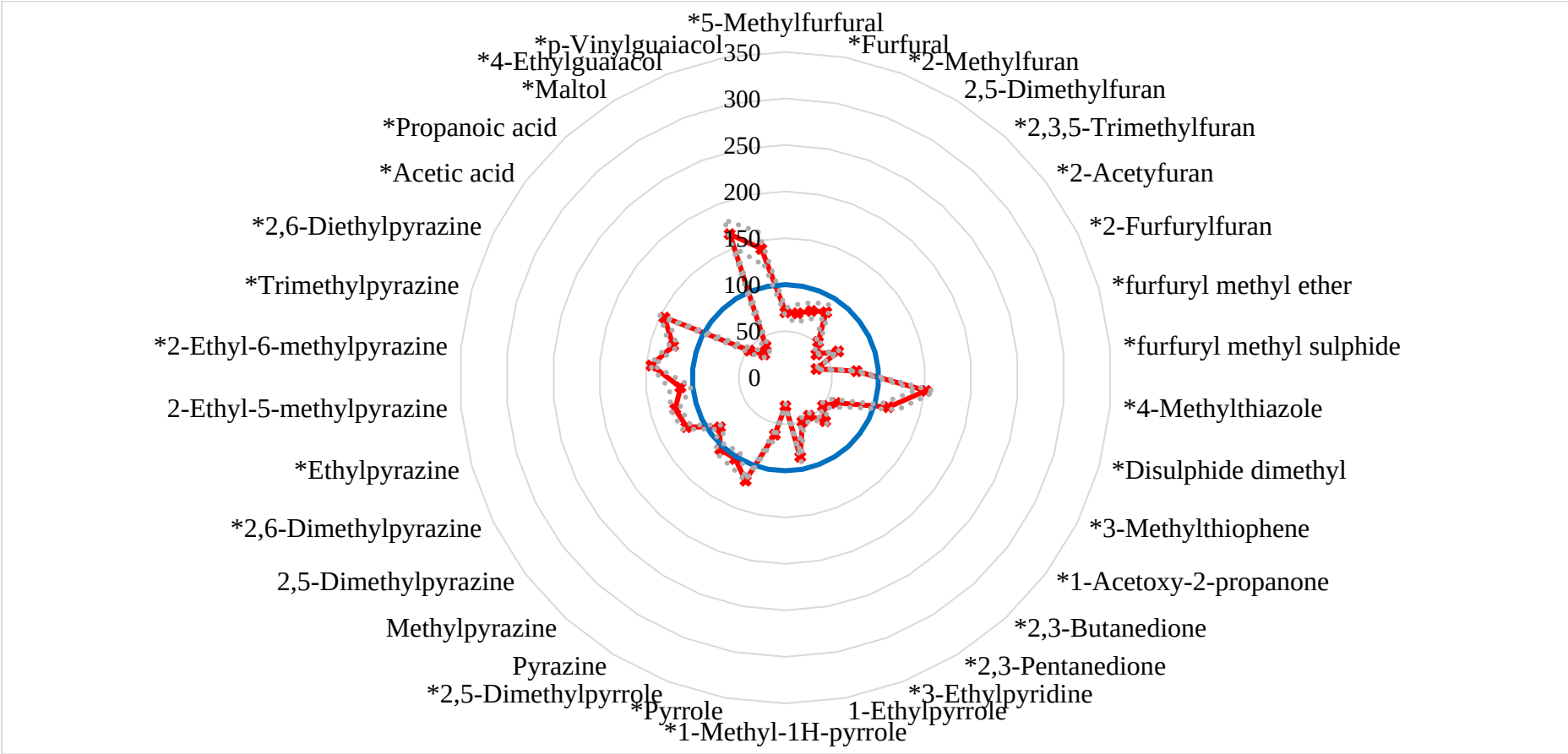


Figure 6.6 Aroma profile between Arabica (blue smooth circle) and 15% glucose treated Robusta (red spider diagram with cross markers) graph with +/− standard deviation showed in grey dotted lines and compounds with ∗ indicated a significant difference between 15% glucose treated Robusta sample and Arabica sample (four replicates each) ($p < 0.05$).

