The University of Nottingham

MODIFYING COFFEE QUALITY BY CHEMICAL MANIPULATION

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ABSTRACT

Chemical modification was applied to a coffee process by-product, silver skin, as well as raw coffee beans, with the aim to improve their aroma quality. Heat treatment in combination with sugar addition or enzyme treatment was applied to silver skin to encourage Maillard reactions. The manipulation applied to silver skin, however, did not give satisfactory results as the treatments neither caused significant increase in coffee aroma levels, nor yielded coffee aroma with quality resembling that of the real coffee. Chemical modification of raw Robusta coffee was carried out using fractionation and reconstitution approaches. The fractionation process involved the use of three types of solvent varying in polarity, dichloromethane (DCM), methanol (MeOH), and water, thus yielding four raw coffee fractions: DCM-soluble, MeOH-soluble, Water-soluble, and residue fractions. The reconstitution process involved wet mixing of the raw coffee fractions, vacuum drying and moisture content adjustment. Several reconstituted coffees were prepared with various proportions of the raw Robusta fractions, roasted and subjected to volatile analysis by Gas Chromatography-Mass Spectrometry (GC-MS). Statistical analysis by Principal Component Analysis (PCA) and the calculation of sum of normalized standard deviation (SNSD) of aroma compounds' odour activity values (OAVs) showed that the reconstituted Robusta that yielded the least variation, in term of aroma profile, from the higher quality coffee, Arabica, was the one composed of 70% d.b. MeOH fraction, 30% d.b. cell-wall material (residue), and 11 % w/w moisture content. The aroma profile of Arabica coffee was used as a reference due to its fine flavour that is commonly considered of better quality compared to that of Robusta (Briandet, Kemsley et al. 1996). Sensory evaluation (by sniffing) employing hedonic pairwise comparison technique confirmed the result from the GC-MS analysis that the aroma quality of the chosen reconstituted

Robusta was improved since its aroma was significantly more preferred to that of the Robusta by the judges (30 people). Non-volatile compound analyses, however, suggested the need for further sensory study that involves tasting/drinking of the brews made with the new reconstituted Robusta for it contained significantly higher contents of bitter/astringent taste compounds, i.e. chlorogenic acids, caffeine and trigonelline, than the original coffees that could also affect the overall sensory quality of the coffee.

TABLE OF CONTENTS

ACKNOWLEDGEMENT i

ABSTRACT ii

CHAPTER 1: INTRODUCTION 1

1.1 LITERATURE REVIEW 2

1.1.1 Coffee as One of the Important World Trade Commodities 2

1.1.2 Coffee Production 4

1.1.3 Coffee Aroma 8

1.1.4 Aroma Quality of Freshly Brewed vs. Soluble (Instant) Coffee 11

1.1.5 Coffee Aroma Recovery: Improving Aroma Quality of Soluble (Instant) Coffee 12

1.1.6 Arabica vs. Robusta 14

1.1.7 Coffee Aroma Formation 22

1.1.8 Coffee Taste Compounds 26

1.1.9 Green Coffee Modification 45

1.1.10 Fractionation and Reconstitution of Coffee 49

1.1.11 Isolation of Coffee Aroma for Volatile Analysis 50

1.1.12 Potential Uses of Coffee Processing By-products: as Coffee Aroma Source? 53

1.1.13 Potential Health Benefits of Coffee Flavour Compounds 55

1.2 OBJECTIVES 61

CHAPTER 2: MATERIALS AND METHODS 62

2.1 DEVELOPMENT OF COFFEE VOLATILE ANALYSIS PROCEDURES 62

2.1.1 Selection of Internal Standard 62

2.1.2 Isolation of Coffee Aromas 63

2.1.3 Gas Chromatography-Mass Spectrophotometry (GC-MS) Analysis 64

2.1.4 Peak Identification and Quantification 65

2.2 DETERMINATION OF COFFEE SILVER SKIN AS A POTENTIAL SOURCE OF COFFEE AROMA 65

2.2.1 Comparison of Contents of Coffee Aroma in Coffee and in the Corresponding Silver Skin 65

2.2.2 Quantification of Aroma Precursors in Coffee Silver Skin 66

2.2.3 Generation of Coffee Aroma from Coffee Silver Skin through Maillard Reactions 68

2.2.4 Determination of Content of Other Functional Component in Coffee Silver Skin 71

2.3 GREEN COFFEE FRACTIONATION AND RECONSTITUTION 72

2.3.1 Development of Fractionation Procedure 72

2.3.2 Development of Reconstitution Procedure 74

2.3.3 Moisture Content Analysis of the Green Beans 77

2.3.4 Determination of Feasibility to Create a New Reconstituted Robusta with Improved Aroma Quality from Robusta fractions 78

2.3.5 Calculation and Statistical Analysis 80

2.4 SENSORY EVALUATION 81

2.4.1 Sample Preparation 81

2.4.2 Sample Presentation 83

2.5 ANALYSIS OF TASTE AND OTHER NON-VOLATILE COMPOUNDS IN THE RECONSTITUTED COFFEE 85

2.5.1 Screening for Key Taste Compounds by Direct Liquid Mass-Spectometry (DL-MS) 86

2.5.2 Total Soluble Solid (TSS) 86

2.5.3 pH and Titratable Acidity (TA) 87

2.5.4 Lipid Analysis 87

2.5.5 Protein Analysis 89

2.5.6 Chlorogenic Analysis by HPLC 90

2.5.7 Sugar Analysis by HPLC 91

2.5.8 Trigonelline and Caffeine Analyses by HPLC 92

CHAPTER 3: DEVELOPMENT OF AROMA COMPOUND ANALYSIS PROCEDURE 94

3.1 INTRODUCTION 94

3.2 RESULTS AND DISCUSSION 95

3.2.1 Internal Standard (IS) Selection 95

3.2.2 Isolation of Coffee Aromas 97

3.3 CONCLUSION 98

CHAPTER 4: INVESTIGATION OF COFEE SILVER SKIN AS A POTENTIAL SOURCE OF COFFEE AROMA 100

4.1 INTRODUCTION 100

4.2 RESULTS AND DISCUSSION 101

4.2.1 Comparison of Aroma Profiles of Coffee and the Corresponding Silver Skin 101

4.2.2 Determination of Possibility to Generate Coffee Aroma from Coffee Silver Skin 102

4.2.3 Generation of Coffee Aroma from Coffee Silver Skin through Maillard Reactions 105

4.2.4 Determination of Content of Other Functional Component in Coffee Silver Skin 111

4.3 CONCLUSION 112

CHAPTER 5: IMPROVING QUALITY OF ROBUSTA COFFEE UTILIZING FRACTIONATION AND RECONSTITUTION APPROACHES 113

5.1 INTRODUCTION 113

5.2 RESULTS AND DISCUSSION 115

5.2.1 Fractionation 115

5.2.2 Reconstitution 120

5.2.3 Analysis of Moisture Content 130

5.2.4 Determination of Feasibility to Create Reconstituted Robusta Coffee with Aroma Quality Comparable to that of Arabica. 130

5.3 CONCLUSION 144

CHAPTER 6: SENSORY EVALUATION 146

6.1 INTRODUCTION 146

6.2 RESULTS AND DISCUSSION 148

6.2.1 Colour Measurement 148

6.2.2 Headspace Volatile Intensity of the Samples Determined by Atmospheric Pressure Chemical Ionization-Mass Spectrometry (APCI-MS) 150

6.2.3 Sensory Evaluation: Hedonic Pairwise Comparison 152

6.3 CONCLUSION 154

CHAPTER 7: ANALYSIS OF NON-VOLATILE COMPOUNDS 156

7.1 INTRODUCTION 156

7.2.1 Screening for Key Taste Compounds by Direct Liquid Mass-Spectrometry (DL-MS) 157

7.2.2 Titratable Acidity 160

7.2.3 Carbohydrate: Total Soluble Solid and Sugar Analyses 164

7.2.4 Analysis of Chlorogenic Acids (CGA) 170

7.2.5 Analysis of Trigonelline and Caffeine 175

7.2.6 Protein Analysis 179

7.2.7 Lipid Analysis 181

7.3 CONCLUSION 183

CHAPTER 8: CONCLUSION AND SUGGESTIONS FOR FUTURE STUDIES 185

8.1 CONCLUSION 185

8.2 SUGGESTIONS FOR FUTURE STUDIES 189

LIST OF TABLES

CHAPTER 1

Table 1.1 Historical indicator coffee prices for year 2009 and the beginning of year 2010 3

Table 1.2 Most important odourants (FD>32) in a freshly filtered coffee brew (FC) and an instant coffee beverage (IC) 9

Table 1.3 Furan and caramel compounds of roasted coffee 15

Table 1.4 Chemical composition of Arabica and Robusta coffees 16

Table 1.5 Contents of mono- and disaccharides in green Arabica and Robusta coffees.. 18

Table 1.6 Odour-active compounds in the thermally treated Cysteine-Ribose solution, treated at 145 °C for 20 min at pH 5 26

Table 1.7 Yields and bitter intensity of fractions isolated from the coffee beverage (CB) by means of high-vacuum distillation, sequential solvent extraction, and ultrafiltration 28

Table 1.8 Chlorogenic acids (CGA) contents in economically relevant Brazillian green and roasted coffee cultivars 32

Table 1.9 Chlorogenic acid lactones (CGL) contents in economically relevant Brazilian green and roasted coffee cultivars 34

Table 1.10 Human taste recognition thresholds of caffeoyl-, feruloyl-, dicaffeoyl-quinides 36

Table 1.11 Contents of trigonelline, nicotinic acid, and caffeine in coffee roasted at different temperature at a constant time (15 min) 37

Table 1.12 Chlorogenic acids and caffeine contents in Ethiopian green coffee beans and coffee beans roasted at different conditions 42

Table 1.13 Acid concentrations in different coffee brands 43

Table 1.14 Effect of grind size on acid concentrations at constant brewing temperature and time (94 °C and 5 min) 44

Table 1.15 Effect of brewing temperature on acid concentrations at constant grind size and time (fine grind for 5 min) 44

Table 1.16 Effect of brewing time on acid concentrations at constant grind size and brewing temperature time (fine grind at 94 °C) 45

Table 1.17 Distribution of carbohydrates, trigonelline, total CGA, and protein in fractions obtained from green Arabica coffee 50

Table 1.18 Some compounds identified in roasted coffee and roasted coffee fractions fractionated with 80% ethanol..... 51

Table 1.19 Chemical composition of coffee silver skin..... 54

Table 1.20 Number of reviews and epidemiological studies on coffee with selected disease..... 58

Table 1.21 Amount of evidence for coffee and selected disease conditions..... 59

Table 1.22 Summary of potential health risks/benefits of coffee drinking..... 60

CHAPTER 2

Table 2.1 Log P's of candidate internal standard (IS) and target coffee aromas..... 62

Table 2.2 Detail of each treatment combination for the experiment: Determination of appropriate solvent extraction method for the coffee sample..... 63

Table 2.3 Full-factorial experimental design for the glucose-added silver skin experiment 69

Table 2.4 Preparation of the enzyme (pancreatin) solutions 70

Table 2.5 Details of the samples in the experiment: Digestion of silver skin by pancreatin enzyme..... 70

Table 2.6 Percentage of each fraction in original green coffees along with the amounts of each fraction weighed to prepare the corresponding reconstituted samples..... 75

Table 2.7 Amounts of water added to the dried reconstituted samples prepared from wet mixing process for moisture content adjustment..... 76

Table 2.8 Combinations of Robusta coffee fractions for the preparation of reconstituted coffee samples 78

Table 2.9 Limitations used for the new set of reconstituted samples..... 79

Table 2.10 Combinations of Robusta coffee fractions for the preparation of new set of reconstituted samples 80

Table 2.11 Composition of the reconstituted coffee samples used in sensory study..... 82

Table 2.12 Temperature profiles used for baking samples for sensory study..... 83

CHAPTER 3

Table 3.1 Concentrations (ppm) of selected coffee aromas recovered by different extraction conditions..... 97

CHAPTER 4

Table 4.1 Concentrations of coffee aromas in roasted and ground (R&G) coffee along 101

with those in the corresponding silver skin.....

Table 4.2 Contents of sugars in Arabica silver skin determined by HPLC method..... 102

Table 4.3 Total reducing sugar analysed by spectrophotometry (expressed as "Maltose equivalent")..... 103

Table 4.4 Sugar composition of light, medium, and dark roasted coffee beans compared to those of green beans 103

Table 4.5 Amino acid contents in green coffee beans and coffee silver skin 104

Table 4.6 The coffee aroma compounds monitored..... 105

Table 4.7 Concentrations of target coffee aromas studied in the original silver skin compared to those in silver skin baked at 215 Cº for 10 min)..... 106

Table 4.8 Percentage of caffeine in roasted Arabica coffee and the corresponding silver skin..... 111

CHAPTER 5

Table 5.1 Relative concentrations (%) of selected coffee aromas in reconstituted coffees prepared by dry mixing approach calculated relative to the contents of the same aromas in the corresponding original Arabica or Robusta..... 123

Table 5.2 Relative concentrations (%) of selected coffee aromas in reconstituted coffees prepared by wet mixing approach calculated relative to the contents of the same aromas in the corresponding original Arabica or Robusta..... 126

Table 5.3 Percentages of moisture content (w/w) in green coffees..... 130

Table 5.4 Sum of standard deviation values of OAVs (SNSD) calculated based on the aroma profiles of the reconstituted samples 133

Table 5.5 Analysis of Variance (ANOVA) table for the mixture linear model..... 136

Table 5.6 Component effect analysis for the mixture linear model..... 138

Table 5.7 Sum of standard deviation values (SNSD) of OAVs of the new set of reconstituted sample..... 141

Table 5.8 Detection thresholds in water of the aroma compounds of interest (µg/l)..... 144

CHAPTER 6

Table 6.1 L a b values from colour measurement by ColorQuest XE Hunter lab on roasted Arabica, roasted Robusta, roasted sample X and roasted sample Y..... 149

Table 6.2 Selected ions monitored along with % variations (expressed as % coefficient of variance, % CV) of the ion intensities detected over time (up to 20 min) by APCI-MS.... 151

Table 6.3 Judges preferences for Arabica, Robusta, sample X and sample Y presented as six pairs of samples using hedonic pairwise-comparison test..... 153

Table 6.4 Some comments by the judges regarding the aroma notes perceived in Robusta and sample Y 154

CHAPTER 7

Table 7.1 Selected taste compounds and their associated ions in liquid phase DL-MS.... 158

Table 7.2 Titratable acidity of raw and roasted Arabica, Robusta and sample Y titrated to pH 6 and 7..... 163

Table 7.3 Total soluble solids (°Brix) in raw and roasted Arabica, Robusta and sample Y 165

Table 7.4 Sugar contents in raw and roasted Arabica, Robusta and sample Y..... 167

Table 7.5 Chlorogenic acid contents in raw and roasted Arabica, Robusta and sample Y 172

Table 7.6 Percentages of the CGAs in the total CGA content of each sample..... 175

Table 7.7 Trigonelline and caffeine contents in raw and roasted Arabica, Robusta and sample Y..... 177

Table 7.8 Protein contents in raw Arabica, Robusta and Sample Y..... 180

Table 7.9 Lipid contents in raw Arabica, Robusta, and Sample Y..... 183

LIST OF FIGURES

CHAPTER 1

Figure 1.1 Schematic of coffee post-harvest processes (wet and dry processes)..... 5

Figure 1.2 Soluble coffee process..... 8

Figure 1.3 Comparison of amino acid content in Arabica and Robusta type green coffee..... 20

Figure 1.4 Maillard reaction pathways..... 24

Figure 1.5 Structure of chlorogenic acids precursors..... 30

Figure 1.6 Proposed reaction pathways leading to the formation of caffeoyl- and feruloyl-quinides upon roasting of coffee beans..... 35

Figure 1.7 Coffee bean structure 53

CHAPTER 2

Figure 2.1 Scheme of the fractionation procedure..... 74

Figure 2.2 Reconstitution Procedure..... 77

CHAPTER 3

Figure 3.1 Stability of 3-pentanone and 2-hexanone under various extraction conditions..... 95

Figure 3.2 Stability of sec-butanol and 2-heptanol under various extraction conditions..... 96

CHAPTER 4

Figure 4.1 Concentrations (ppm) of the target coffee aromas in roasted Arabica coffee, original and baked silver skin (BSS)..... 108

Figure 4.2 Concentrations (ppm) of the target coffee aromas in digested silver skin baked at 200 ºC for 2 h along with those in undigested silver skin (control) baked at the same condition..... 110

CHAPTER 5

Figure 5.1 Concentrations (ppm) of selected coffee aromas in baked Arabica fractions compared to those in original Arabica coffee..... 116

Figure 5.2 Concentrations (ppm) of selected coffee aromas in baked Robusta fractions compared to those in original Robusta coffee..... 117

Figure 5.3 Total content (ppm) of selected coffee aromas in each baked coffee fraction..... 119

Figure 5.4 Concentrations (ppm) of selected coffee aromas in baked reconstituted Arabica prepared by dry mixing approach 121

Figure 5.5 Concentrations (ppm) of selected coffee aromas in baked reconstituted Robusta prepared by dry mixing approach 122

Figure 5.6 Concentrations (ppm) of selected coffee aromas in baked reconstituted Arabica prepared by wet mixing approach 124

Figure 5.7 Concentrations (ppm) of selected coffee aromas in baked reconstituted Robusta prepared by wet mixing approach 125

Figure 5.8 Chromatograms of original and reconstituted Arabica coffee baked at 210 °C for 12.5 min (water was used as solvent for the mixing process) 128

Figure 5.9 Chromatograms of original and reconstituted Robusta coffee baked at 210°C for 12.5 min (water was used as solvent for the mixing process) 129

Figure 5.10 Concentrations (ppm) of selected coffee aromas in baked reconstituted samples 132

Figure 5.11 Bi-plot from Principal Component Analysis (PCA) applied to the concentrations (ppm) of 22 target aroma compounds in Arabica, Robusta, and the reconstituted samples 134

Figure 5.12 Principal Component Analysis (PCA) applied to the concentrations (ppm) of 22 target aroma compounds in Arabica, Robusta, and the reconstituted samples 137

Figure 5.13 Concentration (ppm) of selected coffee aromas in baked reconstituted samples (new set with narrower limitation) 140

Figure 5.14 Bi-plot from Principal Component Analysis (PCA) applied to the concentrations (ppm) of 22 target aroma compounds in Arabica, Robusta, and the reconstituted samples (new set) 142

Figure 5.15 Principal Component Analysis (PCA) applied to the concentrations (ppm) of 22 target aroma compounds in Arabica, Robusta, and the reconstituted samples (new set) 143

CHAPTER 7

Figure 7.1 Peak area of selected ions obtained from Direct-Liquid Mass-Spectrometry (DL-MS) operated in ESI^+ mode 159

Figure 7.2 Typical HPLC chromatogram from the CGA analysis 171

Figure 7.3 Percentage loss of the CGAs in Arabica, Robusta and sample Y after roasting 173

ABBREVIATION

ANOVA	Analysis of Variance
Ara	Arabica
AEDA	Aroma Extract Dilution Analysis
AOAC	Association of Official Agricultural Chemists
APCI-MS	Atmospheric Pressure Chemical Ionization-Mass Spectrometry
BSS	Baked Silver Skin
CA	Caffeic Acid
CFQA	Caffeoylferuloylquinic Acid
CQA	Caffeoylquinic Acid
CGL	Chlorogenic Acid Lactones
CGA	Chlorogenic Acids
CV	Coefficient of Variance
CB	Coffee Beverage
Conc.	Concentration
diCQA	diCaffeoylquinic Acid
DCM	Dichloromethane
diFQA	diFeruloylquinic Acid
DL-MS	Direct Liquid Mass-Spectrometry
d.b.	Dry Basis
ESI	Electrospray Ionization
FA	Ferulic Acid
FQA	Feruloylquinic Acid
FC	Filtered Coffee Brew
FD	Flavour Dilution Factor
GC-MS	Gas Chromatography-Mass Spectometry
glu	Glucose
GC-MS	Gram
HPLC	High Performance Liquid Chromatography
HMWM	High-Molecular-Weight Material
h	Hour
IC	Instant Coffee Beverage

IS	Internal Standard
ICO	International Coffee Organization
ILS	International Laboratory Service
Kg	Kilogram
L	Litre
MRPs	Maillard Reaction Products
m/z	Mass-to-Charge Ratio
MeOH	Methanol
µl	Microlitre
mg	Milligram
ml	Millilitre
mm	Millimetre
min	Minute
n.d.	Not Detectable
OAV	Odour Activity Value
ppm	Part Per Million
p-CoA	p-Coumalic Acid
p-CoQA	p-Coumaroylquinic Acid
PA	Perceived Acidity
pp	Phosphate
PC	Principal Component
PCA	Principal Component Analysis
RI	Refractive Index
R&G	Roasted and Ground Coffee
Ro	Robusta
Rpm	Round per Minute
s	Second
SIM	Selected Ion Monitoring
Sig	Significant
SS	Silver Skin
SD	Standard Deviation
SNSD	Sum of Normalised Standard Deviation
TA	Titratable Acidity

TSS	Total Soluble Solid
v/v	Volume by Volume
w/v	Weight by Volume
w/w	Weight by Weight
w.b.	Wet Basis

CHAPTER 1: INTRODUCTION

To the world, coffee is one of the most important international trade products . The species of coffee dominating the world market are *Coffea Arabica* (Arabica) and *C. Canephora Pierre* (Robusta), which account, respectively, for about 75 and 24% of the total world coffee production (Casal, Alves et al. 2003).

In the coffee market, Arabica is usually more preferred than Robusta. This is due to the fact that Arabica produces a more balanced and subtle flavour when it comes to a cup of coffee. This, therefore, determines the higher commercial value of Arabica (Martin, Pablos et al. 1998; Procida, Campisi et al. 2003).

The dissimilarity in organoleptic properties between the two coffees is mainly attributable to the differences in their chemical composition both before and after the coffee roasting process. Several studies have applied sensory evaluation, instrumental analysis, or the combination of both, to identify these differences. Most methods succeeded in identifying differences (Further details on chemical composition of each coffee species are given in section 1.6).

A number of studies have attempted to improve Robusta sensory properties based on modification of the beans' chemical compositions (Varsanyi, Krajczar et al. 1988; Becker, Schlabs et al. 1991; Imura and matsuda 1992). Approaches used were either by removing particular aromas/precursors, adding the desired flavour/precursors to the coffee, or the combination of the two. All aimed to bring the Robusta sensory quality up to a level close to that of the more preferred specie, Arabica, which should in turn make it become more commercially accepted.

To date, the attempts to improve Robusta to mimic Arabica flavour have had limited success. Evidently, the subject needs further investigation, and therefore has become the focus in this study.

1.1 LITERATURE REVIEW

1.1.1 Coffee as One of the Important World Trade Commodities

As mentioned previously, commercial coffee beverages are made from Arabica, Robusta, or a blend of the two. The two coffees are distinct in sensory quality and, therefore, are different in commercial values (historical indicator prices of coffee are shown in Table 1.1). In general, Robusta has a strong and powerful taste, but also possesses "earthy" and "musty" flavour notes which are undesirable in many consuming countries. Arabica is considered of better quality due to its finer flavour (Briandet, Kemsley et al. 1996) and therefore is more expensive (Martin, Pablos et al. 1998).

The two coffee species require different conditions for their cultivations, i.e. climates, irrigation, soil type. Therefore, the availability of certain types of coffee in different countries/regions can be varied. The difference in availability, thus, has become another factor causing the difference in coffee prices in different countries.

In general, Arabica dominates coffee-growing area in most North, Central, and South American countries, e.g. Costa Rica, Brazil, and Columbia, while Robusta takes up the majority of coffee plantations in African and Asian countries (Smith 1985). African Robusta coffee bean exports now account for approx. one third of the world's coffee crop (Schoenholt 1992).

Table 1.1 Historical indicator coffee prices for year 2009 and the beginning of year 2010. All prices are in US cents per lb.

Monthly Averages	ICO Composite Price	Colombian Mild Arabicas			Brazilian Natural Arabicas			Robustas		
		Market		Daily weighted average	Market		Daily weighted average	Market		Daily weighted average
		New York	Germany		New York	Germany		New York	France	
2009	**115.67**	**180.87**	**174.58**	**177.43**	**111.39**	**116.55**	**115.33**	**77.16**	**74.02**	**74.58**
January	108.39	148.88	137.62	142.32	101.43	111.65	109.18	85.77	82.11	82.74
February	107.6	149.58	140.74	144.55	100.45	109.87	107.69	81.66	79.9	80.22
March	105.87	162	148	154.16	97.48	104.4	102.81	77.48	76.05	76.31
April	111.61	190.94	173.29	181.1	101.46	107.28	105.95	76.5	75.36	75.53
May	123.05	225.58	201	212.05	113.13	120.08	118.4	77	75.2	75.62
June	119.05	195.27	197.14	196.32	109.81	117.1	115.42	75.88	73.16	73.79
July	112.9	192.11	183.71	187.29	104.55	108.79	107.8	74.83	71.09	71.68
August	117.45	181.61	188.36	185.39	114.01	117.71	116.86	75.04	71.76	72.35
September	116.4	169.9	183.39	177.45	114.12	116.82	116.16	77.31	73.04	73.82
October	121.09	175.16	180.55	178.13	122.84	125.16	124.62	76.68	72.86	73.51
November	119.67	180.08	176.48	178.33	126.21	126.11	126.17	73.08	68.86	69.48
December	124.96	199.38	184.67	192.11	131.23	133.68	132.84	74.68	68.86	69.89
2010										
January	126.85	214.55	201.65	207.51	128.11	132.75	131.67	75.09	69.05	70.08
February	123.37	208.36	201.89	204.71	121.61	125.42	124.57	73.49	66.74	67.88
March	125.3	206.37	205.17	205.71	125.28	126.49	126.21	72.53	66.16	67.25

Source: International Coffee Organization (ICO).

1.1.2 Coffee Production

1.1.2.1 From green beans to roasted beans

1.1.2.1.1 Green beans

Traditionally, green coffee is produced by two different methods, known as wet and dry processing. Both methods are aimed at removing the fruit flesh and reducing the water content in the raw coffee beans to about 10-12%. It is well accepted that coffee beans obtained from either method of treatment, (referred to as "washed" or "unwashed"), produce roasted coffee and coffee beverages with very different aroma and taste compositions (Illy and Viani 1995; Bytof, Knopp et al. 2005). A study by Rocha and Maeztu et al. (2003) reported that washed (wet processed) Arabica coffees are characterized by some acidity and intense aroma, while dry-processed Arabica coffees are less acid and have a less marked aroma with a richer body.

A schematic of coffee wet and dry processes is shown in Figure1.1.

The purpose of the fermentation stage in the wet process is to remove the mucilage adhering to the parchment surrounding the bean left from the previous process (pulping) by the action of enzymes. Usually, the beans undergo natural fermentation meaning that degradation by enzymes already present in the mucilage, aided by those arising from yeasts and bacteria which develop concurrently. The process can take up to 72 h, but can be speeded up by adding extra enzyme to it, i.e. pectic enzyme (Smith 1985).

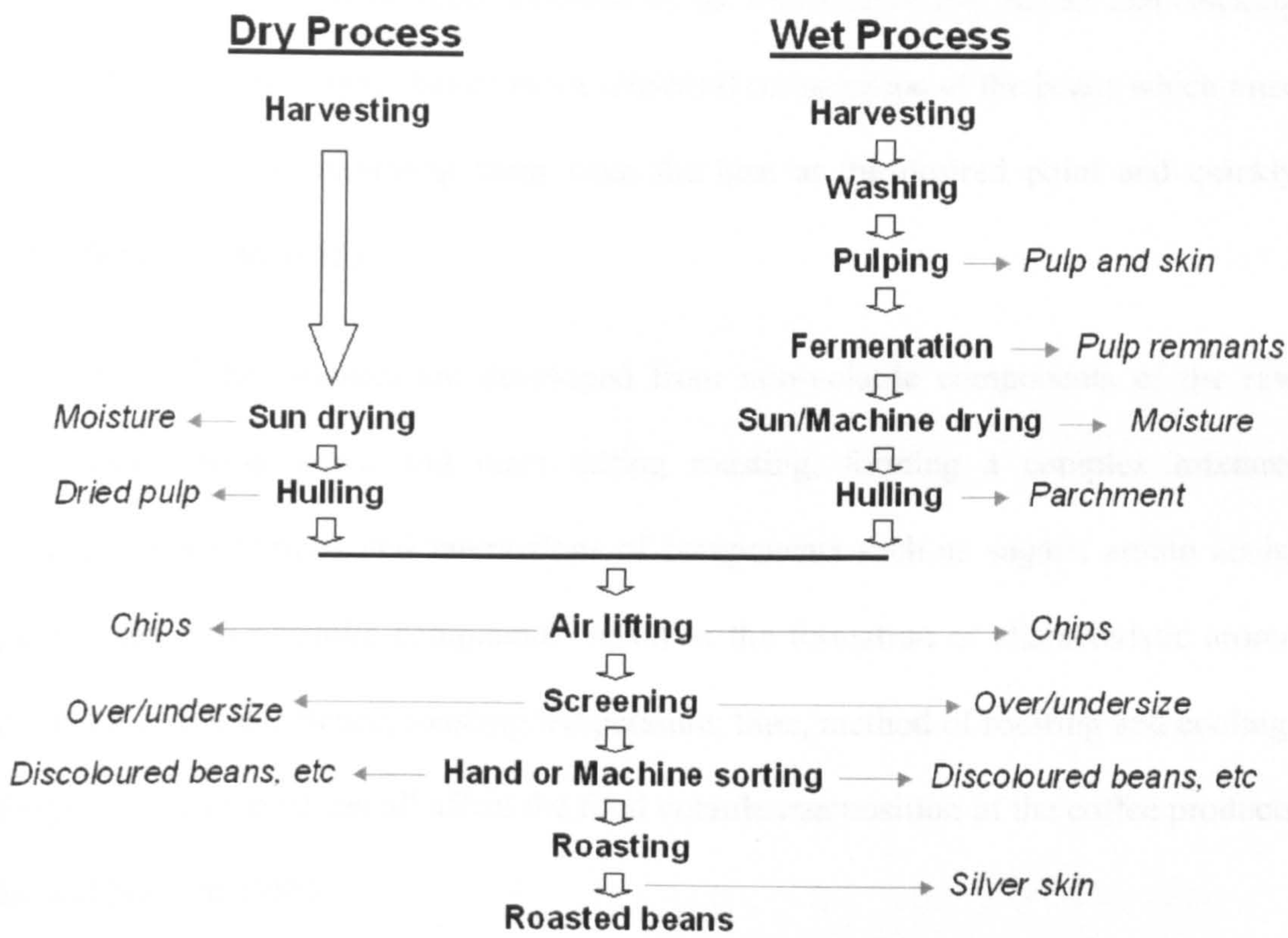


Figure 1.1 Schematic of coffee post-harvest processes (wet and dry processes).

1.1.2.1.2 Roasted beans

Since green beans are not consumed as such and are generally regarded as having no agreeable aroma or flavour, the coffee beans need to be introduced to roasting (following by grinding, and brewing or infusion) before they can be consumed as a beverage. It is the roasting stage where the pleasant aromas of coffee are generated.

During the roasting stage, the beans undergo a two-stage transformation, the first of which involves driving off approximately 12% of free moisture. In this early stage, which takes up about 80% of the roasting time, the green beans gradually change to straw colour then to pale brown. The second stage involves pyrolysis, with swelling of the

beans, and a fairly rapid darkening, followed by the emission of oily smoke and cracking sounds. There is also a rapid change in the chemical composition of the beans which must be halted abruptly by removing them from the heat at the desired point and quickly cooling them (Smith 1985).

Most of the volatiles are developed from non-volatile components of the raw beans, which break down and react during roasting, forming a complex mixture. Pyrolysis, other reactions and interactions of components such as sugars, amino acids, organic acids, and phenolic compounds, result in the formation of characteristic aroma and flavour of coffee. Hence, roasting temperature, time, method of roasting and cooling, and type of roaster used can all affect the final volatile composition of the coffee products (Dart and Nursten 1985).

1.1.2.2 Freshly brewed coffee

Several techniques are used to prepare an aqueous coffee beverage. Techniques vary in brewing temperature, the extent of coffee grinding, how the water is introduced to the coffee, how the liquor is separated from the coffee grounds, and type of brewing equipment (Heath 1988). Although originating from different parts of the world, a number of methods of coffee preparation have been widely adopted.

In the Turkish, or Greek, method the coffee is not filtered from the liquor and this results in a pungent, thick, and muddy brew. With percolation, coffee grounds are continuously exposed to boiling coffee liquor of increasing strength. The Autodrip technique simply pours hot water over grounds in a filter and lets the brew drip out the bottom (this technique is the most popular in the US). The French Press, or Press pot,

requires coffee to be coarsely ground since its filter is not as tight as paper filters. The mesh of the filter allows coffee oils and small undissolved solid particles to pass through. A cup of French-pressed coffee is noticeably fuller, with much more body, and often with more flavour than coffee brewed by most of the other procedures (except espresso), and it often leaves a tell-tale sediment at the bottom of the cup.

Another popular technique for brewing coffee is called Espresso. Illy and Viani (1995) have given a quantitative definition of Italian Espresso; it is a beverage prepared from roasted and ground coffee beans and hot water under pressure. The hot water under pressure is applied for a short time to a compact ground coffee cake by a percolation machine in order to obtain a small cup of a concentrated foamy elixir. The variable ranges are: 6.5 ± 1.5 g for the ground coffee portion, 90 ± 5°C for water temperature, 9 ± 2 bar for inlet pressure, and 30 ± 5 s for percolation time.

1.1.2.3 Instant coffee

Instant coffee has been marketed commercially for almost a hundred years (since~1910 by George Washington). The fast and simple preparation of just mixing the instant powder with hot water led to a good customer acceptance, in particular, in England and Japan (Clarke 1987).

The essentials of soluble coffee manufacture consist of producing a coffee extract by roasting, grinding and extracting the roasted beans, removing the water from the brew either by spray-drying, or freeze-drying, agglomeration, and then packing the product for the market (See Figure 1.2) (Smith 1985).

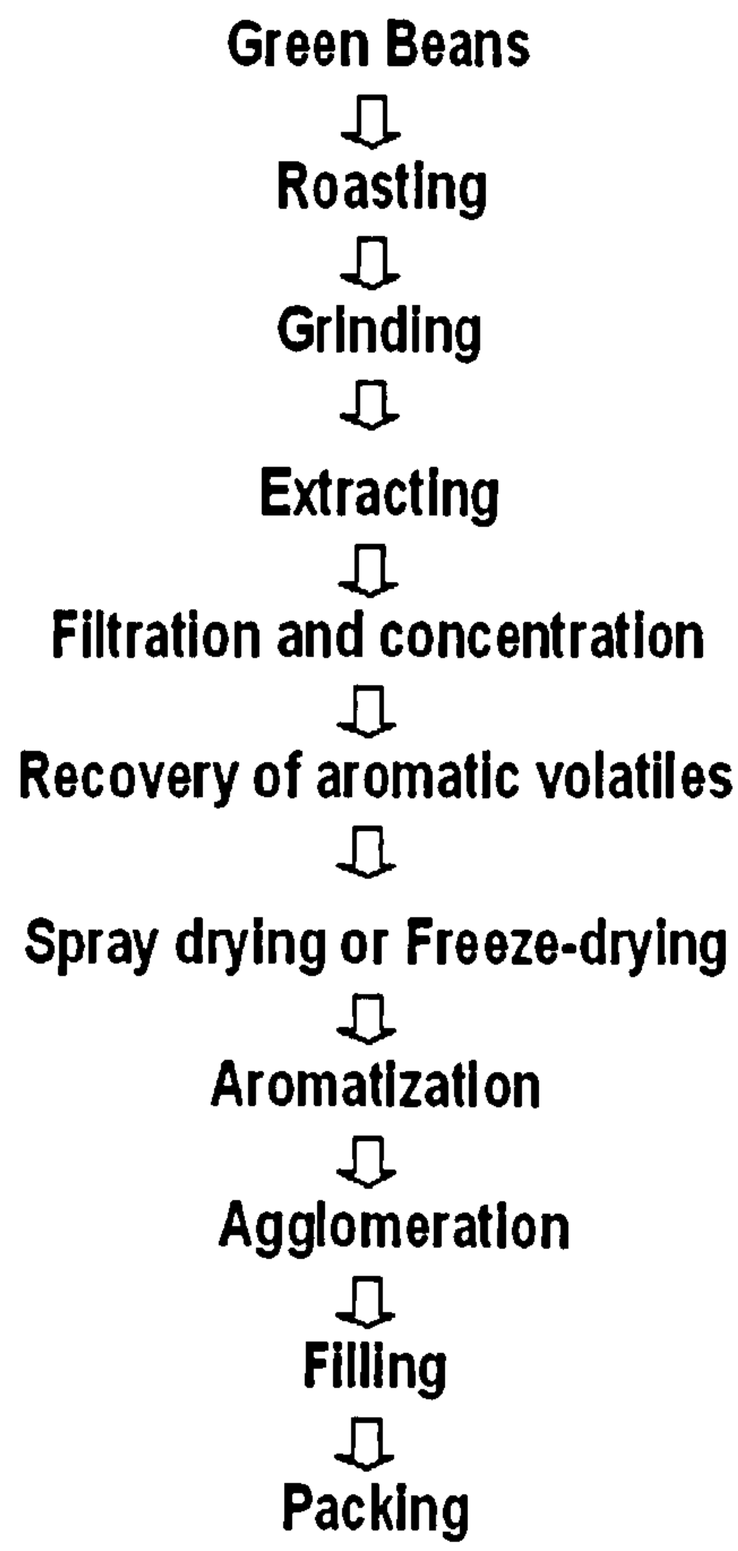


Figure 1.2 Soluble coffee process (Pintauro 1969; Smith 1985).

1.1.3 Coffee Aroma

The pleasant flavour of coffee has been investigated for decades. Coffee flavour results from a complex mixture of aroma compounds along with non-volatile compounds that contribute sourness, bitterness and astringency (Vitzthum 1999).

Numerous studies including those reviewed by Flament (1991) and by Nijssen, Visscher, et al. (1996) have reported more than 800 volatile compounds, with a wide variety of functional groups in coffee headspace (see Table 1.2); most of these compounds are lipophilic (Petracco 2001).

Table 1.2 Most important odourants (FD>32) in a freshly filtered coffee brew (FC) and an instant coffee beverage (IC).

No.	Compound[b]	Odour Quality[c]	FD Factor in	
			FC	IC
1	2,3-butandione[e]	Buttery	32	4
2	1-octen-3-one[e]	mushroom-like	32	4
3	2-methyl-3-furanthiol[e]	meaty	32	1
4	dimethyl trisulfide[e]	sulferous	32	<1
5	2-furfurylthiol[e]	roasty	64	4
6	3-isopropyl-2-methoxypyrazine[e]	earthy	64	64
7	3-ethyl-2,5-dimethylpyrazine[d]	earthy	32	1
8	methional[e]	cooked potato	1024	256
9	2-ethyl-3,5-dimethylpyrazine[d]	earthy	256	32
10	2,3-diethyl-5-methylpyrazine[e]	earthy	256	32
11	3-mercapto-3-methylbutyl formate[e]	catty	1024	64
12	3-isobutyl-2-methoxypyrazine[e]	earthy	32	16
13	2-ethenyl-3,5-dimethylpyrazine[e]	earthy	128	64
14	2-ethenyl-3-ethyl-5-methylpyrazine[e]	earthy	256	4
15	2-/3-methylbutanoic acid[d]	sweaty	256	32
16	2-acetyl-2-thiazoline[e]	roasty	32	<1
17	(E)-B-damascenone[e]	boiled apple-like	≥4096	256
18	2-methoxyphenol[d]	phenolic,burnt	≥4096	256
19	4-ethyl-2-methoxyphenol[d]	phenolic	1024	64
20	4-hydroxy-2,5-dimethyl-3(2H)-furanone(Furaneol)[d]	caramel-like	1024	1024
21	2(5)-ethyl-4-hydroxy-5(2)-methyl-3(2H)-furanone (ethylfuraneol)[e]	caramel-like	1024	256
22	4-methoxyphenol[d]	phenolic	64	1
23	3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon)[e]	spicy	1024	1024
24	4-vinyl-2-methoxyphenol	clove-like	≥4096	256
25	5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone (abhexone)[d]	spicy	≥4096	1024
26	3-methylindole[d]	mothball-like	32	<1
27	vanillin[d]	vanilla-like	1024	128

a Source: Sanz et al. (2002), **b** Compounds which appeared in FC with an FD-factor of at least 32 are given, **c** Odour description assigned during AEDA, **d** The compounds were identified by comparing the RI on capillary DB-5 and DB-FFAP, the odour quality perceived during sniffing and MS data with data of reference compounds, **e** The compounds were identified by comparing the RI on capillary DB-5 and DB-FFAP and the odour quality perceived during sniffing with data of reference compounds.

Although the volatile fraction in coffee is very complex, only the bioactive substances (called key odourants) are responsible for coffee flavour (Grosch 1998). A number of studies have attempted to identify the key aroma compounds that contribute to coffee flavour. Examples of which are by considering compounds' aroma values (Rothe, Thomas et al. 1963), odour units (Guadagni, Buttery et al. 1966), or odour activity values

(OAVs) (Acree, Barnard et al. 1984). These units represent the same value as they are all calculated by dividing the concentration of a volatile compound present in the coffee by its odour threshold.

Another way of expressing the odour significance is through the Flavour Dilution factor (FD) which is a relative measure of odour potency obtained by Aroma Extract Dilution Analysis (AEDA) and is proportional to the OAV of the compound in air since it reflects the ratio of the concentration of the odourant in the initial extract to its concentration in the most dilute extract that can be detected by gas chromatography-olfactometry, GCO (Grosch 1998). A study by Sanz et al. (2002) has reviewed some important coffee aromas along with their corresponding odour qualities and FD values obtained during the AEDA (see Table 1.2)

Charm Analysis™ is also commonly used to identify potent, medium and weak intensity volatile odourants. This technique involves analysis of an aroma extract by gas chromatography-olfactometry (GCO). Instead of OAVs or FD, Charm analysis produces Charm values, areas of chromatographic peaks that are proportional to the odour impact of the chemical in the extract (Acree 1993).

The flavour of different cups of coffee can vary significantly despite similarities in the key aroma compounds. The variation can be due to many factors such as the species and variety of beans, growing location, degree of roasting, and brewing method.

1.1.4 Aroma Quality of Freshly Brewed vs. Soluble (Instant) Coffee

Although the technology of instant coffee production has been improved, it is common experience that the aroma of instant coffee still differs significantly from that of the freshly brewed products (Clarke 1987). This is because instant coffee manufacturing processes are not ideally suitable to producing high quality coffee that are rich in flavour and aroma which maintain the varietal characteristic of the roasted coffee from which they are produced. For several decades, this problem has presented a challenge to soluble coffee technologists to improve aroma quality of soluble coffee to be comparable with that of the freshly brewed product.