6.2.4.5 15% Sucrose Treated Robusta

Figure 6.7 indicated the aroma profile between Arabica and 15 % sucrose treated Robusta. There were no significant difference in the concentration of 7 compounds (2, 5-dimethylpyrazine, 2-ethyl-5-methylpyrazine, methyl pyrazine, pyrazine, 1-ethylpyrrole, 2, 5-dimethylfuran and furfural) between Arabica coffee and 15% sucrose treated Robusta coffee. Both glucose treated Robusta (15% G) and sucrose treated Robusta (15% S) had a similar pattern, apart from the relative concentration of furfural, which showed a significant increase (26%) in 15% sucrose treated Robusta compare with 15% glucose treated Robusta sample.

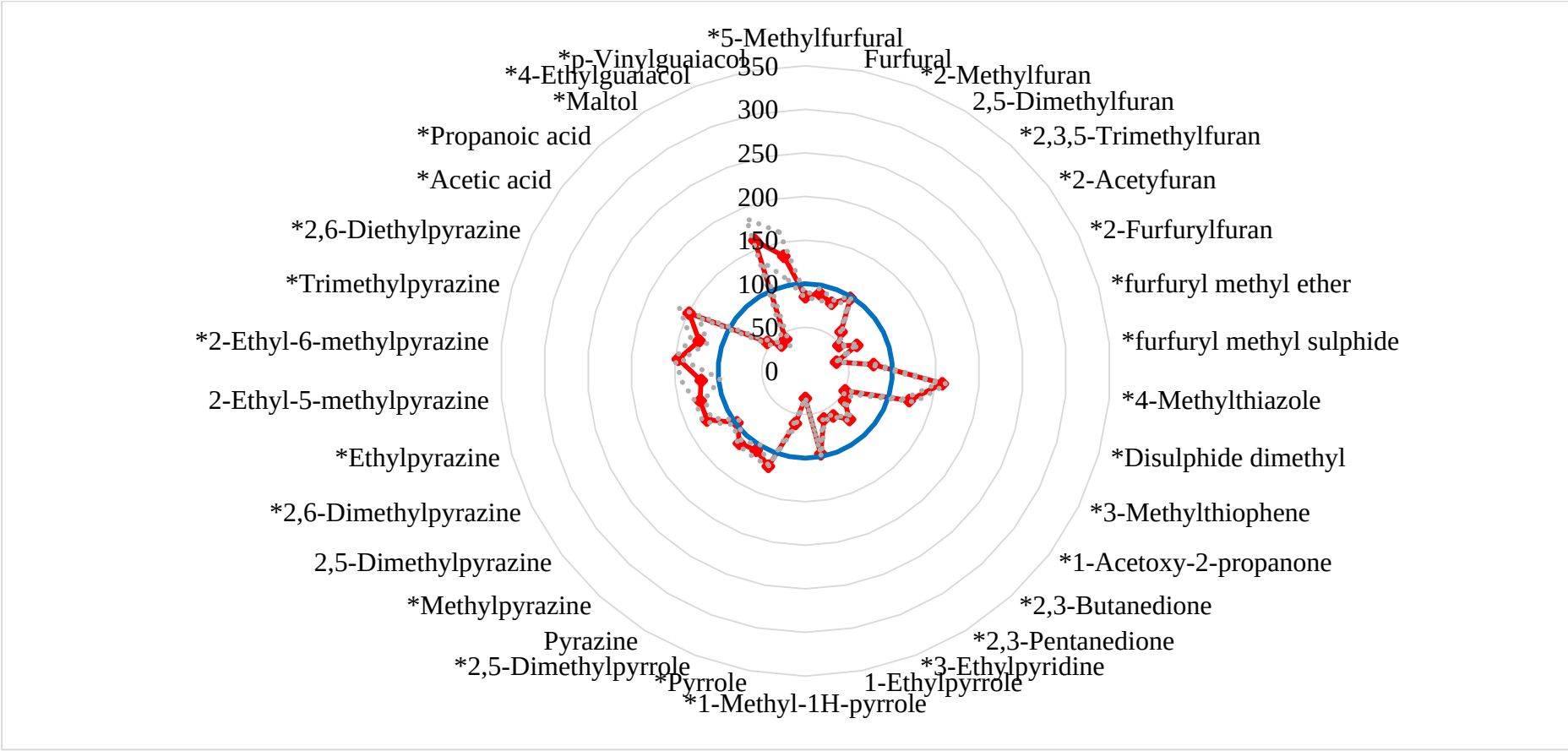


Figure 6.7 Aroma profile between Arabica (blue smooth circle) and 15% sucrose treated Robusta (red spider diagram with rhombus markers); all graph with +/− standard deviation showed in grey dotted lines and compounds with ∗ indicated a significant difference between 15% sucrose treated Robusta sample and Arabica sample (four replicates each) ($p < 0.05$).

The Maillard reaction comprises 3 stages, an initial stage where Amadori products are formed by sugar amine condensation and Amadori rearrangements, an intermediate stage, known to be the most important step for aroma development involving sugar degradation and fragmentation as well as amino acid degradation at elevated temperatures. The final browning stage involves polymerized and unsaturated intermediates, aldol condensation, aldehyde-amine polymerization, which form nitrogenous heterocyclic compounds (Nie, *et al.,* 2013). Research done by Bastos et al 2012 showed that Maillard volatile products can be categorized accordingly. Sugar degradation/fragmentation products consist of furans, pyrans, carbonyls and acids. Aldehydes and sulphur containing compounds under the amino acid degradation products and finally volatiles produced due to further interactions include pyrroles, pyrazines, pyridines, imidazole's.

The significant rise in the ketone, furan and acid compounds in the sugar treated Robusta may be due to the formation of those compounds through carbohydrate pyrolysis and sugar degradation (Flament, 2002). The formations of these furans are suggested to be greatly dependent on the sugar content (Nie, *et al.,* 2013). The sugar treatment level could therefore affect the formation of furans. Pyrazine is known to be predominant in Robusta and is formed by reducing sugars and amino acids following the Maillard reaction (Ehling & Shibamoto, 2005). However, Koehler and Odell discovered that increasing (3 fold) the amounts of sugar added could decrease the concentration of pyrazines generated, and the assumption was that excess sugar affected the reactant ratio hence decreasing pyrazine levels (Koehler, 1970). That could also be the reason for the lower pyrazine levels observed in sugar treated Robusta.

Pyrrole and pyridine concentrations were significantly decreased (around 2-fold) in the sugar treated Robusta (Figure 6.5, 6.6, 6.7). These two groups of compounds are formed as a result of the thermal degradation of Amadori intermediates. The intermediate products can either cyclize to form these nitrogenous heterocyclic compounds, or go to a different route where cleavage and formation of rearranged sugars occur. Due to the rearranged sugars comprising of the intact chain of the starting sugar and the original amine that was liberated, less or different volatile aroma compounds were created (Jousse, Jongen, Agterof, Russell, & Braat, 2002). Moreover, pyrrole and pyridine have also been reported as pyrolysis products of trigonelline (Flament, 2002). The reduced pyrrole and pyridine concentration may be therefore due to the trigonelline leaching out during the pre-treatment process.

Of the three different sugars used to treat Robusta samples (15% fructose, 15% sucrose and 15% glucose), 15% fructose treated Robusta sample was found to be the optimum treatment conditions with the most compounds showing no significant difference compare with Arabica. It indicated that formation of volatile compounds can be affected by the types of sugar involved in the Maillard reaction during roasting process, as also reported by Brands & Van Boekel, 2001. Monosaccharides showed more reactive than disaccharides (van Boekel & Brands, 2005).

For monosaccharides, ketoses such as fructose give rise to the corresponding Heyns compound, whilst the Aldoses such as glucose give rise to the Amadori intermediate compounds (detail illustrated in Figure 6.8 below) (Brands & van Boekel, 2001).