In many cases, the qualitative compositions of freshly brewed and instant coffee were found to be very similar (Semmelroch and Grosch 1995; Sanz, Czerny et al. 2002). The odour intensities of most of the volatile compounds, however, were found to be much lower in instant coffee. A study by Sanz and Czerny (2002) using AEDA technique has shown that the FD, flavour dilution factors, of nearly every compound in soluble coffee were much lower than those of the freshly brewed (see Table 1.2). As shown, the largest differences were determined for some sulfur-containing compounds (e.g., 2-methyl-3-furanthiol, 2-furfurylthiol, 3-mercapto-3-methylbutyl formate) as well as 2-methoxyphenol, 4-ethyl-2-methoxyphenol, 4-vinyl-2-methoxyphenol and vanillin (Sanz, Czerny et al. 2002). Nearly no odourant showed a higher FD factor in the instant coffee compared to freshly brewed coffee.

1.1.5 Coffee Aroma Recovery: Improving Aroma Quality of Soluble (Instant) Coffee

Coffee aroma is a mixture of hundreds of volatile compounds present at concentrations of ppm or ppb, differing widely in terms of chemistry with no specific functional group dominating. Regarding this fact, it is not easy to reproduce such a complex mixture that represents the same aroma quality as that of natural coffee artificially (Bomben, Bruin et al. 1973).

Efforts to maximize retention of natural coffee aroma in coffee end products during coffee production have, therefore, been made. In many studies, the main approaches involve attempts to collect the aroma at early stages of the coffee process, usually prior to the drying stage, where substantial losses are likely to take place. The recovered coffee aroma is then reincorporated back into the coffee product at later stages where it is thought to incur less chance for loss. Alternative approaches may be carried out using selected water removal processes which deliver high retention of aroma constituents, i.e. freeze drying, low temperature spray drying (Bassoli, Sumi et al. 1993), or a combination of the two (Pelt 1979). At different stages, various techniques have been applied.

Regarding aroma collection at earlier stages, some have suggested trapping the aromatic gases released during the roasting and/or grinding processes using aroma absorbents, i.e. activated carbon, or even heating the ground, roasted coffee to release additional aromatic gases which could be trapped. Others showed that passing steam or appropriate solvents (Ortega, Rodriguez et al. 2005) through a bed of ground, roasted

coffee can strip and capture aromatic components. Aromatic oils can also be expressed from spent coffee grounds by exerting pressure on them. Distillation of the aroma vapor released from coffee extract after the brewing process is also common practice.

The collected aroma from these stages, roasting, grinding or extraction, is subsequently added back in later stages in concentrated forms which can be liquid, or solid, i.e. encapsulated. Examples of common practices are incorporating the concentrated aroma to the coffee extract prior to the drying stage (Cale and Imura 1993), or mixing the concentrate with coffee powder to produce aroma-improved soluble coffee prior to packaging (Chmiel, Traitler et al. 1996.).

Synthetic coffee aroma is sometimes used in the situation where the soluble coffee produced contains no significant amount of natural coffee aroma. This normally happens when higher yield of coffee solids is the major goal where high yield is met at the expense of aroma quality. Improvement of instant coffee aroma, therefore, usually relies on synthetic coffee aroma as the price is more reasonable compared to that of natural coffee flavour.

A new technique suggested by Cirkel-Egner and Maier (2001) is mixing the collected aroma with soluble coffee creamer which is mixed with instant coffee just before drinking. They claimed that this optimized stability of the ingredients and resulted in a beverage which closely resembled freshly brewed coffee (Cirkel-Egner and Maier 2001).

1.1.6 Arabica vs. Robusta

As mentioned earlier, 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 possesses a flatter flavour, lacking in taste, and a muddy odour.

Although considered as a low-grade product, Robusta is advantageous in its low cost. It also has more soluble solids in solution and twice the caffeine content of Arabica. Therefore, it has been exclusively used as a filler in blending with at least some of the more expensive Arabica for instant coffee products, supermarket own label packages, large national and local brand name coffees, and mid to low quality institutional and restaurant brands. In the US, coffee blends prepared for food service containing ≥50% Robusta are not uncommon (Izumitani and Yajima 1990; Becker, Schlabs et al. 1991; Schoenholt 1992).

The differences in the sensory characteristics of these coffees are mainly due to their differences in chemical compositions, more quantitative than qualitative (see Table 1.3). Details on the key coffee aromas, as well as chemical compositions of the two coffee types, will be discussed here.

1.1.6.1 Sensory Characters and Aroma Compounds

Regarding the differences in aromas, several works have attempted to discriminate the aromas of the two coffee varieties based on both sensory and instrumental analyses. A study by Leino et al. (1992) reported that the sensory attributes which accounted for the differences observed between roasted Arabica and Robusta

coffee brews were cocoa, papery and brown sugar odours. They found that Robusta coffee had less cocoa and brown sugar notes, but more earthy, papery and burnt odour characteristics than Arabica. Regarding aroma compounds, Arabica coffee was found to contain more dimethyl sulfide, 2-methyl-propanal, butandione, 2,3-pentandione, 2-furaldehyde and 3-methylbutanal than Robusta. However, Robusta contained more phenol, toluene, 1-methylpyrrole, thiophene, 2-hydroxyphenol, 2,5-dimethylpyrazine and furfuryl acetate than Arabica, although the amounts of the compounds were quite small (Leino, Lapvetelaeinen et al. 1992).

Table 1.3 Furan and caramel compounds of roasted coffees (Tressl, Bahri et al. 1978).

Component	Arabica (ppm)	Robusta (ppm)
furfuryl alcohol	300	520
2-furancarboxylic acid	80	55
hydroxymethylfurfural (HMF)	35	10
4-hydroxy-2,5-dimethyl-2H-furan-3-one (furaneol)	50	25
4-ethoxy-2,5-dimethyl-2H-furan-3-one (ethylfuraneol)	8	2
3-methylcyclopentene-1,2-dione (cyclotene)	40	26
2-acetyl-3-hydroxyfuran (isomaltol)	8	1.5
3-hydroxy-2-methyl-4-pyrone (maltol)	39	46
3,5-dihydroxy-2-methyl-4-pyrone (5-hydroxymaltol)	15	6
5,6-dihydro-3,5-dihydroxy-2-methyl-4-pyrone (5,6-dihydro-5-hydroxymaltol)	13	10

1.1.6.2 Chemical Composition: Aroma Precursors

Apart from using sensory descriptors as discriminative tools to separate the two coffees, many studies also attempted to classify coffee varieties based on differences in their chemical compositions.

Table 1.4 Chemical composition (%w/w d.b.) of Arabica and Robusta coffees (Martin, Pablos et al. 1998).

Sample[a]	EXT[b]	POL[c]	AA[d]	TRIG[e]	CHLOR[f]	CAF[g]
1A	27.35	5.0	0.19	1.78	3.2	0.9
2A	27.53	5.2	0.26	1.79	3.4	1.1
3R	23.13	7.5	0.26	1.35	4.9	2.2
4A	27.29	5.0	0.27	1.68	3.9	1.3
5R	25.33	9.5	0.29	0.94	4.2	2.3
6A	23.29	5.6	0.19	1.10	3.7	1.2
7R	26.43	7.8	0.16	0.91	5.6	2.7
8A	27.17	5.9	0.19	1.10	3.9	1.3
9A	26.37	5.9	0.22	1.27	3.5	1.3
10R	27.41	8.2	0.22	0.96	4.6	2.4
11A	25.61	5.2	0.17	1.04	4.1	1.2
12R	27.41	8.4	0.24	1.72	4.7	2.8
13A	24.18	6.1	0.19	1.12	3.7	1.1
14A	22.08	6.4	0.26	1.19	3.4	1.0
15R	23.18	7.4	0.21	1.61	3.8	2.4
16A	24.18	7.2	0.15	1.66	3.7	1.1
17A	28.83	8.2	0.18	1.92	3.7	1.1
18R	24.78	7.8	0.22	1.94	4.6	2.7
19R	24.78	7.0	0.25	1.83	4.7	3.2
20A	28.26	5.8	0.26	1.21	3.9	1.8
21A	28.90	5.7	0.25	1.14	3.1	1.2

a A: Arabica, R: Robusta, **b** EXT: Contents of aqueous extract, **c** POL: Total polyphenols, **d** AA: Total free amino acids, **e** TRIG: Trigonelline, **f** CHLOR: Cholorogenic acids, **g** CAF: Caffeine.

In green coffee beans, polysaccharides, lipids, and proteins appear to be the major constituents (Underwood and Deatherage 1952). Minor components such as free amino acids, trigonelline, chlorogenic acids (CGA), free sugars (mainly sucrose) and others are, however, also found to be relevant (MacDonald and Macrae 1985). Chemical compositions of the two coffees are shown in Table 1.4.

Among all compounds in green coffee, the carbohydrates and amino acids are considered to be the main components that play important roles, i.e. act as precursors, in the formation of the colour and aroma during coffee roasting (Murkovic and Derler 2006). The importance of each aroma precursor is discussed in more detail here.

1.1.6.2.1 Carbohydrate

It was reported by Prodolliet et al. (1995) that sucrose is the main carbohydrate occurring in green coffee. The finding is in agreement with a later study done by Murkovic and Derler (2006) who found sucrose to dominate carbohydrate in green coffee with a concentration of up to 90 mg/g (mean =73 mg/g) in Arabica beans, whereas, a significantly lower amount was found in Robusta beans (mean 45 mg/g). The content of sucrose in the beans was found to be up to 9% of the total weight. Also, this agreed with the values previously reported by Clark (2003) (8% of sucrose in Arabica and 4 % in Robusta). The other carbohydrates (galactose, glucose, and fructose), however, were found to occur at significantly lower concentrations with mannitol found only in Arabica beans (Murkovic and Derler 2006) (see Table 1.5).

Regarding higher-molecular weight polysaccharides, Arabinogalactan was found to be the main polysaccharide in the water-soluble high-molecular-weight fraction in green coffee (De Maria, Trugo et al. 1994). Besides Arabinogalactan, Bradbury and Halliday (1990) also identified mannan, cellulose, and minor amounts of other polysaccharides. Arabinogalactan was reported in a later study by De Maria et al. (1996a) to be the main polysaccharide involved in the formation of furans in roasted coffee.

Table 1.5 Contents of mono- and disaccharides in green Arabica and Robusta coffees (mg/g d.b.) (Murkovic and Derler 2006).

Sugar	Arabica	Robusta
Mannitol	n.d. – 0.9	n.d.
Fucose	n.d.	n.d.
Arabinose	n.d.	n.d.
Galactose	<0.1 – 0.6	<0.1 – 0.6
Sucrose	63-90	36-56
Glucose	n.d. – 2.2	<0.3 – 1.6
Mannose	n.d.	<0.3
Fructose	n.d. – 1.9	n.d. – 5.9

n.d.: below limit of detection

In addition, Bradbury and Halliday (1990) characterized the major polysaccharides, Arabinogalactan and mannan, and reported that the Arabinogalactan on hydrolysis gave arabinose (19.8% w/w), galactose (48.2% w/w), mannose (0.8% w/w) and rhamnose (1.1% w/w). In contrast, the mannan on hydrolysis gave mannose (94% w/w), galactose (3.3% w/w) and glucose (1.7% w/w).

Comparing the two coffee varieties, no marked difference in the total polysaccharide content was observed between green Arabica and Robusta coffee beans: neither the structures of the galactomannans nor the arabinogalactans in the two green coffee varieties differ significantly (Fischer, Reimann et al. 2001). This finding is in agreement with that of Nunes and Coimbra (2002) who reported 1.85% content, on a dry and defatted weight basis, of the total polysaccharides in Robusta green coffee and found similar values in their green Arabica coffee using the same extraction procedure. However, in the same study, the content of Arabinogalactan extracted from green Robusta was found to be higher than that extracted from Arabica. Therefore, this seems

to be a characteristic that distinguishes Robusta from Arabica green coffees (Nunes and Coimbra 2002).

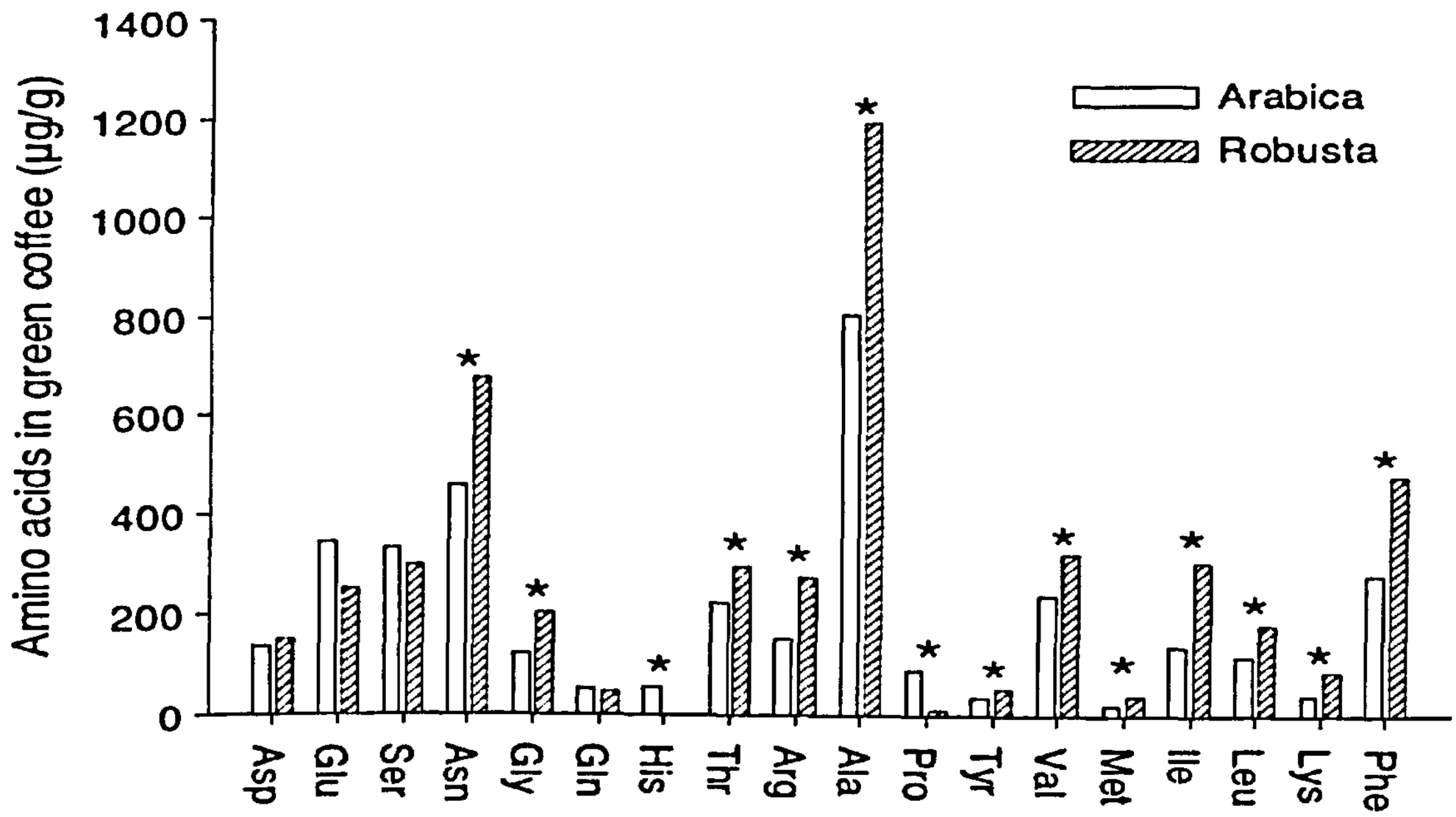
1.1.6.2.2 Amino Acids, Peptides, and Protein

Murkovic and Derler (2006) reported that the concentration of free amino acids is, in general, higher in Robusta than in Arabica. Alanine was found as the most predominant amino acid in coffee followed by asparagine. Alanine contents were found at 1200 μg/g and 800 μg/g in Robusta and Arabica, respectively. While, asparagine was found at 680 μg/g and 360 μg/g in Robusta and Arabica, respectively (Murkovic and Derler 2006). Their finding was, however, different from the previous finding from Casal et al. (2005) who found that glutamic acid, asparagine, and gamma-aminobutyric acid (GABA) were the three main amino acids in both Arabica and Robusta green coffees (Casal et al. 2005). Murkovic and Derler (2006) also suggested that most of the amino acids occur at higher concentrations in Robusta beans than in Arabica with aspartic acid, glutamic acid, serine, and glutamine present in comparable concentrations in both types of green coffee. The only amino acid they found to be higher in Arabica was proline (see Figure 1.3).

Casal et al. (2005) explained, however, that the level of glutamic acid in Robusta can be higher when roasting was applied at lower temperature, as described earlier by Clifford (1985) and Illy & Viani (1995).

Casal et al. (2003) suggested that multivariate analyses on concentrations of free amino acids can be used as a tool for discrimination among coffee species, with a special reference to L-glutamic acid, L-tryptophan, and pipecolic acid. Some studies have also

shown that these compounds can be used for discrimination between green coffees subjected to different postharvest processes. Amino acid levels observed after acid hydrolysis can also be used for the same purposes, although displaying less discriminatory power.



*significant differences

Figure 1.3 Comparison of amino acid content in Arabica and Robusta type green coffee (Murkovic and Derler 2006).

Besides free amino acids, a model roasting study by Ludwig et al. (2000) showed that peptides also play an important role as coffee aroma precursors. Regarding the contents in green coffees, the authors indicated that Arabica and Robusta were not significantly different in peptide content, but were significantly different in peptide composition. Peptides with weakly acid pI values were mainly found in the Robusta, while peptides from Arabica samples ranged evenly from the weakly acidic up to the weakly basic pH range. Apparent molecular weight of the peptides in the two coffee

varieties lay between 4 and 10 kDa. Both coffees were found to contain protease, however differences in the enzyme patterns of Arabica and Robusta, as obtained by electrophoresis, were observed (Ludwig et al. 2000).

Apart from amino acids and peptides, protein is also another source that contributes to coffee aroma formation. Nunes and Coimbra (2002) reported that protein accounted for 36% of the green coffee's high-molecular-weight material (HMWM), while the content decreased to 11-13% after the same coffee was roasted (Nunes and Coimbra 2002).

It was reported that the major proteins present in green Arabica infusions had molecular weights of 58 and 38 kDa. However, from roasted coffees, only a defined band with $\leq$ 14 kDa and a diffuse band with > 200 kDa were observed (Nunes and Coimbra 2001).

1.1.6.2.3 Other Possible Flavour Precursors

Besides higher-molecular-weight components, low molecular weight compounds such as trigonelline and chlorogenic acids (CGA) are also considered important coffee aroma precursors. The degradation of trigonelline was found to yield a range of volatiles such as pyridines and pyrroles (Viani and Horman 1974), while CGA and the esters of quinic acid, were found to contribute to phenols, e.g. guaiacol, 4-vinylguaiacol, and phenol , in roasted coffee (Clifford and Willson 1985).

It was proposed that the superiority of Arabica in beverage quality could be accounted for by its lower level of CGA in the beans compared to that in the Robusta as

the CGA is known to contribute to the final acidity, astringency and bitterness in the final cup.

Many studies have tried to correlate levels of CGA to the final cup quality, most have agreed that CGA level has an inverse association with coffee quality, with higher CGA content being observed in lower quality samples (Silva, 1999; Farah et al. 2006). Bertrand et al. (2003) suggested that the large difference in CGA contents of these two species can be considered one of the factors responsible for flavour differences between the two species.

1.1.7 Coffee Aroma Formation

1.1.7.1 Factors, Reactions, and Mechanisms

During coffee processing, roasting is the key step responsible for coffee aroma development and is the stage where several chemical reactions take place. The reactions involved are Maillard and Strecker reactions, sugar caramelization, degradation of trigonelline, chlorogenic acids (CGA) and polysaccharides. Among these reactions, the Maillard reaction is regarded to be the main one. It involves reactions between reducing sugars and amino acids. These precursors, as mentioned earlier, are found in green coffee beans as a complex mixture of various soluble components, such as glucose, fructose, galactose, and sucrose. Rhamnose and arabinose were also claimed to play parts in the caramelisation and Maillard process since their amounts were found to decrease during roasting (Tressl 1989). Belitz and Grosch (1999) revealed the loss of total amino acid content of 30% during the roasting process where lysine, serine, threonine, arginine, histidine, methionine and cystine were found to degrade to a high extent.

In addition to precursor content in the beans, other factors that influence flavour formation are pH, water activity, as well as temperature and time (Reineccius 1990).

Evidence from Madruga and Mottram (1995) and Hofmann and Schieberle (1998) showed that important sulfur-containing compounds in meat such as 2-methyl-3-furanthiol, 2-furfurylthiol, and 2-methyl-3-(methyldithiol)furan are preferably formed at a pH of 3-4. Oh et al. (1991) found that a superior roasted /meaty character was obtained in an aqueous system in their study. Schieberle and Hofmann (1998) found that drying (as compared to aqueous conditions) yielded higher amounts of key odourants with roasty notes such as 2-furfurylthiol, 2-ethyl-and 2-ethynyl-3-5-dimethylpyrazine, whereas the meat-like sulfur compounds were found in comparable lower concentrations in their cysteine/ribose model system. Regarding water activity, evidence by Leahy and Reineccius (1989) indicated pyrazine formation was optimal at an a_w of about 0.75.

In Maillard reactions, there are three main chemical pathways starting with imine formation between a reducing sugar and an amino acid (Figure 1.4). The Amadori compound (derived from aldose sugars) and Heyn's products (derived from ketose sugars) are rearrangement products and important intermediates of the early phase of the Maillard reaction.

As shown in Figure 1.4, route A leads to the formation of 1- and 3-deoxyosones, which upon cyclisation, reduction, dehydration and/or reaction with hydrogen sulfide result in heterocyclic aroma compounds. Route B is characterized by fragmentation of the sugar chain through retro-aldolisation or alpha or beta-cleavage. By aldo-condensation of two sugar fragments or a sugar fragment and an amino acid fragment, heterocyclic aroma

compounds are generated upon cyclisation, dehydration and/or oxidation reactions. Alternatively, the fragments can react with hydrogen sulfide and form very potent alicyclic flavour substances. Route C involves the so called Strecker degradation of amino acids, which is catalysed by dicarbonyl or hydroxycarbonyl compounds. The reaction is a "decarboxylating transmination" and the resulting Strecker aldehydes are potent flavour compounds. Strecker aldehydes can also be formed directly from Amadori or Heyn's rearrangement products.

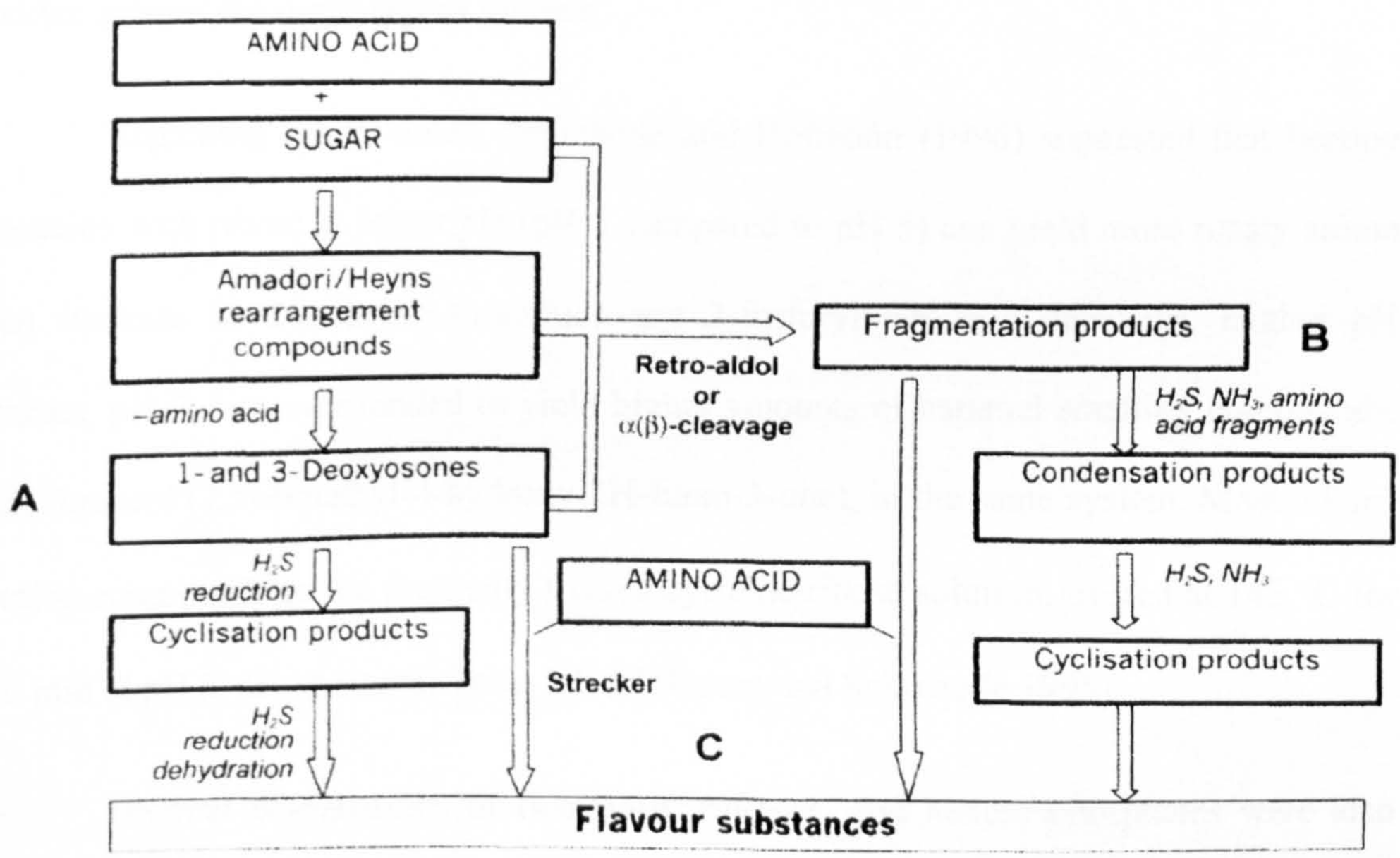


Figure 1.4 Maillard reaction pathways (Kerler and Winkel 2002).

Given that a specific coffee flavour is desired, a strong understanding of the routes involved in aroma formation is required. This can first be achieved by evaluating the character impact compounds of the desired flavour or model system using a combination of sensorial and instrumental analysis (e.g. based on the odour activity value

concept) (Kerler and Winkel 2002). By identifying the sensorially important end products, the important precursors and chemical pathways can also be identified. Once the precursors and the processing conditions are right, certain food flavours can be mimicked (Lane and Nursten 1983).

1.1.7.2 Model Systems for Specific Coffee Aromas

Several studies have developed model systems in an attempt to generate specific aromas from specific precursors. Some model systems involving formation of important coffee aromas are described as follows.

Regarding roasty notes, Schieberle and Hofmann (1996) suggested that heating cysteine with ribose at lower pH (pH 3 compared to pH 5) can yield more roasty aroma (an increase in 2-methyl-3-furanthiol and 2-furfurylthiol was detected). Higher pH values, pH 7, however tended to yield higher amounts of caramel-smelling compounds, i.e. furaneol (2,5-dimethyl-4-hydroxy-2H-furan-3-one), in the same system. Most odour-active compounds in the thermally treated cysteine-ribose solution, treated at 145 °C for 20 min at pH 5, are shown in Table 1.6 (Hofmann and Schieberle 1995).

Thermal degradations of rhamnose, fructose, and hexose-phosphates were also shown to form furaneol (Blank, Devaud et al. 1996). Zabetakis et al. (1996) proposed that the deoxysugar, 6-deoxy-D-fructose, was the main precursor of furaneol-glucoside, one of the furaneol derivatives present in strawberry. Their experiment found that 6-deoxyhexoses with an aldo group (6-deoxy-D-galactose, 6-deoxy-L-galactose and 6-deoxy-L-mannose) have a smaller effect on the amount of furaneol-glucoside formed than the 6-deoxyhexose with a keto group (6-deoxy-D-fructose).

Regarding pyrazines, De Maria et al. (1996b) reported that pyrazines are mainly formed by pyrolysis of hydroxyl amino acids, higher molecular weight aroma precursors . The finding is in agreement with that reported before by Baltes and Bochmann (1987).

L-ascorbic acid can also be utilized as a precursor for the production of variety of flavour and aroma constituents, i.e. in Strecker degradation reactions with free amino acids in heated model systems. It was found that, under basic conditions, the condensation reactions of L-ascorbic acid with lysine can create a high number of N-containing heterocyclic compounds (Rogacheva, Verhe et al. 1996).

Table 1.6 Odour-active compounds in the thermally treated Cysteine-Ribose solution, treated at 145 °C for 20 min at pH 5 (Schieberle and Hofmann 1996).

Odourants	Flavour Dilution
2-furfurylthiol	512
3-mercapto-2-pentanone	512
2-methyl-3-furanthiol	256
5-acetyl-2,3-dihydro-1,4-thiazine	256
3-mercapto-2-butanone	128
bis(2-methyl-3-furyl)disulfide	128
2-acetyl-2-thiazoline	64
2-hydroxy-5-methyl-3(2H)-furanone	64
4-hydroxy-2,5-dimethyl-3(2H)-furanone	32

1.1.8 Coffee Taste Compounds

Apart from aroma compounds which are known to be responsible for the sensory quality of coffee, taste compounds, mainly non-volatile, also play an important role in the overall quality of coffee that should also be taken into consideration when it comes to quality evaluation.

In green coffee, most compounds are in the non-volatile state and the sensory notes caused by them are not prominent. However, these substances are precursors of

important volatile and non-volatile flavour compounds that will be developed during roasting. These non-volatile compounds, therefore, are important factors determining final sensory quality of coffee brew.

In the previous chapter, volatile compounds and their contribution to coffee sensory quality have been discussed. Hence, this section will focus only on the non-volatiles and their direct functions in coffee quality.

Buffo and Cardelli-Freire (2004) have summarized the key non-volatile compounds found in roasted coffee which are:

- Caffeine
- Trigonelline and its two non-volatile derivatives: nicotinic acid and N-methylnicotinamide
- Protein and peptides
- Polysaccharides: cellulose, hemicelluloses, arabinogalactan, and pectin
- Humic acids or melanoidins
- Carboxylic acids: citric, malic, acetic
- Chlorogenic acids (O-caffeoylquinic acids)
- Lipids
- Minerals: Potassium (40% w/w of the total mineral)

Each of the compounds contributes to different functions in coffee brew. For instance, polysaccharides and lipid contribute to texture, i.e. viscosity, of coffee as well as play role in the retention of volatiles (most coffee aromas are located in coffee oil; Rizzi et al. (2004). Melanoidins contribute mainly to the colour, whereas most of the rest of the compounds are responsible for the taste sensations in coffee brew.

Table 1.7 Yields and bitter intensity of fractions isolated from the coffee beverage (CB) by means of high-vacuum distillation, sequential solvent extraction, and ultrafiltration (Frank, Zehentbauer et al. 2006).

Sample	Amount (g/100 g)	Yield[b] (%)	Perceived bitter intensity[c]
Coffee beverage (CB)[a]	23	100	4.0
CB volatiles	n.d	n.d	0
CB non-volatiles	n.d	n.d	4.0
CB fraction I	0.41	1.8	0
CB fraction II	2.10	9.1	3.5
CB fraction III	2.21	9.6	0.5
CB fraction IV	18.26	79.4	0
CB fraction II, >1 kDa	0.1	0.5	0
CB fraction II, <1 kDa	2.00	8.7	3.5

[a]Beverage was freshly prepared by percolating ground, roasted, decaffeinated coffee (54 g) with water (1100 ml, 80 °C).
[b]Yields were determined by weight.
[c]The bitter taste intensity of aqueous solutions (pH 5.0) containing the individual coffee fractions in "natural" concentrations were judged on a scale from 0 (not detectable) to 5.0 (strongly detactable).

Regarding coffee taste, three main taste sensations found in coffee are bitterness, astringency and sourness. A comparative sensory analysis by Frank et al. (2006) found that green coffee only possesses astringency and sourness when brewed. Bitterness, however, was found to be pronounced in the beverage prepared from roasted coffee. Their fractionation experiment also discovered that this coffee bitterness was exclusively located in the non-volatile fraction (as shown in Table 1.7).

1.1.8.1 Coffee Key Taste compounds

Since bitterness, astringency and sourness are claimed as key tastes present in coffee, compounds believed to be responsible for the coffee taste sensations are discussed in detail here.

1.1.8.1.1 Chlorogenic acids (CGA) and Lactones

CGA have a marked influence on roasted coffee sensory character (Farah, Monteiro et al. 2006). They are involved in the formation of bitterness and astringency of coffee due to their decomposition into phenolic compounds upon roasting. As a consequence, their composition in coffee is very important in determining coffee cup quality (Moreira, Trugo et al. 2001).

CGA are particularly abundant in the coffee plant (Streuli 1970; Amorin, Teixeira et al. 1974), especially in green beans as well as in roasted coffee beans, soluble coffee powders, and coffee brews (Clifford and Willson 1985). CGA are esters of trans-cinnamic acids, such as caffeic, ferulic and p-coumaric acids, with (-)-quinic acid (QA) (Figure 1.5) (Clifford 2000). Frank et al. (2007) reported that CGA precursors, i.e. caffeic acid, can generate intense bitterness reminiscent of the bitter taste of a strongly roasted espresso-type coffee.

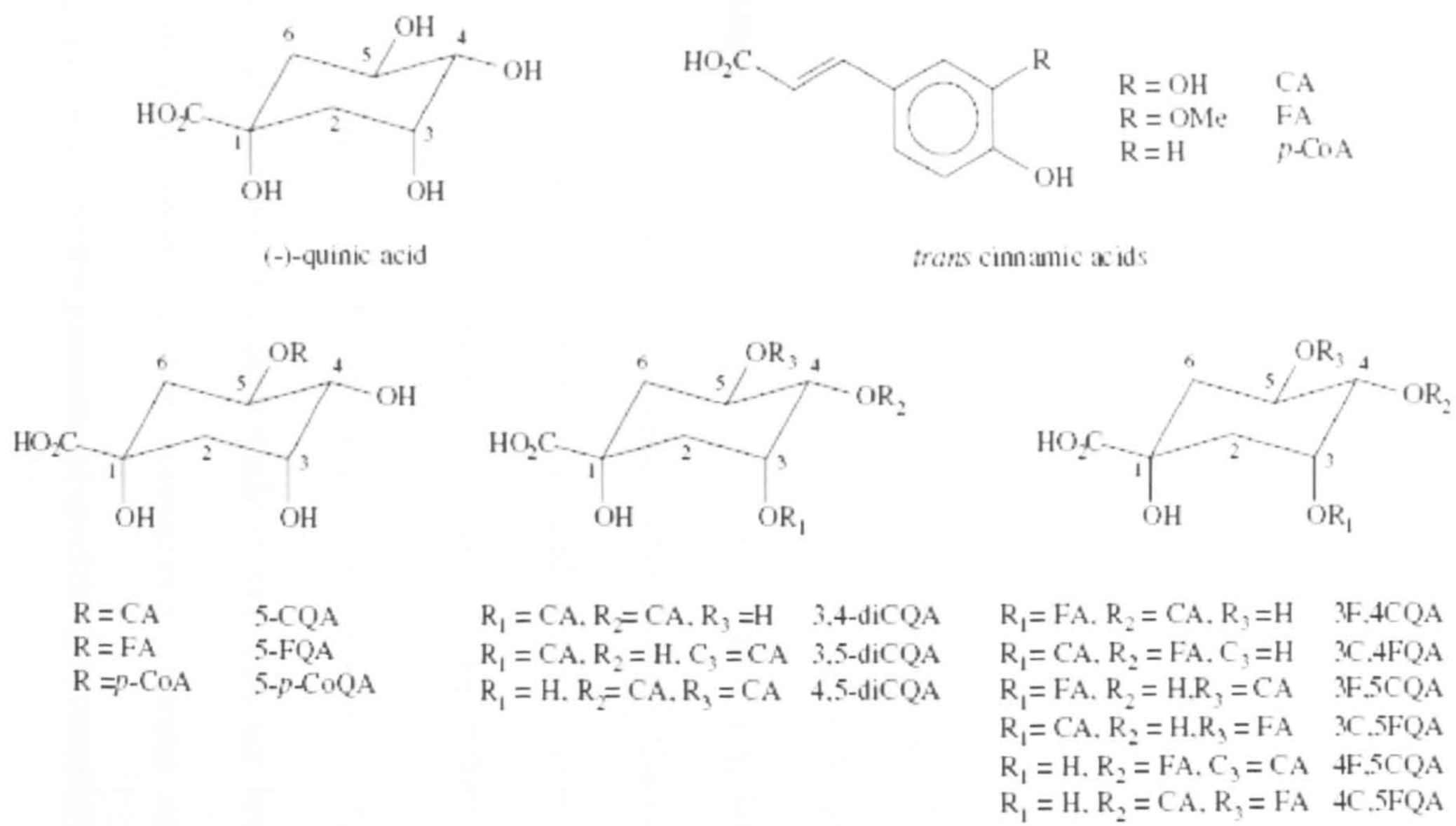


Figure 1.5 Structure of chlorogenic acids precursors – quinic acid, caffeic acid (CA), ferulic acid (FA), p-coumaric acid (p-CoA) – followed by CGA main subclass: caffeoylquinic acids (CQA), feruloylquinic acids (FQA), p-coumaroylquinic acids (p-CoQA) (Example of 5-isomers for CGA monoesters), dicaffeoylquinic acids (diCQA) and caffeoylferuloylquinic acids (CFQA). IUPAC numbering system (IUPAC, 1976) is used for chlorogenic acids (Perrone et al. 2008b).

During roasting, CGA are largely degraded, via thermohydrolysis. Thus, quinic acid is released. Frank et al. (2007) reported that quinic acid exhibited an "aspirin-like" bitter taste at a threshold concentration of 10 ppm. As the concentration of it in roasted coffee exceeded its taste threshold concentration by a factor of 20, quinic acid, therefore, was also claimed as one of the taste compounds contributing to bitterness in coffee (Maga 1978; McCamey, Thorpe et al. 1990).

Six major classes of CGA have been reported. They are caffeoylquinic acids (CQA), dicaffeoylquinic acids (diCQA), feruloylquinic acids (FQA), p-coumaroylquinic acids (pCoQA), caffeoylferuloylquinic acids (CFQA), and feruloylcaffeoylquinic acids (FCQA). The first three classes represent around 98% of the total CGA found in coffee (Clifford and Staniforth 1977). Each class of CGA contains three isomers differing in the number and identity of the acylating residues (Ky, Louarn et al. 1999). Recently, minor classes such as diferuloylquinic acids (diFQA), di-p-coumaroylquinic acids (di-p-CoQA), dimethoxycinnamoylquinic acids and others, which together constitute less than 1% of total CGA content, have also been identified (Clifford, Knight et al. 2006).

Due to the abundance of CGA and their effect on sensory character in coffee, several attempts have been made to correlate the levels of CGA with beverage quality particularly trying to find correlations between specific sensory attributes, such as astringency, and the presence of specific CGA isomers.

Ky et al. (1999) proposed that CGA of coffee beans modifies cup taste through both direct and indirect effects. The direct effect is due to the presence of CGA after roasting. For example, diCQA is known to increase astringency (Ohiokpehai et al. 1982).

Table 1.8 Chlorogenic acids (CGA) contents in economically relevant Brazillian green and roasted coffee cultivars (Perrone, Farah et al. 2008).