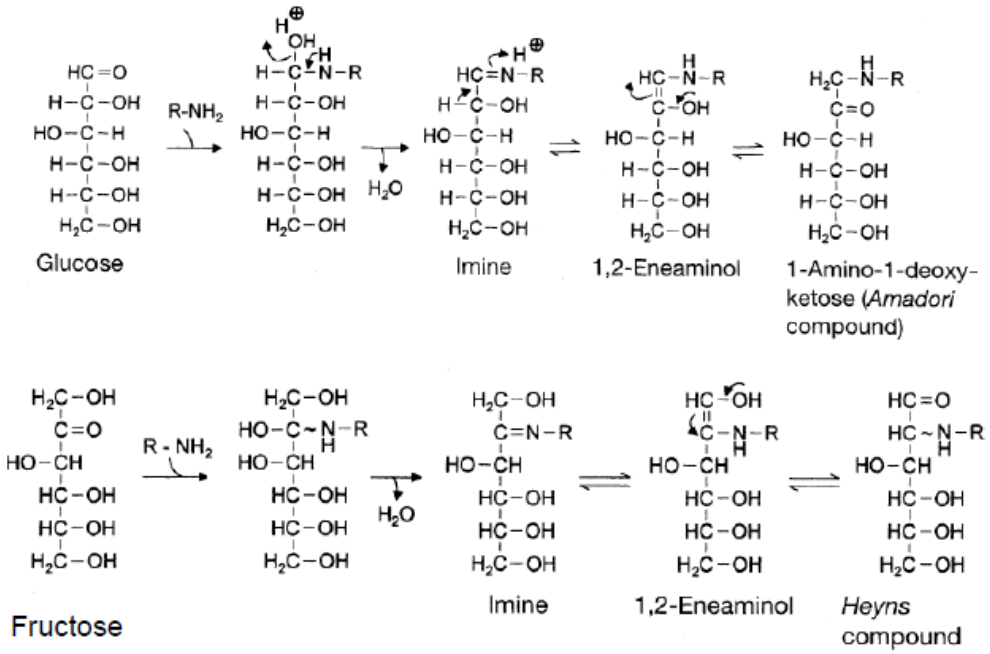


Figure 6.8 Glucose and fructose involved in the initial phase of Maillard reaction, which correlated to Amadori and Heyns products (Fennema, 1996).

There are conflicting reports in the literature regarding the issue of reactivity of sugars. Several studies (Baxter, 1995; Spark, 1969) support that glucose is more reactive, while other researches claim that fructose is more reactive (Kato, Yamamoto, & Fujimaki, 1969; Mauron, 1981; Suarez, Etlinger, Maturana, & Weitman, 1995; Walton, McPherson, & Shilton, 1989). Further studies indicated that the relative reaction rates vary for both glucose and fructose depending on the reaction conditions (Brands & van Boekel, 2001; Laroque, Inisan, Berger, Vouland, Dufossé, & Guérard, 2008; Rewicki, Kersten, Helak, Nittka, & Tressl, 2005).

In our study, 15% fructose treated Robusta generated more furans, ketones, aldehydes and acetic acid compared with 15% glucose treated Robusta, which agreed with the study worked on the flavour precursors in the Maillard reaction done by Kraehenbuehl (Kraehenbuehl, Davidek, Devaud, & Mauroux, 2010). On the other hand, formation of pyrazines showed significantly decreased in 15%

fructose treated Robusta compared with 15% glucose treated Robusta. No significant difference can be observed in the 15% fructose treated Robusta compared with Arabica. As discussed above, only 15% fructose treated Robusta sample will be use for the sensory evaluation.

6.2.5 Results of Sensory Evaluation

Fructose treated (at 15%) Robusta coffee was blended with up to 80% Arabica coffee and compared with the Arabica control. The results of the numbers of correct responses in a sensory triangle test evaluation of brewed coffee are shown in Table 6.9. According to ISO4120:2007, samples were classed as being similar to Arabica if the number of correct responses was less than 40 out of 98.

Participants could not tell a difference between Arabica and Arabica containing 20% Robusta blend, but once the blending ratio increased to 40% Robusta, participants could tell that the aroma was significantly different from the 100% Arabica sample (Chapter 5). Interestingly, when comparing Arabica with 15% fructose treated Robusta blended with Arabica, participants could not discriminate between the two samples, no matter the percentage of the blending (from 20% to 80% blends). The sensory evaluation results are consistent with the volatile analysis which show that the 15% fructose treated samples made the Robusta aroma profile closer to Arabica, and enable an increase in blending ratio from 20% Robusta 80% Arabica to 80% treated Robusta 20% Arabica .

Table 6.9 Results of triangle tests (the total number of correct response, conclusion and P value).

Sample 1	Sample 2	No. of Correct Reponses out of 98	Binomial P value	Significant at 0.05
Arabica	80% Arabica + 20% Robusta	**37**	0.2	no
Arabica	60% Arabica + 40% Robusta	46	0	yes
Arabica	80% Arabica + 20% treated Robusta	**34**	0.42	no
Arabica	60% Arabica + 40% treated Robusta	**33**	0.51	no
Arabica	40% Arabica + 60% treated Robusta	**39**	0.11	no
Arabica	20% Arabica + 80% treated Robusta	**39**	0.11	no
Arabica	0% Arabica + 100% treated Robusta	58	0	yes

** Proportion of discrimination =30%, β=0.05; blended samples are similar to Arabica, correct number should be less than 40*

6.2.6 Influence of Accelerated Shelf-life Storage on the Volatile Compounds

The relative percentage of each group of volatile compounds present in the headspace of the three coffee samples stored for six weeks at 35 ºC is shown in Figure 6.10. The relative aroma difference during storage was normalised to (100%) its original level in each coffee. The use of relative abundance in Figure 6.10 was used to avoid different starting points for Arabica, Robusta and treated Robusta coffee before storage as these two varieties might contain different amounts of the volatile compounds after roasting.

For Arabica, all compounds significantly decreased over the storage period between 25% - 60% ($p < 0.05$). The only exception was acid that increased around two-fold over the six weeks' time. The concentrations of total pyrroles, ketones, aldehydes, furans reduced significantly during six week stored at 35 ºC in Arabica, non-treated Robusta and 15% Fructose treated Robusta. However, the non-treated Robusta

coffee bean was significantly more stable when compared with Arabica coffee bean and 15% Fructose treated Robusta. Non-treated Robusta showed no significant difference in the pyrazines after six weeks stored at 35 ºC when compare with the control.

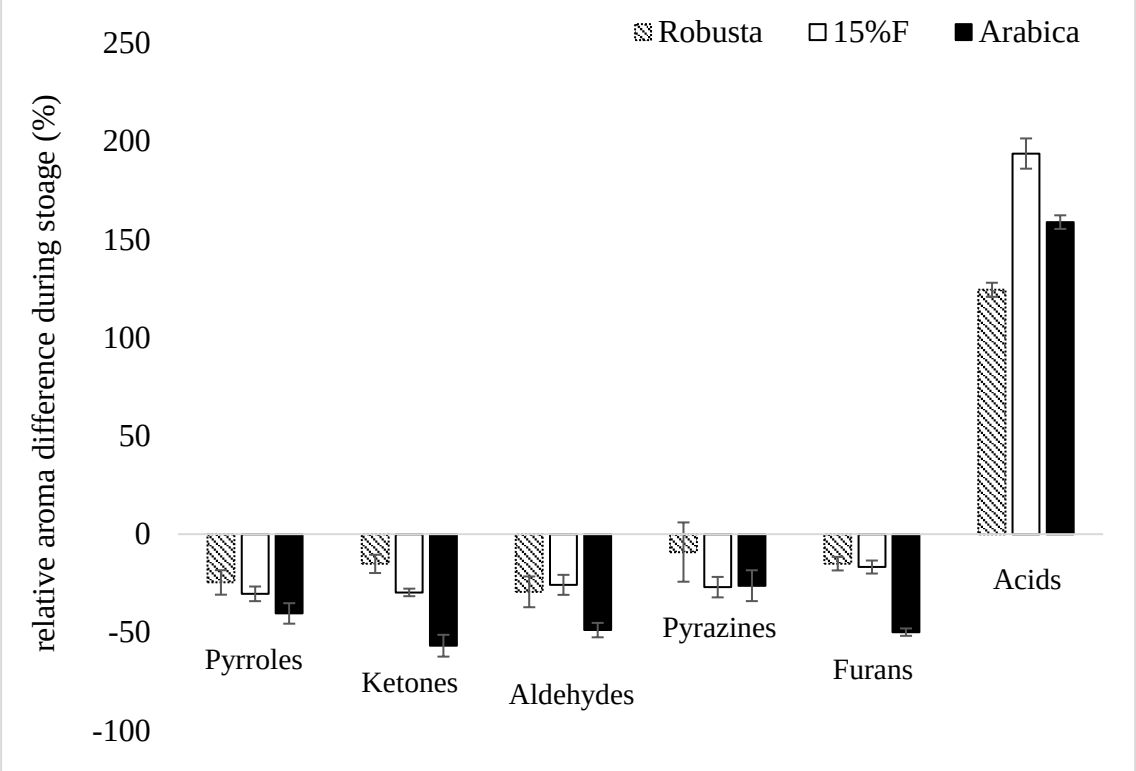


Figure 6.10 Relative aroma difference (%) during six week storage at 35 ˚C. Each volatile compounds relative concentration for each stored coffee was normalised to its original level in respective coffee stored at -80 ˚C, which was regarded as 100% (four replicates each).