Roasting condition[b]	Weight loss (%)	3-CQA	4-CQA	5-CQA	3-FQA	4-FQA	5-FQA	3-*p*-CoQA	4-*p*-CoQA	5-*p*-CoQA	3,4-diCQA	3,5-diCQA	4,5-diCQA	3,4-diFQA	CFQA
C. arabica cv. Mundo Novo															
Green	0.0	667.2 ± 15.4	807.5 ± 13.2	3611.2 ± 63.1	9.6 ± 0.6	31.3 ± 0.9	192.0 ± 9.6	11.4 ± 0.2	8.0 ± 0.5	49.5 ± 2.3	262.1 ± 14.4	269.1 ± 15.9	316.6 ± 27.6	0.7 ± 0.1	31.2 ± 7.2
1	11.5	1096.6 ± 26.4	1344.7 ± 40.8	2736.7 ± 73.2	43.2 ± 0.8	58.3 ± 4.4	179.0 ± 3.8	12.6 ± 0.9	11.6 ± 1.0	44.2 ± 0.4	198.3 ± 4.5	154.6 ± 3.3	232.7 ± 11.8	0.8 ± 0.0	16.6 ± 0.1
2	13.6	720.8 ± 13.0	868.4 ± 18.2	1656.6 ± 29.3	31.5 ± 1.2	49.1 ± 0.6	143.8 ± 8.8	8.4 ± 1.5	6.8 ± 2.4	28.8 ± 1.4	103.4 ± 4.2	80.2 ± 3.2	118.6 ± 13.7	0.8 ± 0.0	10.3 ± 0.7
3	15.4	433.1 ± 2.5	493.3 ± 1.6	942.0 ± 11.4	18.9 ± 0.8	33.2 ± 0.3	104.1 ± 1.5	4.7 ± 0.8	5.9 ± 0.9	19.2 ± 1.0	48.1 ± 0.9	33.4 ± 1.3	49.6 ± 0.1	0.5 ± 0.0	5.4 ± 4.3
4	16.1	324.9 ± 2.4	388.4 ± 5.4	673.6 ± 3.1	13.6 ± 0.3	26.6 ± 0.7	80.7 ± 0.0	3.8 ± 0.3	ND[c]	15.8 ± 3.2	33.1 ± 1.2	21.9 ± 1.4	32.9 ± 0.5	0.4 ± 0.0	1.8 ± 0.0
5	19.9	51.8 ± 0.8	55.2 ± 1.5	93.3 ± 2.5	11.5 ± 0.4	7.2 ± 0.9	15.3 ± 1.0	ND	ND	ND	ND	1.4 ± 0.0	2.2 ± 0.5	ND	ND
C. arabica cv. Catuaí Vermelho															
Green	0.0	618.0 ± 1.7	770.9 ± 13.4	3357.4 ± 55.3	6.7 ± 0.2	25.7 ± 1.9	160.9 ± 2.2	10.3 ± 0.3	6.1 ± 0.3	38.8 ± 0.7	190.3 ± 2.0	156.9 ± 3.1	164.6 ± 4.2	0.9 ± 0.1	13.4 ± 1.0
1	10.4	1030.1 ± 10.5	1253.4 ± 20.2	2451.9 ± 16.6	20.2 ± 0.9	51.0 ± 0.3	148.6 ± 2.1	13.4 ± 0.6	11.2 ± 1.0	38.1 ± 1.4	146.9 ± 2.8	109.2 ± 2.2	161.2 ± 1.9	1.7 ± 0.2	7.8 ± 0.3
2	11.7	807.5 ± 1.1	971.0 ± 0.4	1905.1 ± 12.1	16.7 ± 1.2	38.2 ± 1.3	139.2 ± 4.3	9.4 ± 4.8	9.6 ± 4.1	39.0 ± 0.6	94.5 ± 5.3	69.9 ± 3.3	102.4 ± 7.2	1.4 ± 0.0	4.6 ± 0.3
3	14.1	495.3 ± 1.8	592.6 ± 1.3	1150.6 ± 8.2	9.4 ± 1.2	29.7 ± 1.2	109.9 ± 3.2	5.5 ± 0.4	ND	28.1 ± 1.2	46.8 ± 3.0	36.2 ± 3.8	54.8 ± 7.3	0.8 ± 0.1	6.0 ± 2.6
4	15.0	328.0 ± 18.9	393.4 ± 25.2	761.4 ± 54.5	7.1 ± 0.2	21.4 ± 1.0	77.4 ± 5.0	1.5 ± 0.6	ND	19.8 ± 1.7	26.2 ± 0.5	20.2 ± 2.5	29.7 ± 4.2	0.5 ± 0.2	1.3 ± 0.1
5	17.9	70.4 ± 3.8	82.4 ± 5.0	118.9 ± 29.4	ND	7.0 ± 2.9	18.0 ± 1.8	ND	ND	3.0 ± 0.4	ND	1.2 ± 0.2	4.4 ± 1.1	ND	0.3 ± 0.0
C. canephora cv. Conillon															
Green	0.0	1065.6 ± 9.5	1277.2 ± 13.6	4114.0 ± 39.7	32.6 ± 0.2	57.5 ± 1.0	519.7 ± 6.0	10.6 ± 0.3	11.8 ± 0.1	30.0 ± 6.2	429.1 ± 11.8	283.5 ± 7.9	586.4 ± 17.7	2.9 ± 0.1	136.5 ± 5.6
1	10.3	1308.4 ± 0.1	1666.5 ± 15.9	3175.5 ± 4.1	115.3 ± 2.3	139.0 ± 1.7	411.1 ± 2.7	9.4 ± 0.3	11.5 ± 0.5	20.6 ± 1.0	269.6 ± 5.0	195.8 ± 2.4	503.5 ± 0.8	5.9 ± 0.2	113.9 ± 1.0
2	11.7	1064.6 ± 38.8	1324.9 ± 47.4	2460.5 ± 85.7	108.1 ± 2.6	125.3 ± 4.1	362.6 ± 7.2	8.0 ± 0.3	9.2 ± 1.3	19.1 ± 1.5	181.2 ± 23.5	138.8 ± 12.2	370.9 ± 48.2	5.9 ± 0.3	76.7 ± 5.4
3	13.2	711.0 ± 27.2	894.4 ± 32.4	1534.3 ± 52.9	184.8 ± 2.8	105.3 ± 0.2	289.0 ± 2.6	5.9 ± 0.1	5.9 ± 0.4	16.3 ± 1.1	94.5 ± 3.8	73.8 ± 6.8	221.9 ± 8.6	7.2 ± 0.3	57.1 ± 2.0
4	13.9	563.4 ± 25.6	706.7 ± 21.4	1145.1 ± 37.5	155.3 ± 6.0	89.5 ± 4.2	223.5 ± 1.0	5.0 ± 0.1	ND	14.3 ± 0.7	61.1 ± 3.6	48.5 ± 8.0	163.4 ± 6.9	5.2 ± 0.7	39.0 ± 0.3
5	16.8	95.9 ± 2.5	106.9 ± 0.5	174.9 ± 1.6	49.6 ± 2.0	24.6 ± 0.3	51.4 ± 2.7	1.6 ± 0.3	ND	ND	3.9 ± 0.3	2.3 ± 0.2	30.0 ± 1.1	2.3 ± 0.1	ND

[a] Results are shown as the means of extractions in duplicates ± standard deviation, expressed as mg/100 g of coffee dry weight, except for 3,4-diFQA, which is expressed as mg/100 g of coffee dry weight.

[b] 1 = 170 °C, 6 min; 2 = 170 °C, 8 min; 3 = 170 °C, 12 min; 4 = 170 °C, 15 min; 5 = 200 °C, 15 min.

[c] Not detected.

The indirect effects are due to molecular changes during roasting which can have positive or negative influence. Degradation of CGA into phenolic derivatives (Leloup, Louvrier et al. 1995) and the inhibition of pyrazine formation by the Maillard reactions (Guyot, Christophe et al. 1997) have negative effects. On the contrary, the CGA reaction with trigonelline, sucrose, and amino acids, which enriches headspace volatile profiles, is a positive effect (De Maria, Trugo et al. 1994).

Chlorogenic acid lactones (CGL) have also been claimed to contribute intense bitterness to roasted coffee in many studies. The institute for Coffee Studies (2001) reported that approximately half of the CGA compounds lose a molecule of water during roasting, thereby forming an internal ester bond that results in a mixture of non-acidic quinolactones (quinides). Perrone et al. (2008) proposed that among the lactones generated from roasting, cinnamoyl-1,5-γ-quinolactones (CGL) are the main CGA lactones found in roasted coffee, being produced through the formation of an intramolecular ester bond between positions 1 and 5 of quinic acid (Farah, de Paulis et al. 2005).

Regarding the CGL formation mechanism, an experiment by Frank et al. (2008) showed that through trans-esterification, epimerization, and lactonization reactions (Frank, Zehentbauer et al. 2006) (see Figure 1.6), the non-bitter 5-O-caffeoylquinic acid in the raw beans is converted upon roasting into the five bitter-tasting CGL compounds, i.e. 5-O-caffeoyl-muco-γ-quinide, 3-O-caffeoyl-γ-quinide, 4-O-caffeoyl-muco-γ-quinide, 5-O-caffeoyl-epi-δ-quinide, and 4-O-caffeoyl-γ-quinide. Similarly, the bitter tasting 3-O-feruloyl-γ-quinide and 4-O-feruloyl-γ-quinide were found to be generated from O-feruloylquinic acids, and the bitter-tasting 3,4-O-dicaffeoyl-y-quinide, 3,5-O-dicaffeoyl-

epi-δ-quinide, and 4,5-O-dicaffeoyl-muco-γ-quinide were found to be generated from the corresponding O-dicaffeoylquinic acids (Frank, Zehentbauer et al. 2006).

Table 1.9 Chlorogenic acid lactones (CGL) contents in economically relevant Brazilian green and roasted coffee cultivars (Perrone, Farah et al. 2008).

Roasting condition[b]	Weight loss (%)	3-CQL	4-CQL	3-FQL	4-FQL	3-*p*-CoQL	4-*p*-CoQL	3,4-diCQL
C. arabica cv. Mundo Novo								
Green	0.0	ND[c]	ND	5.6 ± 1.5	ND	ND	ND	ND
1	11.5	11.51 ± 3.5	77.6 ± 1.6	16.8 ± 1.1	15.0 ± 0.7	2.4 ± 0.3	1.6 ± 0.0	3.0 ± 0.9
2	13.6	170.1 ± 4.0	107.6 ± 6.5	25.2 ± 0.6	19.2 ± 0.1	3.5 ± 0.3	2.2 ± 0.9	9.4 ± 0.0
3	15.4	183.2 ± 4.6	118.8 ± 0.7	31.4 ± 2.8	23.8 ± 0.4	4.0 ± 0.2	4.5 ± 1.0	9.9 ± 0.6
4	16.1	161.4 ± 1.0	106.8 ± 1.4	27.3 ± 0.4	22.0 ± 1.2	3.9 ± 0.0	3.9 ± 0.6	8.4 ± 0.7
5	19.9	32.5 ± 0.4	18.0 ± 1.2	10.3 ± 1.4	7.6 ± 1.1	2.0 ± 0.1	0.9 ± 0.2	ND
C. arabica cv. Catuaí Vermelho								
Green	0.0	ND	ND	ND	ND	ND	ND	ND
1	10.4	106.4 ± 0.9	69.9 ± 1.8	14.5 ± 0.0	10.3 ± 0.0	1.6 ± 0.4	1.5 ± 0.4	8.3 ± 1.4
2	11.7	164.2 ± 0.2	109.7 ± 3.5	21.5 ± 5.6	18.8 ± 1.0	3.1 ± 1.9	2.0 ± 1.0	12.5 ± 1.8
3	14.1	215.8 ± 0.5	133.4 ± 0.3	36.8 ± 5.6	26.2 ± 4.0	3.6 ± 0.1	3.8 ± 0.7	11.3 ± 0.9
4	15.0	172.1 ± 7.9	107.6 ± 5.0	23.8 ± 0.3	30.7 ± 2.2	3.2 ± 0.2	3.1 ± 0.8	7.3 ± 1.1
5	17.9	47.7 ± 2.5	26.6 ± 3.5	15.0 ± 0.2	10.7 ± 0.1	2.0 ± 0.3	2.5 ± 0.4	2.6 ± 1.4
C. canephora cv. Conillon								
Green	0.0	ND	ND	ND	ND	ND	ND	ND
1	10.3	153.2 ± 2.1	111.4 ± 0.8	132.0 ± 0.4	31.5 ± 0.4	2.8 ± 0.1	4.8 ± 0.6	20.7 ± 0.1
2	11.7	210.4 ± 1.3	147.2 ± 0.9	143.7 ± 3.5	43.9 ± 0.9	3.7 ± 0.3	5.5 ± 0.5	17.5 ± 2.1
3	13.2	235.3 ± 5.7	163.9 ± 5.1	135.6 ± 4.3	56.6 ± 0.7	4.7 ± 1.1	5.4 ± 0.0	18.4 ± 0.0
4	13.9	218.3 ± 0.9	146.8 ± 1.8	133.4 ± 1.1	66.2 ± 10.8	4.4 ± 0.1	5.4 ± 0.1	10.2 ± 1.2
5	16.8	54.8 ± 0.2	31.6 ± 2.6	52.9 ± 0.6	28.5 ± 3.3	2.0 ± 0.8	3.2 ± 0.4	ND

[a] Results are shown as the means of extractions in duplicates ± standard deviation, expressed as mg/100 g of coffee dry weight.
[b] 1 = 170 °C, 6 min; 2 = 170 °C, 8 min; 3 = 170 °C, 12 min; 4 = 170 °C, 15 min; 5 = 200 °C, 15 min.
[c] Not detected.

The tastes of these quinides were described as being strongly different from the taste quality of other bitter reference compounds such as caffeine or quinine. They were claimed to impart an astringent-like mouth-coating sensation (Frank, Blumberg et al. 2008). Moreover, depending on the chemical structure, the bitter threshold levels of these lactones ranged between 9.8 and 180 μmol/l as determined by human sensory study (Frank, Zehentbauer et al. 2006).

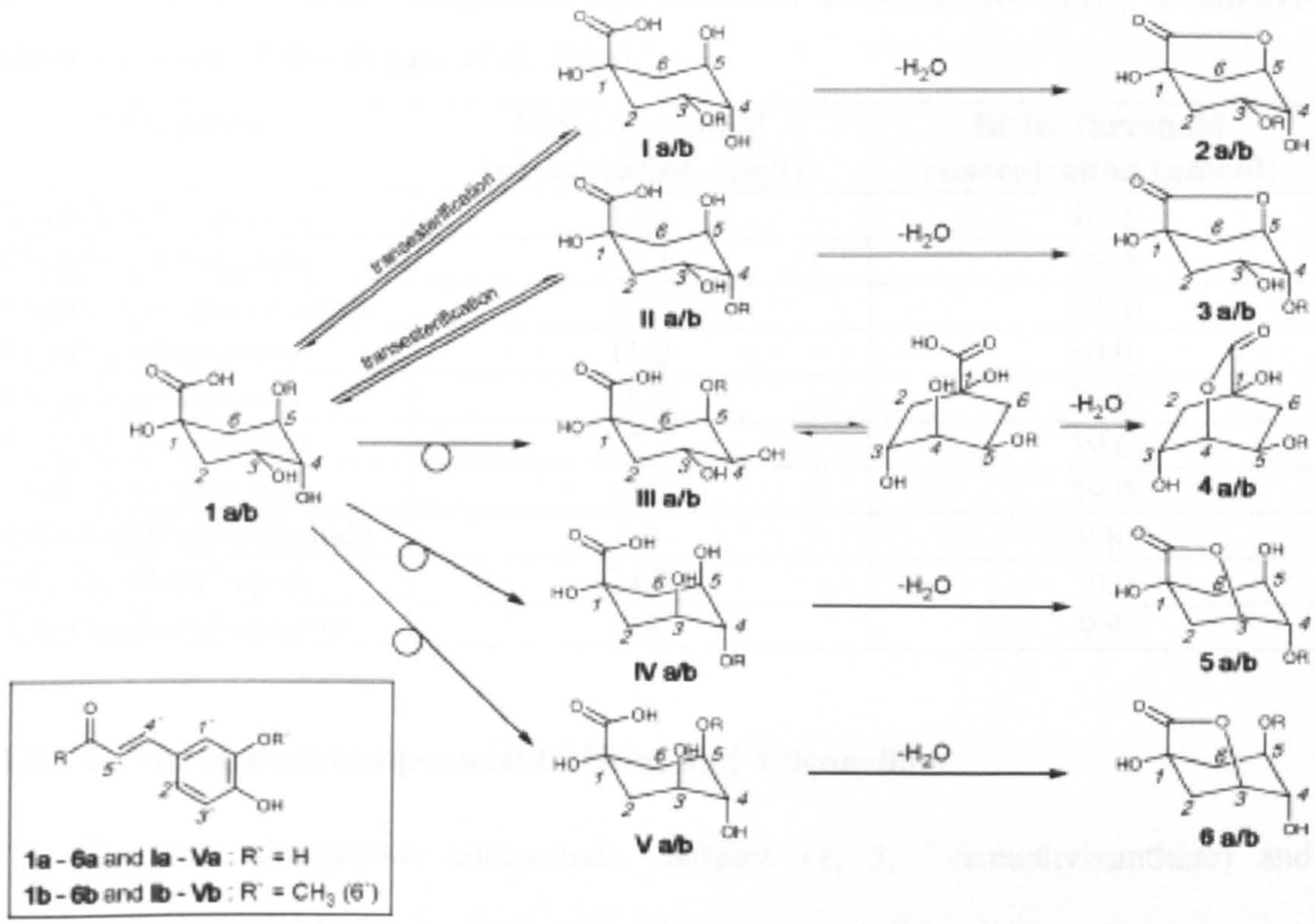


Figure 1.6 Proposed reaction pathways leading to the formation of caffeoyl- and feruloyl-quinides upon roasting of coffee beans (Frank, Zehentbauer et al. 2006).

In addition to CGA and CGL, (Frank, Blumberg et al. 2007) reported that thermal breakdown of the caffeoyl moiety of O-caffeoylquinic acids and /or the corresponding quinides can generate 4-vinylcatechol oligomers which are found to contribute lingering, harsh, bitter-taste to coffee beverage as well.

Table 1.10 Human taste recognition thresholds of caffeoyl-, feruloyl-, dicaffeoyl-quinides (Frank, Zehentbauer et al. 2006).

Compound	Bitter threshold concentration (mg/l)	Bitter threshold concentration (µmol/l)
3-O-caffeoyl-γ-quinide	13.4	40.0
4-O-caffeoyl-γ-quinide	12.1	36.0
5-O-caffeoyl-epi-δ-quinide	60.5	180.0
4-O-caffeoyl-muco-γ-	11.2	30.0
5-O-caffeoyl-muco-γ-	9.7	29.0
3-O-feruloyl-γ-quinide	13.7	39.0
4-O-feruloyl-γ-quinide	13.7	39.0
3,4-O-dicaffeoyl-γ-quinide	4.8	9.8
3,5-O-dicaffeoyl-epi-δ-	24.9	50.0
4,5-O-dicaffeoyl-muco-γ-	4.8	9.8

1.1.8.1.2 Nitrogenous compounds: Caffeine and Trigonelline

The two nitrogenous compounds, caffeine (1, 3, 7-trimethylxanthine) and trigonelline (N-methylbetaines of pyridine-3-carboxylic acid) (Casal, Oliveira et al. 2000b) have been claimed to play important roles in bitterness contribution in coffee drinks.

More than 20 years ago, the first systematic analytical and sensory studies revealed that the alkaloids, caffeine and trigonelline, accounted for a maximum of 10-30 and 1% respectively, of the total bitter taste intensity of a coffee beverage (Chen 1979). Another study by Voilley (Voilley, Sauvageot et al. 1980), however, suggested that caffeine only accounted for around 10% of the perceived coffee bitterness.

Macrae (1989) reported that caffeine possessed a distinct bitter taste with a taste threshold of only 75-155 mg/l (60-200 mg/l). Hardwick (1977) suggested that the bitterness of caffeine can be weakened when polyphenols are introduced (McCamey, Thorpe et al. 1990). The roasting process, however, was reported to have only a little effect on levels of caffeine in coffee (see Table 1.11).

Table 1.11 Contents of trigonelline, nicotinic acid, and caffeine (mg/kg d.b.) in coffee roasted at different temperatures at a constant time (15 min) (Casal, Oliveira et al. 2000b).

	Trigonelline		Nicotinic Acid		Caffeine	
	Arabica	**Robusta**	**Arabica**	**Robusta**	**Arabica**	**Robusta**
Green	8.91	6.32	0.03	0.02	12.36	20.84
140 °C	8.47	6.37	0.06	0.06	14.37	22.12
160 °C	8.31	5.86	0.08	0.05	15.18	21.71
180 °C	8.29	5.78	0.06	0.04	13.57	19.81
200 °C	7.80	5.43	0.07	0.06	13.87	19.93
220 °C	5.57	4.20	0.13	0.06	12.95	19.88
240 °C	0.49	0.97	0.17	0.13	10.96	19.25

Regarding trigonelline, Ordynsky (1965) reported that its bitterness can be perceived at a concentration of 0.25%. The loss of trigonelline strongly dependent upon the degree of roast and associated with the formation of nicotinic acid, a water-soluble B vitamin known as niacin (see Table 1.11), through trigonelline demethylation during the roasting process (Perrone, Farah et al. 2008). This nicotinic acid produced during coffee processing is found to be highly bioavailable, in contrast to natural sources where it is present in the bound form (Trugo 2003). Coffee is, therefore, considered one of the significant sources of niacin in the diet (Trugo and Macrae 1985).

Apart from being a taste compound, trigonelline is also regarded as one of the key coffee aroma precursors contributing, upon roasting, to the formation of important coffee aromas including furans, pyrazine, alkyl-pyridines and pyrroles (Ky, Louarn et al. 2001).

As regards the amounts of these nitrogenous compounds in coffee (as shown also in Table 1.11), Robusta beans were found to contain higher amounts of caffeine compared to Arabica. Arabica, on the other hand, was found to be richer in trigonelline than Robusta. Since trigonelline is known as one of the key coffee aroma precursors,

some authors {Casal, 2000a #271; Ky, 2001 #290} speculated that the higher trigonelline content in Arabica green bean could partially explain its better cup quality when compared to that of Robusta.

In addition, Casal et al. (2000) suggested that levels of caffeine and trigonelline can be used to discriminate between Arabica and Robusta coffees. Nicotinic acid contents, however, were not found to be significantly different between the two coffees in their study. Therefore, it was not recommended as a factor that discriminated Arabica and Robusta coffees.

1.1.8.1.3 Carboxylic acids

Carboxylic acids of coffee are also claimed to be part of the coffee organoleptic profile (Blanc 1977). They provide acid taste, i.e. astringency and/or sourness, to coffee. The acidity of coffee is typically a highly valued quality especially in Central American and some East African coffee. Sourness, however, is an extreme of acidity and can be considered a coffee defect (Coffeeresearch.org).

A review by Woodman (1985) regarding the role of carboxylic acids in coffee infusions and the importance of acidity to taste and flavour has shown that lightly roasted coffee contained a "fine" acidity which was "clean" and "quick off" the palate. Darkly roasted coffee, on the other hand, contained little or no acidity hence allowing coffee bitterness to become pronounced. Maier (1987) suggested that the increase in bitterness can help reduce the undesired sourness in coffee. All in all, it might be possible to say

that the darker the roast, the lower the acid content (Woodman 1985) and, as a consequence, the less sourness in coffee.

A review by Blanc (1977) (since 1956) revealed that a mixture of five acids, citric, malic, lactic, pyruvic, and acetic, represented the main part of the aliphatic carboxylic acid fraction in both soluble and roasted coffee. Citric, malic, and acetic acids, however, have been claimed as the key ones due to the high contents as well as their low pK_a values.

A study by Maier et al. (1984) on the contribution of individual acids to the sour taste of coffee drinks reported 22 acids that, altogether, contributed 93% and 75% to the titratable acidity of roasted coffee and coffee extract, respectively. Acetic and citric acids were claimed to be the main contributors. However, only citric acid was found to be present above its threshold value for sour taste in their coffee samples. Acetic acid, on the other hand, was found to be present just under its threshold. High-molecular-weight acids and malic acids were also found to participate appreciably as contributors to acidity. Although, all other acids present in small amounts (below 3% each), the author believed that they are also parts of the sour taste of coffee beverage (Flament 2002b).

Regarding determination of perceived acidity (PA), there is no doubt that hydrogen ion concentration (related directly to pH) is associated with PA, and correlation between PA and pH of coffee beverage has been found in many studies (Sivetz and Desrosier 1979; Pangborn 1982; Griffin and Blauch 1999). However, many studies have found only moderate correlation between sour taste and pH. Total acidity of a coffee brew, expressed as titratable acidity (TA), has been demonstrated to show better

correlation to sourness than pH (Bahre and Maier 1996). Maier et al. (1983) found a correlation coefficient (r^2) of a linear regression between acid taste and pH to be 0.53, while r^2 of linear regression between acid taste and TA were found to be 0.89 and 0.92 for TA titrated to pH 7 and pH 6, respectively.

As regards acid contents, Blanc (1977) reported that a typical medium roast coffee consisted of 0.30%, 0.22%, 0.13%, 0.07%, and 0.27% of citric, malic, lactic, pyruvic and acetic acid, respectively (Clarke 2001). At very light roasts, concentrations of these acids increased to around 1.58%, while they could drop down to 0.71% with dark roasting (Coffeeresearch.org).

In Arabica coffee, the intensity of acid taste is generally found to be higher than that in Robusta. Flament (2002) proposed that this could be due to the higher content of acids in roasted beans, the somewhat higher proportion of free acid groups, as well as the greater sensory effectiveness of the prevailing individual acids.

1.1.8.1.4 Other compounds contributing to taste in coffee

Apart from the major taste compounds already mentioned, there are also other minor compounds that have been found to have potential in being part of the coffee taste.

Chen (1979) suggested that several heterocyclics, such as furfuryl alcohol, 5-hydroxymethyl-2-furanaldehyde, pyrazines and various trigonelline thermolysis products, are potential bitter-tasting substances in roasted coffees. Shibamoto et al. (1981) noted furfuryl alcohol contributed a burnt and bitter taste to coffee.

Frank et al. (2006) reported a series of cis- and trans-configured 2,5-diketopiperazines as bitter constituents of roasted coffee. An earlier study by Ginz and Engelhardt (2000) also found that PRO- and PHE-based diketopiperazines (DKPS) gave a bitter taste at concentrations ranging from 10 to 50 ppm when tasted in aqueous solutions. Five PRO-based DKPS have been identified by them in roasted coffee proteins and roasted coffee itself. The author, however, still could not come to a conclusion regarding the contribution of these heterocyclic compounds to the coffee bitter taste (Frank et al. 2006).

Besides bitterness and acid tastes, other taste sensation i.e. metallic (salty) is sometimes noticed in coffee. This metallic taste was claimed to be caused by the presence of numerous organic and mineral acids and their metallic salts (Illy and Viani 1995).

All in all, it is worth noting that all these taste sensations found in coffee brew, i.e. sour, metallic, bitter and astringent, are attributable to only about one percent of the total dissolved solids present in the coffee drink (Buffo and Cardelli-Freire 2004).

1.1.8.2 Factors Affecting Content of Taste Compounds in Coffee

In the same way as aroma compounds, the contents of taste compounds in coffee are also affected by many factors e.g. coffee species, variety, geographical origin, agricultural processes, as well as industrial processes (Casal, Oliveira et al. 1998; Casal, Oliveira et al. 2000b; Ky, Louarn et al. 2001; Oosterveld, Harmsen et al. 2003a). Table 1.13 shows CGA concentrations in different coffee brands/types.

Roasting plays an important role in the contents of taste compounds in roasted coffee beans. Moon et al. (2009) observed that total CGA were reduced in accordance with the intensity of roasting conditions in their experiment (see Table 1.12). The loss of

trigonelline was also found to be strongly dependent upon the degree of roasting and associated with the formation of nicotinic acid. Caffeine, however, was found to be minimally affected by roasting (Casal, Beatriz Oliveira et al. 2000a).

Table 1.12 Chlorogenic acids and caffeine contents (mg/g d.b.) in Ethiopian green coffee beans and coffee beans roasted at different conditions (mean ± SD, n=3) along with pH (Moon et al. 2009).

Compound	Green	Light roast	Medium	City roast	French roast
3-CQA	2.08±0.05	5.45±0.10	3.37±0.07	0.38±0.01	0.07±0.01
5-CQA	50.70±1.61	15.11±0.35	9.27±0.18	0.79±0.01	0.16±0.01
4-CQA	4.28±0.13	7.56±0.23	4.87±0.10	0.59±0.02	0.11±0.01
4-FQA	3.58±0.08	1.28±0.03	0.89±0.02	n.d	n.d
5-FQA	0.29±0.00	0.56±0.02	0.42±0.01	n.d	n.d
3,4-diCQA	1.01±0.05	0.65±0.01	0.29±0.01	n.d	n.d
3,5-diCQA	5.75±0.30	0.56±0.00	0.21±0.03	n.d	n.d
4,5-diCQA	1.33±0.14	0.92±0.01	0.40±0.01	n.d	n.d
Total CQA	69.09±2.36	32.09±0.75	19.72±0.43	1.76±0.04	0.34±0.03
pH	5.65	4.86	5.01	5.88	6.14
Caffeine	12.75±0.35	12.05±0.42	12.52±0.28	13.42±0.68	13.31±0.31

Regarding extraction conditions, McCamey et al. (1990) proposed that the perceived bitter taste in the mouth from coffee is correlated to the extent of extraction which, apart from roasting, is dependent upon the mineral content of the water, water temperature, time, grind size, and brewing procedure. Voilley et al. (1980) found that bitterness is reduced in coffee brewed with either soft or hard water relative to distilled water (McCamey et al. 1990). A study by the Technical Unit of the International Coffee Organization reported acid levels in coffee were affected by different extraction conditions. This involved differences in particle sizes of ground roasted coffee, water temperature, and extraction times (see Table 1.14-1.16).

Table 1.13 Acid concentrations in different coffee brands (Fujioka and Shibamoto 2008).

BRAND	Concentration (mg/g of ground coffee)[a]									Total
	3-CQA	4-CQA	5-CQA	3-FQA	4-FQA	5-FQA	3,4-DCQA	3,5-DCQA	4,5-DCQA	
Regular										
A	1.32 ± 0 02	1.44 ± 0 02	2.13 ± 0 04	0.82 ± 0.00	0.84 ± 0.01	0 89 ± 0.01	0.47 ± 0.00	0.40 ± 0.00	0 28 ± 0.00	5 26 ± 0 09
B	2 01 ± 0.03	2.28 ± 0 03	3 23 ± 0.05	1.29 ± 0.00	0.21 ± 0.00	0 27 ± 0.00	0.11 ± 0.00	0 67 ± 0 00	0.73 ± 0 00	8.39 ± 0.12
C	2 93 ± 0 01	3 34 ± 0 01	4 62 ± 0 01	0.98 ± 0.00	0.53 ± 0 02	0.75 ± 0.03	0 22 ± 0.00	0.12 ± 0.00	0.16 ± 0.00	12.8 ± 0.08
D	2 77 ± 0.02	3.13 ± 0 02	4 48 ± 0.04	0.98 ± 0.00	0.34 ± 0.00	0.47 ± 0 01	0.18 ± 0.00	0.01 ± 0.00	0.11 ± 0.00	11.7 ± 0.10
E	3 95 ± 0.06	4.56 ± 0.07	6 27 ± 0.14	0.13 ± 0.00	0 62 ± 0.01	0.89 ± 0 03	0.30 ± 0.01	0.19 ± 0.01	0.20 ± 0 01	17.1 ± 0.34
F	3.78 ± 0.01	4.27 ± 0.01	7.06 ± 0 01	0.12 ± 0 00	0.29 ± 0 00	0.54 ± 0 02	0.30 ± 0.00	0.22 ± 0 00	0 28 ± 0.00	16 9 ± 0.05
G	3 43 ± 0.01	3 89 ± 0.01	6 06 ± 0.01	0 13 ± 0.00	0 28 ± 0 00	0.45 ± 0 00	0 27 ± 0 00	0.19 ± 0.00	0.25 ± 0 00	15 0 ± 0.04
Decaffeinated										
A	0.45 ± 0.00	0 51 ± 0 00	0.82 ± 0 00	0 04 ± 0.00	0 09 ± 0.00	0.13 ± 0 00	0.03 ± 0.00	0 02 ± 0.00	0.01 ± 0.00	2.10 ± 0.01
B	0.83 ± 0.00	0.90 ± 0.00	1.43 ± 0 01	0.06 ± 0 00	0.16 ± 0 00	0.26 ± 0.00	0.07 ± 0.00	0.04 ± 0.00	0 05 ± 0.00	3 80 ± 0.02
C	3.22 ± 0 01	3.54 ± 0.01	5.77 ± 0 01	0.11 ± 0 00	0 70 ± 0 00	1.22 ± 0.00	0.40 ± 0.00	0.26 ± 0.00	0.41 ± 0.00	15.6 ± 0.04
D	3 42 ± 0 02	3.78 ± 0.02	6.23 ± 0 03	0.10 ± 0.00	0.53 ± 0.00	0.99 ± 0.01	0.40 ± 0.00	0.27 ± 0.00	0.41 ± 0.00	16.1 ± 0.08
E	3 26 ± 0 05	3.59 ± 0.03	5.75 ± 0.03	0.12 ± 0 01	0.60 ± 0.02	1.09 ± 0.03	0.36 ± 0.01	0 23 ± 0.01	0.37 ± 0.00	15 4 ± 0.19

[a] Values are means ± SD ($n = 3$).

Regarding species, Ky et al. (1999) found CGA content in green coffee beans varied between species from 0.14% d.b. in *Coffea ehamnifolia Bridson* to 9.90% d.b. in *C. sessiliflora Bridson*. Robusta coffee was found to contain higher levels of both caffeine and chlorogenic acids (CGA) than Arabica (McCamey et al. 1990). Casal et al. (2000b) also showed that trigonelline and caffeine contents in Arabica and Robusta were significantly different ($p<0.99$), both in green and roasted states. Robusta beans were found to contain the highest concentration of caffeine compared to Arabica, while Arabica was found to be richer in trigonelline than the former. The author, hence,

proposed the possibility of using caffeine and trigonelline contents to discriminate these two coffee varieties (See Table 1.11) (Casal et al. 2000b).

Table 1.14 Effect of grind size on acid concentrations at constant brewing temperature and time (94 °C and 5 min).

Acids	Course Grind Size (mg/l)	Fine Grind (mg/l)	Extra Fine Grind (mg/l)
Lactic Acid	109.67	194.50	308.33
Acetic Acid	242.67	225.67	209.00
Citric Acid	325.00	461.00	440.00
Malic Acid	119.33	137.00	163.67
Phosphoric	68.33	77.33	82.00
Quinic Acid	435.33	495.00	510.00
Chlorogenic	700.00	1,064.67	1,177.00
Palmitic Acid	5.03	5.90	3.63
Linoleic Acid	6.27	5.97	4.50

Source: ICO sensory.

Table 1.15 Effect of brewing temperature on acid concentrations at constant grind size and time (fine grind for 5 min).

Acids	70°C (mg/l)	94°C (mg/l)	100°C (mg/l)
Lactic Acid	121.00	194.50	187.33
Acetic Acid	151.33	225.67	187.00
Citric Acid	388.33	461.00	332.00
Malic Acid	131.00	137.00	122.00
Phosphoric Acid	86.33	77.33	80.00
Quinic Acid	348.33	495.00	383.33
Chlorogenic Acids	872.67	1,064.67	1,067.67
Palmitic Acid	3.26	5.90	6.53
Linoleic Acid	3.83	5.97	8.30

Source: ICO Sensory.

Table 1.16 Effect of brewing time on acid concentrations at constant grind size and brewing temperature time (fine grind at 94 °C).

Acids	1 min (mg/l)	5 mins (mg/l)	14 mins (mg/l)
Lactic Acid	56.67	194.50	125.67
Acetic Acid	261.00	225.67	242.00
Citric Acid	343.33	461.00	355.33
Malic Acid	109.33	137.00	100.33
Phosphoric	75.00	77.33	75.67
Quinic Acid	525.00	495.00	556.67
Chlorogenic	955.00	1,064.67	988.33
Palmitic Acid	4.97	5.90	5.87
Linoleic Acid	6.70	5.97	6.37

Source: ICO Sensory.

1.1.9 Green Coffee Modification

1.1.9.1 General Improvement

In literature, three common approaches used to modify raw coffee for an overall sensory improvement in final coffee products have been identified:

- Breaking down macromolecules to components that are more readily available for aroma generation reactions, i.e. polysaccharides to mono/di-saccharides, protein to amino acids, by acid/base hydrolysis or enzyme treatment.
- Addition of aroma compounds/precursors.
- Removal of specific aroma compounds or precursors with potential to cause undesired flavour/aroma in final products.

Regarding enzyme treatment, early experiments by Hashimoto and Fukumoto during 1969-1971 utilized mannanase, mannosidase, galactanase from the Koji culture of *Rhizopus niveus* and *Aspergillus niger* to hydrolyze coffee's mannan, manno-oligosaccharides, and arabinogalactan, respectively. Moreover, a later work by Puhlmann and Sobek in 1986 revealed that treating green coffee with an aqueous suspension of 0.01-1 wt. % of polysaccharide-degrading enzyme for 0.5- 4 h at 50-75 °C before roasting with/ without the addition of reducing sugar can help reduce roasting time. The application resulted in better coffee sensory quality, less roasting loss due to the reduction of the exothermic phase that causes gas formation, and therefore a higher yield resulted.

A study by Small and Asquith (1989) suggested a method to improve flavour and reduce bitterness of coffee product by treating green or partially roasted beans with a solution containing cell-wall-degrading, cell-storage-component-digesting, or phenol oxidase enzymes. The suggested technique provided the roasted coffee product with a character of "toddy", described as mild and aromatic with a sweet and caramel-like taste, caused by an increase in levels of aldehydes, diketones, pyrazines, and caramel compounds such as furaneol. They claimed that the preferred "toddy" character is caused by a reduction in the level of certain undesirable flavour components including guaiacols, as well as other phenolic compounds. A process to remove the guaiacols and other bitter phenolic compounds was also suggested in their study. The roasted and ground coffee produced by this method has increased levels of extractable brew solids, as well as increased flavour strength. Some sugars were also added in the treatment solution (2.5-50% by weight of the solution). Other food-grade aroma/flavour precursors were also included, e.g. amino acids, organic phosphates, sodium hydroxide, ammonium carbonate,

ammonium hydroxide and etc. However, no evidence of sensory evaluation was found in this document.

The use of proteolytic enzyme to digest proteins and peptides in green coffee beans was studied by Uhlig et al. (1971). They suggested treating raw coffee beans with a neutral or slightly acidic solution of an ammonium salt and a proteolytic enzyme to help improve flavour and digestibility of low-quality coffee. Suitable ammonium salts recommended by them were sulfate, bisulfate, neutral or acidic phosphate or acetate. The enzyme used comprised one or more protease mixed with amylase and glycosidase. Addition of small amounts of protein hydrolysates, 0.01-0.5% by wt. coffee, was also suggested as an option.

Some evidence showed that tannin can remarkably improve coffee quality. A work by Izumitani and Yajima (1990) suggested that adding or treating green beans with tannin could relieve the bitterness and so the taste of a coffee drink could be improved. The suggested process involved spraying tannin onto green beans surface (0.01-3% by weight), allowing the sprayed beans to stand at room temp (0.5-2.0 h.) before roasting. The improvement on flavour and colour was also confirmed by their sensory evaluation (10 panelists by scoring). Adding tannin to roasted coffee, however, did not show any improvement to the flavour of coffee.

1.1.9.2 Improvement of Robusta coffee

A number of studies have focused on the modification of Robusta coffee as it is considered of lower quality when compared to Arabica. Some studies have applied steam

with/without pressure to the raw Robusta beans and improvements in organoleptic properties were noticed.

Becker et al. (1991) indicated that the "earthy and musty" flavour notes in Robusta are undesirable in many countries and therefore are an issue for the coffee industry. They, therefore, suggested treating raw Robusta coffee beans with steam at elevated temperature and pressure to remove 2-methylisoborneol, a compound responsible for the undesirable earthy and musty aromas of Robusta, from the beans before roasting. The process involved increasing the water content of Robusta green beans, then treating the beans with saturated steam at a temperature of 135 -140 °C at a pressure or 3-4 bar for 75-90 min.

Besides steam and pressure, organic acids are sometimes used in combination. Varsanyi et al. (1988) explained that pretreatment of Robusta green beans by saturated steam under pressure with one or more organic acid(s) can help remove unpleasant off-tastes and odour of the Robusta as well as refining its irritating and bitter taste. After the pretreatment, any of the traditional roasting processes could be used and roastability up to optimum quality is facilitated. It was claimed that the coffee obtained can be used without limitation in coffee blends with no deterioration in taste (Varsanyi, Krajczar et al. 1988).

Another approach to reduce the bitter taste from Robusta in products made from blends of Arabica and Robusta was proposed by Imura and Matsuda (1992). They suggested applying distillation and partial (varietal) steaming (where only the Arabica portion of the blend is stripped to recover aromas) in order to overcome the problem.

With these techniques, the coffee products containing fractionated (distilled) steam aroma concentrates showed an overall improvement in quality and decreased bitterness and harshness. Partial steaming also permitted enhancement of desirable Arabica coffee characteristics and reduction in Robusta coffee notes in soluble coffee products. Consumer testing of the products suggested that acceptability of samples prepared using both techniques was increased (Imura and Matsuda 1992).

1.1.10 Fractionation and Reconstitution of Coffee

Fractionation and reconstitution studies can provide an idea of how the components in a sample contribute to the final properties of the sample. Extensive studies on these subjects have been done mostly on bakery and dairy products. However, evidence has shown that, to an extent, fractionation has also been applied to coffee. The most relevant studies on coffee fractionation were carried out by De Maria et al. (1994-1996). Their early study in 1994 used aqueous ethanol (80% v/v) to extract water-soluble fractions from green Arabica to obtain low and high-molecular weight fractions. Flavour precursors were present in both fractions (see Table 1.17). Their later study in 1996 investigated coffee volatiles formed during roasting of the Arabica green coffee fractions (see Table 1.18). The fractions studied were water-insoluble that were rich in polysaccharides, proteins and lipid. Their fractionation methods were based on water solubility and molecular mass. Head space method and high resolution Gas Chromatography-Mass Spectrometer (GC-MS) were applied for their aroma analyses (See Table 1.24 for a list of aroma compounds formed from each heated coffee fraction)

Regarding reconstitution of coffee, no relevant evidence has yet been observed.

Table 1.17 Distribution of carbohydrates, trigonelline, total CGA, and protein in fractions obtained from green Arabica coffee (De Maria, Trugo et al. 1994).

	Fractions (g%)	
	80% ethanol-soluble	**80% ethanol-insoluble**
Galactose	Tr*	21.2
Arabinose	Tr	18.1
Mannose	Tr	6.9
Glucose	Tr	1.9
Xylose	Tr	1.9
Sucrose	17.4	-
Trigonelline	5.3	-
Total CGA	33.2	-
Protein (6.25)	7.5	40

*Tr: trace.

1.1.11 Isolation of Coffee Aroma for Volatile Analysis

Aroma compound analysis can be carried out in two manners: sensory evaluation and instrumental analysis. Each has its own advantages and disadvantages. Sensory evaluation can be highly subjective; however it is the only tool that measures human response to the test product which is what we ultimately want to measure (Meilgaard, Civille et al. 1999). While instrumental analysis gives a more unbiased and reproducible result, it cannot measure response caused by psychological component in human. Therefore, it is not uncommon to find that results obtained from these two different analyses do not agree with each other.

In instrumental aroma analysis, one of the important steps is aroma isolation. The two most common procedures reported in the literature for the isolation of the aromatics are sampling the headspace and extraction of the sample itself (Parliment 2001).

Table 1.18 Some compounds identified in roasted coffee and roasted coffee fractions fractionated with 80% ethanol (De Maria, Trugo et al. 1994).

Compounds		Fractions	
	Roasted coffee	80% ethanol-soluble	80% ethanol-insoluble
1-methylpyrrole	+++	++++	++
5-methyl-2-vinylfuran	+++	+++	(+)
1-ethylpyrrole			(+)
pyridine	++	++++	++
pyrazine	+++		+++
2-methyltetrahydro-3-furanone	++	++++	
methylpyrazine	++++		++++
2,5-dimethylpyrazine	+		+++
2,6-dimethylpyrazine	+		++
ethylpyrazine	++		++
2,3-dimethyl-pyrazine	(+)		+
2-ethyl-6-methylpyrazine	(+)		++
2-ethyl-5-methylpyrazine	(+)		++
trimethylpyrazine	+++		++
2-methyl-3-(2H)-furanone	+++	+++	
2,6-diethylpyrazine	(+)		++
2,3-diethylpyrazine			+
acetic acid		+++	
2-furfural	+++	++++	(+)
furfural methanoate	++		
Pyrrole		++++	
2-methyl-pyrrole		++	
5-methyl-2-furfural	+++	+++	++
2-formyl-1-methylpyrrole	+++	+++	
2-acetyl-1-methylpyrrole	++	++	
2-furfural-alcohol	+++	++++	+++
Cyclotene	+	++	
2-methoxy-phenol	+	++	(+)
phenol	+	++	(+)

*Results are average of duplicate determinations; (+) Trace, + very low conc., ++ low conc., +++ high conc., ++++ extremely high conc.