The aroma of 15% fructose treated Robusta was more stable during 6 weeks storage compared with Arabica, as most of the volatiles in Arabica coffee showed a greater loss over storage when compared to the treated Robusta. The only exception was the total acid (include acetic acid and propanoic acid) that 15% fructose treated Robusta generated 35% more acids compare with Arabica during the six weeks stored. The preceding results suggested that the thermal decomposition could be happening during storage. The formation of acetic acid can be due to degradation

of small to medium chained carbohydrates such as glucose, sucrose and fructose (Illy & Viani, 1995). The higher fructose content may result in a greater acid release in the roasted coffee (Farah, 2012; Rewicki, Kersten, Helak, Nittka, & Tressl, 2005). Moreover, as mentioned in Chapter 5 that volatile acids increased may be due to the oxidation of the volatile compounds (such as furfural, acetaldehyde) during coffee storage period (Bengis, 1936; Elder, 1937). As 15% fructose treated Robusta coffee generated around 25% more furfural compared with Arabica (Figure 6.5). Therefore, higher volatile acids formation during coffee storage could also be explained by additional oxidation.

6.3 Conclusions

In conclusion, this project has successfully developed a sugar pre-treatment method for Robusta coffee and result in an improved aroma profile that is closer to Arabica's aroma. Analytical results showed that the 15% fructose treated Robusta coffee had optimum aroma profile to Arabica coffee. Sensory test results validate this finding, which proved that 15% fructose treated Robusta had a similar perceived aroma as Arabica. The maximum permissible blending proportion of Robusta increased from 20% for the non-treated coffee to 80% for the 15% fructose treated coffee. The treatment of Robusta with fructose before roasting clearly alters its aroma profile and allows it to be blended with Arabica in greater proportions. Moreover, the aroma stability of 15% fructose treated Robusta was more stable after roasting compared with Arabica.

Chapter 7 General Discussion, Conclusion and Suggestions for Future Studies

7.1 General Discussion and Conclusion

This thesis evaluated different green bean pre-treatment to improve the similarity of Robusta coffee aroma compared with Arabica coffee aroma. However, there were many variation that can affect the quality of our study.

The first variation comes from coffee species and origins, which can result in different aroma profile. In my study, all green beans were from single origin at the same harvested year 2014. The first step in the whole experiment was to identify the difference between Arabica and Robusta in our coffee samples. In chapter 3, Robusta green beans showed higher protein, CGA, and caffeine content, while Arabica showed higher trigonelline, sucrose and lipid content, which is concordant with Farah's and Illy & Viani's work. Arabica and Robusta green beans were roasted in light, medium and dark level. Arabica beans tend to have more furans, ketones and acids compared with Robusta. In contrast, Robusta coffee generated more pyrazines, phenolic compounds and sulphur-containing compounds after roasting. (Chapter 3)

The second challenge is coffee roasting variation that is known with large batch to batch variation in industry. Traditional industrial drum roaster are relatively simple machines commonly used in the coffee industry. The major challenge to using a traditional industrial drum roaster in this study was the variation from batch to batch and between different sample sizes (from 1kg to hundreds of kg at a time). Instead of using a traditional industrial drum roaster, a convection oven was used to roast the green beans in this study to minimize the batch to batch variation and reduce the sample size (to 25 g). Prior to the main study, a preliminary test was carried out

to select the coffee beans (location on the tray) with minimum variation after roasting. Roasting variation of 5% - 16% (across 14 volatile compounds, 3 replicates each) was due to the roasting location at the edge of the baking tray. Roasting variation can be minimized by remove the inter-batch variations in the later studies (exclude the samples at the edge of the baking tray detail showed in chapter 3).

Modifying the roasting condition to bring Robusta coffee aroma closer to Arabica coffee aroma was evaluated in this study (chapter 3). A D-optimal design was used in our study to model the Robusta coffee aroma formation with different roasting times and temperatures. This was a novel approach to match Robusta coffee aroma with Arabica coffee. Compared with the previous study done by Ku Madihah et al (2012) who used a central composite design (5 roasting time-temperature profiles with 6 replicates in the middle), D-optimal design (15 different roasting time-temperature profiles with 3 replicates each) in our study is more suitable as each parameter is automatically selected, to maximise quadratic and cubic modelling options. Results indicated that Robusta green beans roasted at 200 ºC for 20 min are closer to the dark roasted Arabica (chapter 3). This roasting condition was further applied in chapters 3, 4, 5 and 6.

Another challenge is that complexity of aroma compounds can be generated during coffee roasting. Despite hundreds of volatiles were found in roasted and ground coffee. Fifty-seven volatile compounds were selected at a range of functional groups (which include furans, aldehydes, ketones, pyrazines, ethers, acids, acetates, alcohols, phenolic compounds, sulphur-containing compounds and heterocyclic N-containing compounds), which all have been previously reported in the literatures with significant contribution to coffee aroma.

Since all the major variation were carefully controlled, different pre-treatment methods were evaluated. Regarding the objective of this study, firstly, the effect of cold plasma pre-treatment on the aroma generation within Robusta coffee was tested in chapter 4. Although the treated Robusta aroma profile was not comparable to Arabica coffee aroma, the results indicated a significant increase (25% - 60%) ($p < 0.05$) in 15 volatile compounds include furans, ketones, sulphur-containing compounds and phenolic compounds in the treated Robusta. Previous studies applied the cold plasma technique to enhance texture and nutrient content, mainly on wheat flour, lettuce and fruit juice products (Almeida, *et al.*, 2015; Bahrami, Bayliss, Chope, Penson, Perehinec, & Fisk, 2016; Grzegorzewski, Ehlbeck, Schlüter, Kroh, & Rohn, 2011; Rodríguez, Gomes, Rodrigues, & Fernandes, 2017). This is the first study that demonstrated the relationship between cold plasma pre-treatment and aroma generation within Robusta coffee. Although cold plasma treatment does not make Robusta coffee closer to Arabica, the cold plasma approach did alter the aroma formation in treated Robusta.

Secondly, the effect of pre-soaking treatment was evaluated. During this process important water soluble precursors are leached into water, hence compromising the flavour generation potential of the roasted coffee. This study to investigate how much water-soluble precursors were lost during pre-soaking of green coffee beans and the resulted aroma analysis illustrated significant aroma loss. Pre-soaking with an optimised soaking time and temperature was developed as a method in chapter 4. The proposed pre-soaking condition (2 h for 20 ºC) was used in subsequent chapters with further modifications to reduce the Arabica-Robusta aroma differences. Then different chemicals were used to pre-soak green Robusta coffee. Results indicated that both aroma profile of lysine pre-treated Robusta and sodium

carbonate treated Robusta are further away from Arabica. Acetic acid pre-soaked Robusta did alter the aroma formation during the roasting process and was the closest to Arabica. Chapter 5 further describes the work on the acetic acid treatment.

Acetic acid pre-treated Robusta coffee provided an improved aroma profile (from analytical results) that is closer to Arabica coffee aroma. Sensory test (sniff) results validated this finding, which showed that acid treated Robusta had a similar perceived aroma as Arabica. The maximum permissible blending proportion of Robusta increased from 20% for the non-treated coffee to 60% for the 4% acetic acid and 80% for the 2% of acetic acid treated coffee. The treatment of Robusta with acetic acid before roasting clearly alters its aroma profile and allows it to be blended with Arabica in greater proportions. However, the limitation was that participants were only asked to sniffing only. This was due to the fact that the treatment process was carried out in the non-food grade lab. Further studies involving for consuming these coffee brews could be carried out if food grade facilities are used in future.

In order to launch a new product, it is essential to determine the product shelf life after any treatment in the industry. The stability results indicated a significant difference in volatile compound stability with different varieties (i.e. Arabica and Robusta) and noticeable differences were shown by the treatment process. Most of the volatiles in Arabica coffee showed a greater loss over storage when compared to the non-treated Robusta. Whereas, the treated Robusta coffee bean was significant more stable than both Arabica and non-treated Robusta.

While the approach to study coffee using X-ray analysis was previously reported by Frisullo *et al.,* 2012, the impact of pre-treated Robusta coffee bean micro-

structures as measured by X-ray analysis has never been reported in the literature. The treated Robusta coffee beans and Arabica coffee beans had significantly higher porosity compared to the non-treated Robusta coffee beans in the centre. Treated Robusta coffee beans showed the highest porosity in the centre of the beans, which helped to explain the results of aroma stability (treated Robusta more stable). As a consequence, the pre-treated and roasted Robusta appears to have a longer shelf life compared with Arabica and non-treated Robusta. X-ray analysis proved to be a useful tool that can be applied in the coffee industry.

Another approach using fructose pre-treatment to improve aroma profile in Robusta coffee has been successfully developed. Analytical results showed the sugar treated Robusta coffee had a closer aroma profile to Arabica coffee. Sensory test results validated this finding, and proved that 15% fructose solution treated Robusta had a similar perceived aroma as Arabica. The maximum permissible blending proportion of Robusta increased from 20% for the non-treated coffee to 80% for the 15% fructose treated coffee. The treatment of Robusta with fructose before roasting allows Robusta to be blended with Arabica in greater proportions. Although the 15% fructose treated Robusta showed less aroma stability than non-treated Robusta during 6 weeks storage, the aroma of 15% fructose treated Robusta was still more stable compared with Arabica.