Regarding extraction, various extraction methods exist. The choice of extraction method depends on the type of food and the information needed (Sarrazin, Quere et al. 2000). No single technique will prove optimal for every sample, and evaluations should

be made to ensure that decomposition and loss of desired components do not occur (Parliment 2001).

As regards solvent extraction, solvents most commonly used are diethyl ether, diethyl ether/ pentane mixture, hydrocarbons, freons, and methylene chloride. The latter two are claimed to have the advantage of being nonflammable. Methylene chloride has been reported as a satisfactory general purpose solvent although it is somewhat toxic and is an animal carcinogen (Parliment 2001). Liquid carbon dioxide is considered non-toxic and inexpensive. It is reported to have solvent properties similar to diethyl ether and to be particularly selective for esters, aldehydes, ketones, and alcohols.

In the case of coffee, it is ideal that the extract obtained for the analysis should represent the initial coffee in terms of its sensory characteristics. A study by Sarrazin et al. (2000) compared coffee extracts obtained from five different extraction methods, using supercritical carbon dioxide (SC-CO_2), simultaneous distillation extraction, oil recovery under pressure and vacuum steam-stripping with water or with methylene chloride. Their result revealed that vacuum steam-stripping with water followed with methylene chloride extraction and concentration provided the most representative extracts of the roasted and ground coffee as measured by sensory evaluation. Another study by Ramos et al. (1998) found that extraction by different methods: SC-CO_2 extraction, liquid-liquid extraction with pentane or methylene chloride, and headspace solid-phase micro-extraction (HS-SPME) yielded different compositions of the corresponding extracts. Their conclusion, however, noted that SC-CO_2 extraction provided aroma extracts with high olfactory resemblance to the original brewed coffee in their optimized condition.

1.1.12 Potential Uses of Coffee Processing By-products: as Coffee Aroma Source?

Coffee process by-products include coffee berry materials, coffee pulp, mucilage, parchment, wash water, husks, residues from roasting process i.e. chaff, residues from extraction of instant coffee, spent-ground (Camaggio- Sancineti and Nicoletti 1995) (see Figure 1.7 for the structure of coffee bean).

Management of coffee process by-products is not only important regarding environmental, but also economical aspects of waste disposal and by-product utilization (Mbura and Mwaura 1996).

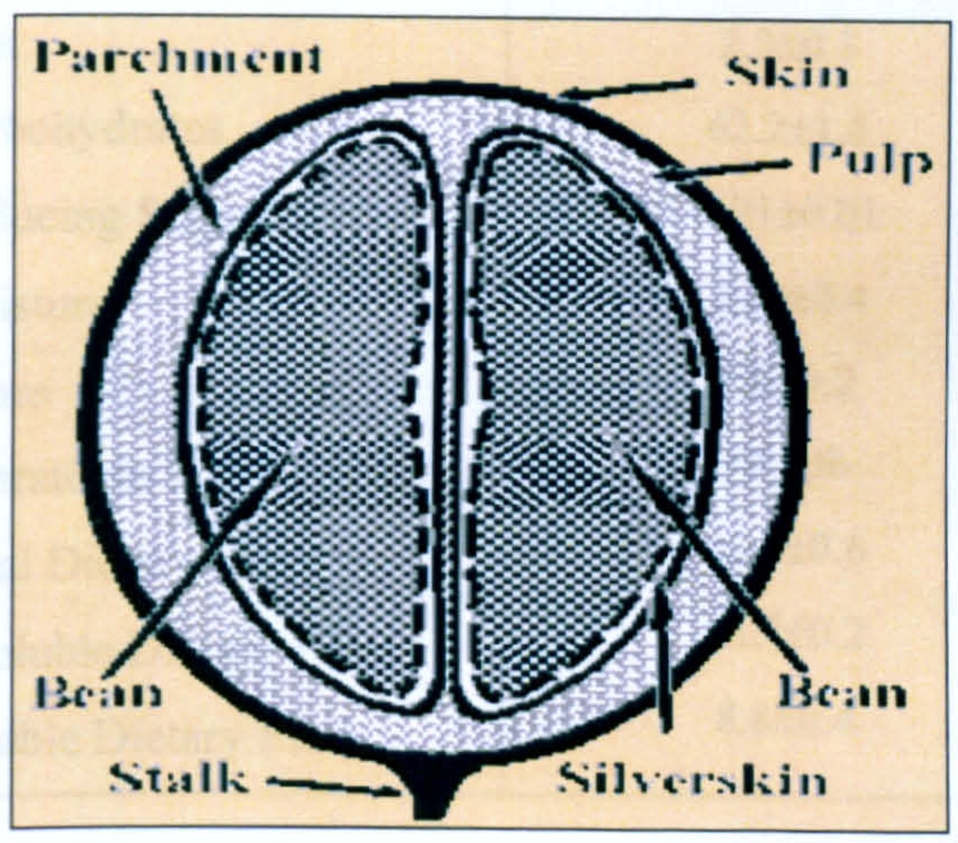


Figure 1.7 Coffee bean structures.

As regards utilization, coffee pulp has been used for the production of charcoal briquettes (after sun-drying), organic manure for methane, ethanol, wine, and vinegar productions, and for extraction of caffeine, pectin and sugars (Ananda-Alwar and Ramaiah 1986; Mbura and Mwaura 1996). Coffee mucilage is also used for production of pectin or vinegar. Applications of coffee husk and parchment include production of

furfural or recovery of coffee aroma. Coffee spent-grounds can be compressed to yield coffee oil, or used for furfural production (Rao and Natarajan 1974; Ananda-Alwar and Ramaiah 1986). Evidence from Rao and Nararajan (1974) has shown that in addition to the uses for extraction of furfural, as a microbiological medium, or in the fermentation industries, husk, pulp, and spent ground are also utilized as adjuncts for blending with roasted coffee powder (roasted husk up to 30%).

Table 1.19 Chemical composition of coffee silver skin (Borrelli, Esposito et al. 2004).

Parameters	g/100g of product
Protein	18.6±0.6
Fats	2.2±0.1
Carbohydrates	62.2±1.6
Reducing Sugars	0.21±0.01
Moisture	7.30±0.4
Ashes	7.0±0.2
Ochratoxin A	<4 ppb
Total Dietary Fiber	62.4±0.6
Insoluble Dietary Fiber	53.7±0.2
Soluble Dietary Fiber	8.8±0.4

With respect to coffee silver skin, coffee silver skin is a by-product from coffee roasting process. Because the coffee silver skin is so thin and attached so well to the coffee beans, it usually cannot be completely removed by the green bean process and tends to stay with the coffee beans up until roasting. The roasting causes the silver skin to crack off because the silver skin does not expand like the inner coffee bean when roasted.

Coffee silver skin, apart from being used as a fuel or fertilizer (Saenger and Hartge 2001), has recently been proposed by Borrelli et al. (2004) as a new potential functional ingredient as their study found it contained a high content of soluble dietary fiber, yielded marked antioxidant activity, as well as showing potential prebiotic activity.

1.1.13 Potential Health Benefits of Coffee Flavour Compounds

A considerable amount of research looked into the relationship between coffee and health with both adverse and beneficial effects investigated.

Brewed coffee has been reported to contain large number of health-beneficial antioxidants. These include those present naturally in green coffee, i.e. polyphenolic compounds, as well as those generated by the roasting process, e.g. volatile heterocyclic compound formed as Maillard reaction products (MRPS). The antioxidant phenolics in coffee are of the hydroxycinnamic acid family, such as caffeic, coumaric, chlorogenic and ferulic acids (Dorea and da Costa 2005). Caffeine and its catabolic products might contribute to this activity as well (Azam, Hadi et al. 2003). Chlorogenic acids (CGA) are found up to 10% in green coffee beans (ICS research, 2002), and have been claimed to be the main contributor to antioxidant activity in coffee (Charurin, Ames et al. 2002). Richelle et al. (2001) claimed that among coffee, cocoa and tea, coffee has the highest antioxidant activity on a cup-serving basis

During roasting, most natural polyphenols are destroyed. Nevertheless, the products of chemical reactions occurring during roasting, in turn, become prevailing contributors to the antioxidant activity in coffee (Nicoli, Anese et al. 1997). Nicoli et al. (1997) found the highest antioxidant properties to occur in medium-roasted coffee brews. Similar result was found by Richelle et al. (2001) who discovered that the proportion of

total antioxidant activity due to the coloured HMM fraction increased with roasting for the light- and medium-roasted coffees but not for the dark-roasted sample. Torrefacto roast, coffee roasting with an addition of sugar at the end of the process, was found to increase antioxidant capacity while decreasing redox potential. This is claimed to be due to the formation of MRPs which have reducing properties (Andueza, Cid et al. 2004).

Regarding coffee variety, roasted Arabica was found to present lower antioxidant capacity than Robusta. This was assumed to be due to higher percentage of chlorogenic acids found in Robusta ground coffee than Arabica (Andueza et al. 2004).

Although evidence has shown the potential of coffee as a good source of antioxidant, understanding of the protective role of dietary antioxidants still requires a better characterization of the antioxidant composition as well as quantitative data on their in vivo absorption, their tissue distribution, their metabolism, and their biological actions (Hollman and Katan 1997; Scalbert and Williamson 2000).

A recent experiment by Mauro and Maria (2009) showed that coffee had higher potential as an antioxidant among the different plant foods by in vitro testing. However, despite the promising evidence in vitro, in vivo studies are scarce and inconclusive, and thus do not allow any conclusions on the possibility of coffee to strengthen antioxidant defense in humans. Higdon and Frei (2006) suggested that CGA and caffeic acids are extensively metabolized in the body and thus may not contribute as much antioxidant activity in vivo as the metabolites often have lower antioxidant activity than the parent compounds.

Regarding possible effects on human diseases, the preponderance of scientific evidence suggests that moderate coffee consumption (3-5 cups/day) may be associated

with reduced risk of certain disease conditions (Dorea and da Costa 2005; Higdon and Frei 2006). A review by Clemens and Coughlin (2007) has revealed some of the diseases and effects from coffee as follows.

- **Reduce risk of type 2 diabetes:**

  Consumption of 5+ cups/ day has been associated with improved glucose regulation and tolerance and a substantially lower risk of type 2 diabetes (35-75%) in diverse populations in the United States, Europe, and Japan. Caffeine-containing and decaffeinated coffees have been shown to give similar protective effects, which may be attributed to natural polyphenolic antioxidants, lignans, and magnesium.

- **Reduce risk of cancer:**

  This is by its natural polyphenolic antioxidants (chlorogenic acid derivatives) and its heat-produced antioxidants (Maillard reaction products, including volatile heterocyclic compounds and brown melanoidin polymers). Studies in many countries have shown that coffee is actually the major individual source of dietary antioxidants (exceeding wine, tea, chocolate, and individual fruits and vegetables).

- **Reduce risk of liver disease**

- **Reduce risk of Parkinson's disease:**

  This is due to the neuroprotective effect of caffeine. Some research in neuropharmecology suggests one cup of coffee (80-140 mg of caffeine) can halve the risk of the disease.

- **Cardiovascular Disease risk:**

  A 20-year prospective cohort study with approximately 130,000 men and women without a history of coronary heart disease or cancer did not provide any evidence that coffee increased the risk of coronary heart disease.

Table 1.20 Number of reviews and epidemiological studies on coffee with selected disease (Binns, Lee et al. 2008).

Disease	Reviews (epidemiological studies)	Cohort	Case–control	Cross-sectional	Total epidemiological studies
Cancers					
Breast cancer	2	9	25	0	34
Pancreatic cancer	5	14	31	0	45
Ovarian cancer	2	2	15	0	17
Renal cancer	2	3	13	0	16
Thyroid	1	0	12	0	12
Prostate	2	8	8	0	16
Urinary tract cancer	7	10	78	0	88
Colorectal	4	14	35	0	49
CVD					>100*
Other conditions					
Type 2 diabetes	5	16	0	11	27
Parkinson's disease	2	8	12	0	20
Liver cirrhosis	0	4	3	0	7
Reproductive adversities	24				>100*
Bone health	1	13	5	22	40
Gallstones	0	3	7	2	12
Rheumatoid arthritis	0	4	2	1	7

*For conditions with over 100 epidemiological studies, the number of studies was not counted.

Others effects include increasing mental alertness and, cognitive functions, wakefulness, and physical stamina, while reducing the risk of Alzheimer's disease, kidney stones, gallstones, depression and suicide (Clemens and Coughlin 2007). There is also evidence that some compounds found in coffee may be harmful or unsuitable for certain individuals, including those with, or at risk for, osteoporosis (Barrett-Connor, Chang et al. 1994), pregnant women (Fenster, Hubbard et al. 1997), and epilepsy patients (Bonilha and Li 2004).

Currently, the Food Standards Agency recommends that pregnant women should limit their consumption of caffeine to a maximum of 300 mg per day. Although there is no official recommendation for the amount of coffee or caffeine consumed per day for the general population, research appears to indicate that moderate levels of consumption (up to five cups per day) do not pose any risk to health.

Table 1.21 Amount of evidence for coffee and selected disease conditions (Binns, Lee et al. 2008).

Evidence	Decreases risk	No relationship	Increases risk
Convincing		Breast cancer	
Probable		Pancreatic cancer	
		Ovarian cancer	
		Renal cancer	
		Thyroid cancer	
		Prostate cancer	
		Urinary tract/bladder cancer	
		CVD	
		Reproductive adversities	
		Bone health	
Possible	Colorectal cancer	Gallstones	
	Type 2 diabetes	Rheumatoid arthritis	
	Parkinson's disease		
	Liver cirrhosis		
Insufficient			

In conclusion, coffee drinking seems to be a non-harmful habit for individuals who drink it regularly and in moderation. In fact, it may even be beneficial for most people. It should be noted that regardless of preparation and whether or not it contains caffeine, coffee is a good source of antioxidants (Taylor and Demmig-Adams 2007).

Table 1.22 Summary of potential health risks/benefits of coffee drinking (Taylor and Demmig-Adams, 2007).

Health concerns	Potential risks/benefits	Conditions	Overall assessment
Cardiovascular disease	Reduced or no risk (but increased risk with family history)	Filtered coffee reduces risk; in combo with smoking increases risk	+/−?
Physical fitness	Reduced leg muscle pain, no diuretic effect or electrolyte imbalance	Caffeinated coffee best studied (effects of antioxidants unknown)	+
Neurodegenerative diseases	Decreased risk for Alzheimer's and Parkinson's	Decreased risk for Parkinson's for caffeinated coffee only	+
Epilepsy	May increase number of seizures	Effect of decaf coffee unknown	−
Reproduction and fetal viability	May increase risk for abortion during 1st, but may decrease maternal stress during 3rd trimester	No effect of decaf coffee on fetal viability.	+/−
Ovarian cancer	No risk	For coffee drinking only	0
Rheumatoid arthritis	No risk; may reduce inflammation		+?
Osteoporosis	Possible increased risk	Risk may be offset by drinking one glass of milk per day	+/−?
Type 2 diabetes	Decreased risk	Only ground (but not instant) coffees lower risk, due to chlorogenic acid	+
Gallstone disease	Decreased risk	Caffeinated coffee only	+
Chronic liver disease	Decreased risk	Decaf coffee not tested	+
Colorectal cancers	Decreased risk for rectal cancer; no risk for colon cancer	Decaf coffee linked to decreased risk of rectal cancer	+?

Notes: Results are summarized in the overall assessment as positive (+), neutral (0), and/or negative (−)

1.2 OBJECTIVES

There are two main objectives in the thesis:

1. To make use of a coffee process by-product, silver skin, as a source for natural coffee aroma production through chemical modification and Maillard reactions. The resulting coffee aroma is aimed to be used to reinforce the aroma of coffee products with poor aroma quality, i.e. instant coffee, as well as to be marketed as "natural" coffee flavour to other industries that natural coffee flavouring is preferred over the synthetics. The results of the experiments related to this objective are demonstrated in Chapters 3 and 4.
2. To improve sensory quality of Robusta coffee by chemical modification through refractionation and reconstitution approaches. The resulting modified Robusta is aimed to be used as an Arabica replacement/alternative as Arabica is normally more expensive and can be less available in some countries, e.g. in Africa and Asia. The results of the experiments related to this objective are demonstrated in Chapters 3, and 5 to 7.

CHAPTER 2: MATERIALS AND METHODS

2.1 DEVELOPMENT OF COFFEE VOLATILE ANALYSIS PROCEDURES

A review of the scientific literature has provided useful information on techniques suitable for coffee volatile analysis. However, since no single technique will prove optimal for every sample (Parliment 2001), some techniques have been adapted and applied to analyze the samples in this experiment. The suitability of the procedures in terms of simplicity and information provided was evaluated. Details on each step of the method development are as follow.

2.1.1 Selection of Internal Standard

Internal standards (IS) were selected based on the compound's physicochemical property, log P. Candidate Internal Standards were those with log P values falling within the ranges of log P's of key coffee aroma compounds (see Table 2.1).

Table 2.1 Log P's of candidate internal standards (IS) and target coffee aromas.

Candidate IS	log P*	Coffee Aroma	log P*
3-pentanone	0.99	furanmethanethiol	1.96
2-hexanone	1.38	furanmethanol(furfuryl)	0.28
2-heptanol	2.31	2-methylbutanal	1.23
2-butanol	0.61	3-methylbutanal	1.23
		2,3,5-trimethylpyrazine	0.95
		pyridine	0.65
		2,5-dimethylpyrazine	0.63
		2,6-dimethylpyrazine	0.54
		2-ethyl-5-methylpyrazine	1.52
		2,5-dimethyl-3-ethylpyrazine	2.07
		furfuryl acetate	1.45
		furaneol	0.82

*Source: ChemDraw.

IS solution was prepared in methanol by adding 10 μL of each candidate IS into a 10 ml volumetric flask and making up to the mark with methanol. The mixture was put on a shaker for 15 min. The concentration of each compound in the standard solution obtained was 1000 ppm.

IS solution (200 μL) was added to 2 g of each sample (roasted and ground coffee) prior to aroma extraction to allow calculation of extraction efficiency.

2.1.2 Isolation of Coffee Aromas

Solvent extraction techniques were chosen to isolate coffee aroma from the samples in this study. Three different types of solvent varying in solvent properties, e.g. polarity, solubility, miscibility, and boiling point, were employed. Comparison was made among the extracts obtained from the three solvents. Effect of extraction time was also investigated. A full-factorial experimental design was carried out for this experiment yielding 6 treatment combinations (runs) in total (see Table 2.2).

Table 2.2 Detail of each treatment combination for the experiment: determination of appropriate solvent extraction method for the coffee sample.

Treatment	Solvent	Time	Extraction Temp. (C°)
1	Water	1 h	Ambient
2	Water	overnight	Ambient
3	DCM	1 h	Ambient
4	DCM	overnight	Ambient
5	MeOH	1 h	Ambient
6	MeOH	overnight	Ambient

For all trials, two grams of Arabica roasted & ground coffee, provided by a coffee factory from Thailand, were mixed with 20 ml of one of the three solvents in a Pyrex tube. Tubes containing the mixtures were placed on a roller or a shaker for either 1 h, or

overnight at ambient temperature according to the experimental design. Samples were subsequently filtered with no. 4 Whatman® filter paper into the tubes and were subjected to Gas-Chromatography Mass Spectrometry (GC-MS) for volatile analysis. An exception was made for the aqueous extracts as they needed to be re-extracted with Dichloromethane (DCM) prior to injection to the GC. This is due to the high boiling point of water which can cause trap blockage in the GC column, preventing complete separation of volatiles in the sample, as well as causing high pressure in the GC-MS system. As a consequence, all aqueous extracts were re-extracted with 1 ml of DCM on a roller for 30 min in the same tubes, and the obtaining DCM phase was then subjected to GC-MS analysis.

2.1.3 Gas Chromatography-Mass Spectrophotometry (GC-MS) Analysis

All extracts were transferred to auto-sampler vials (Chromacol®, UK) before being subjected to GC injection. One micro liter of each extract was injected in splitless mode using an AS 3000 autosampler (Thermo Scientific) into the injector port (250 °C) of a Trace GC ULTRA (Thermo Scientific) equipped with DSQ mass spectrometer (Thermo Scientific) operating in full scan mode, from 20 to 250 *m/z*. The column was ZB-5, 30 m × 0.25 mm i.d. × 1 μm film thickness, supplied by Phenomenex. Helium was the carrier gas (15 psi). The initial oven temperature was 40 °C; this was maintained for 5 mins and then programmed to 180 °C at the rate of 3 °C /min, then at 6 °C /min until the oven reached 220 °C.

2.1.4 Peak Identification and Quantification

Peak identifications were made by comparing GC retention times to those of standard compounds. Confirmation was done by selected ion monitoring and matching spectra of the target compounds, generated from GC-MS, with those from the MS library.

Quantification was calculated and expressed as ppm (in original sample) on relative basis to the concentration of the internal standard, 2-heptanol.

2.2 DETERMINATION OF COFFEE SILVER SKIN AS A POTENTIAL SOURCE OF COFFEE AROMA

2.2.1 Comparison of Contents of Coffee Aroma in Coffee and in the Corresponding Silver Skin

A 2-g portion of each sample, roasted and ground coffee or milled silver skin, was introduced into a 100-ml Pyrex tube. A hundred micro liter of internal standard solution, (1000 ppm solution of 2-heptanol in methanol), was added to the sample followed by the addition of 20 ml purified water (Purelite®) for extraction. The tube, with the mixture of sample in it, was immediately capped and put on a roller mixer for 1 h at ambient temperature. After one hour, the mixture was filtered using no.4 Whatman® filter paper, re-extracted with 1 ml of DCM for 30 min before the DCM phase was transferred to a GC auto-sampler vial (Chromacol®, UK) and subjected to GC analysis.

Quantification of all coffee aromas was performed relative to concentration of the spiked internal standard.

2.2.2 Quantification of Aroma Precursors in Coffee Silver Skin

Since key precursors of Maillard reactions are amino acids and reducing sugars, an investigation on the amounts of total amino acids and reducing sugars in coffee silver skin was carried out in this experiment.

2.2.2.1 Determination of Reducing Sugar Content

Two methods were performed to analyze sugar content in this part. The first one was a measurement of total reducing sugars expressed as "Maltose Equivalent", while the second analyzed contents of individual sugars in the sample using a HPLC technique.

2.2.2.1.1 Sample Preparation

Roasted and ground coffee and milled silver skin was extracted using the aqueous method at ambient temperature as described previously in 2.1.2. However, the extraction was carried out without the addition of internal standard. The spent ground was re-extracted with 20 ml of Purelite® water for 1 h. The two extracts obtained were combined and subjected to filtration through C18 SPE cartridge (Sep-Pak®). The final solution was transferred to an aluminum tray and put in a freeze dryer for 4 days. The freeze-dried sample was subsequently diluted with 4 ml of Purelite® water and the sample was ready for sugar analysis. The sample was divided into two parts, one for each of the two assays which were carried out in triplicate.

2.2.2.1.2 Spectrophotometer Method: as Maltose Equivalent

The oxidizing reagent (3,5-dinitrosalicylic acid; DNSA) solution was prepared by mixing together 10 g DNSA, 200 ml of 2 M NaOH, and 500 ml of distilled water.

Sodium potassium tartrate (30 g) was slowly added to the mixture with gentle stirring and heating. The obtained solution was brought up to 1 liter using distilled water. The solution was stored at 4 °C in a dark place until used.

Coffee and silverskin extract (300 μL each) was pipetted into 300 μL DNSA solution in an Eppendorf tube (sample: DNSA ratio is 1:1). A blank, DNSA with phosphate buffer (pH 6.9), was also prepared.

The samples (in Eppendorf tubes) were heated at 100 °C to facilitate colour development, and were immediately cooled after 5 min. The samples were diluted by a factor of 10 before being subjected to spectrophotometry, LKB Brochom Ultraspec 4050 UV/Vis spectrometer, set to measure the absorbance at 540 nm. A serie of maltose solutions with known concentrations was made for calibration curve development.

2.2.2.1.3 HPLC Assay

The samples were injected into the HPLC with the 20 μL injection loop. The mobile phase was 80% Acetonitrile 20% Water (degassed prior to use). The flow rate was 3 ml/min. Column was 250 x 4.6 mm ID, Spherisorb NH_2 with 5 um particle size. Wyatt Refractive Index (RI) Detector was used for detection.

Quantifications for the two assays were performed using standard curves.

2.2.2.2 Determination of Amino Acid Contents

The analysis of total amino acid of the coffee silver skin was done by International Laboratory Services (ILS) using acid hydrolysis approach.

2.2.3 Generation of Coffee Aroma from Coffee Silver Skin through Maillard Reactions

Due to the low amount of coffee aromas recovered from the silver skin, an attempt to generate more coffee aromas from the silver skin was established. Modification of the silver skin matrix by adding reducing sugar or digesting components in the silver skin using enzyme and heat treatment was carried out.

2.2.3.1 Heat Treatment

Milled silver skin (10 g) was divided into two 5 g portions. The first part was subjected to baking at either 150 °C or 200 °C for 50 mins in an aluminum baking tray, while the other part was wetted with approx. 1.5 g of distilled water before being subjected to baking in the same manner. The coffee aromas in the baked silver skin were isolated and analyzed by aqueous extraction and GC-MS analysis as previously described in 2.1.

2.2.3.2 Addition of Reducing Sugar: Glucose

Due to the low level of reducing sugars in coffee silver skin, some glucose was added to the silver skin before heat treatment was applied. Factors involved in this experiment were the percentage of glucose added, percentage of moisture, and degree of temperature applied. A full-factorial experimental design was established (see Table 2.3). Coffee volatile analysis was carried out with aqueous extraction and GC-MS as described in 2.1. Changes in coffee aroma levels were monitored.

2.2.3.3 Enzyme Treatment: Pancreatin

An attempt to digest protein and carbohydrate in coffee silver skin in order to increase the levels of Maillard-reaction precursors, reducing sugars and amino acids, was made. Pancreatin enzyme (catalogue no. 416745000, Acros Organics) was used in this study as it is a combination of protease, amylase, and lipase. Concentrations of the enzyme as well as digesting temperatures used were varied.

Table 2.3 Full-factorial experimental design for the glucose-added silver skin experiment.

Sample	20% Moisture (w/w)	% Glucose added (d.b)	Baking Temp. (°C)
1	√	20	150
2	X	20	150
3	√	40	150
4	X	40	150
5	√	20	200
6	X	20	200
7	√	40	200
8	X	40	200
Control	X	0	X

*All samples, except the control, were baked for 50 min.

2.2.3.3.1 Preparation of Enzyme Stock Solution

Pancreatin enzyme (1 g) was dissolved in 100 ml phosphate buffer (pH 7). The solution was centrifuged (2000 g) at 0 °C for 20 min. The supernatant was kept at 4°C until used for digestion.

2.2.3.3.2 Preparation of Samples

To provide approximately 0.1, 0.5, and 1% of pancreatin enzyme to the coffee silver skin (w/w), three levels of enzyme solution were prepared as shown in Table 2.4.

Table 2.4 Preparation of the enzyme (pancreatin) solutions.

Solution	ml of enzyme stock solution	ml of phosphate buffer (pH 7)
1	1.67	98.3
2	8.3	91.7
3	16.7	83.3

Ten samples were prepared in 100 ml Pyrex tubes. Each tube contained 5 g of milled silver skin mixed with either distilled water or one of the enzyme solutions. Details are as follows (Table 2.5).

Table 2.5 Details of the samples in the experiment: Digestion of silver skin by pancreatin enzyme.

Sample	Sample	Note
1, 6	Control (w)	5 g sample + 30 ml distilled water
2, 7	Control (pp)	5 g sample + 30 ml pp buffer (pH 7)
3, 8	Silver skin with 0.1% enzyme (w/w)	5 g sample + 30 ml solution 1
4, 9	Silver skin with 0.5% enzyme (w/w)	5 g sample + 30 ml solution 2
5, 10	Silver skin with 1% enzyme (w/w)	5 g sample + 30 ml solution 3

*w: in water, pp: in phosphate buffer (pH 7).

2.2.3.3.3 Enzyme Digestion

The samples were incubated on a roller in an oven set at either 37 °C, for sample 1-5, or 80 °C, for samples 6-10. Digestion time for all the samples was 4 h.

2.2.3.3.4 Heat Treatment

After digestion, each sample was transferred to an aluminum baking tray and subjected to baking at 200 °C for 2 h.

2.2.3.3.5 Aroma Compound Analysis

Changes in contents of key coffee aromas were monitored by GC- MS analysis. The aqueous extraction procedure and GC-MS analyses were carried out in the same manner as described in section 2.1.

2.2.4 Determination of Content of Other Functional Component in Coffee Silver Skin

2.2.4.1 Caffeine

Comparison between caffeine contents of the roasted Arabica coffee and the corresponding silver skin was made.

2.2.4.1.1 Sample Preparation

The method was modified from Zou and Li (2006).

Silver skin (milled) and roasted coffee (ground) were extracted with aqueous method as described in 2.1. The spent ground, however, was re-extracted with another 20 ml of Purelite® water for another 1 h on a roller. The two aqueous extracts were combined. The resulting combined extract was then re-extracted with 2 ml of DCM on a roller for another 30 min. An aliquot (25 μL) of the DCM layer was then transferred to a 1.5-ml auto-sampler vial and was mixed with 1 ml of ethyl acetate. The resulting mixture was then subjected to HPLC analysis.

DCM (20 ml) was used to extract the spent ground from the previous aqueous extractions to collect the remaining caffeine in the spent ground. The extraction was left overnight. The resulting DCM extract was concentrated down to 5 ml under nitrogen.

The DCM extract was then mixed with ethyl acetate in an auto-sampler vial and analyzed using the same HPLC method.

2.2.4.1.2 Caffeine Analysis by HPLC

The HPLC method used in this study was suggested by Phenomenex; the Acetaminophen, USD method. The column used was Luna, 5μ C18 (2). The mobile phase was a mixture of water, methanol, and acetic acid with a ratio of 68:23:3. Flow rate was 1.5. ml/min. The UV detector was set at 275 nm.

Quantification was performed using caffeine standard curves.

2.3 GREEN COFFEE FRACTIONATION AND RECONSTITUTION

Because of the hard texture of green coffee beans and with the purpose of avoiding the heat development that could occur during the grinding of green coffee, the green beans were frozen in liquid nitrogen before being ground using a laboratory mill. The obtained ground green coffee was then sieved with a sieve no. 710 and stored at 4°C until used.

2.3.1 Development of Fractionation Procedure

The fractionation procedure used in this study was adapted from various fractionation techniques previously described by various authors (De Maria, Trugo et al. 1994; De Maria, Trugo et al. 1996a; Nunes and Coimbra 2002; Grassberger, Schieberle et al. 2003).

Three types of solvents varying in polarities were chosen for serial fractionations. They were dichloromethane (DCM), methanol (MeOH), and water. Their polarity indices are 3.1, 5.1, and 9, respectively(Byers 2003)

The extracts obtained from different solvents were expected to contain compounds with different chemical properties, i.e. molecular weight and polarity, and thus to form different coffee volatiles when heated. The procedure is described as follows.

Ground green coffee sample (50 g) was weighed in a 500-ml Erlenmeyer flask. DCM (400 ml) was then added. The mixture was stirred for 1 h and centrifuged at 100g for 20 min. The residue was re-extracted with DCM twice under the same condition (3 extractions in total). The DCM of the pooled supernatant was removed by rotary evaporator. The distilled DCM obtained after the evaporation was reused. The evaporated (dried) fraction was stored in a closed container at 4 °C until used. The residue (residue 1) was air-dried overnight under the fume hood.

Next, the residue (residue 1) was mixed with 400 ml of MeOH, stirred for 1 h, and centrifuged (100g, 20 min). The extraction was done twice. The MeOH of the pooled supernatant was also removed by rotary evaporator. The obtained condensed MeOH was also kept for reuse. The residue (residue 2) was air-dried before being subjected to water extraction. The obtained MeOH fraction (dried) was stored in a closed container at 4 °C until used.

Water extraction was modified from a technique by Maria et al. (1996). The residue (residue 2) was extracted with 20 ml of 80 °C reverse osmosis filtered water (Purelite® water) in a shaking water bath for 1 h twice. After separation (centrifuged at 100g, 20 min), the pooled supernatant and the final residue (residue 3) were freeze-dried and stored at 4 °C until used (See Figure 2.1).

Each fraction (5 g) was then heated (210 °C, 9 min), extracted (as described in 2.1.2), and subjected to volatile analysis by GC-MS (see section 2.1.3).

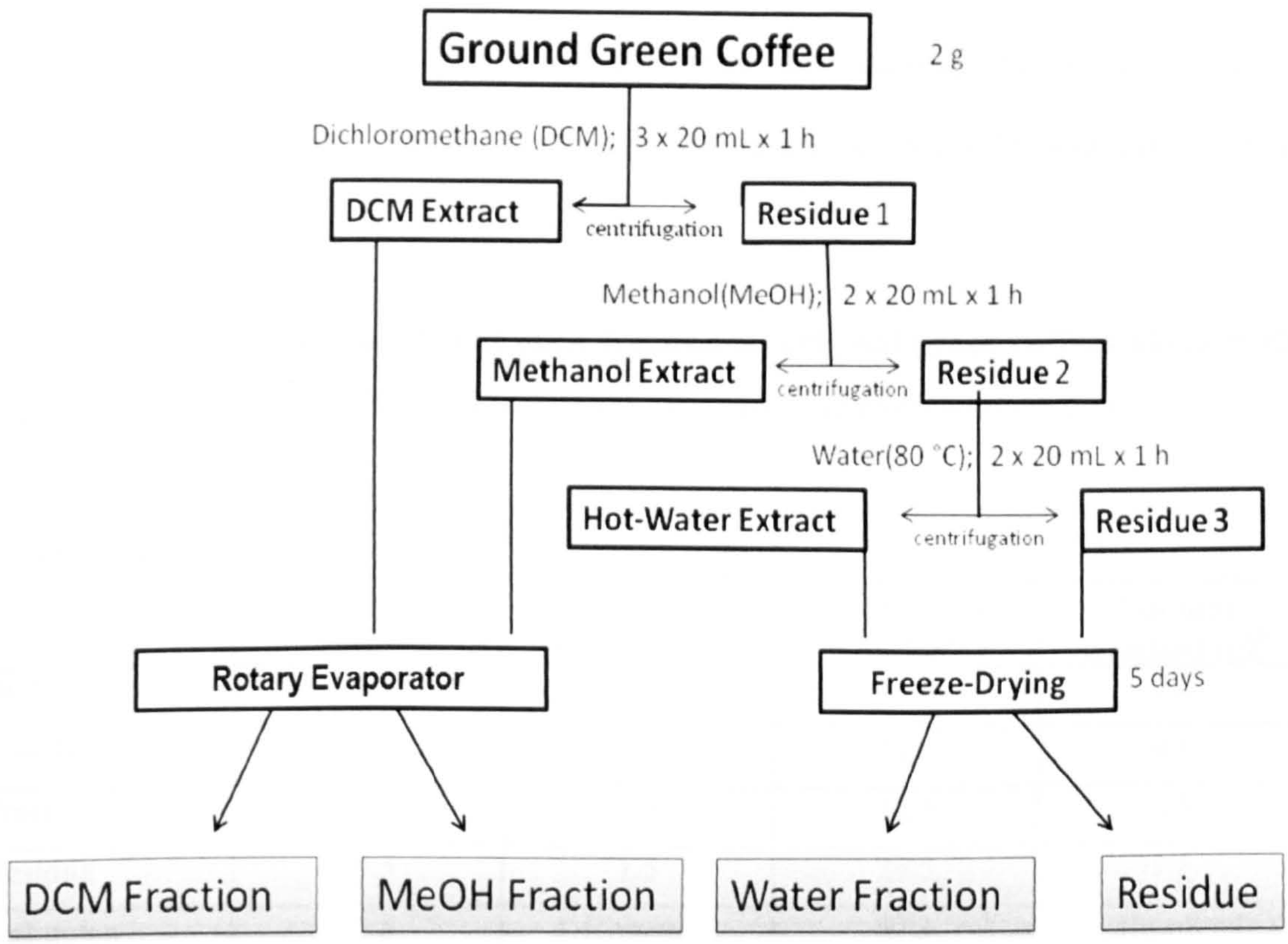


Figure 2.1 Scheme of the fractionation procedure.

2.3.2 Development of Reconstitution Procedure

Two approaches were carried out in this experiment, dry and wet mixings.

2.3.2.1 Dry Mixing

All dried fractions, DCM, MeOH, Water, and Residue fractions, were weighed together in an aluminum tray. The weight of each fraction was calculated proportional to the percentage of the fraction found in the original green coffee and to make a total weight of 5 g for each reconstituted sample (see Table 2.6). Purelite® water (0.5 g) was also added to each sample for moisture content adjustment. The unknown parts, however, were omitted. Therefore, all the samples' final weights were lower than 5 g. All fractions were, then, mixed thoroughly in a laboratory grinder for 1 min. Two grams of each reconstituted sample was transferred to a glass tube, covered with aluminum foil, baked at 210 °C for 12.5 min, aqueous extracted (as described in 2.1.2), and subjected to volatile analysis by GC-MS (see section 2.1.3).

Table 2.6 Percentage (% d.b.) of each fraction in original green coffees along with the amounts (g) of each fraction weighed to prepare the corresponding reconstituted samples.

Fraction	Arabica		Robusta	
	% in green coffee[a]	Amount weighed (g)[b]	% in green coffee[a]	Amount weighed (g)[b]
DCM	23.3	1.2	24.0	1.2
MeOH	12.2	0.6	11.5	0.6
Water	12.4	0.6	12.7	0.6
Residue	29.5	1.5	29.5	1.5
Unknown	22.6	1.1(omitted)	22.3	1.1(omitted)
Total	100	5	100	5

[a]Data were averages of at least 3 replicates.

[b]The amounts were calculated based on a total of 5-g weight of each reconstituted sample.

2.3.2.2 Wet Mixing

Each fraction (weighed according to Table 2.6) was dissolved in 2 ml of one of the following solvents, water (Purelite®), MeOH, and 20% MeOH in water (Purelite®). The dissolved fractions were then mixed together in a blender for 1 min. The blended slurries were then transferred to aluminum trays and dried in a vacuum oven overnight at 40 °C to remove all excess solvents (see Figure 2.2 for the reconstitution process using water to dissolve all fractions).

Table 2.7 Amounts of water (g) added to the dried reconstituted samples prepared from wet mixing process for moisture content adjustment.

Sample	Wet mixed in	(g) Tray	(g) Tray + Dried sample (after vacuum drying)	(g) Dried sample (D)	(g) Water added (W)
Reconstituted Arabica	Water	1.36	4.76	3.4	0.44
Reconstituted Robusta	Water	1.36	4.21	2.85	0.35
Reconstituted Arabica	MeOH	1.37	4.36	2.99	0.39
Reconstituted Robusta	MeOH	1.37	4.46	3.09	0.37
Reconstituted Arabica	MeOH/Water	1.36	4.73	3.37	0.44
Reconstituted Robusta	MeOH/Water	1.38	4.46	3.08	0.37

*W values were calculated based on the formulation: W = (M*D)/(100-M), where M = 11.5 and 10.8 for Arabica and Robusta, respectively.

Moisture contents were adjusted by adding aliquots of Purelite® water to the dried reconstituted samples with a pipette, using a balance to measure the amounts by weight. The amount of water added to each sample was calculated based on the % moisture contents of the green beans determined according to section 2.3.3, 11.5 and 10.8 % for Arabica and Robusta, respectively. The amounts of water added are shown in Table 2.7. The moistened reconstituted samples were again blended in a laboratory grinder to ensure even distribution of the water in the sample. Two grams of each sample was, then,

transferred to a glass tube, covered with aluminum foil, baked at 210 °C for 12.5 min, aqueous extracted (as described in 2.1.2), and subjected to volatile analysis by GC-MS (see section 2.1.3).

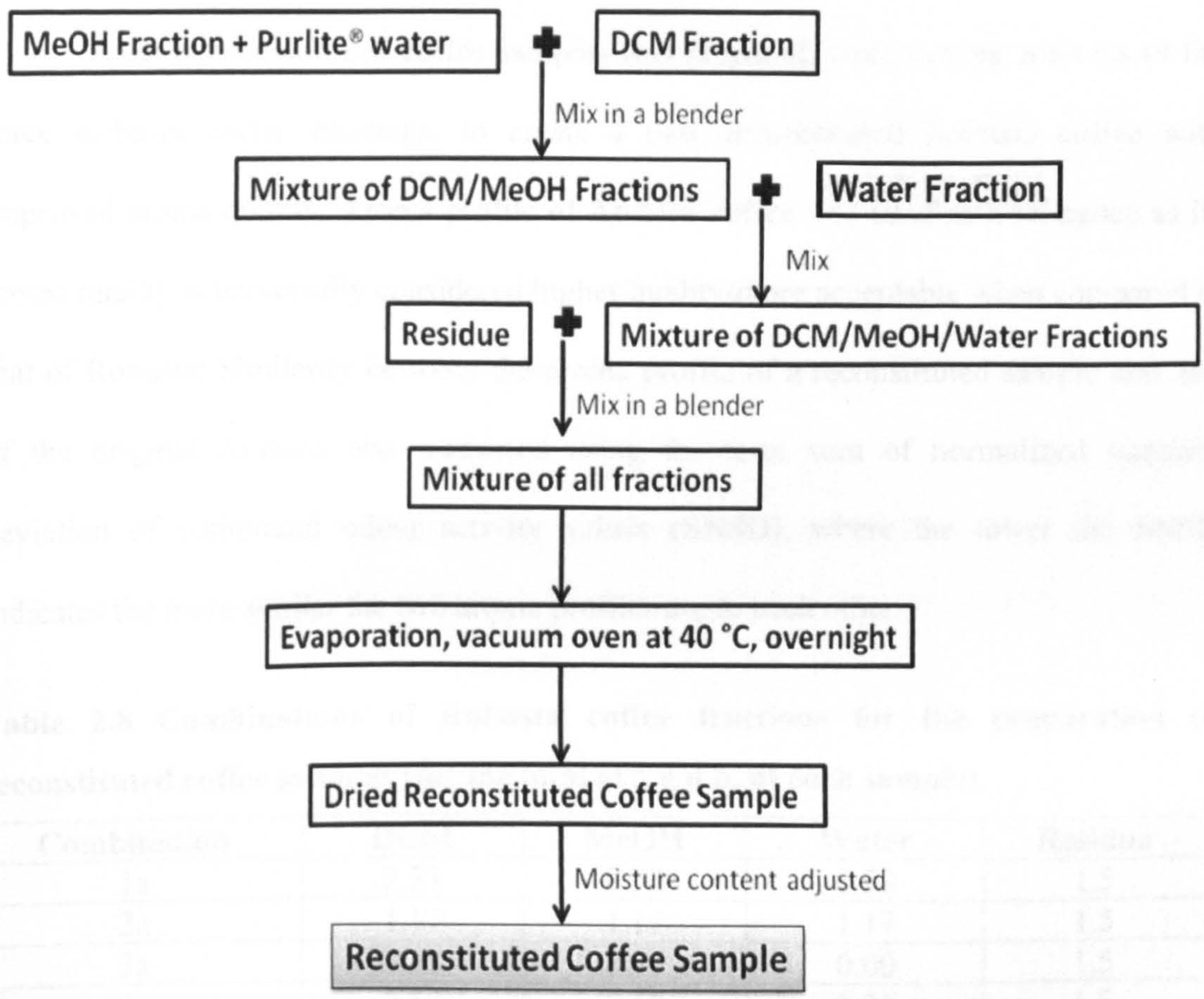


Figure 2.2 Reconstitution procedure.