In conclusion, the main novelty in this work lies in combined use of chemical profiling, sensory testing and structural analysis to elucidate new approaches for green bean pre-treatment. Two novel approaches, acetic acid pre-treatment and fructose pre-treatment were proved to improve the similarity of Robusta coffee aroma compared with Arabica coffee aroma and improved flavour stability in the treated Robusta coffee. Both approaches proved that it is feasible to apply

laboratory-derived knowledge and adapt scientific techniques in a commercial context. It has also demonstrated how the research findings can be transferred into real life.

7.2 Suggestions for Future Studies

- The scope of this thesis is to develop novel methods to improve Robusta flavour being more like Arabica in terms of aroma. On the other hand, the taste of coffee also plays an important role in the quality of coffee. So it will be interesting to explore the impact on various taste compounds in further detailed studies.
- Currently the coffee beans were either single-origin wet processed Robusta green beans from Vietnam or high grade Arabica green beans from Aberdares, Mount Kenya. However, due to the different regions may have different post-harvest treatment, it will be interesting to use both Arabica and Robutsa green bean from the same region (exactly same processing way). Process adjustment for the treatment to increase the feasibility for industrial manufacturing, i. e. to facilitate industrial machinery as well as to reduce processing cost which could involve:

  - Roasting could be performed on a real roaster (eg. IKAWA home roaster), not in an oven.
  - The use of food grade treatment chemicals for consumer safety.
  - Instead of using freeze drying process, modifying the treatment time could further reduce processing cost.

Appendix

Table A1 The sources of all chemicals and purity used as standards in the thesis

	Standards	Supplier	Purity
1	Acetic Acid	Sigma-Aldrich	≥ 99%
2	2,3-Butanedione	Sigma-Aldrich	97%
3	2,5-Dimethylpyrazine	Sigma-Aldrich	≥ 98%
4	3-Ethylpyridine	Sigma-Aldrich	≥ 99%
5	2,5-Dimethylfuran	Sigma-Aldrich	99%
6	2,3-Dimethylpyrazine	Sigma-Aldrich	99%
7	4-Ethyl guaiacol	Sigma-Aldrich	≥ 98%
8	2-Ethyl,5-methylpyrazine	Sigma-Aldrich	≥ 98%
9	Guaiacol	Sigma-Aldrich	≥ 98%
10	Hexanal	Sigma-Aldrich	≥ 98%
11	3-Heptanone	Sigma-Aldrich	98%
12	Pyrrole	Sigma-Aldrich	98%
13	5-Methylfurfural	Sigma-Aldrich	98%
14	Maltol	Sigma-Aldrich	≥ 99%
15	2,3- Pentanedione	Sigma-Aldrich	97%
16	2-Methylthiophene	Sigma-Aldrich	98%
17	3-Penten-2-one	Sigma-Aldrich	≥ 95%
18	Methylpyrazine	Sigma-Aldrich	≥ 99%
19	Furfural	Sigma-Aldrich	≥ 99%
20	2-Furanmethanol	Sigma-Aldrich	98%
21	Phenol	Sigma-Aldrich	≥ 99%
22	2,5-Dimethylpyrrole	Sigma-Aldrich	98%
23	Sucrose	Sigma-Aldrich	≥ 99.5%
24	D-(+)-Glucose	Sigma-Aldrich	≥ 99.5%
25	D-(−)-Fructose	Sigma-Aldrich	≥ 99%
26	Trigonelline	Sigma-Aldrich	Reference Standard (USP)
27	Caffeine	Sigma-Aldrich	≥ 99%
28	(Chlorogenic acid) CGA	Sigma-Aldrich	≥ 95%
29	L-lysine	Sigma-Aldrich	≥ 98%
30	Sodium Carbonate	Sigma-Aldrich	≥ 99%

Table A2 R^2 values and calibration equations for the analytical data

	R^2	Calibration Equations
Sucrose by LC-MS	0.9796	y = 1968.5x + 23334
Glucose by LC-MS	0.9774	y = 4286.6x + 329047
Fructose by LC-MS	0.9789	y = 8142.4x + 153627
bovin serum albumin (BSA) by standard Assay Kit	0.9976	y = 0.0008x + 0.0231
Trigonelline by HPLC	0.9438	y = 14.473x + 1217.6
Caffeine by HPLC	0.9627	y = 27.068x + 1856.7
CGA by HPLC	0.9648	y = 20.945x + 3953.1

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