2.3.3 Moisture Content Analysis of the Green Beans

Moisture contents of the beans were determined according to the method suggested by Mazzafera (1999).

Five grams of ground green coffee was weighed in an aluminum tray, left in a 105 °C oven for 48 h., kept in desiccators for 1 h before being reweighed.

2.3.4 Determination of Feasibility to Create a New Reconstituted Robusta with Improved Aroma Quality from Robusta fractions

A set of reconstituted coffee samples was prepared, with varying amounts of the three Robusta coffee fractions, to create a new reconstituted Robusta coffee with improved aroma quality. Aroma profile of Arabica coffee was used as a reference as its aroma quality is universally considered higher quality/more acceptable when compared to that of Robusta. Similarity between the aroma profile of a reconstituted sample and that of the original Arabica was measured using the term sum of normalized standard deviation of compound odour activity values (SNSD), where the lower the SNSD indicates the more similar the two aroma profiles are to each other.

Table 2.8 Combinations of Robusta coffee fractions for the preparation of reconstituted coffee samples (for the total of 5 g d.b. of each sample).

Combination	DCM	MeOH	Water	Residue
1a	2.33	0.58	0.58	1.5
2a	1.17	1.17	1.17	1.5
3a	1.75	1.75	0.00	1.5
4a	0.58	0.58	2.33	1.5
5a	3.50	0.00	0.00	1.5
6a	3.50	0.00	0.00	1.5
7a	1.75	0.00	1.75	1.5
8a	0.58	2.33	0.58	1.5
9a	0.00	3.50	0.00	1.5
10a	0.00	0.00	3.50	1.5
11a	1.75	1.75	0.00	1.5
12a	0.00	1.75	1.75	1.5
13a	0.00	3.50	0.00	1.5
14a	0.00	0.00	3.50	1.5

Combinations of Robusta coffee fractions produced 14 reconstituted coffee samples based on a mixture design with (D-optimal option) using Design Expert software (Stat-Ease) (Table 2.8). The amounts used for DCM, MeOH, and water fractions were set to vary from 0 to 70% (d.b.), while the amount of residue fraction was set constant at 30% (d.b.) for all reconstituted samples. Composition of the reconstituted samples is shown in Table 2.8.

All samples were prepared according to the wet reconstitution procedure described in section 2.3.2.2 using water to dissolve all fractions prior to mixing (see Figure 2.2). Moisture contents of all the samples were adjusted to approx. 11% (w/w) based on the equation W = (M*D)/(100-M), where M = 11, D = g dried mass, and W = g water to be added. Two grams of each reconstituted sample were, then, weighed in a glass tube, covered with aluminum foil, baked at 210 °C for 12.5 min, aqueous extracted (as described in 2.1.2), and subjected to volatile analysis by GC-MS (see section 2.1.3).

Table 2.9 Limitations used for the new set of reconstituted coffee samples.

Fraction	Limitation (% d.b.)
DCM	0 – 30%
MeOH	35 -70 %
Water	0 – 23 %

Another set of reconstituted samples were also created with narrower limitations as shown in Table 2.9. The new set of reconstituted samples was prepared according to Table 2.10.

The reconstituted samples (new set) were, then, moisture content adjusted, baked, aqueous extracted in the same fashion previously done with the first set, and subjected to volatile analysis by GC-MS (see section 2.1.3).

2.3.5 Calculation and Statistical Analysis

Similarity between the aroma profiles of a reconstituted sample and the original Arabica was determined using the term "sum of normalized standard deviation" (SNSD) of the compounds' odour activity values (OAVs). The OAVs were calculated by dividing the concentration of each aroma compound by its threshold value in water. An SD of the OAVs was calculated from the OAVs of each compound found in the baked original Arabica and a baked reconstituted sample. Normalization of the SDs (NSD) was done by dividing the SDs by their mean OAV. The obtained NSDs of all the targeted coffee aromas were then summed up to make the SNSD value.

Table 2.10 Combinations of Robusta coffee fractions for the preparation of new set of reconstituted samples (for the total of 2.5 g d.b. of each).

Combination	DCM fraction	MeOH	Water	Residue
1b	0.36	1.24	0.14	0.75
2b	0.62	1.06	0.07	0.75
3b	0.20	1.27	0.29	0.75
4b	0.00	1.75	0.00	0.75
5b	0.00	1.46	0.29	0.75
6b	0.88	0.88	0.00	0.75
7b	0.58	0.88	0.29	0.75
8b	0.39	1.07	0.29	0.75
9b	0.44	1.31	0.00	0.75
10b	0.44	1.31	0.00	0.75
11b	0.00	1.46	0.29	0.75
12b	0.00	1.75	0.00	0.75
13b	0.73	0.88	0.14	0.75
14b	0.18	1.50	0.07	0.75
15b	0.88	0.88	0.00	0.75

Experimental design and ANOVA analyses were carried out using the programs Design Expert 7.1 (Stat-Ease) and MINITAB™ V.15. The software UNSCRAMBLER 9.6 was used for Principal Component Analysis (PCA).

2.4 SENSORY EVALUATION

The sensory method "pairwise-comparison" was chosen for this study. The sensory panel was conducted on 30 people who were students and staff members of the University of Nottingham, Sutton Bonington campus. All were regular coffee drinkers.

Since there were four samples, Arabica, Robusta, Sample X and Y (details of samples are explained in section 2.4.1), there were six possible pair-wise combinations. Each judge was, therefore, introduced to six pairs of samples (one pair at a time). The judges were asked to sniff the samples in the order presented (from left to right) and tick the sample whose smell was preferred by them. The presentation order of the 6 pair-wise sequence was random, so was the order of the samples in a pair. The sensory panel was carried out following the experimental design generated by the program FIZZ.

2.4.1 Sample Preparation

Two reconstituted samples, those with lowest SNSD values, were chosen to be tested in the sensory study. They are referred to as sample X and sample Y. Compositions of sample X and Y are described in Table 2.11.

The raw reconstituted coffee samples were prepared using the wet mixing method explained in section 2.3.2.2. However, bigger batches of samples were prepared since the sensory study needed higher amount of samples than that needed for the instrumental

analysis. The preparation steps were the same as the small-scale procedure (See Figure 2.2). However, the amounts of the fractions and solvent (aqueous) used were adjusted according to the quantity of the final samples needed.

Table 2.11 Composition of the reconstituted coffee samples used in the sensory study

Sample	% MeOH fraction (d.b.)	% DCM fraction (d.b.)	% Residue fraction (d.b.)	% Moisture Content (w/w)
X	35	35	30	11
Y	70	0	30	11

* **d.b:** dry weight basis

Due to the capacity of the lab oven used, approximately 20 g of each sample was prepared each time. For sample X, 7 g of each MeOH and DCM fractions (a total of 14 g solid fraction) were mixed with 12 g of water (Purelite®). After the fractions were dissolved, 6 g of the residue fraction was added to the mixture and blended thoroughly in a lab blender until it became homogeneous. The blended slurries were then transferred to an aluminum tray and dried in a vacuum oven overnight at 40 °C. For sample Y, the preparation process was carried out in the same fashion as done with the sample X, except that 14 g of MeOH fraction was used instead of 7 g and no DCM fraction was involved. The amounts of residue fraction and water used were still 6 g and 12 g, respectively.

Moisture contents of these two samples were set constant at 11% (w/w). It is the average percentage of moisture contents found in Arabica and Robusta green beans from the experiment in section 2.3.3. The moisture content adjustment procedure is described in section 2.3.2.2.

After moisture adjustment, the samples were subjected to baking in a lab oven. Since all the four samples, Arabica, Robusta, Sample X and Y, are different, they required different baking temperature profiles to produce samples with the same degree of roast (determined by colour for this experiment). After a preliminary experiment to determine appropriate temperature profiles for the samples, four different temperature profiles suitable for baking each sample were established (see Table 2.12).

Table 2.12 Temperature profiles used for baking samples for the sensory study.

Sample	Temperature (°C)	Time (min)
Arabica	210	13.5
Robusta	210	15.0
Sample X	210	11.0
Sample Y	210	9.5

*These temperature profiles were specifically established for samples weighed approximately 20 g.

After baking, all samples were subjected to colour measurement to ensure they had been roasted/baked to the same degree of roast before they were used for the sensory evaluation. The colour measurement was carried out using L a b scale by ColourQuest XE, Hunter Lab machine.

2.4.2 Sample Presentation

One gram of each coffee sample was weighed and infused with 5 ml of hot water (95 °C) in a brown bottle (30 ml) capped with a white screw lid. The bottle was shaken and warmed in a water bath (72 °C) for approximately 1 min to allow volatiles from the samples to equilibrate. The samples were then introduced to the judges for sniffing.

Due to a limited number of samples, samples were reused (within the same judge) during the sensory panel. Therefore, after being introduced and sniffed by the judges, the samples were put back into the water bath (72 °C) for at least 1 min (but not more than 20 min). This was to warm up the samples, as well as to allow more volatile built-up in the headspace before being reintroduced to the judge.

Since the samples were reused (for 3 times), an analysis determining consistency of the volatile intensity in the bottle head space over time was carried out. The change of the headspace volatile intensity over time was followed using Atmospheric Pressure Chemical Ionization- Mass Spectrometry (APCI-MS). Detail of the analysis is explained in section 2.4.2.1.

The sensory test was carried out in sensory booths. All sample bottles were wrapped with cardboard sleeves to prevent the heat from the bottles contacting the judges' hands. As mentioned, six pairs of samples were introduced to each judge, one pair at a time. The time in-between introduction of the pairs was 2 min. This was to allow time for the judges to breathe in fresh air and to clear the smell of the previous samples before sniffing the next ones. The sample presentation was done in random order and followed the experimental design created by FiZZ program. The session lasted approximately 20 min for each judge. Statistical analysis was done using paired-t test and was also carried out by the FiZZ program.

2.4.2.1 Determination of Headspace Volatile Intensity over Time by Atmospheric Pressure Chemical Ionization-Mass Spectrometry (APCI-MS)

The Platform II quadrupole mass spectrometer fitted with the APCI-MS interface (Micromass Ltd, UK) was used in this study.

The infused coffee samples (5 ml) were placed in 30-ml bottles capped with one-port lids. After an equilibration of 1 min in a water bath (set at 72 °C), the head space was sampled through the port on the lid into the APCI-MS with a sampling flow of 10 ml/min. The sampling was done for 10 s each time. After each sampling, the bottles were left open for 5 s to mimic the situation where some of the volatiles were lost during sniffing by the judges. The samples were then capped and put back onto the water bath to re-equilibrate before being sampled again for another 2 times.

Selected Ion Monitoring (SIM) mode was used and headspace values were obtained from the ion trace using Masslynx 3.2 software (Micromass Ltd, UK). The ions (*m/z*) monitored were 42, 43, 57, 58, 79, 81, 86, 94, 95, 96, 98, 100, 107, 108, 109, 110, 121, 122, 124, 126, 135, 136, 137, 140, 150, 151 and 152.

2.5 ANALYSIS OF TASTE AND OTHER NON-VOLATILE COMPOUNDS IN THE RECONSTITUTED COFFEE

In addition to the previous GC-MS analysis and sensory evaluation carried out to measure quantity and quality of the "aroma", analyses on "taste" compounds were also applied to the coffee samples since it is also a significant factor determining final quality of coffee. Details of the taste compounds experiments are as follows.

As there are many taste compounds in coffee and it is not possible to analyze all of them, a preliminary experiment to screen for the key taste components was set up. Since taste compounds are non-volatile, the use of Direct Liquid Mass-Spectrometry (DL-MS) was employed for this purpose. The detail of the experiment is explained in section 2.5.1.

According to the result from the sensory study, only three samples were subjected to the taste compound experiments: Arabica, Robusta, and sample Y (composition of sample Y is shown in Table 2.11). All were ground and screened through sieve no. 710 to ensure particle uniformity prior to all the following experiments.

2.5.1 Screening for Key Taste Compounds by Direct Liquid Mass-Spectometry (DL-MS)

All roasted samples (0.5 g each) were dissolved in 5 ml of 50:50 methanol: water (Purelite®) in 15-ml bottles. The samples (in the bottles) were extracted on a roller for 1 h. The mixtures were then filtered with no. 1 Whatman® filter paper prior to injection into the DL-MS. Aliquots (20 µl) of each sample extract were injected into the source of the mass spectrometer via an injection loop. The scanning was done from *m/z* 100 to 500 both in positive and negative ionization modes.

2.5.2 Total Soluble Solid (TSS)

The three roasted samples (0.5 g each) were extracted with 5 ml of Purelite® water for 1 h on a roller, filtered with no.4 Whatman® filter paper, then subjected to TSS

analysis using a refractometer (ATAGO, Japan). The results were expressed as degree Brix.

2.5.3 pH and Titratable Acidity (TA)

Both green and roasted coffee samples were subjected to this experiment. Hence, six samples were analysed.

One gram of each samples was extracted with 10 ml of 80:20 methanol:water in a 30-ml tube on a roller for overnight (room temp). The extracts were then filtered with no.4 Whatman® filter paper before being subjected to pH and titratable acidity analyses.

pH of the extracts were measured using a pH meter (Inolab®)

TA of the extracts was measured using the method modified from AOAC Official Method 920.20. The titration used 0.1 M NaOH as a titrant. The extracts from green samples were 4-fold diluted, while the roasted samples were 10-fold diluted prior to the titration. The titration was carried out in replicate using 20 ml of each diluted sample extract each time. Measurement was done at two end-points, at pH 6 and 7. The end-points were determined using a pH meter (Inolab®).

The results were expressed as volume (ml) of the alkali solution (0.1 M NaOH) required to neutralize acidity of 100 g test sample to the points where pH equals 6 or 7.

2.5.4 Lipid Analysis

The method was modified from that of Murphy and Cummins (1989).

All samples (raw) were dried in a vacuum oven set at 40 °C. The samples were dried overnight or until the weights remained constant to ensure complete removal of free moisture. The dried samples were then subjected to the analysis.

Dried samples (0.2 g each) were weighed into 1.5-ml centrifuge vials and mixed with 500 µl isooctane for extraction. Chrome steel beads, 2.5 mm (BioSpec), were added to each vial to help disintegrate the cells of the samples while being beaten in a mini bead beater (MBB) (set at speed 48). The beating was carried out for 30 s. The vials were, then, centrifuged at 13,000 rpm for 5 min. The supernatant (300 µl) was pipetted to an Eppendorf® tube. Extraction of the same sample was carried out with the same process for another 2 times (each time also with 500 µl of isooctane). However, the volume of the supernatant pipetted was 450 µl instead of 300 µl for the second and the third extractions. The supernatants obtained from the three extractions were pooled and were transferred (500 µl) to Bijou bottles for drying. The supernatants (in the Bijou bottles) were placed on a hot plate with temperature set to gradually increase from 100 to 180 °C for 60 min to dry out the solvent. The Bijou bottles, containing dried lipid component, were then weighed. The solid residues from the extraction were kept at -20 °C in the same vials for future protein analysis.

Calculation for lipid content (%):

$$Lipid\ (g) = \frac{\{[wt.\,dry\ lipid\ \ with\ bijou\ bottle\ (g)] - wt.\,empty\ bottle\ (g)\}}{500} \times [300 + (2)(450)]$$

$$Lipid\ content\ (\%) = \frac{[Lipid\ (g)] \times 100}{wt.\,dry\ original\ sample\ (g)}$$

2.5.5 Protein Analysis

The Bicinchoninic acid protein assay (BCA) (Smith, Krohn et al. 1985) was employed for this experiment.

The defatted solid residues (from the previous lipid analysis) were weighed (together with the vials and caps) and placed on a vial heater set at 60 °C for 30 min or until the samples became completely dry (caps left open). The dried samples (with vials and caps) were then reweighed and mixed with 1 ml of 2% Sodium Dodecyl Sulfate solution (SDS). The samples were heated again on a vial heater for 30 min at 60 °C, vortex-mixed for 1 min and centrifuged for 3 min at 13000 rpm. The obtained supernatants were transferred (10µl) to Eppendorf tubes and 100-fold diluted by mixing with 1 ml of 2% SDS solution. The diluted samples were again transferred (50 µl) to new Eppendorf tubes and mixed with 1 ml of standard working reagent (SWR) which without sample was used as a blank. The obtained solutions (in Eppendorf tubes) were heated on a vial heater for 30 min at 60 °C until the colour of the solutions turned purple. The solutions were then pipetted to 1.5-ml cuvettes and subjected to spectrophotometry. The measurement was carried out at 562 nm.

Note: SWR solution was made by mixing 10 ml of bicinchonic acid with 200 µl of 4% (w/v) cupric sulphate ($CuSO_4.5H_2O$).

Calculation for protein content (%):

$$\textbf{Protein content (\%)} = \left[\frac{(A562/C) \times 100\ (dilution)}{(g)\ dry\ original\ sample}\right] \times 100\% \times \left[\frac{1\ (g)}{1{,}000{,}000\ (\mu g)}\right]$$

$$= \left[\frac{(A562/C) \times (1/100)}{(g)\ dry\ original\ sample}\right]$$

Note: A562: Absorbance at 562 nm

C: Constant value (slope) from linear regression of the standard curve;

Equation: Y = C*X; where Y = Absorbance at 562 nm, C = slope, and X = Conc. of standard (µg /ml)

2.5.6 Chlorogenic Analysis by HPLC

The method was modified from that of Moon et al. (2009).

One gram of each sample (both raw and roasted) was mixed with 50 ml of 70:30 MeOH:H_2O in a 100-ml centrifuge tube. The mixtures (in the tubes) were extracted 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 0.1 ml 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. Precipitate was separated by centrifuging at 4000 rpm for 10 min. After the solutions were decanted and filtered with Acrodisc syringes with 0.2 µM Sartorius stedim Minisart filter, the samples were stored at 4 °C until HPLC analysis.

Quantitative analyses of Chlorogenic acids (CGA) were performed using an Agilent 1100 model HPLC system equipped with Phenomenex C-18, 5μ column (250 mm × 3 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 from 0 to 3 min, then linearly increased to 75:25 at 45-50 min and then to 50:50 at 53-57 min. The flow rate was 0.8 ml/min. The detector was set at 325 nm; injection volume was 10 μl.

Concentrations of the investigated CGA's were calculated based on the regression equation obtained from a standard curve made using 5-Caffeoylquinic acid (5-CQA) standard solutions at 50, 100, 500, 1000 and 2500 ppm.

2.5.7 Sugar Analysis by HPLC

The method was modified from that of Chavez-Servin et al. (2004).

Both raw and roasted coffees (0.5 g each) were weighed and mixed with 5 ml of 50:50 MeOH:H_2O in 15-ml centrifuge tubes for extraction. The extraction was carried out on a roller for 1 h at room temperature. The extracts were, then, filtered with no.4 Whatman® filter papers before being transferred (1 ml) to new tubes. The extracts (1 ml) were treated with 50 μl Carrez I solution, vortex-mixed for 1 min, then treated with 50 μl Carrez II solution and vortex mixed again for 1 min. Acetonitrile (1 ml) was then added. The mixtures were left to stand for 10 min and centrifuged at 4000 rpm for 10 min. The supernatants were filtered through C18 cartridges (Phenomenex Strata™-X) previously conditioned with 2 ml of MeOH and 2 ml of Purelite® water. The filtered extracts were

forced through 0.45 μm filters (Minisart®) and transferred (1 ml) to 2-ml tubes. The tubes were placed on a tube heater (set at 60 °C) until all the solvent dried out. Solvent (200 μl) 50:50 MeOH:H_2O was added to each sample, vortex-mixed for one min before being subjected to HPLC sugar analysis.

Regarding HPLC conditions, chromatographic separation was undertaken with an isocratic elution mobile phase of 80% acetonitrile, and degassed before use. The flow-rate was set at 1 ml/min, the volume injected was 5μl. Column temperature was maintained at 25 °C. The HPLC system was Agilent 1100 model equipped with Phenomenex Luna 5μ NH_2 100A column (250 mm × 2 mm) and a refractive index (RI) detector (Jasco, RI-2031 Plus).

Peaks were identified by comparing retention times to those of sugar standards. Standard curves were made of fructose, glucose, and sucrose standards (Sigma Aldrich®). Each was made at five levels of concentration: 1, 2.5, 5, 7.5, and 10 mg/ ml. All dissolved in 50:50 MeOH:H_2O. The respective peak areas were used for the quantitative analysis.

2.5.8 Trigonelline and Caffeine Analyses by HPLC

Trigonelline and caffeine were analyzed simultaneously using the method modified from that of Casal et al. (1998).

Both raw and roasted coffees (0.5 g each) were mixed with 10 ml of water (Purelite®) in 15-ml tubes and were extracted on a roller at room temperature for 1 h. The samples (in the same tubes) were then placed in a shaking water bath set at 50 °C for a further 30 min extraction. The extracts were then allowed to cool down, filtered through

no.4 Whatman® filter paper, 4-fold diluted and filtered again through 0.2 μm filter paper before being subjected to HPLC analysis.

HPLC analysis was carried out using a HPLC unit consisting of two PU pumps (Jasco PU-980 and PU-1580), and a variable wavelength detector (Spectro Monitor®3000). A Phenomenex C-18 Luna 5μ column (250 × 3 mm) was used. The solvent system used was a gradient of phosphate buffer pH 4.0 (A), prepared with 5% of potassium dihydrogen orthophosphate (Fisher) 0.2 M, and methanol (B). The gradient was set as follows: 0'-7% B, 4'- 9% B, 6'-25% B, 13'-29% B, and 21'-50% B with a flow rate of 0.7 ml/min. Injection volume was 5μl. Detection was accomplished at 265 nm for both trigonelline and caffeine.

Peaks were identified by comparing retention times to those of caffeine and trigonelline standards (Sigma Aldrich®). Standard curves were made from the standard compounds prepared at 100, 250, 500, 750, and 1000 ppm. All dissolved in Purelite® water. The respective peak areas were used for quantitative analysis.

CHAPTER 3: DEVELOPMENT OF AROMA COMPOUND ANALYSIS PROCEDURE

3.1 INTRODUCTION

As previously stated in section 1.7, one of the important steps in aroma compound analysis, especially in instrumental analysis, is aroma isolation. Since one of the main purposes of this study was to recover as much as possible the coffee aroma from the coffee process waste, silver skin, the conventional solvent extraction method was chosen over the headspace type extraction.

There have been a number of studies that applied solvent extraction to the study of coffee aroma (Ramos, Valero et al. 1998; Sarrazin, Quere et al. 2000). However, as there is no single technique proved to be optimal for every sample (Parliment 2001), development of a method suitable for aroma extraction of the coffee sample of interest was needed.

In this study three types of solvent were used for extraction: water, methanol (MeOH), and dichloromethane (methylene chloride or DCM). The three solvents differ in polarity with polarity indexes of 9, 5.1 and 3.1, respectively (Byers 2003). It was expected that the groups of aroma compounds extracted by these solvents be different due to the differences in the solvents' polarities and, as a consequence, should produce extracts that are dissimilar in aroma quality. In this experiment, each extraction involved the use of 20 ml solvent to extract 2-g of roasted and ground coffee at ambient tempterature (see section 2.1.2 for the procedure). Quantification of the aroma

compounds was based on the use of an internal standard, 2-heptanol. Recoveries of target coffee aromas by different extraction conditions were compared and the results are illustrated in the following sections.

3.2 RESULTS AND DISCUSSION

3.2.1 Internal Standard (IS) Selection

Stability, reproducibility, as well as log P, the lipophilicity parameter: log [n-octanol/water partition coef.], were criteria used for IS selection (see sections 2.1.1 for detail on internal standard (IS) selection procedure). An ideal IS is a compound whose log P falls within the range of log P's of the interested coffee aromas (see Table 2.1 for log P's of candidate IS's), and that is stable in the extraction conditions used, which should subsequently produce reliable and reproducible results.

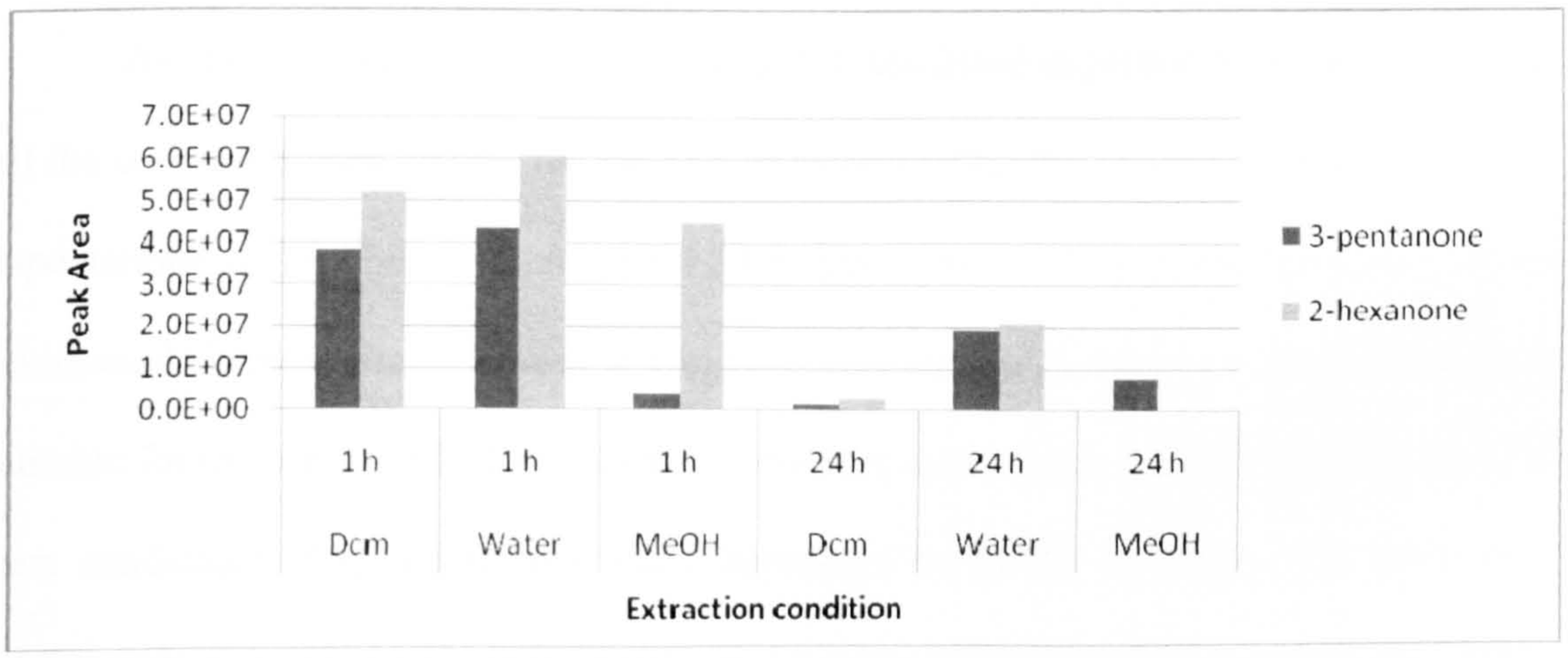


Figure 3.1 Stability of 3-pentanone and 2-hexanone under various extraction conditions.

As mentioned in 2.1.1, four candidate IS's were studied. Their performance under selected extraction conditions were monitored (see Figures 3.1 and 3.2).

The experiment was divided into two parts. The first part was a study of 3-pentanone and 2-hexanone, while the second part was for the other two compounds, sec-butanol and 2-heptanol.

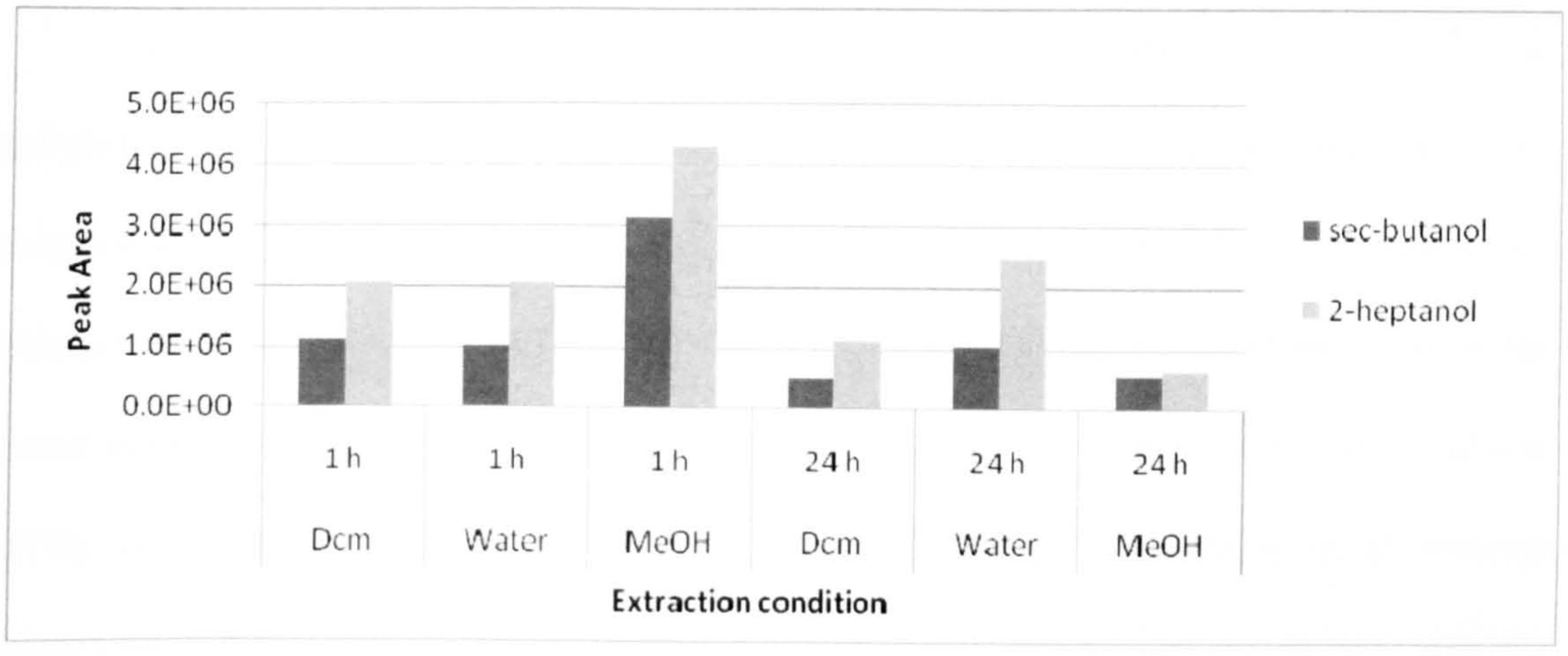


Figure 3.2 Stability of sec-butanol and 2-heptanol under various extraction conditions.

As seen in Figure 3.1, 3-pentanone and 2-hexanone degraded over time in almost all the conditions used, except for the MeOH extract where the system seemed to recover 3-pentanone slightly better when left for 24 h. Since the result indicated instability of the compounds in most circumstances, it suggested that neither of these two compounds were suitable for the use as an IS for this study. Another experiment was then carried out with new candidates IS's, sec-butanol and 2-heptanol. As shown in Figure 3.2, these two compounds were obviously more stable over longer extraction time in water and DCM. MeOH extraction, however, still caused significant degradation of both compounds over time. The result, therefore, indicated the potential of MeOH causing degradation of other aroma compounds of interest. As to IS selection, sec-butanol and 2-heptanol seemed to

exhibit equivalent behavior. However, since log P of 2-heptanol is closer to those of the coffee aromas of interest, 2-heptanol was selected as an IS for future experiments.

3.2.2 Isolation of Coffee Aromas

A preliminary study showed that higher extraction temperatures, up to 35 °C, did not yield a significant increase in coffee aroma recovery in any of the three solvents used, compared to the extraction at ambient temperature. Therefore, the factor "temperature" was omitted. As mentioned earlier, the three solvents, differing in polarities, used in the experiment were water, MeOH and DCM. Each extraction was done using 20 ml of one of the solvents to extract 2-g ground sample. All extraction was carried out at ambient temperature both for 1 h and overnight (see section 2.1.2 for the procedure). Results regarding the effects of solvent and extraction time on the amount of the target aromas recovered are illustrated in Table 3.1.

Table 3.1 Concentrations (ppm) of selected coffee aromas recovered by different extraction conditions.

Solvent	Time	methyl-pyrazine	3-furan methanol	2,5-dimethyl-pyrazine	2,6-dimethyl Pyrazine	2-furan methanol acetate	trimethyl pyrazine
Water	1 h	16.5 bc	128.4 d	34.5 c	164.8 c	78.1 c	8.6 a
DCM	1 h	6.8 a	15.4 a	9.7 a	15.0 a	17.9 a	5.8 a
MeOH	1 h	8.1 a	38.1 b	11.9 a	39.8 b	31.7 b	7.8 a
Water	24 h	14.0 b	134.8 d	37.5 c	141.8 c	67.4 c	8.5 a
DCM	24 h	20.6 c	75.6 c	18.4 b	57.2 b	26.7 b	2.4 a
MeOH	24 h	11.0 ab	79.8 c	27.2 c	40.8 b	21.5 b	4.9 a
Solvent effect		√	√	√	√	√	**X**
Time effect		**X**	√	√	**X**	**X**	**X**

*All pairwise comparisons were made by Tukey's Test at 95% confidence level. Means with the same letter are not significantly different.

** The results were based on 2 replicates.

Comparisons were statistically made based on ANOVA and Tukey's test at 95% confidence level.

As seen in Table 3.1, longer extraction with DCM and MeOH produced higher recovery of the target aromas. With DCM extraction, the increases were significant for most of the aromas. While, with MeOH extraction the significant increases were only found on 3-furanmethanol and 2,5-dimethylpyrazine. Extraction by water, however, showed to well maintain the recovery levels of all the target compounds over time.

Regarding the effect of solvent (see Table 3.1), aqueous extraction was statistically proved to produce the highest recovery for all the coffee aromas studied. MeOH extraction for 1 h was found to give better aroma recovery than DCM extraction, however the recovery was still far less than that given by aqueous extraction. The result is in agreement with the finding from a study by Ortega and Rodriguez (2005) who found coffee flavour more predominant in the aqueous extract when compared to alcoholic extract at the extraction time of 15-52 min.

The extraction by water (aqueous) for 1 h was, therefore, chosen as a method for coffee aroma isolation for the future experiments.

3.3 CONCLUSION

2-Heptanol was chosen as an internal standard due to its stability in the extraction conditions applied. Aqueous extraction was found to give the best recovery of the selected coffee aromas in the coffee samples studied. Therefore, this extraction method

along with the use of 2-heptanol as an internal standard was chosen as a method to apply to all future aroma compound analysis in this study.

CHAPTER 4: INVESTIGATION OF COFEE SILVER SKIN AS A POTENTIAL SOURCE OF COFFEE AROMA

4.1 INTRODUCTION

Silver skin is the thin, innermost skin of the coffee fruit (see Figure 1.8). It clings to the dried coffee beans until it is either removed by polishing or floats free during roasting and becomes what roasters call silver skin (Davids 2001). In the coffee industry, coffee silver skin is recognized as a process waste and is normally handled by using it as fuel, fertilizer (Saenger and Hartge 2001), or animal feed.

Borrelli et al. (2004) investigated the potential to use this process by-product as a functional ingredient and found that silver skin possesses great antioxidant properties, contains high amounts of dietary fiber and has potential prebiotic activity. Chemical composition of silver skin is shown in Table 1.19.

As the objective of this study was to try to initiate an alternative way to add value to this coffee waste by using it as a source for natural coffee flavouring agent, an investigation on the possibility to generate coffee aroma from the silver skin was established. Although there has been no evidence for the use of coffee silver skin as a source for coffee aroma, the possibility of this material being used for this purpose relies on the fact that it contains high content of Maillard reaction precursors, protein and carbohydrate (see Table 1.19), which are regarded as compounds necessary for the generation of coffee aroma.

The attempt to generate more coffee aroma from silver skin was carried out through chemical modification and Maillard reaction (see section 2.2 for detail regarding the procedure). This involved application of heat treatment and modification of silver skin chemical composition by addition of chemicals and/or enzyme treatment.

4.2 RESULTS AND DISCUSSION

4.2.1 Comparison of Aroma Profiles of Coffee and the Corresponding Silver Skin

An instrumental aroma compound analysis by GC-MS was carried out on Arabica, Robusta, and their silver skin (method is discussed in section 2.2). All samples were Thai coffee and were obtained from a coffee company in Thailand. Comparison of the aroma profiles of the coffees and their corresponding silver skin was made (sec 2.2.1).

Table 4.1 Concentrations (ppm) of coffee aromas in roasted and ground coffee (R&G) along with those in the corresponding silver skin.

Aroma Compounds	R&G coffee	Silver skin
methylpyrazine	13.4	0.4
3-furanmethanol	149.4	8.6
2,5-dimethylpyrazine	34.0	0.8
2,6-dimethylpyrazine	169.0	2.8
2-furanmethanol acetate	71.7	0.7
Trimethylpyrazine	12.2	0.5

*Numbers are averages of 2 replicates.

Since not all coffee aroma compounds found in roasted coffee were found in the corresponding silver skin, only concentrations of the compounds found in both samples are presented and compared here (see Table 4.1). As seen, large differences between

concentrations of the selected compounds in roasted coffee and those in silver skin were observed. The differences were found to vary from 30 to 150 times less in the silver skin depending on the aroma compounds. Further investigation on the possibility to generate more coffee aromas from this coffee by-product was then carried out.

4.2.2 Determination of Possibility to Generate Coffee Aroma from Coffee Silver Skin

Due to the low concentrations of coffee aromas found in the silver skin, attempts to find whether it was possible to generate more coffee aromas from the sample matrix were made. Since Maillard reactions are the main reactions involved in coffee flavour development, quantification of Maillard-precursors, sugars and amino acids, in silver skin were carried out (see section 2.2.2 for methods).

Table 4.2 Contents of sugars in Arabica silver skin determined by HPLC.

Sugar	g/100g sample*	R^2 of each standard curve
Fructose	0.077	0.9751
Glucose	0.129	0.9816
Galactose	0.202	0.9737
Sucrose	0.120	0.9764
Maltose	0.094	0.9241
Lactose	0.185	0.8152
Total	0.808	
Total reducing sugar as reported by Borrelli et al. (2004)	0.21±0.01	

*Numbers are averages of 3 replicates.

Quantification of sugars was carried out using two different types of assay; total reducing sugar by spectrophotometry (expressed as "maltose equivalent") and individual sugar analysis by HPLC.

Table 4.3 Total reducing sugar analysed by spectrophotometry (expressed as "Maltose equivalent").

Sample	Average g total reducing sugar/100 g sample	SD
Silver skin	2.38	0.112

*The average value is based on 4 replicates.
**R^2 of maltose standard curve is 0.9836.

As observed in Table 4.2 and 4.3, total reducing sugar measured by the spectrophotometer assay gave a much higher value than that given by the sum of individual sugar contents analyzed by the other method. This was as expected as the principle of the spectrophotometer assay was based on the reduction of 3,5-dinitrosalicylic acid by any reducing groups available in the sample, which yields colour for spectrophotometry measurement. This value, hence, might have included other reactions that come from non-sugar reducing groups, i.e. from phenolic compounds, and thus causing the value to be higher than those given by the other method as well as those suggested from the literature, (0.21 g/100g sample; Borrelli, Esposito et al. 2004).

Table 4.4 Sugar composition of light, medium, and dark roasted coffee beans compared to those of green beans (mol %).

Sugar	Green	Light	Medium	Dark
Rhamnose	1	1	1	1
Arabinose	10	7	6	5
Xylose	1	1	1	1
Mannose	42	48	51	51
Galactose	24	23	22	21
Glucose	18	15	16	18
Total sugar (% w/w)	46	42	41	37

*Source: Oosterveld et al. (2003b).
** Fructose was found as trace (Fischer, Reimann et al. 2001).

The sum of individual reducing sugar concentrations analyzed by HPLC, was higher than that suggested by Borrelli et al. (2004). This difference might be due to the variation among samples coming from different regions, and probably different processing conditions. In either circumstance, the levels of total reducing sugar in silver skin were still much lower than that of the green coffee beans (see Table 4.4). This suggested that some modification on silver skin matrix's chemical composition to increase levels of reducing sugars may be needed in order to create a system that would be more suitable for the generation of coffee aromas.

Table 4.5 Amino acid contents in green coffee beans and coffee silver skin (g/100g).

Amino acids	Arabica green beans (free amino acids)[a]	Brazilian Arabica green beans (total amino acids)[b]	Silver skin (total amino acids)[c]
Aspartic	0.051	0.96	0.89
Serine	0.032	0.50	0.49
Glutamic	0.112	1.83	0.91
Glycine	0.008	0.61	0.59
Histidine	0.004	0.23	0.22
Arginine	0.009	0.53	0.23
Theonine	0.005	0.34	0.39
Alanine	0.055	0.47	0.54
Proline	0.035	0.61	0.65
Cystine	n/a	n/a	0.2
Tyrosine	0.007	0.46	0.33
Valine	0.018	0.47	0.47
Methionine	n/a	0.23	0.13
Lysine	0.006	0.62	0.26
Iso-Leucine	0.008	0.36	0.43
Leucine	0.012	0.94	0.71
Phenylalanine	0.016	0.63	0.55
Total	**0.378**	**9.81**	**7.99**

[a] Tressl et al. (1983).
[b] Contents after hydrolysis (Thaler and Gaigl 1963).
[c] Contents after hydrolysis analysed by International Laboratory Service (ILS).

Regarding amino acids, the total amino acid profile of the silver skin (see Table 4.5) closely resembles that of green Arabica coffee beans reported by Tressl et al. (1982), both in terms of quality and quantity. This indicated potential of the silver skin as a material for coffee flavour generation. Efforts to produce more coffee aromas from the silver skin through Maillard reactions were then carried on in the next experiments.

4.2.3 Generation of Coffee Aroma from Coffee Silver Skin through Maillard Reactions

As the quantification of Maillard-reaction precursors in the previous experiment (see sections 2.2.2 and 4.2.1) suggested the possibility to generate more coffee aroma from the silver skin, the silver skin were, then, subjected to various treatments: baking, addition of reducing sugar and digestion by enzyme (see section 2.2.3 for more details). Changes in the aroma profile of the silver skin after being chemically (see Figures 4.2 and 4.3) and thermally treated (Table 4.7) were monitored.

Table 4.6 The coffee aroma compounds monitored.

Odorant	Odour
2(3)-methylbutanal	Malty
Alkylpyrazines	Roasty, Toasted, Cooked
3(2)-furanmethanol (furfuryl alcohol)	Burnt
2-furancarboxaldehyde (2-furaldehyde)	Bread, Almond, Sweet
2-furanmethanol acetate (fufuryl acetate)	Fruity, Banana-like

The key coffee aroma compounds monitored and their associated sensory characters are shown in Table 4.6. These represent end products of the major pathways in

the Maillard reactions. Methylbutanal is not only an important aroma compound but is a marker for the Strecker reaction. Alkylpyrazines represent the secondary Maillard products, while the furan products are markers of sugar degradation (Nursten 2005).

The first experiment applied heat treatment alone to see if it helped trigger Maillard reactions in the silver skin matrix. In this experiment, the baking temperature was arbitrarily set to 215 °C in order to mimic the temperature of coffee roasting process that commonly ranges from 200 to 240 °C. A preliminary study showed that leaving the sample in the oven longer than 15 min caused the sample to burn. Thus, 10 min was chosen as time for which the heat treatment was applied. This heating time also conformed to time normally applied for coffee roasting with home coffee roasting apparatus, i.e. 4-20 min.

Table 4.7 Concentrations (ppm) of target coffee aromas in original silver skin compared to those in silver skin baked at 215 C° for 10 min.

Sample	Trial	methyl pyrazine	2-furan methanol	2,5-dimethyl pyrazine	furfuryl acetate	Trimethyl pyrazine
Original Silverskin	1	0.85	0.59	1.97	0.37	0.70
Original Silverskin	2	2.21	0.86	2.89	0.41	0.75
	MEAN	**1.53 a**	**0.73 a**	**2.43 a**	**0.39 a**	**0.72 a**
Baked	1	4.99	0.56	4.23	0.40	1.28
Baked Silverskin	2	1.69	1.09	1.76	0.23	0.62
	MEAN	**3.34 a**	**0.82 a**	**2.99 a**	**0.31 a**	**0.95 a**

*Means with the same letter are not significantly different at 95% confidence level.
** Means were calculated based on 2 replicates.

The result (see Table 4.7) showed that heating at 215 °C for 10 min did not cause significant change in levels of any of the marker coffee aromas. The change in methylpyrazine levels seemed significant but inter-sample variation was large and, therefore, the change was not statistically significant. The result, thus, indicated that applying only heat treatment to the sample was not enough to stimulate Maillard reactions in this silver skin. Further attempts to modify silver skin matrix were carried out by adding some reducing sugar (glucose) into the matrix prior to heating. Lower heating temperatures with longer heating time were also applied to the sample to see if this would help. The effects of these factors were monitored, and the findings were illustrated in Figure 4.1.

Heating was carried out at two different temperatures, 150 and 200 °C. Heating time was set to 50 min. As shown (Figure 4.1), baking silver skin material (BSS) at 200 °C with glucose (glu) at both levels, (20 and 40% w/w), caused a noticeable increase in furans, furfuryl acetate, 2-furancarboxaldehyde, and 3-furanmethanol, but not pyrazines. Adding moisture (20% w/w water) into the system, however, seemed to cause reduction in aroma concentrations in all conditions. All silver skin samples subjected to heating at 150 °C contained less aroma than the original unheated silver skin. This result suggested that 150 °C was high enough to cause losses of aroma volatiles already present in the silver skin, however it was also too low to stimulate Maillard reactions to produce more coffee aromas.

With respect to the increase of furans upon heating at 200 °C, Heyns et al. (1996) reported furanic compounds as the principle decomposition products in the thermal

degradation of D-glucose and sugar polymers which is in agreement with a study by Sheldon et al. (1986) who also found furans in their heated cysteine/glucose model system. Regarding these evidences, it is likely that the increase of furans found in our experiment was merely due to caramelization and Maillard reaction of the glucose added, and not caused by an intrinsic composition of the silver skin itself. Otherwise, an increase in aroma concentration should at least have been detected when original silver skin, without glucose, was heated (as shown in the result of the previous experiment: Table 4.7).

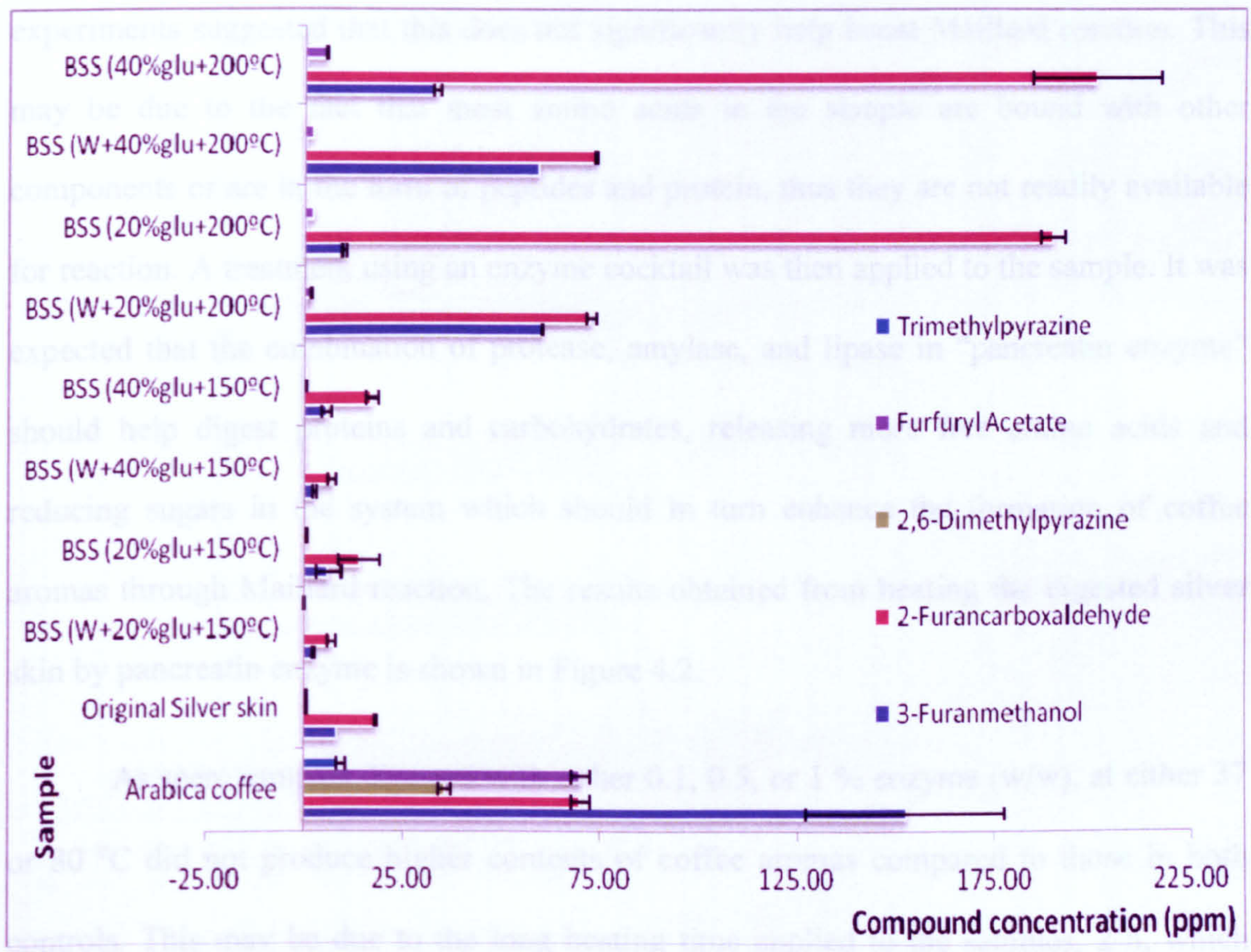
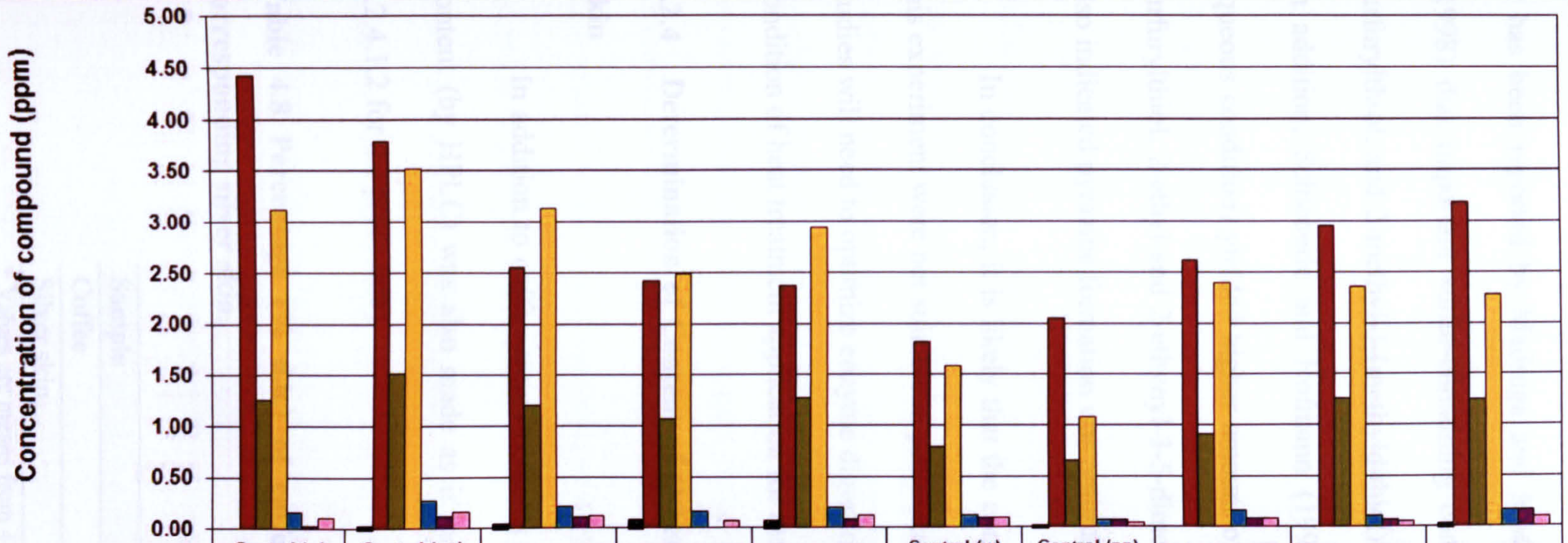


Figure 4.1 Concentrations (ppm) of the target coffee aromas in roasted Arabica coffee, original and baked silver skin (BSS). Glu represents glucose addition (% w/w), W represents water addition (20% w/w).

To sum up, it is clear that the amount of reducing sugars in the silver skin itself is not enough to stimulate more coffee aromas through Maillard reaction upon heating. Adding glucose, however, still did not provide coffee aroma profile resembling that of the roasted coffee as it showed to help produce only specific classes of aroma compounds, i.e. furans, but not others, i.e. pyrazines.

Another attempt to modify silver skin matrix was done using enzyme treatment to hydrolyze the protein fraction. Although total amino acid profile of the silver skin closely resembles that of the green coffee (as reported in Table 4.5.), results from previous experiments suggested that this does not significantly help boost Maillard reaction. This may be due to the fact that most amino acids in the sample are bound with other components or are in the form of peptides and protein, thus they are not readily available for reaction. A treatment using an enzyme cocktail was then applied to the sample. It was expected that the combination of protease, amylase, and lipase in "pancreatin enzyme" should help digest proteins and carbohydrates, releasing more free amino acids and reducing sugars in the system which should in turn enhance the formation of coffee aromas through Maillard reaction. The results obtained from heating the digested silver skin by pancreatin enzyme is shown in Figure 4.2.

As seen, samples digested with either 0.1, 0.5, or 1 % enzyme (w/w), at either 37 or 80 °C did not produce higher contents of coffee aromas compared to those in both controls. This may be due to the long heating time applied to the samples, 2 h, which caused losses of the volatile substances. pH and water activity of the sample matrices while being heated are probably other factors inhibiting aroma generation in the system.



	Control (w) (37)	Control (pp) (37)	0.1% enz (37)	0.5% enz (37)	1% enz (37)	Control (w) (80)	Control (pp) (80)	0.1% enz (80)	0.5% enz (80)	1% enz (80)
3-Methylbutanal	0.00	0.03	0.04	0.08	0.07	0.00	0.01	0.00	0.00	0.04
3-Furaldehyde	4.42	3.79	2.55	2.42	2.37	1.81	2.05	2.61	2.96	3.18
3-Furanmethanol	1.26	1.52	1.21	1.07	1.26	0.79	0.64	0.91	1.25	1.24
2-Furancarboxaldehyde	3.11	3.52	3.13	2.48	2.94	1.58	1.08	2.39	2.36	2.28
2,6-Dimethylpyrazine	0.16	0.27	0.21	0.15	0.20	0.12	0.07	0.16	0.11	0.15
2-Furanmethanol Acetate	0.03	0.12	0.10	0.00	0.09	0.09	0.07	0.08	0.07	0.15
Trimethylpyrazine	0.10	0.16	0.13	0.07	0.13	0.09	0.04	0.08	0.06	0.10

Sample

*Controls with "w" and "pp" are undigested silver skin in water and phosphate buffer system (pH 7), respectively.

** Numbers in parentheses (37 and 80) are temperature (°C) used for digestion.

Figure 4.2 Concentrations (ppm) of the target coffee aromas in digested silver skin baked at 200 °C for 2 h along with those in undigested silver skin (control) baked at the same condition.

It has been reported by Madruga and Mottram (1995) and Hofmann and Schieberle (1998) that important sulfur-containing compounds such as 2-methyl-3-furanthiol, 2-furfurylthiol, and 2-methyl-3-(methyldithiol)furan are preferably formed at a pH of 3-4. In addition, Schieberle and Hofmann (1998) found that drying (as compared to in aqueous condition) yielded higher amount of key odorants with roasty notes such as 2-furfurylthiol, 2-ethyl-and 2-ethynyl-3-5-dimethylpyrazine. Leahy and Reineccius (1989) also indicated pyrazine formation was optimal at an a_w of about 0.75.

In conclusion, it is likely that the conditions used to heat the digested samples in this experiment were not suitable in promoting formation of coffee aromas. Hence, future studies will need to optimize enzyme digestion condition for the silver skin, as well as the condition of heat treatment application for the digested matrix.

4.2.4 Determination of Content of Other Functional Component in Coffee Silver Skin

In addition to coffee aroma contents in the silver skin, quantification of caffeine content (by HPLC) was also made as it may be useful for future studies (see section 2.2.4.1.2 for the procedure).

Table 4.8 Percentage (% d.b.) of caffeine in roasted Arabica coffee and the corresponding silver skin.

Sample	% caffeine (d.b.)
Coffee	0.76
Silver skin	0.46

* Values are means from 4 replicates.
**R^2 of caffeine calibration curve is 0.9968.

As seen in Table 4.8, caffeine content of the silver skin is approximately half that of the roasted coffee. This indicated that, apart from being a source for coffee aroma production, silver skin may also be used as a caffeine source that might be valuable to many industries, i.e. as ingredients for energy beverage or cosmetic industries.

4.3 CONCLUSION

The coffee process by-product, silver skin, did not contain substantial amounts of coffee aromas, 30 – 150 times less than those in roasted coffee. Although total amino acid analysis revealed reasonable amount of amino acids in the sample, stimulation of Maillard reaction by applying heat treatment alone to the sample did not cause a significant increase in coffee aromas. The low quantity of Maillard-reaction precursors; free amino acids and reducing sugars, in the matrix is the likely reason that inhibits aroma generation through Maillard reaction. Modification of silver skin matrix composition by sugar addition only yielded the increase of certain coffee aroma, i.e furans. Therefore, it did not help generate aromas from silver skin with profile resembling that of the original coffee. The attempt to increase levels of free amino acids and reducing sugars by enzyme digestion was carried out to a certain level. However, the results were still not satisfactory. Further investigation to find a suitable condition for the enzyme treatment as well as the heat application is needed.

CHAPTER 5: IMPROVING QUALITY OF ROBUSTA COFFEE UTILIZING FRACTIONATION AND RECONSTITUTION APPROACHES

5.1 INTRODUCTION

Robusta coffee, *Coffea robusta,* is often considered inferior in sensory quality as compared to Arabica. However, as Robusta coffee is less susceptible to diseases/severe climates, it is easier to maintain and hence cheaper to produce. In some regions where climates are not very suitable for Arabica, i.e. Southeast Asia, Robusta is predominant. Robusta is usually limited to lower grade coffee blends or as a filler (wikipedia.org).

A numbers of studies (most registered as patents), have tried to chemically modify Robusta beans to improve its sensory quality. Becker et al. (1991) indicated that the "earthy and musty" flavour notes in Robusta are undesirable in many countries and therefore are an issue for the coffee industry.

As mentioned in section 1.9, common approaches to modify coffee are: addition of desired components (e.g. aroma precursors), removal of unwanted aromas, break-down of macromolecules to smaller components, or combinations of the approaches.

Fractionation-reconstitution techniques have been extensively applied to bakery/dairy products. It is believed to provide an idea of how components in a sample contribute to the final properties of the sample. For example, Courtin et al.(1999) applied these fractionation and reconstitution approaches to trace the role of endoxylanases in

bread-making. Given that the roles of components in the sample matrix are known, and proportions of components in the matrix can be adjusted, i.e. via reconstitution, improvement of the desired property in the final product should be possible. Rogers and Hoseney (1989) applied the techniques to cracker flours and have successfully improved the quality of the poor quality flours.

In coffee, the only relevant published studies on coffee fractionation are those carried out by De Maria et al. (1994-1996). Their main objectives, however, were only to determine flavour precursors present in each fraction and the volatiles formed from them. Therefore, there was no further application of reconstitution, nor an attempt to improve quality of the coffee in their studies.

The objective of this experiment was, therefore, to make use of these techniques to improve the sensory quality of the poorer quality coffee, Robusta. In order to achieve this goal, development of procedures to fractionate and reconstitute raw coffee samples was carried out first. Then the samples were chemically modified via reconstitution techniques and quality improvement was determined both instrumentally, i.e. GC aroma compound analysis (results shown in this Chapter), and sensorially (results shown in Chapter 6). Instrumental analyses of taste compounds in the reconstituted samples were also carried out and the results are shown in Chapter 7).

In addition, a more recent study by Rubach et al. (2010) has applied fractionation to trace components in coffee responsible for the symptoms of gastric irritation caused by coffee consumption in some people.

5.2 RESULTS AND DISCUSSION

5.2.1 Fractionation

In this study, three solvents; dichloromethane (DCM), methanol (MeOH), and water, were used to extract chemical components from the ground green coffees (see section 2.3 for method). The extracts were dried under vacuum condition (by rotary evaporator), and the remaining dried materials were, therefore, used for the reconstitution process (see section 5.2.2). It was expected that these solvents, varying in their polarity, should yield different groups of flavour precursors in different coffee fractions. In this experiment, the dried materials obtained from the DCM, MeOH, and water extracts were referred to as DCM fraction, MeOH fraction, and water fraction, respectively. The remaining undissolved material from the extractions was referred to as residue fraction.

From the results from volatile analysis by GC-MS (see Figure 5.1, 5.2, and 5.3), it was found that different aroma profiles were obtained from different coffee fractions after heating (210 °C, 9 min). This, therefore, indicated that the chemical compositions among the four fractions, DCM fraction, MeOH fraction, water fraction, and residue, were different.

As illustrated in Figure 5.1 and 5.2, water fractions from both Arabica and Robusta yielded aroma profiles that were the closest, compared to the other fractions, to those of their corresponding original coffees both qualitatively and quantitatively. The MeOH fraction of Arabica, however, yielded the highest levels of furans compared to those given by the other fractions of the same coffee (or even those given by the original Arabica) (see Figure 5.1). Since furans are known as products of sugar degradation, it is

anticipated that the high levels of furans formed from the MeOH fractions could be due to the high levels of sugars in the MeOH fraction. This assumption could be supported by a study by De Maria et al. (1994) who found higher level of sugar in their alcohol-extracted fraction (ethanol). They suggested that most compounds present in the ethanol soluble fractions were low-molecular-weight flavour precursors, i.e. mono, di-saccharides, and small amino acids, which undergo Maillard reactions more readily than the higher-molecular weight components (reported to be less soluble in alcohol). The higher molecular weight components, i.e. polysaccharides and proteins, however were reported to be more soluble in water (De Maria, Trugo et al. 1996a).

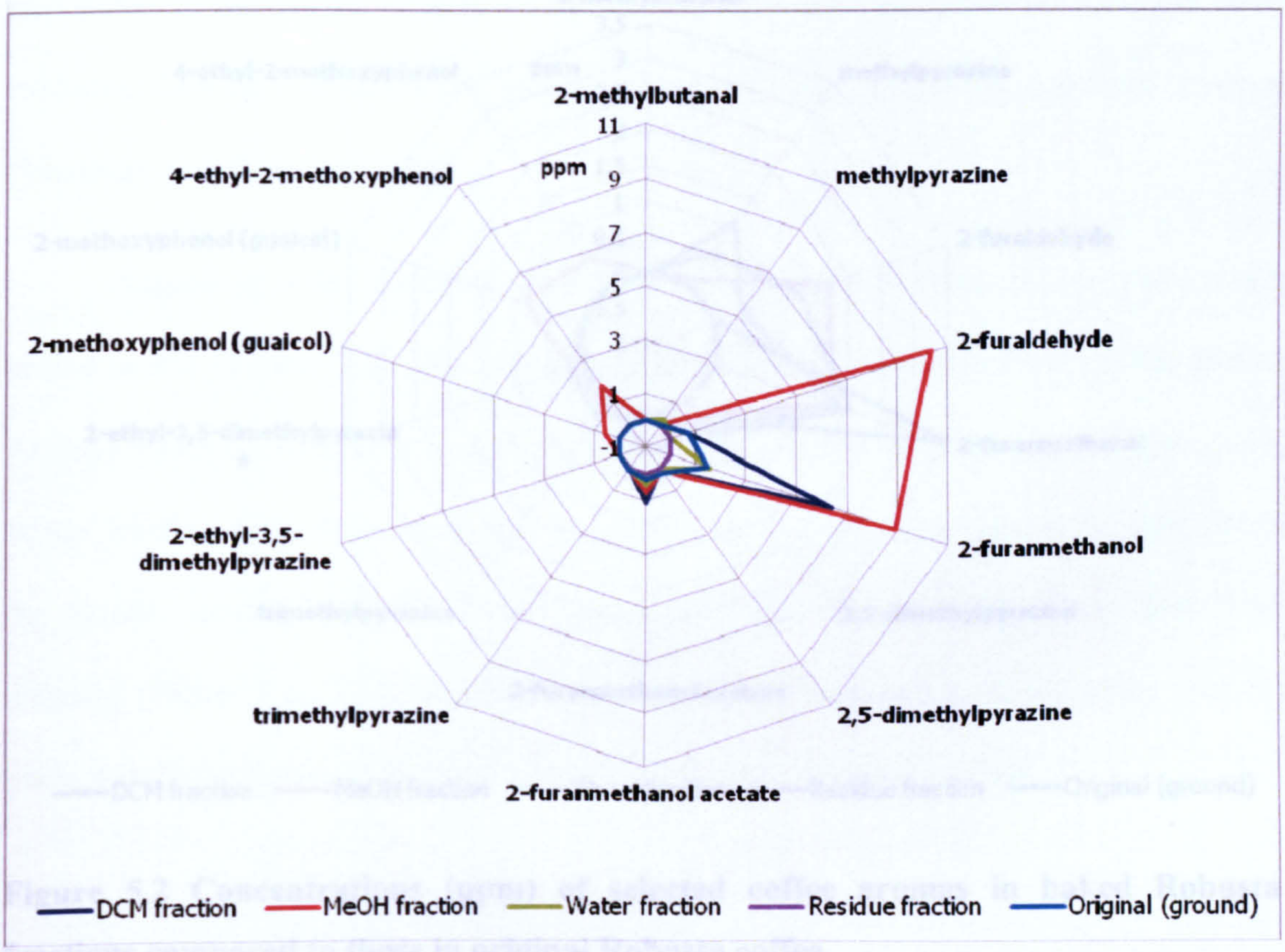


Figure 5.1 Concentrations (ppm) of selected coffee aromas in baked Arabica fractions compared to those in original Arabica coffee.

Comparing the aroma profile of Arabica to that of Robusta (Figure 5.1, 5.2), it was found that levels of furans formed from the Arabica MeOH fraction were higher than those yielded by the MeOH fraction of Robusta. The differences found were likely to be caused by the fact that Arabica, naturally, is richer in sugar content than Robusta (Murkovic and Derler 2006) and could have led to higher concentration of sugars in its MeOH extract, hence its MeOH fraction. This, therefore, could have been the reason causing higher levels of furans formed from Arabica MeOH fraction when compared to the levels yielded by the Robusta MeOH fraction.

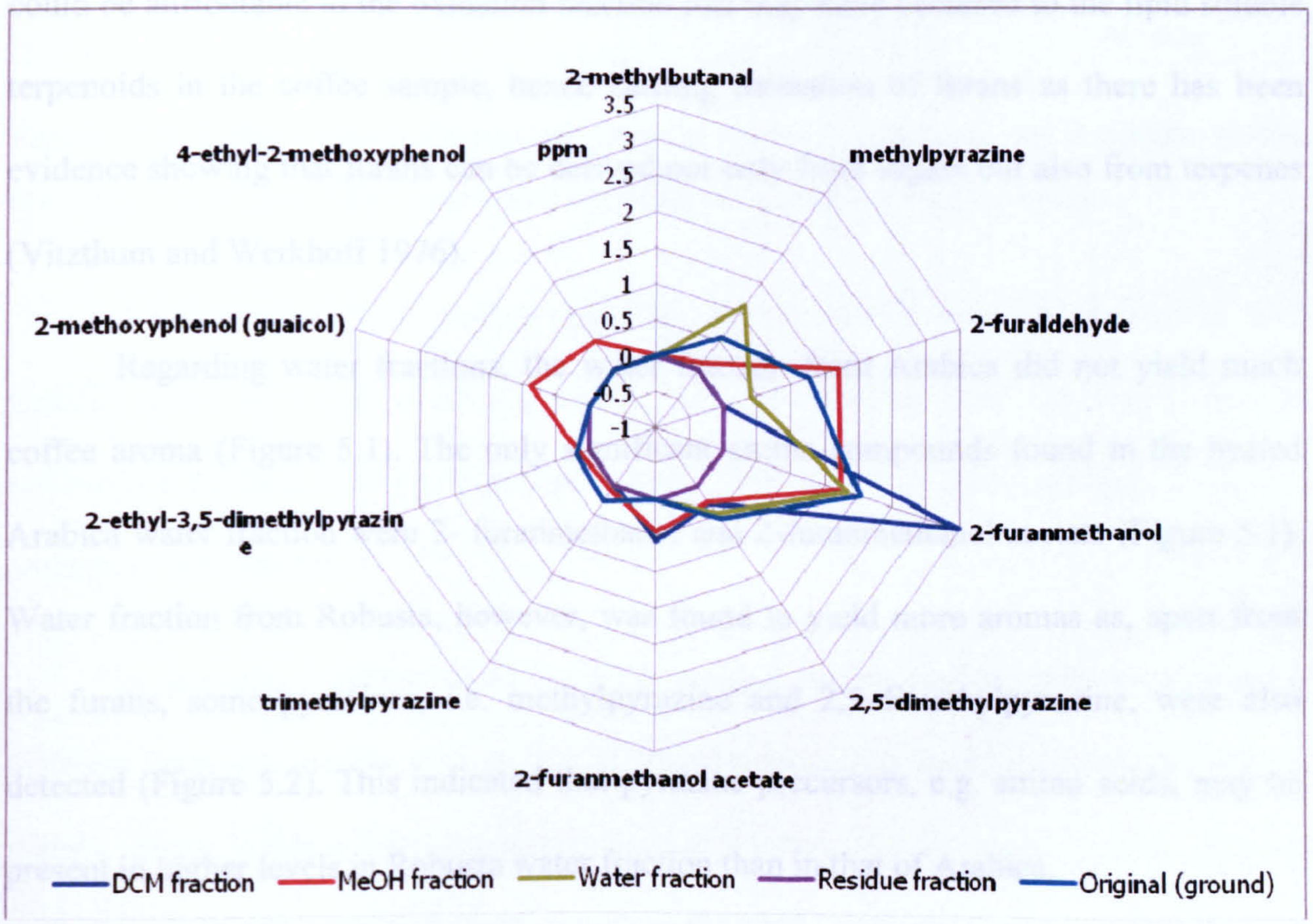


Figure 5.2 Concentrations (ppm) of selected coffee aromas in baked Robusta fractions compared to those in original Robusta coffee.

With regard to DCM fraction, DCM has been extensively used for defatting applications in cereal products, i.e. wheat flour (Grassberger, Schieberle et al. 2003). Therefore, it was expected that DCM fractions of the green coffee would contain primarily of lipid. De Maria et al. (1996a) reported that lipids contribute very little aroma formation in roasted coffee. Thus, it was anticipated that the DCM fractions would yield no significant level of any coffee aromas when heated. The results in this study, however, showed that the DCM fractions both from Arabica and Robusta yielded high levels of furans, especially 2-furanmethanol (see Figure 5.1, 5.2). An explanation for the result could be attributable to the oxidation reaction that may have occurred to the lipid soluble terpenoids in the coffee sample, hence causing formation of furans as there has been evidence showing that furans can be derived not only from sugars but also from terpenes (Vitzthum and Werkhoff 1976).

Regarding water fractions, the water fraction from Arabica did not yield much coffee aroma (Figure 5.1). The only significant aroma compounds found in the heated Arabica water fraction were 2- furanmethanol and 2-furanmethanol acetate (Figure 5.1). Water fraction from Robusta, however, was found to yield more aromas as, apart from the furans, some pyrazines, i.e. methylpyrazine and 2,5-dimethylpyrazine, were also detected (Figure 5.2). This indicated that pyrazine precursors, e.g. amino acids, may be present in higher levels in Robusta water fraction than in that of Arabica.

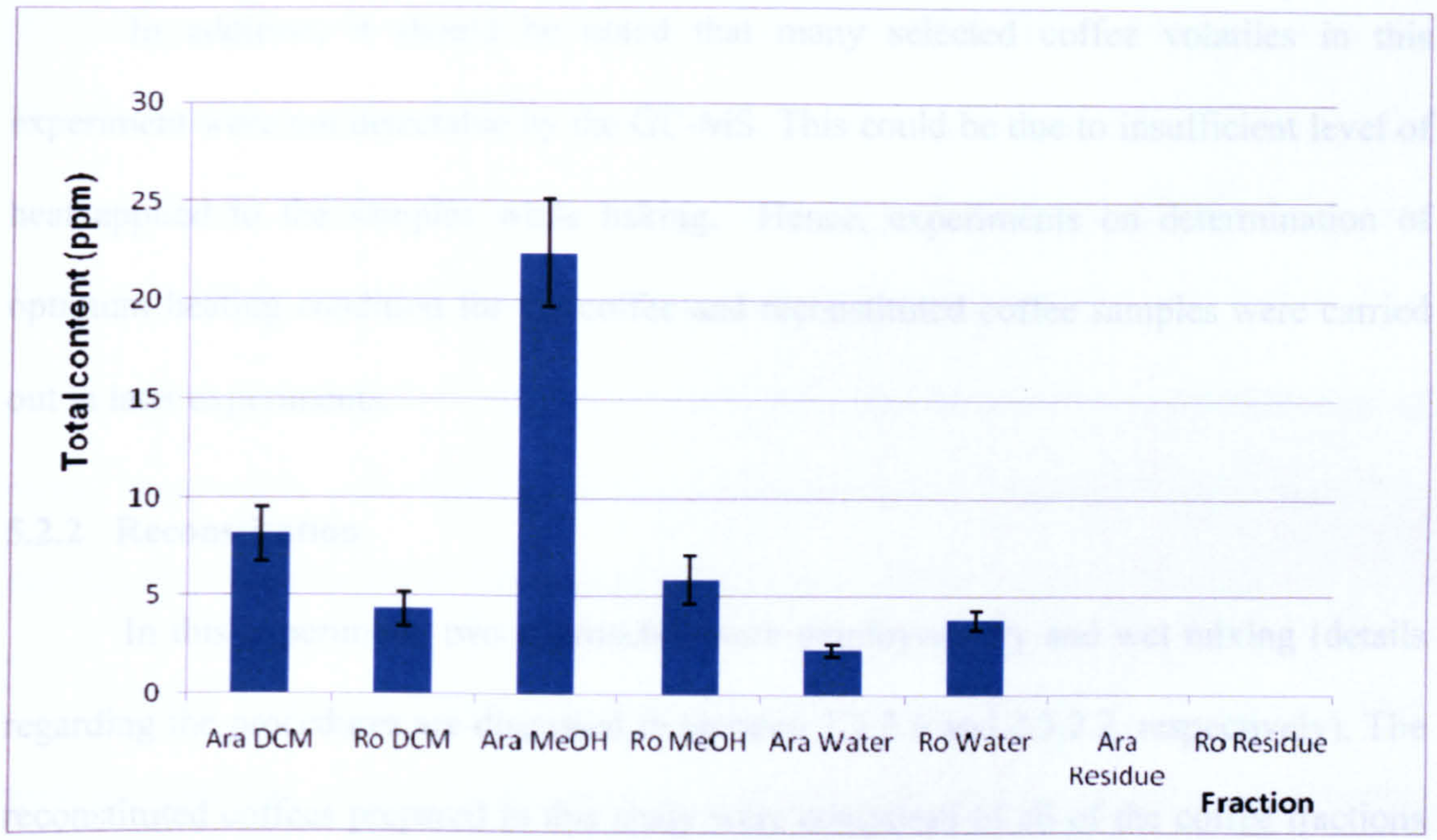


Figure 5.3 Total content (ppm) of selected coffee aromas in each baked coffee fraction. "Ara" and "Ro" represent Arabica and Robusta, respectively.

Residues from both coffees (that had been extracted with DCM, MeOH, and water) yielded no aroma at all (Figure 5.1, 5.2). The finding, thus, confirmed that the residue is composed mostly of cell-wall material fiber with only trace amounts of volatile precursors still present.

In general, fractions from Arabica yielded aroma profiles that were largely different from one another with its MeOH fraction producing the highest level of aroma, mainly furans. Robusta fractions, except residue fraction, yielded aroma profiles varying in the types of compounds formed, however the total contents of the volatiles formed were not found to be that much different among all fractions (see Figure 5.3).

In addition, it should be noted that many selected coffee volatiles in this experiment were not detectable by the GC-MS. This could be due to insufficient level of heat applied to the samples while baking. Hence, experiments on determination of optimum heating condition for the coffee and reconstituted coffee samples were carried out in later experiments.

5.2.2 Reconstitution

In this experiment, two approaches were employed; dry and wet mixing (details regarding the procedures are discussed in sections 2.3.2.1 and 2.3.2.2, respectively). The reconstituted coffees prepared in this study were composed of all of the coffee fractions obtained from the previous fractionation experiment (see Table 2.6 for composition of the reconstituted samples). The amounts of each fraction used to prepare the reconstituted samples were calculated based on the amount (dry weight percentage) of each fraction found in their corresponding original coffee determined in the previous experiment (see Table 2.6). Relative concentrations of the selected coffee aromas in the reconstituted samples prepared from different approaches (expressed as % aroma concentrations relative to the concentrations of the same aromas in the corresponding original coffees) were determined (see Tables 5.1 and 5.2).

5.2.2.1 Dry Mixing

With this approach, all fractions (weighed according to Table 2.6) were dry mixed in a laboratory grinder for 1 min. The mixtures (2 g each) were, then, baked at 210 °C for 12.5 min, extracted, and subjected to volatile analysis by GC-MS (see section 2.3.2.1 for full detail regarding the procedure). According to Figure 5.4 and 5.5, it seemed that the

dry mixing approach was more suitable for making reconstituted Arabica than Robusta. The results (Figure. 5.4) showed that the reconstituted Arabica yielded an aroma profile that was similar, both quantitatively and qualitatively, to that of the original Arabica (see Table 5.1). While the aroma profile of the reconstituted Robusta, although followed the pattern of the original Robusta (Figure 5.5), contained much lower concentrations of some compounds, i.e. methylpyrazine, 2-furaldehyde, 2-furanmethanol, and 2,5-dimethylpyazine, than the levels present in the original Robusta (see Table 5.1).

These results suggested that, although the original state of the coffee structures were not obtained, typical development of coffee volatiles could still occur when coffee flavour precursors were present regardless of the form of the coffee (whole beans vs. ground).

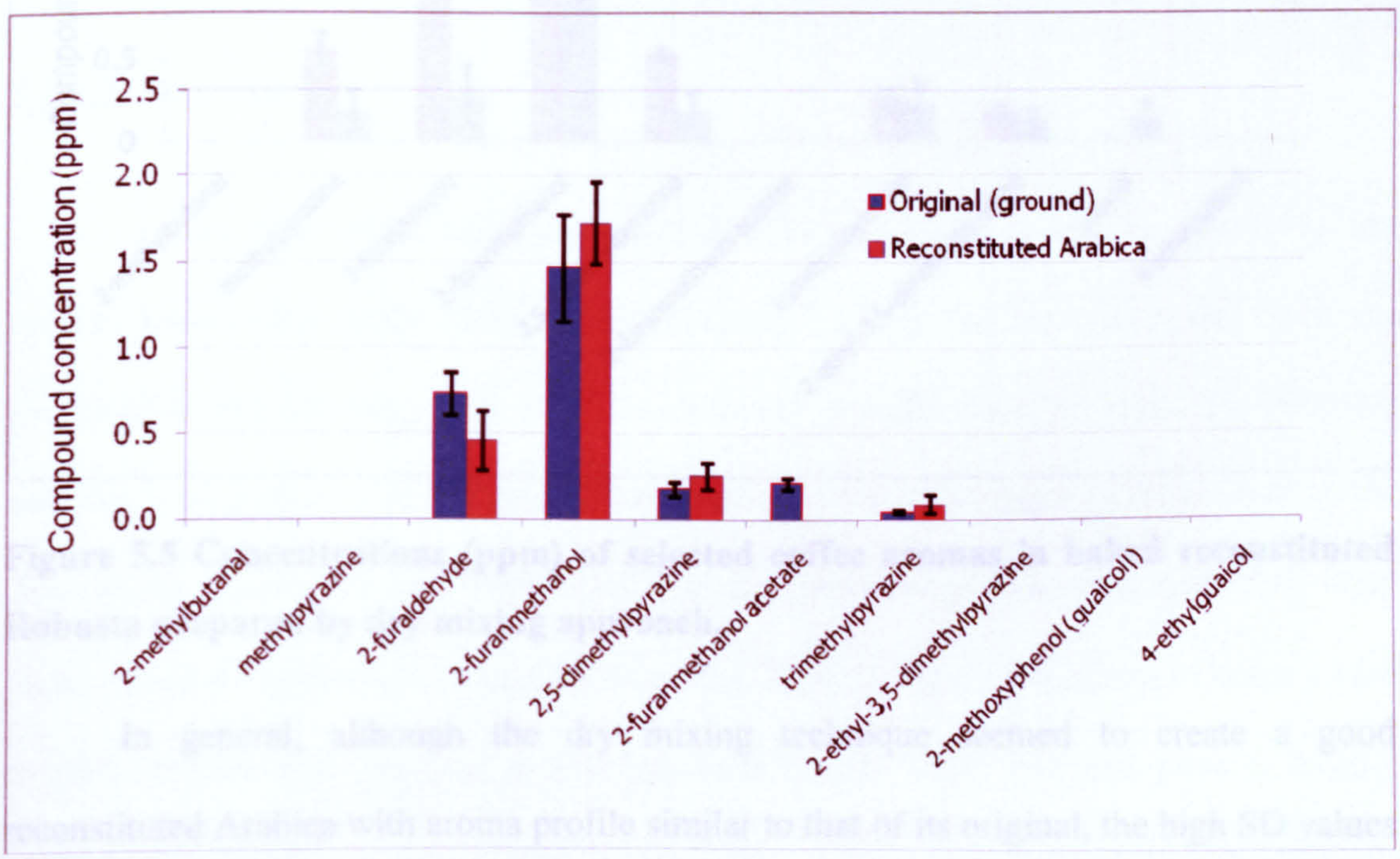


Figure 5.4 Concentrations (ppm) of selected coffee aromas in baked reconstituted Arabica prepared by dry mixing approach.

Considering the reproducibility of the method, standard deviation values (SD) of both reconstituted Arabica and Robusta (illustrated as error bars in Figure. 5.4 and 5.5) were relatively high, when compared to those of the original coffee. This suggested the lack of homogeneity of the reconstituted samples. For example, the cause for the low levels of some compounds in the reconstituted Robusta could be due to the deficiency of a lipid layer, believed to help protect volatile loss in food, in some part of the sample and therefore causing more loss of some volatiles when exposed to heat treatment.

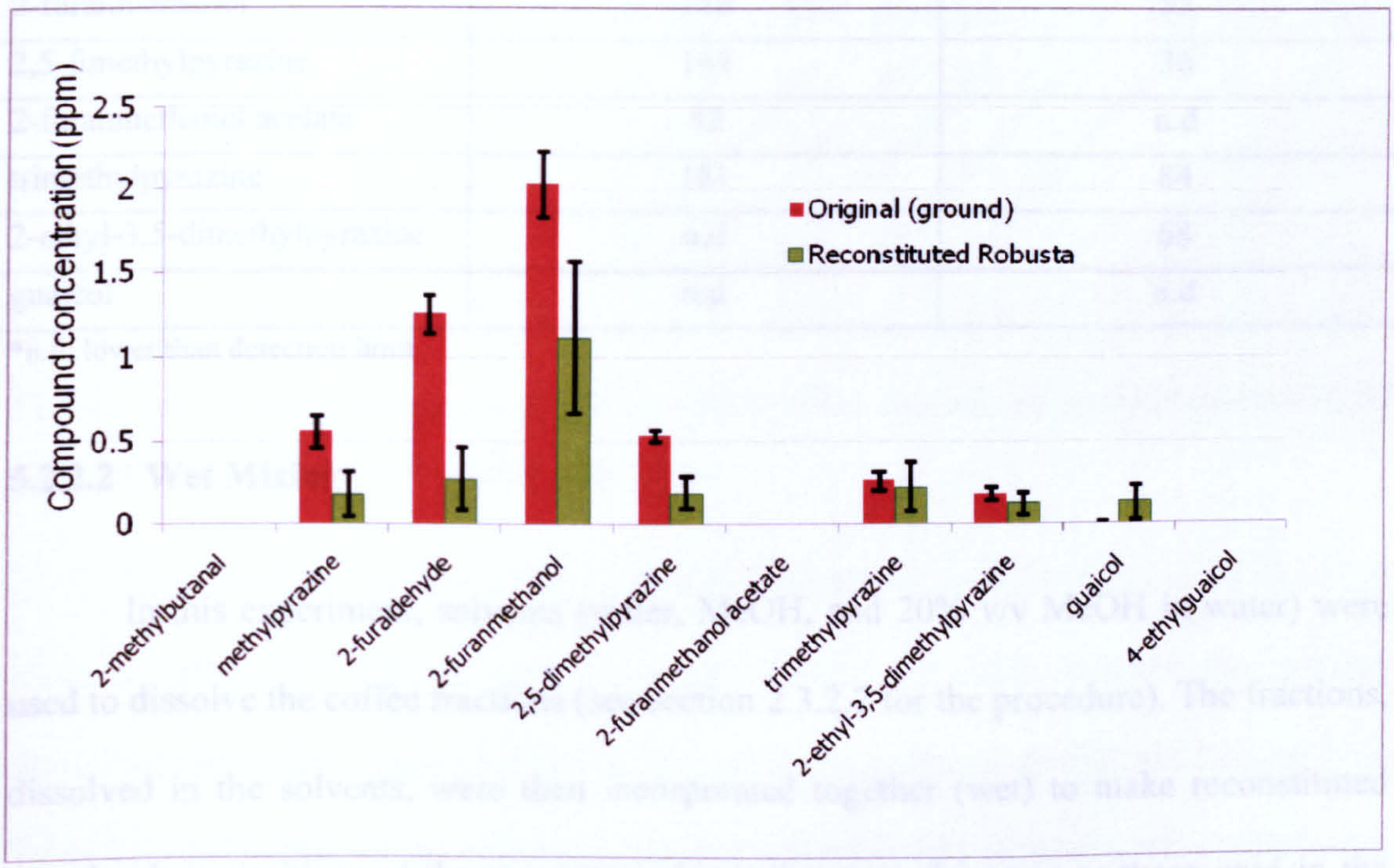


Figure 5.5 Concentrations (ppm) of selected coffee aromas in baked reconstituted Robusta prepared by dry mixing approach.

In general, although the dry mixing technique seemed to create a good reconstituted Arabica with aroma profile similar to that of its original, the high SD values suggested the lack of reproducibility of the method. Therefore, a more suitable approach

to create reconstituted samples was needed. Further attempts at reconstitution were, then, carried out involving the use of solvents to dissolve coffee fractions prior to mixing.

Table 5.1 Relative concentrations (%) of selected coffee aromas in reconstituted coffees prepared by dry mixing approach calculated relative to the contents of the same aromas in the corresponding original Arabica or Robusta.

Compound	Reconstituted Arabica	Reconstituted Robusta
2-methylbutanal	n.d	n.d
methylpyrazine	n.d	33
2-furaldehyde	63	22
2-furanmethanol	118	55
2,5-fimethylpyrazine	144	36
2-furanmethanol acetate	82	n.d
trimethylpyrazine	181	84
2-ethyl-3,5-dimethylpyrazine	n.d	68
guaicol	n.d	n.d

*n.d: lower than detection limit.

5.2.2.2 Wet Mixing

In this experiment, solvents (water, MeOH, and 20% v/v MeOH in water) were used to dissolve the coffee fractions (see section 2.3.2.2 for the procedure). The fractions, dissolved in the solvents, were then incorporated together (wet) to make reconstituted samples (compositions of the reconstituted samples were the same as those used in the dry mixing experiment; see Table 2.6). The wet reconstituted samples were then dried under vacuum condition, moisture adjusted (see Table 2.7 for the amounts of water added to each sample), and subjected to heat treatment at 210 °C for 12.5 min prior to volatile analysis by GC-MS.

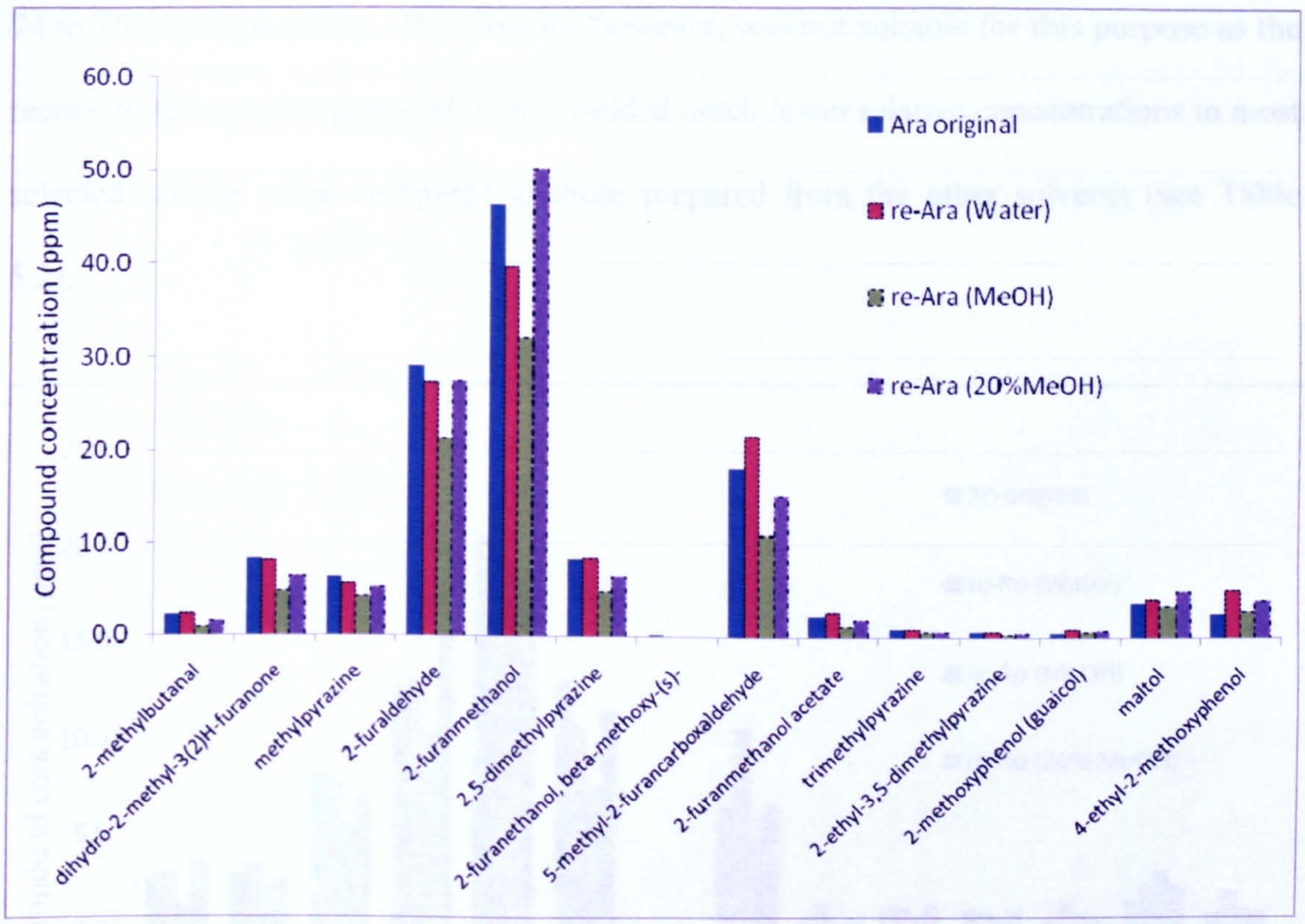


Figure 5.6 Concentrations (ppm) of selected coffee aromas in baked reconstituted Arabica prepared by wet mixing approach.

In general, it was found that mixing wet fractions that were dissolved with either water or 20% v/v MeOH in water produced reconstituted coffee samples that yielded aroma profiles highly resemble those of their original coffees (see Figures 5.6 and 5.7). Table 5.2 shows that the reconstituted Arabica samples made by wet mixing with water and 20% MeOH yielded relative concentrations of the aromas (% relative to the concentrations of the same aromas in the original Arabica) ranging from 86 to 205%, and 66 to 163%, respectively. Whereas, the reconstituted Robusta samples prepared using water and 20% MeOH yielded relative concentrations of the aromas (% relative to the concentrations of the same aromas in the original Robusta) ranging from 84 to 252%, and

74 to 152%, respectively. Pure MeOH, however, was not suitable for this purpose as the reconstituted samples prepared with it yielded much lower relative concentrations in most selected aromas when compared to those prepared from the other solvents (see Table 5.2).

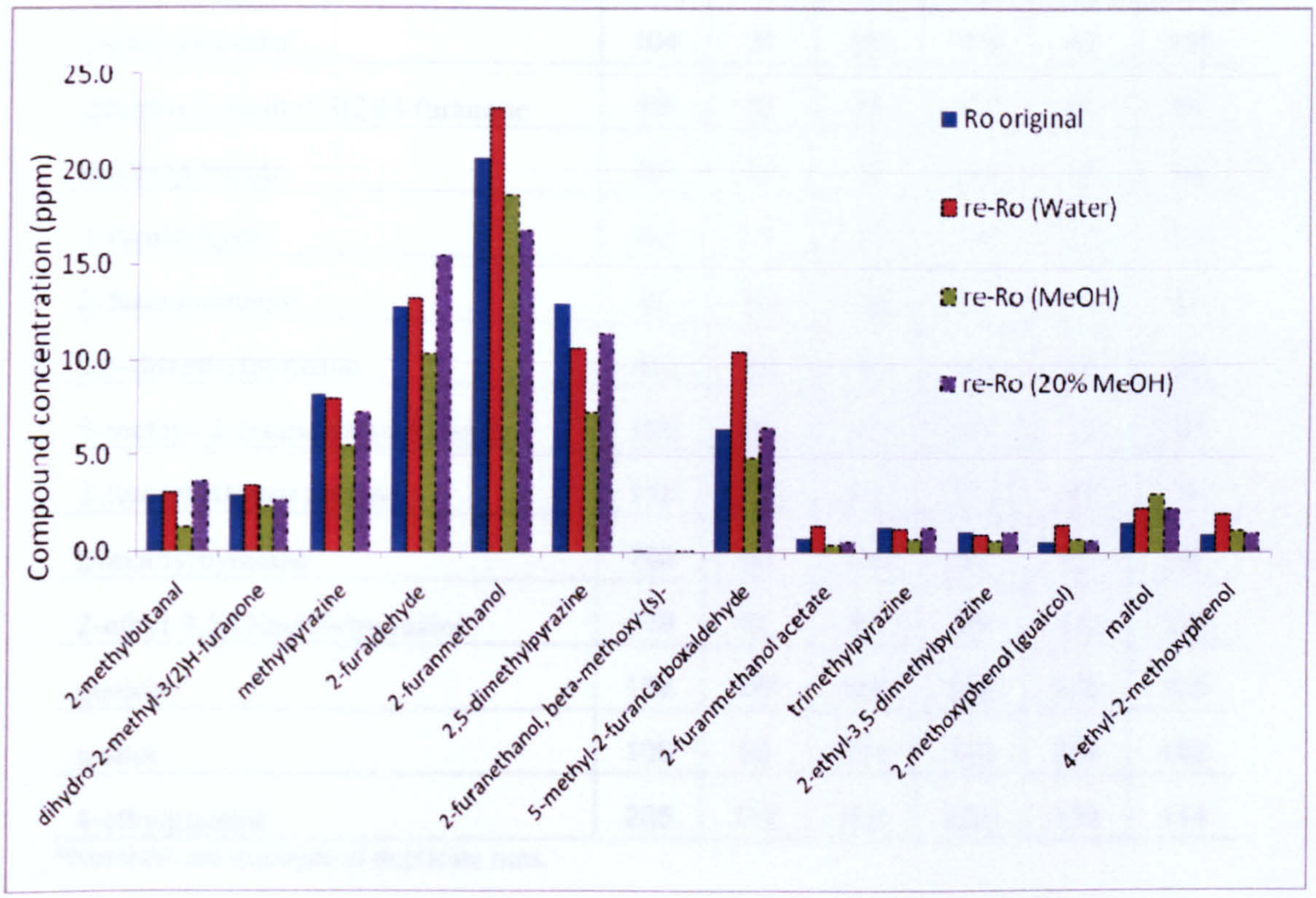


Figure 5.7 Concentrations (ppm) of selected coffee aromas in baked reconstituted Robusta prepared by wet mixing approach.

It should be noted that the high relative concentrations of phenols in this experiment, i.e. guaicol and 4-ethyl guaicol, could be due to their very low concentrations present in the samples that could have caused detection errors.

Table 5.2 Relative concentrations (%) of selected coffee aromas in reconstituted coffees prepared by wet mixing approach calculated relative to the contents of the same aromas in the corresponding original Arabica or Robusta

Compound	Reconstituted Arabica*			Reconstituted Robusta*		
	Water	MeOH	20% MeOH	Water	MeOH	20% MeOH
2-methylbutanal	104	37	66	105	42	125
dihydro-2-methyl-3(2)H-furanone	99	57	78	118	82	91
methylpyrazine	89	65	83	98	67	89
2-furaldehyde	94	73	94	106	81	121
2-furanmethanol	86	69	108	111	91	82
2,5-dimethylpyrazine	103	58	79	84	56	88
5-methyl-2-furancarboxaldehyde	119	60	84	162	77	102
2-furanmethanol acetate	118	50	80	179	57	74
trimethylpyrazine	106	60	78	84	57	99
2-ethyl-3,5-Dimethylpyrazine	119	61	81	86	59	104
guaicol	182	127	162	252	125	108
maltol	109	93	133	143	204	152
4-ethylguaicol	205	112	163	221	130	114

*Numbers are averages of duplicate runs.

In conclusion, the reconstitution procedure by wet mixing approach carried out in this experiment provided reconstituted coffees with aroma profiles that were highly comparable to their corresponding original coffees for both Arabica and Robusta. The reconstituted coffees obtained (when baked) yielded aroma profiles that were qualitatively (see the chromatograms in Figure 5.8 and 5.9) and quantitatively (determined by relative concentrations of the coffee aromas in the reconstituted samples calculated relative to the concentrations of the same aromas in the original coffees)

similar to those of their corresponding roasted originals. As a result, the wet mixing procedure was considered appropriate and was chosen to be used for coffee reconstitution in future experiments. Pure water was chosen as it is non-toxic, highly available, as well as more economical as opposed to 20% v/v MeOH in water.

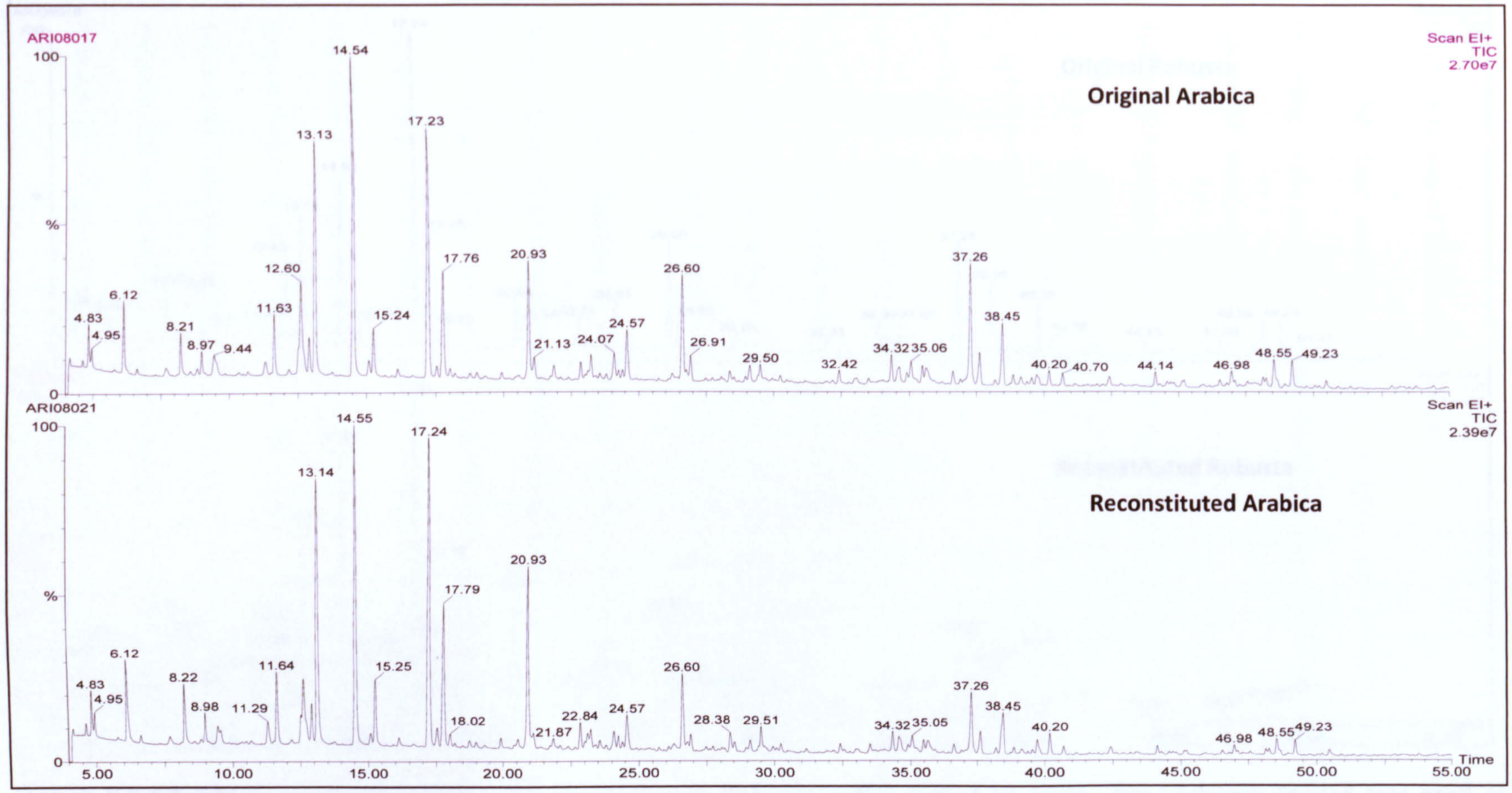


Figure 5.8 Chromatograms of original and reconstituted Arabica coffee baked at 210 °C for 12.5 min (water was used as solvent for the mixing process).

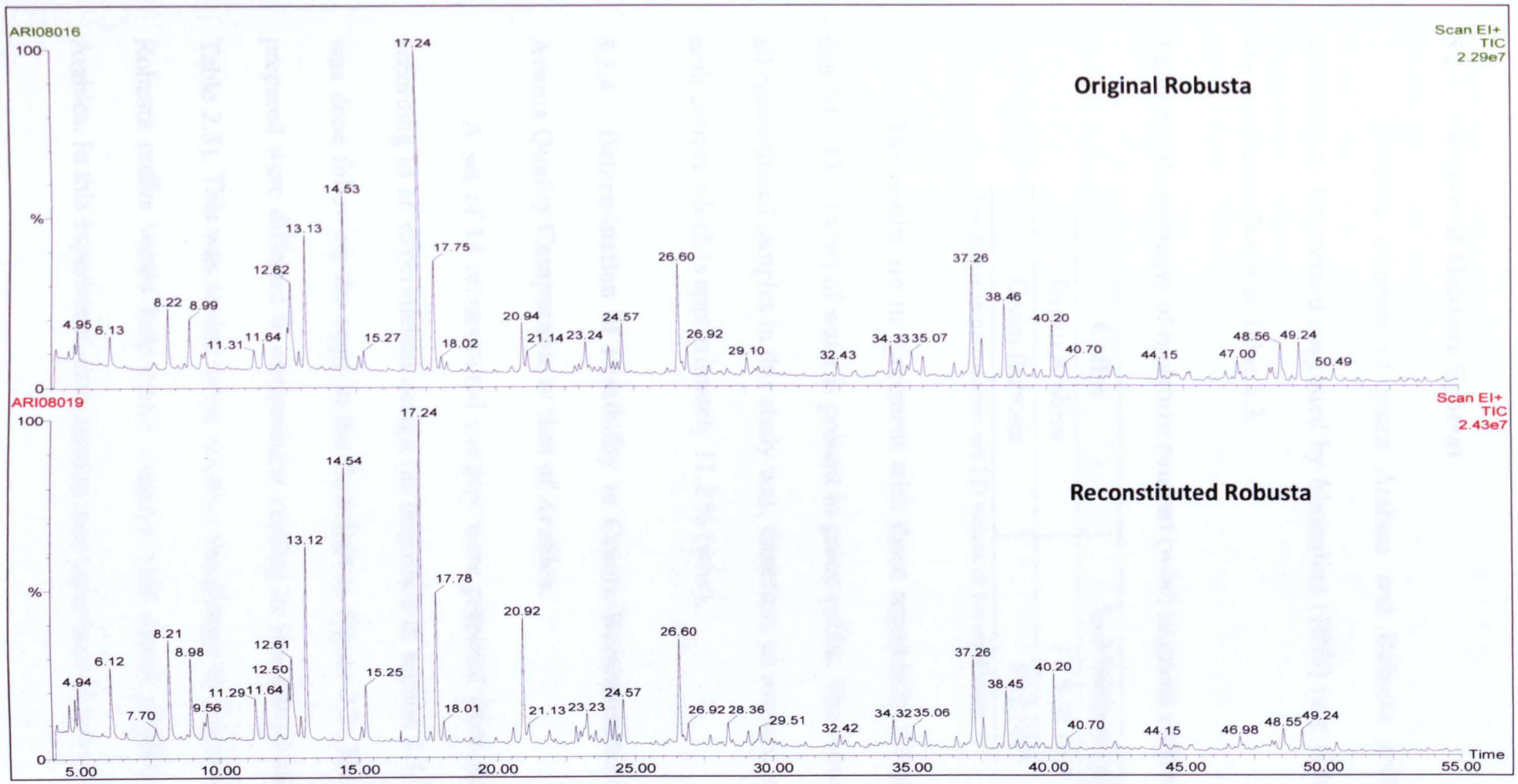


Figure 5.9 Chromatograms of original and reconstituted Robusta coffee baked at 210°C for 12.5 min (water was used as solvent for the mixing process).

5.2.3 Analysis of Moisture Content

Moisture contents of green Arabica and Robusta coffees were determined according to the method suggested by Mazzafera (1999) (see section 2.3.3 for method). The results are shown in Table 5.3.

Table 5.3 Percentages of moisture content (w/w) in green coffees.

Coffee	% Moisture (w/w)
Green Arabica	11.5 (0.14)*
Green Robusta	10.8 (0)*

*Numbers in parentheses are SD values of two replicates.

The results are in agreement with those reported by Clarke and Macrae (1985) that 10 – 13% (w/w) of water is present in green coffee. The moisture content applied to all reconstituted samples in this study was, therefore, an average of the contents found in both coffees which is approximately 11.2 % (w/w).

5.2.4 Determination of Feasibility to Create Reconstituted Robusta Coffee with Aroma Quality Comparable to that of Arabica.

A set of 14 reconstituted samples were prepared from Robusta coffee fractions according to an experimental design (as described in section 2.3.4). Sample preparation was done following the steps in the flowchart in Figure 2.2. The reconstituted samples prepared were different in composition varying in amounts of each coffee fraction (see Table 2.8). This was to determine whether the change in proportions of the fractions in Robusta coffee would help create samples with aroma profiles similar to that of the Arabica. In this experiment, each sample was composed of two or more fractions with the

percentage of residue (cell wall material) set constant at 30% (d.b.) for all samples. The percentages of DCM, MeOH, and water fractions in the samples, however, were designed to vary from 0 to 70% (d.b.). Aroma compound analysis by GC-MS was done on 22 compounds which were 3-methylbutanal, 2-methylbutanal, 2,3-pentanedione, dihydro-2-methyl-3(2)H-furanone, pyridine, methylpyrazine, 2-furaldehyde, 2-furanmethanol, 1-(acetyloxy)-2-propanone, beta-methoxy-(s)-2-furanmethanol, 2,5-dimethylpyrazine, 5-methyl-2-furaldehyde, 2-furanmethanol acetate, 2-ethyl-5(6)-methylpyrazine, trimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, furaneol, 2-methoxyphenol (guaicol), 2-methylisoborneol, 4-ethyl-2-methoxyphenol (4-ethylguaicol), 2-methoxy-4-vinylphenol, vanillin. However, only compounds found to show distinct trends were illustrated in Figures 5.10 and 5.11.

Principal Component Analysis (PCA) was applied to the concentrations (ppm) of all 22 compounds. Calculation of the sum of normalized standard deviation (SNSD) of odour activity values was done on 16 compounds, omitting dihydro-2-methyl-3(2)H-furanone, 1-(acetyloxy)-2-propanone, beta-methoxy-(s)-2-furanmethanol, 5-methyl-2-furaldehyde, 2-furanmethanol acetate, and furaneol due to the unavailability of their threshold values (see Table 5.8 for aroma thresholds of the compounds of interest used for SNSD calculation).

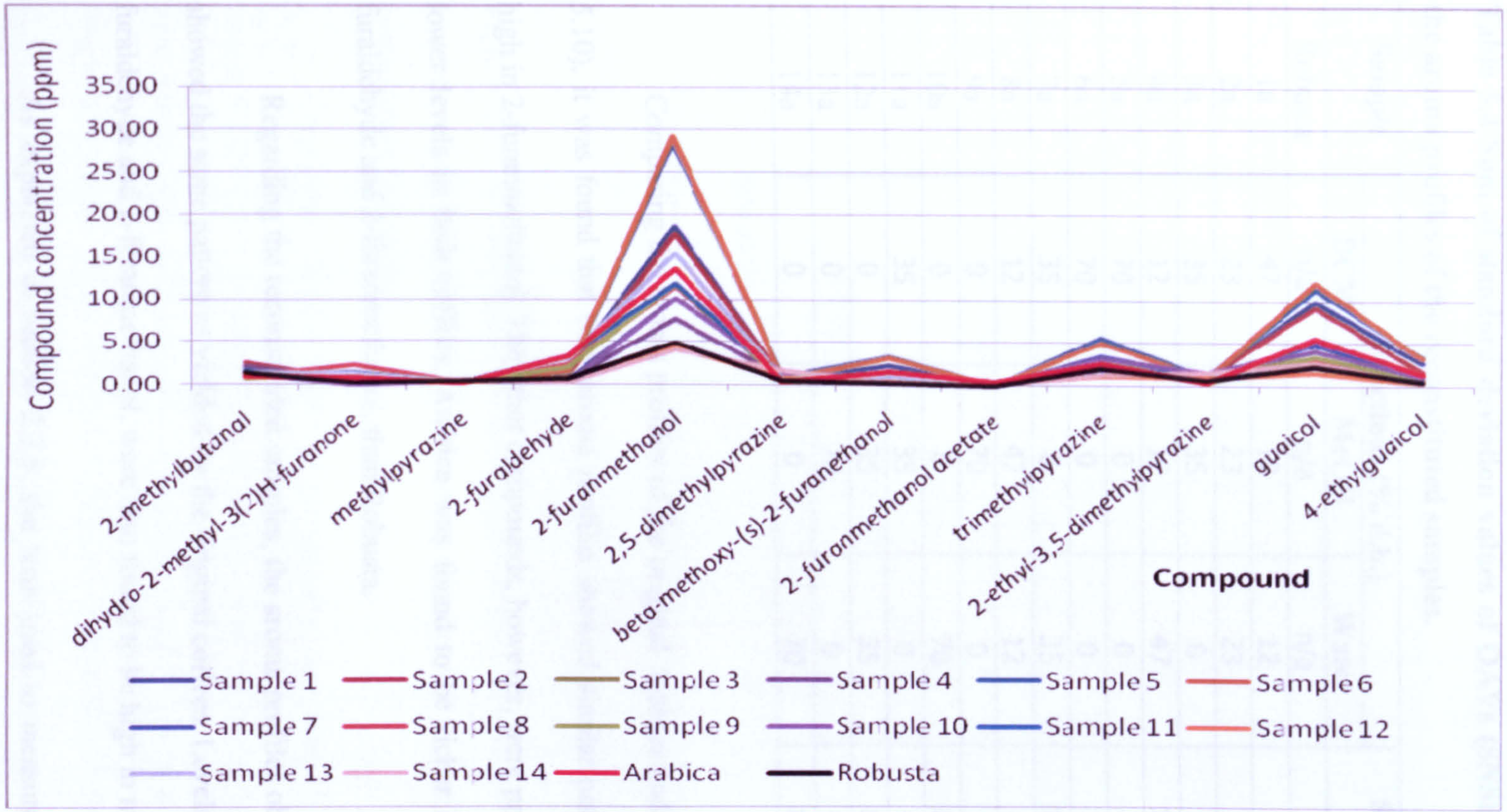


Figure 5.10 Concentrations (ppm) of selected coffee aromas in baked reconstituted samples.

Table 5.4 Sum of standard deviation values of OAVs (SNSD) calculated based on the aroma profiles of the reconstituted samples.

Sample	Fractions (% d.b.)			SNSD	Ranking
	DCM	MeOH	Water		
Robusta	n/a	n/a	n/a	8.05	7
1a	47	12	12	6.60	4
2a	23	23	23	8.20	8
3a	35	35	0	5.88	**1**
4a	12	12	47	10.47	13
5a	70	0	0	8.93	10
6a	70	0	0	6.87	6
7a	35	0	35	10.51	11
8a	12	47	12	8.36	9
9a	0	70	0	7.03	**5**
10a	0	0	70	11.24	14
11a	35	35	0	6.84	**3**
12a	0	35	35	10.04	12
13a	0	70	0	6.60	**2**
14a	0	0	70	13.09	15

Comparing the aroma profiles of the original Arabica and Robusta coffee (Figure 5.10), it was found that both aroma profiles showed similar patterns in that both were high in 2-furanmethanol. The other compounds, however, were present in relatively much lower levels in both coffees. Arabica was found to be richer in furans, especially 2-furaldehyde and 2-furanmethanol, than Robusta.

Regarding the reconstituted samples, the aroma profiles obtained after baking also showed the same pattern as yielded by the original coffees. Levels of furans, especially 2-furaldehyde and 2-furanmethanol, were also found to be high in most samples.

As explained in section 2.3.5, the term used to measure similarity between an aroma profile obtained from a reconstituted sample and that of the original Arabica is called the sum of normalized standard deviation (SNSD) of the compounds' odour

activity values (OAVs). Since the term represents the variation between two aroma profiles, a lower SNSD value shows greater similarity between two aroma profiles.

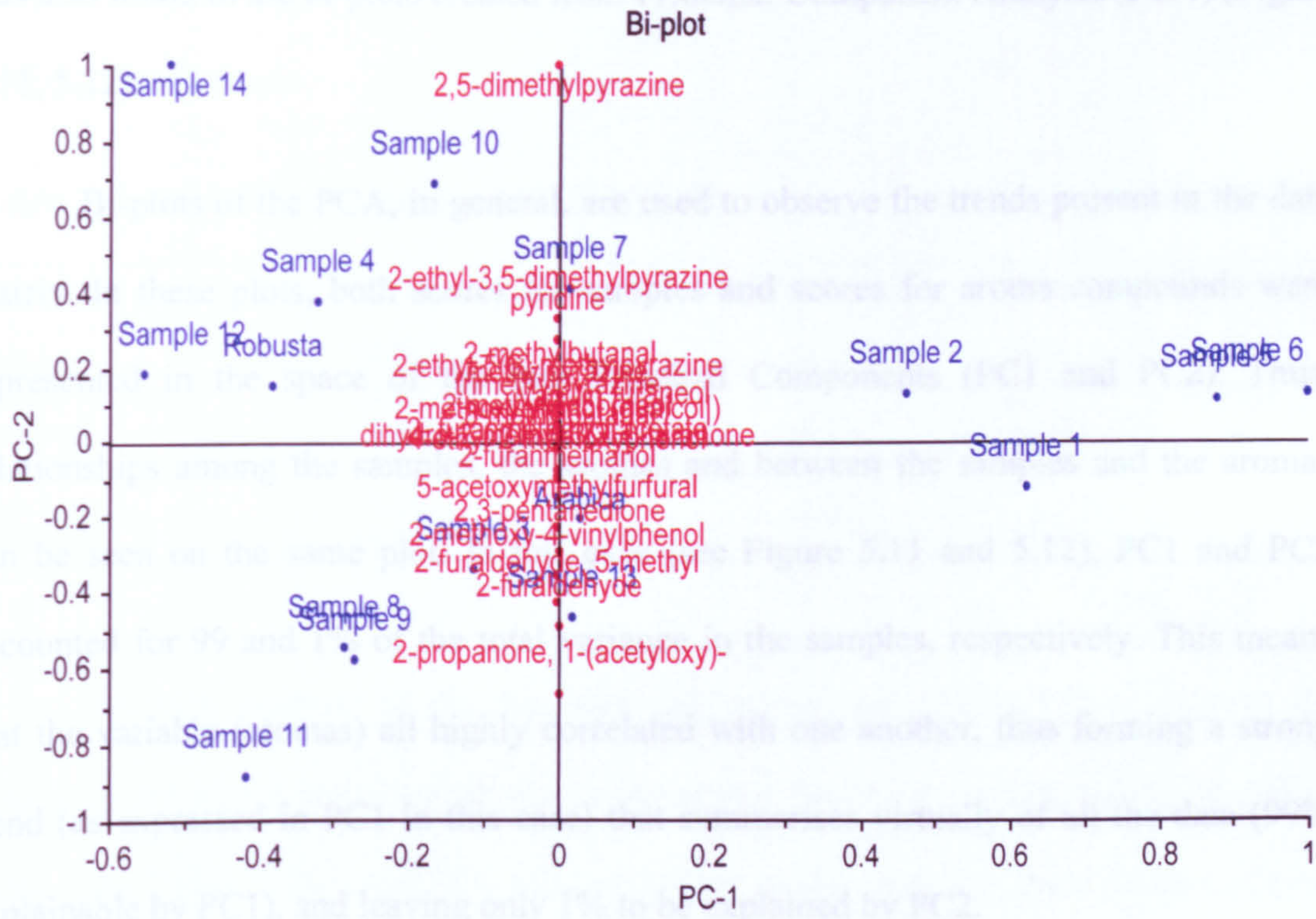


Figure 5.11 Bi-plot from Principal Component Analysis (PCA) applied to the concentrations (ppm) of 22 target aroma compounds in Arabica, Robusta, and the reconstituted samples.

Considering SNSD values of all samples (see Table 5.4), sample 3a gave the lowest SNSD value. Its replicate, sample 11a, also had a low SNSD value and was the third lowest value, indicating that this sample should yield an aroma profile with higher resemblance to that of the original Arabica, as compared to Robusta. Sample 13a and its replicate, sample 9a, were also found to have low SNSD values (rank 2 and 5), respectively. Given the relatively low SNSD values of these samples (3a, 9a, 11a, and 13

a), compared to that given by Robusta (at rank 7), these samples were, then, expected to possess aroma quality that was more similar to that of Arabica than Robusta. Agreement was also found in the bi-plots created from Principal Component Analysis (PCA) (Figure 5.11, 5.12).

Bi-plots of the PCA, in general, are used to observe the trends present in the data matrix. In these plots, both scores for samples and scores for aroma compounds were represented in the space of the two Principal Components (PC1 and PC2). Thus, relationships among the samples, the aromas and between the samples and the aromas can be seen on the same plot. In this case (see Figure 5.11 and 5.12), PC1 and PC2 accounted for 99 and 1% of the total variance in the samples, respectively. This means that the variable (aromas) all highly correlated with one another, thus forming a strong trend (as expressed in PC1 in this case) that summarises virtually of all the data (99% explainable by PC1), and leaving only 1% to be explained by PC2.

As seen in the plot (Figure 5.11), Arabica coffee was positively correlated to the levels of furans, i.e. 2-furaldehyde, 2-furanmethanol and 2-furanmethanol acetate, while inversely correlated to most of the pyrazines' contents, i.e. 2,5-dimethylpyrazine and 2-ethyl-3,5-dimethylpyrazine. Robusta, however, was inversely correlated to the contents of most furans and some ketones and phenol, while positively correlated to pyrazines and pyridine. Regarding the relationship among the samples, sample 3a, and 13a were found to be the closest to Arabica in the bi-plot space than the rest with sample 3a locating the nearest to Arabica which is in agreement with the result from SNSD values.

The results from both PCA and SNSD values, therefore, indicated that the aroma profiles of these samples have shifted closer towards that of Arabica than the rest of the samples and should, therefore, have aroma qualities that are more similar to that of Arabica than Robusta.

Table 5.5 Analysis of variance (ANOVA) table for the mixture linear model.

Source	Sum of Squares	df	Mean Square	F Value	p-value (Prob > F)
Model	51.96	2	25.98	28.41	<0.0001
Linear Mixture	51.96	2	25.98	28.41	<0.0001
Residual	10.06	11	0.91		
Lack of Fit	3.53	7	0.50	0.31	0.9163
Pure Error	6.53	4	1.63		
Cor Total	62.02	13			

ANOVA analysis of the data was also carried out (Table 5.5) to observe the effects of the coffee fractions' levels on the aroma similarity, as determined by SNSD value (see Table 5.6). ANOVA mixture linear model with the three factors (DCM, MeOH and water fractions) as predictor variables and SNSD value as a dependent variable (response) indicated that the variation of the SNSD values among the samples can significantly be explained by the variation in amounts of the coffee fractions (p-value < 0.0001 with 95% confidence level) (see Table 5.5). Considering the effects of the components (see Table 5.6), all components, DCM, MeOH and water fractions were found to significantly affect the SNSD value, p-values were 0.0019, 0.0070 and < 0.0001, respectively. The direction of the effects of DCM and MeOH components were found to be negative as opposed to that of water whose direction of the effect was found to be positive. This indicated that the higher the DCM and/or MeOH fraction in the sample, the lower the variation of the sample from Arabica (SNSD value). On the other hand, a

higher variation from Arabica (SNSD) is expected to be obtained when higher level of water fraction is present.

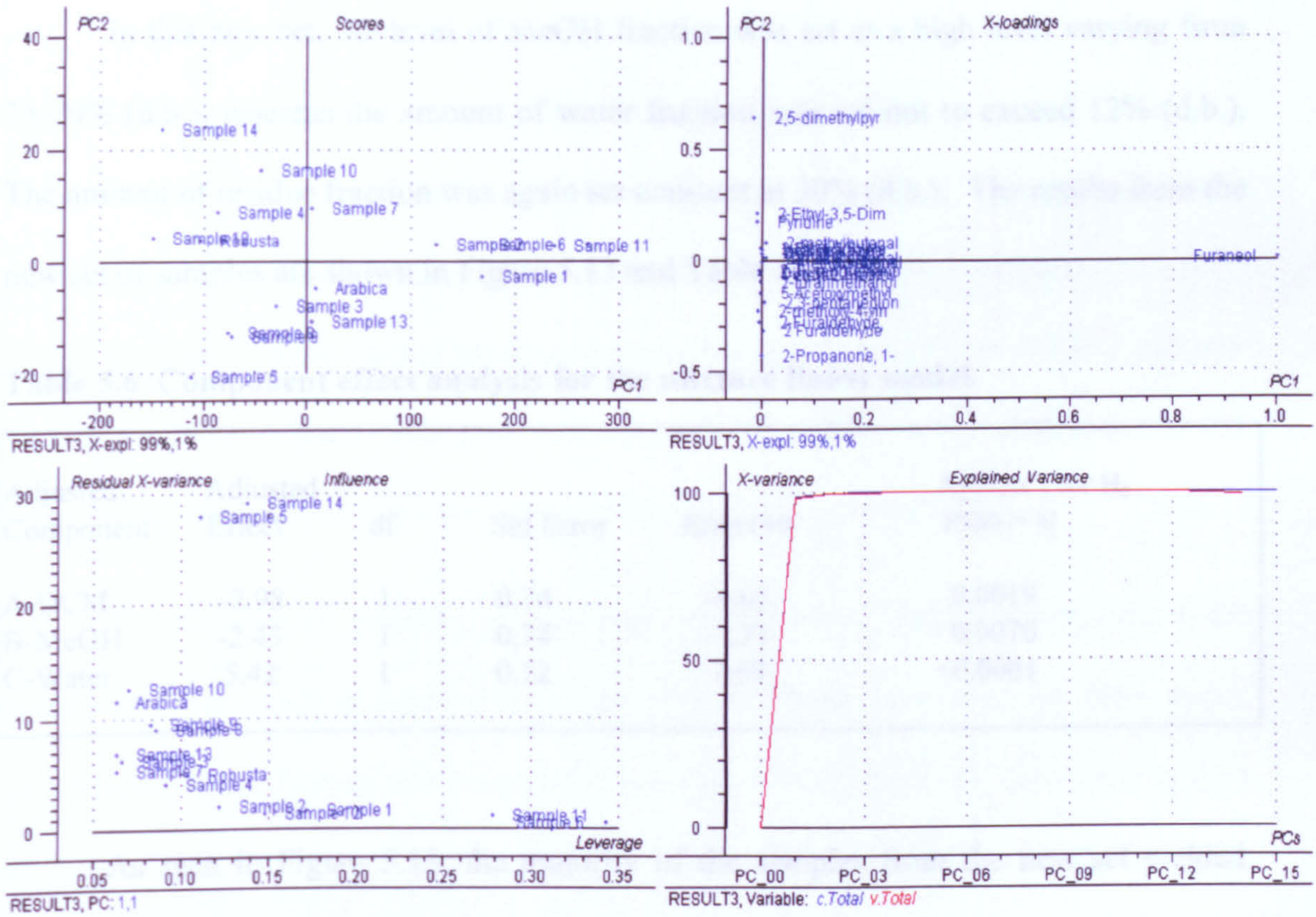


Figure 5.12 Principal Component Analysis (PCA) applied to the concentrations (ppm) of 22 target aroma compounds in Arabica, Robusta, and the reconstituted samples.

In order to verify the finding, another set of reconstituted samples was created with narrower limitations (see Table 2.10). As suggested by the component effect analysis from the mixture linear model (Table 5.6), the new set of samples was supposed to be made with high level of DCM, and MeOH fractions and low levels of water fraction in order to obtain low SNSD values. Despite this fact, the level of DCM fraction was limited to 35% maximum (d.b.) in this new sample set. This was to avoid any negative

effect that could occur in the samples due to lipid oxidation (as DCM fraction contains high level of lipid).

In this new set, the level of MeOH fraction was set at a high level varying from 35-70% (d.b.), whereas the amount of water fraction was set not to exceed 12% (d.b.). The amount of residue fraction was again set constant at 30% (d.b.). The results from the new set of samples are shown in Figure 5.13 and Table 5.7.

Table 5.6 Component effect analysis for the mixture linear model.

Adjusted Component	Adjusted Effect	df	Std Error	Effect=0	Approx t for H_0 Prob > |t|
A-DCM	-2.98	1	0.74	-4.05	0.0019
B-MeOH	-2.43	1	0.74	-3.31	0.0070
C-Water	5.42	1	0.72	7.53	<0.0001

As seen in Figure 5.13, the majority of the samples from the new set yielded aroma profiles with patterns more likely to follow that of Arabica than Robusta. Considering furans and pyrazines, the major classes of coffee volatiles (Van Straten, Maarse et al. 1983), most samples contained higher levels of furans and lower levels of most pyrazines when compared to the levels found in the original Robusta. Given that Arabica was reported to contain higher amounts of furans, and lower amounts of pyrazines than Robusta, especially 2,5-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, and 2,3-diethyl-5-methylpyrazine (Tressl, Bahri et al. 1978; Leino, Lapvetelaeinen et al. 1992; Grosch, Semmelroch et al. 1993), this finding, therefore, is a good indication that

the aroma intensities caused by furans and pyrazines of the reconstituted samples have shifted closer towards that of the Arabica.

Regarding the furanone, dihydro-2-methyl-3(2)H-furanone, the amount was approx. 2 times higher in Arabica than Robusta (Figure 5.13). Tressl et al. (1978) reported that, compared to Robusta, the concentrations of furanones were approx. 2 to 4 times higher in Arabica. Given this fact, the increase in levels of dihydro-2-methyl-3(2)H-furanone in most reconstituted samples in this second set (Figure 5.13) could also be another good indicator indicating that the aroma intensity of this compound in the reconstituted samples have become closer to that of Arabica than Robusta.

The contents of phenols, guaicol and 4-ethylguaicol, although supposed to be present at lower levels in Arabica (Dart and Nursten 1985), seemed to not differ much between the two coffees in this experiment (see Figure 5.12 and 5.13). This could be due to the fact that phenols are usually present at fairly low concentrations and, therefore, might have caused errors in quantification.

Regarding the SNSD values yielded by this second set of reconstituted samples (Table 5.7), samples 6b and 12b were found to give the lowest and the second lowest SNSD values. In fact, these two samples were also found to give the lowest and the second lowest SNSD values in the first set as well. Although their replicates, sample 15b and 4b, fell in rank 5 and 7, respectively, still they showed relatively much lower SNSD values when compared to the value yielded by Robusta. Robusta, in this second set, was found to yield the highest SNSD value compared to the rest. Agreement was also found in the bi-plots from PCA (Figure 5.14) in that Robusta seems to locate the furthest away

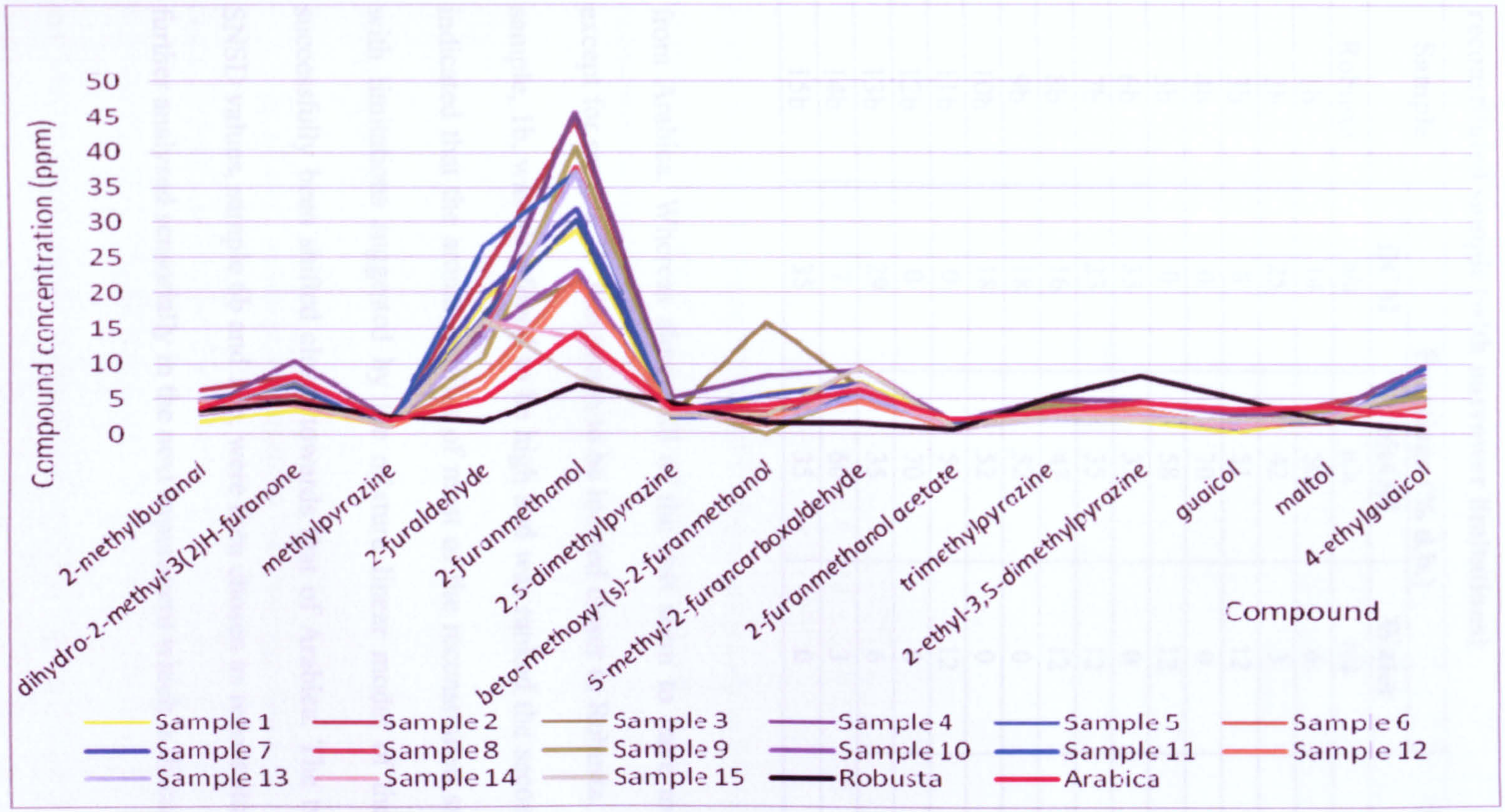


Figure 5.13 Concentration (ppm) of selected coffee aromas in baked reconstituted samples (new set with narrower limitations).

Table 5.7 Sum of standard deviation values (SNSD) of OAVs of the new set of reconstituted sample (with narrower limitations)

Sample	Fractions (% d.b.)			SNSD	Ranking
	DCM	MeOH	Water		
Robusta	n/a	n/a	n/a	10.41	16
1b	14	50	6	9.23	15
2b	25	42	3	8.06	11
3b	8	51	12	8.46	12
4b	0	70	0	6.18	7
5b	0	58	12	7.28	10
6b	35	35	0	3.70	**1**
7b	23	35	12	6.81	9
8b	16	43	12	8.55	13
9b	18	52	0	4.51	3
10b	18	52	0	6.79	8
11b	0	58	12	8.68	14
12b	0	70	0	4.01	**2**
13b	29	35	6	4.88	4
14b	7	60	3	6.00	6
15b	35	35	0	5.73	5

from Arabica. Whereas almost all of the rest seem to have moved closer to Arabica, except for sample 1b that seems to be located closer to Robusta. The SNSD value of this sample, 1b, was also found to be high and was ranked the second highest. These results indicated that the aroma profiles of most of the reconstituted samples in this set, made with limitations suggested by the mixture linear model of the first sample set, have successfully been shifted closer towards that of Arabica. The two samples with lowest SNSD values, sample 6b and 12b, were then chosen as reconstituted coffee samples to be further analysed sensorially in the next experiment which is discussed in Chapter 6.

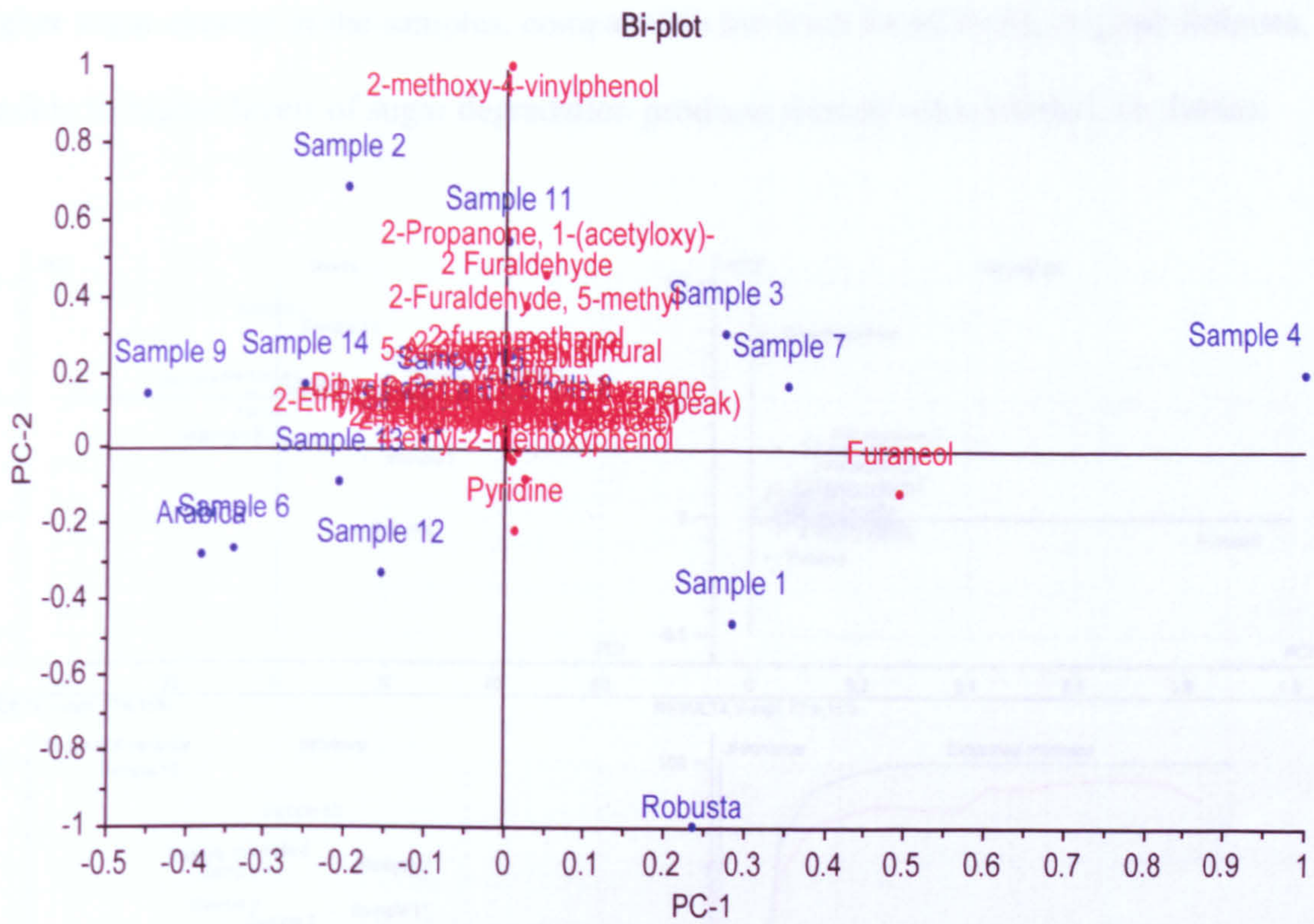


Figure 5.14 Bi-plot from Principal Component Analysis (PCA) applied to the concentrations (ppm) of 22 target aroma compounds in Arabica, Robusta, and the reconstituted samples (new set).

According to Table 2.10, these two samples were composed of 30% (d.b.) of residue fraction with either 70% (d.b.) of MeOH fraction, or half DCM and half MeOH fractions (35% d.b. each).

In general, possible explanations for the suggestion by the mixture linear model could be owing to the fact that Arabica, by nature, contains higher amount of sugars than Robusta (Smith 1985; Murkovic and Derler 2006). The low SNSD values obtained from the MeOH-fraction-rich reconstituted samples, hence, could be due to the relatively

higher sugar content in the samples, compared to the level found in the original Robusta, leading to higher levels of sugar degradation products formed when roasted, i.e. furans.

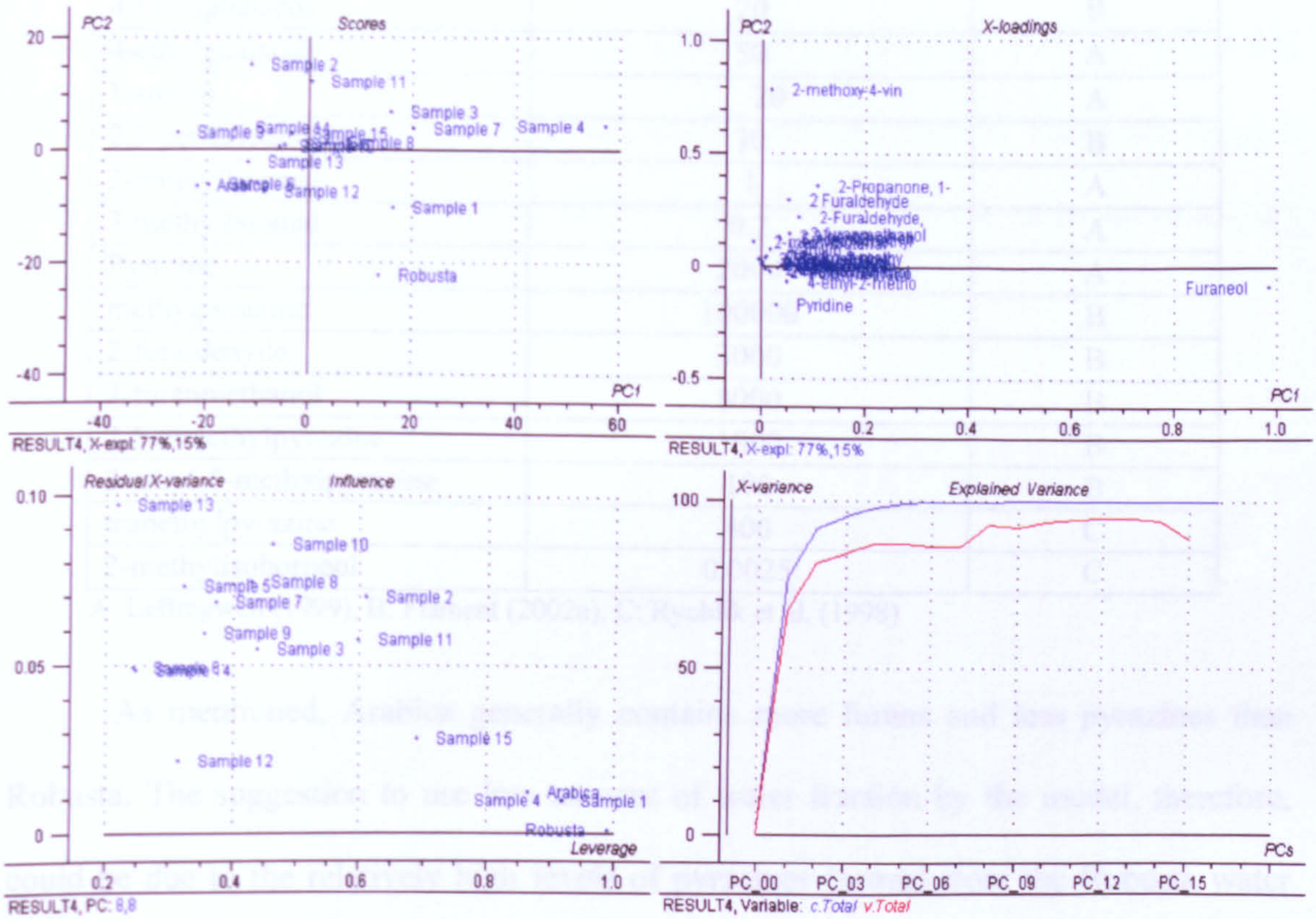


Figure 5.15 Principal Component Analysis (PCA) applied to the concentrations (ppm) of 22 target aroma compounds in Arabica, Robusta, and the reconstituted samples (new set).

Although DCM fractions are expected to contain lipid and lipid contributes very little aroma formation in roasted coffee (De Maria et al. 1996a), the suggestion for the effect of the DCM fraction in the linear model could be due to the fact that most coffee aromas are non-polar and therefore the presence of lipid may help retain the coffee volatiles in the coffee matrix.

Table 5.8 Detection thresholds in water of aroma compounds of interest (µg/l).

Compound	Threshold in water (µg/l)	Source
2-ethyl-3,5-dimethylpyrazine	1	A
Guaiacol	3	A
4-vinylguaiacol	20	B
4-ethylguaiacol	50	A
Vanillin	20	A
2,3-pentanedione	30	B
2-methylbutanal	1	A
3-methylbutanal	0.2	A
Pyridine	2000	A
methylpyrazine	100000	B
2-furaldehyde	3000	B
2-furanmethanol	8000	B
2,5-dimethylpyrazine	1800	B
2-ethyl-5-methylpyrazine	100	B
trimethylpyrazine	400	C
2-methylisoborneol	0.0025	C

A: Leffingwell (1999), B: Flament (2002a), C: Rychlik et al. (1998)

As mentioned, Arabica generally contains more furans and less pyrazines than Robusta. The suggestion to use low amount of water fraction by the model, therefore, could be due to the relatively high levels of pyrazines formed from the Robusta water fraction (see result from section 5.2) which might be the reason causing higher variation from Arabica.

5.3 CONCLUSION

A procedure for fractionating and reconstituting coffee that yields satisfactory recovery of coffee aromas has been developed. Although the original form of coffee was not attained (whole beans vs. ground form), the contribution of the chemicals in the coffee fractions to coffee aroma development was found to be unaffected.

With regards to the reconstituted samples, the reconstituted Robusta coffees whose aroma profiles were the least different from that of the original Arabica, as determined by SNSD value, were the ones composed of 30% (d.b.) of cell-wall material (residue) and 11.2% (w/w) moisture content either with 35% (d.b.) of DCM fraction and 35% (d.b.) of MeOH fraction or with 70% (d.b.) of MeOH fraction. These two samples were referred to as sample X and sample Y, respectively, and were subjected to sensory analysis in the later Chapters.

CHAPTER 6: SENSORY EVALUATION

6.1 INTRODUCTION

Sensory evaluation was carried out to determine whether human perception (by sniffing) on the selected reconstituted samples agrees with the results obtained from the previous instrumental analysis (in Chapter 5). Since the aroma profiles of the selected reconstituted samples, sample X and Y, were found to be the least different from that of Arabica coffee [as determined by SNSD values (see section 2.3.5) and Principal Component Analysis (refer to section 5.5)], it was expected that the aroma qualities of these reconstituted Robusta coffees be comparable to that of the Arabica, or if not, have been improved and become more desirable than that of the original Robusta. A preliminary sensory study using difference-from-control test was conducted to determine similarity between the aromas of the selected reconstituted samples and that of the Arabica. The results obtained from the test, however, showed that the aroma qualities of the reconstituted samples were much different from that of the Arabica and even more different than the original Robusta itself. The explaination for this conflict could be due to the fact that the methods used to determine aroma profile similarity (either by PCA or SNSD calculation) was based only on 16 - 22 aroma compounds (out of > 800 compounds of coffee aromas), therefore resulting in the methods not being effective enough to predict similarity between aroma profiles. A sensory test to determine acceptance/preference on the coffee samples was, then, performed to determine if the aroma qualities of the reconstituted samples have been improved and whether they are preferred to that of the original Robusta regardless of their large differences in aroma

quality from Arabica. The technique "pairwise-comparison" was chosen and employed hedonistically for this purposes. The sensory panel was conducted on 30 people who were students and staff members of the University of Nottingham, Sutton Bonington campus. All were regular coffee drinkers. Details of the experimental procedure are discussed in section 2.4.

According to the results from the previous instrumental experiments (see section 5.6), the two chosen reconstituted Robusta samples had the following compositions:

Sample X: 30% (d.b.) of cell-wall material (residue), 35% (d.b.) of DCM fraction and 35% (d.b.) of MeOH fraction

Sample Y: 30% (d.b.) of cell-wall material (residue) and 70% (d.b.) of MeOH fraction

Both samples were adjusted to have moisture contents of 11 % (w/w).

In addition, due to the limited amount of reconstituted sample available, samples were reused within the same judge during the sensory panel (discussed earlier in section 2.4.2). This means that each sample was re-warmed three times during the sensory analysis. To ensure that this had no significant effect on the aroma profile, an analysis determining consistency of the volatile intensity in the bottle head space over time was carried out. The analysis was done using Atmospheric Pressure Chemical Ionization-Mass Spectrometry (APCI-MS) (see section 2.4.2.1). The result of the analysis is discussed in section 6.2.2. Colour measurement was also done in order to ensure all

samples were roasted to the same degree prior to sensory analysis (result is discussed in section 6.2.1).

6.2 RESULTS AND DISCUSSION

6.2.1 Colour Measurement

Due to the difference in chemical composition, different samples needed different roasting/ baking conditions in order to produce samples with comparable degree of roast (see Table 2.12).

In coffee, the degree of roast is commonly determined by colour and/or weight loss of the roasted beans. However, due to the small amount of samples used in the experiment, weight loss measurements were inaccurate and, therefore, only colour measurement was carried out.

Prior to sensory evaluation, the colour of the four samples; Arabica, Robusta, sample X and sample Y, were measured. This was to ensure that all the samples had been roasted to the same degree of roast that should, as a consequence, verify that their flavour have been developed to the same degree.

The colour measurement was carried out using the ColorQuest XE Hunter Lab instrument. The measured colour was expressed in terms of "L" (lightness; 0 = dark and 100 = bright), "a" (negative = greenness and positive = redness) and "b" (negative = blueness and positive = yellowness).

Table 6.1 L a b values from colour measurement by ColorQuest XE Hunter lab on roasted Arabica, roasted Robusta, roasted sample X and roasted sample Y.

Sample	Dark- Light	Green – Red	Blue – Yellow
	L	A	B
Roasted Arabica (210 °C, 13.5 min)	39.3 a	3.2 c	1.9 b
Roasted Robusta (210 °C, 15.0 min)	39.3 a	2.7 bc	1.7 b
Roasted X (210 °C, 11.0 min)	40.0 a	2.6 ab	1.9 b
Roasted Y (210 °C, 9.5 min)	39.1 a	2.3 a	1.0 a

*All pairwise comparisons were made by Tukey's Test at 95% confidence level. Means with the same letter show no significant difference. The results were based on 3 replicates.

As seen in Table 6.1, all roasted samples possessed the same level of darkness/lightness as the statistical analysis, at 95% confidence level, showed no significant difference among the "L" values of the samples. Roasted Arabica, however, showed a significantly higher red hue than roasted samples X and Y, but not more than roasted Robusta. Roasted sample Y was found to be the least yellow among all the samples.

Since the Hunter Colour "L" value is generally used to define the degree to which the beans have been roasted (Gutwein and Kirkpatrick 1998), only the "L" value was taken into consideration here. As all the roasted samples showed no significant difference in "L" values, these samples were assumed to have been roasted to the same degree and were, then, qualified to be used in the sensory analysis.

6.2.2 Headspace Volatile Intensity of the Samples Determined by Atmospheric Pressure Chemical Ionization-Mass Spectrometry (APCI-MS)

As discussed above, due to the limited number of samples, samples were reused (within the same judge) during the sensory panel. Therefore, the analysis of headspace aroma intensity of the samples over time was conducted (see section 2.4.2 for the procedure). The analysis was done on the samples left in a water bath (72 °C) over different lengths of time (from 1 min to 20 min). The headspace intensity of the samples was measured using APCI-MS. The selected ions monitored are shown in Table 6.2.

As seen in Table 6.2, the sample that showed the highest level of change (as measured by the highest average % CV) was sample Y with an average % CV of 24.6 %. This result could be due to the low lipid level in the sample Y as it contained no DCM fraction, the fraction that contains mostly of lipid. As lipid is a component that helps retain aromas in coffee and other foods, the lack of lipid content in sample Y could have increased the volatile loss over time, causing higher variations when the sample was re-heated over time. Overall, the variation (% CV) of the ions' intensities varied from 1.1% to 60.9%.

In general, over 93% of the headspace ion intensities measured was found to vary within 30% CV provided that the samples were warmed over time at 72 °C for upto 20 min.

Table 6.2 Selected ions monitored along with % variations (expressed as % coefficient of variance, % CV) of the ion intensities detected over time (up to 20 min) by APCI-MS.

Ion	Variation of ion intensity (%CV)			
	Arabica	Robusta	Sample X	Sample Y
43	13.3	35.1	16.8	26.0
57	13.5	27.9	10.3	23.9
58	59.1	42.9	25.0	49.3
79	1.4	18.0	8.6	40.5
81	5.5	1.6	10.1	4.1
86	14.2	1.8	5.1	38.4
94	1.7	14.2	23.5	25.3
95	2.3	10.6	14.7	14.5
96	2.0	11.7	9.2	22.6
98	4.6	5.1	15.5	18.7
100	3.2	3.5	13.3	26.3
107	23.3	11.1	16.1	19.6
108	5.1	16.7	18.7	24.5
109	7.8	10.0	17.0	22.2
110	5.0	4.8	16.2	20.6
121	58.0	3.9	16.6	13.2
122	13.1	8.8	16.0	23.4
124	6.9	4.5	17.4	22.5
126	3.6	11.3	18.1	26.5
135	60.9	1.1	20.1	19.7
136	18.1	11.7	29.8	24.4
137	3.2	11.1	14.0	29.9
140	7.3	13.0	17.5	27.3
150	4.7	11.7	22.3	28.8
151	19.1	7.6	17.1	23.2
152	4.8	5.5	15.7	23.1
Average	**13.9**	**11.7**	**16.3**	**24.6**

* Each % CV was calculated from the intensities of a particular ion ion in a sample that was warmed in a water bath (72°C) and subjected to APCI-MS for 3 times at varied lengths of time (from 1 min to 20 min).

6.2.3 Sensory Evaluation: Hedonic Pairwise Comparison

Six pairs of samples were introduced to each judge (30 judges in total) to sniff and decide which sample they preferred in each pair (as described in section 2.4.2). Results are shown in Table 6.3. As seen, on the basis of preference, the samples may be ranked in order of decreasing preference as Arabica, sample Y, sample X, and Robusta. When statistical analysis (paired t test) was applied to the results (also shown in Table 6.3), it was found that at the 95% confidence level, sample Y was significantly more preferred to Robusta and sample X, but not more than Arabica as there was no significant difference found between them. This could mean that sample Y and Arabica are about equally desirable (based on sniffing). Regarding sample X, no differences were significant when comparing sample X to Arabica or Robusta indicating that by sniffing, sample X was as desirable as Arabica and as Robusta. However, when comparing between Arabica and Robusta, a significant difference was found and, as expected, Arabica was found to be significantly more preferred to Robusta.

In general, the results showed that the aroma of a reconstituted sample made of Robusta fractions, sample Y, was more accepted than that of the original Robusta. Another reconstituted Robusta, sample X, may not have been preferred to Robusta, however it was not found to be significantly less preferred to Arabica as well. Overall, the result from the sensory evaluation has suggested that the aroma quality of these two reconstituted coffees made of Robusta fractions through fractionation and reconstitution procedures have been improved to be about as equally desirable as that of Arabica.

Table 6.3 Judges preferences for Arabica, Robusta, sample X and sample Y presented as six pairs of sample using hedonic pairwise-comparison test.

Test	Product	Answers	Significant. (risk)
1	No answer Robusta Arabica	0 8 22	0.0161*
2	No answer Robusta Sample X	1 12 17	0.4583
3	No answer Robusta Sample Y	0 8 22	0.0161*
4	No answer Arabica Sample X	0 18 12	0.3616
5	No answer Arabica Sample Y	0 16 14	0.8555
6	No answer Sample X Sample Y	0 7 23	0.0052**

* Significantly different, p = 0.05.
** Highly significantly different, p = 0.01.

Since only sample Y showed to be significantly preferred to Robusta, it was chosen as a sample with a comprehensible improvement in aroma quality and was then subjected to taste compound analyses (see Chapter 7).

In addition, considering the comments from judges regarding the aroma notes perceived by sniffing from sample Y and Robusta (Table 6.4), it was found that sweet notes seem to be predominant in sample Y, while, as usual, burnt notes were predominant in Robusta. This is as expected since sample Y contains higher levels of sugars (from MeOH fraction) and, as a consequence, can produce more furans which are normally

associated with sweet-like aromas. Taking into account the results from the sensory test along with the comments, it could also give an implication that the judges may prefer coffee that smells sweet to coffee that smells burnt.

Table 6.4 Some comments by the judges regarding the aroma notes perceived in Robusta and sample Y.

Robusta	Sample Y
Burnt	Fruity, Vanilla, Sweet
Bland	Rounded
Burnt popcorn	Good aroma, Hint of coffee
Bean-like	More coffee aroma
Acrid	Like filtered coffee
Rich	Medicinal
Sharp	Natural, Well-roasted
	Strong aroma with bitter smell
	Sweet and feels as mixed with herbs
	Smooth, Sweet like treacle

6.3 CONCLUSION

The colour measurement confirmed that all four samples used in the sensory study, Arabica, Robusta, sample X and sample Y, had been roasted to the same roast degree prior to the use in the sensory test. This was taken as verification that the flavour of all samples had been developed to the same degree, and thus should not cause bias during sensory evaluation. The evaluation of the change in headspace intensity of the samples heated over time (from 1 to 20 min) has shown that the variations of the intensity

of most target ions fell within 30% CV. A sensory evaluation using pairwise comparison approach was carried out to measure relative acceptance of the four coffee samples; Arabica, Robusta, sample X and sample Y. The results indicated that the two chosen reconstituted Robusta coffees, sample X and sample Y, were about as equally as desirable to Arabica to the judges. The result from the sensory evaluation, as a consequence, suggested that the modification of Robusta coffee through fractionation and reconstitution processes has increased the aroma quality level of the Robusta coffee to become closer to that of the Arabica. However, since only sample Y was found to be significantly more preferred to Robusta, via sniffing, sample Y was the only reconstituted Robusta coffee considered as a sample with a comprehensible aroma improvement and, therefore, subjected to further taste compound analysis.

CHAPTER 7: ANALYSIS OF NON-VOLATILE COMPOUNDS

7.1 INTRODUCTION

In scientific literature, less attention has been devoted to the non-volatile components of coffee flavour when compared to that given to the volatile components (Buffo and Cardelli-Freire 2004). Besides volatile compounds, non-volatile components in coffee also play important roles in the final cup quality, i.e. for taste and texture (body and mouth-feel in the case of coffee). According to a study by Frank et al. (2006), using high-vacuum distillation technique to fractionate a coffee beverage into a volatile and a non-volatile fraction, it was found that the bitterness of the coffee beverage was exclusively located in the non-volatile fraction (see Table 1.7).

Since the analyses of the volatile (aroma) compounds had already been carried out in the previous experiments (Chapters 5 and 6), the experiments in this part were focused on the non-volatiles components of coffee.

In this Chapter, the analyses focused both on the non-volatiles that contribute directly to the taste and body/mouth-feel in coffee: i.e. caffeine, chlorogenic acids (CGA), carboxylic acids, carbohydrate, and lipid, as well as on those regarded as important precursors of coffee volatile and non-volatile flavour compounds developed during Maillard reactions, i.e. protein, carbohydrate, and trigonelline (refer to section 2.5 for methods). It was expected that the results from the non-volatile analyses (discussed in sections 7.2.2 – 7.2.8) would help predict the taste quality of the chosen reconstituted Robusta coffee, sample Y (see composition in Table 2.11), as well as help explain the

findings from Chapters 5 and 6 regarding the volatile compounds found in sample Y after roasting.

As there are many non-volatile compounds in coffee and it is not possible to analyze all of them, a preliminary experiment to screen for relevant compounds (mainly responsible for the coffee taste) was set up. The use of Direct Liquid Mass-Spectrometry (DL-MS) was employed (see 2.5.1 for method) and the result is shown in 7.2.1.

It should also be noted that the only reconstituted coffee subjected to the non-volatile analyses in this experiment was sample Y as it was the reconstituted Robusta coffee that performed best in terms of aroma quality (according to the sensory evaluation in Chapter 6). The non-volatile analyses were also carried out on the original Arabica and Robusta coffee samples for comparison purposes.

7.2 RESULTS AND DISCUSSION

7.2.1 Screening for Key Taste Compounds by Direct Liquid Mass-Spectrometry (DL-MS)

As the three common taste sensations in coffee are bitterness, astringency and sourness, the screening by DL-MS was aimed at the compounds believed to contribute in a major way to these tastes, i.e. caffeine, trigonelline, chlorogenic acids (CGA) and their derivatives. The compounds studied and their associated ions monitored are shown in Table 7.1.

Table 7.1 Selected taste compounds and their associated ions in liquid phase DL-MS

Compound	Ion (*m/z*)
Caffeine	195 [a]
Trigonelline	138 [a]
Caffeoyl quinides	337.1 [b]
Feruloylquinic acid	369.2 [b]
Feruloyl quinides	351 [b]
p-Coumaric acid	165 [b]
Ferulic acid	195 [b]
Caffeic acid	181 [b]
Dimethoxycinnamic acid	209 [b]
Dicaffeoyl quinides	499.1 [b]

[a] data from Perrone et al (2008).
[b] data from Frank et al (2006).

Since most of the compounds analyzed by DL-MS are best monitored in positive-ion mode (ESI^+), the peak areas from positive-ion spectra are illustrated (see Figure 7.1).

As shown in Figure 7.1, the three samples studied, Arabica, Robusta and sample Y, contained considerable amounts of the ions associated with trigonelline (*m/z* 138), caffeine (*m/z* 195), CGA and its derivatives (*m/z* 165, 351, 369), especially ion (*m/z*) 138 whose intensity was found high in all the samples, and outstandingly in sample Y. Ion (*m/z*) 195 which is associated with caffeine and ferulic acid, known as one of the key precursors of CGA (Frank et al. 2006), was also found abundant in all the samples. Its intensity, however, was found relatively much higher in Arabica and sample Y than in Robusta. The intensities of the ions (*m/z*) 165 and 351 (both associated with CGA derivatives) were the highest in Arabica, while the rest of the ions were not much different among the three samples.

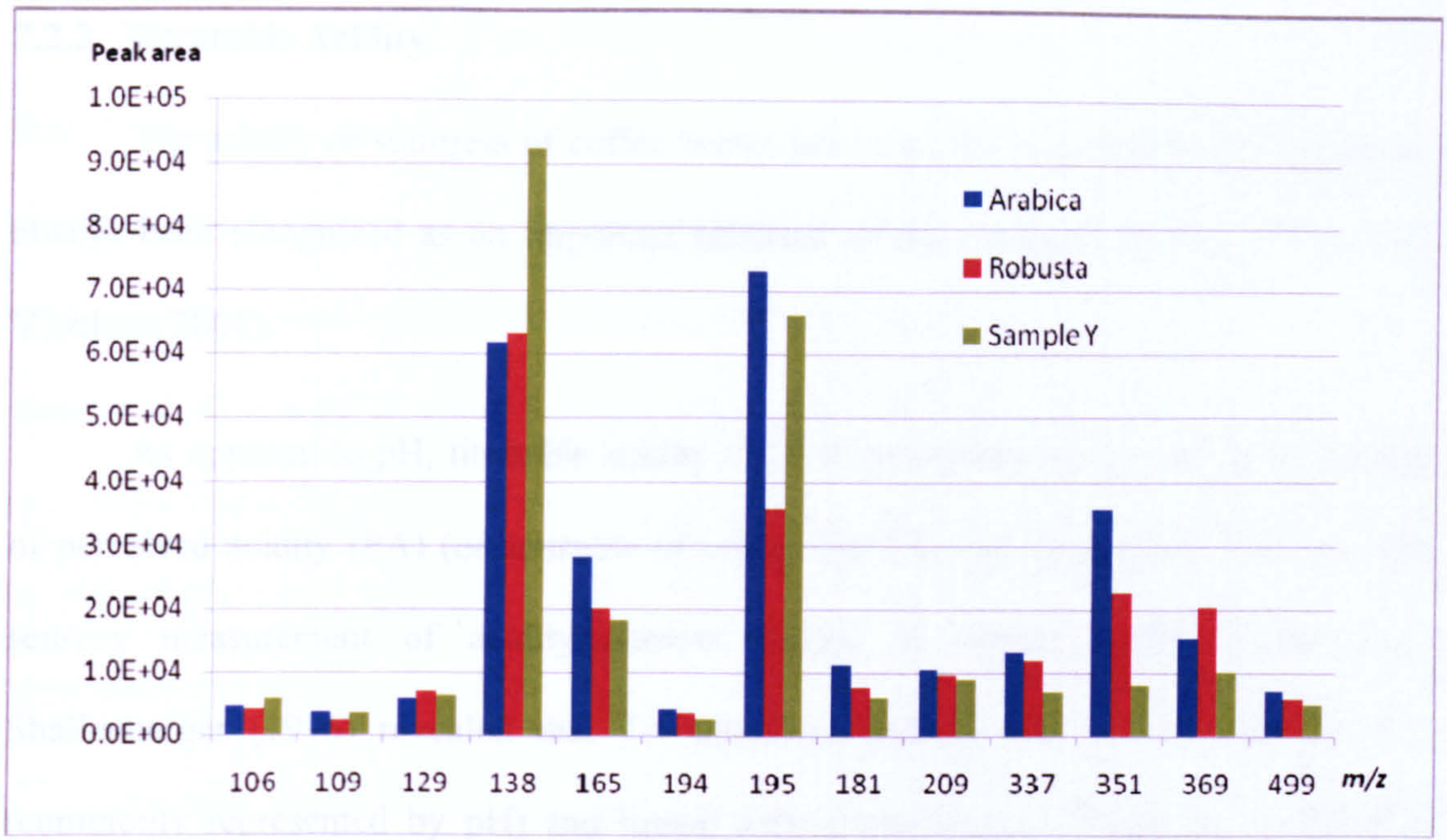


Figure 7.1 Peak area of the selected ions obtained from Direct-Liquid Mass-Spectrometry (DL-MS) operated in ESI$^+$ mode.

In general, the results obtained gave an indication that the three samples studied could contain relatively much more trigonelline and caffeine than CGA and its derivatives. However, as DL-MS was a quick method that analyzes non-volatiles by measuring the ions associated with the compounds of interest, the information given could not provide conclusive information as to how much of a particular compound there was in the samples. For that reason, in addition to the analyses of the main non-volatile components in coffee, i.e. total acids, sugars, protein and lipids (discussed in sections 7.2.1, 7.2.2, 7.2.6 and 7.2.7, respectively), analyses of CGA, caffeine, and trigonelline by High Performance Liquid-chromatography (HPLC) were also carried out (discussed in sections 7.2.4-7.2.5) in order to obtain more accurate information about the contents of these bitter-tasting compounds present in the coffee samples.

7.2.2 Titratable Acidity

The acidity or sourness of coffee brews has (together with aroma and bitterness) always been recognized as an important attribute of their sensory quality (Clarke and Vitzthum 2001).

As opposed to pH, titratable acidity (TA) is recommended for the determination of perceived acidity (PA) (or sourness of coffee) as TA correlates better than pH with sensory measurement of acidity/sourness (Bahre & Maier, 1996). Furthermore, Shallenberger (1996) revealed that TA measures protons that are in both the free (commonly represented by pH) and bound forms, and both of which are believed to contribute to perceived sourness as acid is "titrated" in mouth by the alkali present in saliva.

In this experiment, the acid titration was carried out to 2 end-points, pH 6 and pH 7 (see 2.5.3 for method). Titration to pH 6 was suggested by Maier et al. (1983) as they found the TA titration to pH 6 to have the best correlation to acid taste when compared to the TA's titrated to pH 7 and pH 8. They explained that because human saliva around the tongue has a pH in the region of 6, thus sour taste could be related to neutralization of acids in the mouth. Further titration to pH 7 was carried out due to the high initial pH values of some sample extracts that were already higher than 6, and therefore making titration (by alkali) to pH 6 impossible.

The acidity values were expressed as volume (ml) of the alkali solution (0. 1 M NaOH) required to neutralize the acidity of 100 g test sample to the end-points (results shown in Table 7.2).

As seen in Table 7.2, the initial pH values of all raw coffee extracts were lower than those of their corresponding roasted coffee extracts, indicating the loss of acids upon roasting. This finding was as expected as, the darker the roast of coffee, the lower the acid content remaining (Woodman 1985). pH values of the raw Arabica and Robusta samples, 5.45 and 5.52, respectively, were slightly lower than the average pH value of raw coffee beans reported by Charurin et al. (2002) which was 5.8. This slight difference in pH values could be due to many factors, i.e. differences in sample preparation procedures and sources of coffee samples.

The pH value of raw Y extract (5.10) was the lowest among the three coffee extracts (pH values of raw Arabica and Robusta extracts were 5.45 and 5.52, respectively). Since Maillard reaction can be slowed down at lower pH (deMan 1999), this low pH value of the raw Y sample could be a factor causing drawbacks in flavour development during heat treatment of the sample. This is because at lower pH there are more free protons available in the food matrices which can interact and bond with the basic amino group of the amino acids to form $-NH_3^+$, which is inactive and, therefore, will not react with reducing sugars for condensation (in model systems) which is the first step of Maillard reactions. However, as other factors, i.e. moisture content, levels of flavour precursors in the sample and degree of roast, also affect the formation of coffee flavour, these factors should also be taken into consideration in addition to the pH value of the coffee.

Regarding the roasted coffees, pH values of the roasted Arabica and Robusta extracts, (5.62 and 6.51, respectively), were slightly higher than those reported in

literature which are 4.9-5.5 for roasted coffee extract (Werner and Kohley 1965). An explanation for this disagreement could be due to the high dilution factor used for the roasted coffee extracts in this experiment (10-fold as recommended by AOAC) that may have weakened the acidity in the extracts, and thus caused higher pH values than normal.

The pH value of the roasted Y extract (5.90) was not significantly different from that of the roasted Arabica extract (5.62), while the pH value of the roasted Robusta extract (6.51) was significantly higher than those of the other two coffee extracts. This indicated that the amount of free protons in the brew prepared from the roasted Y should be comparable to that in the brew prepared from the roasted Arabica than to that in the brew prepared from the roasted Robusta.

It might be interesting to note that a small change in pH in a coffee brew can affect the sour/metallic taste ratio in the coffee. Nevertheless, if the levels of bitter and astringent taste compounds are predominant in the coffee brew system, the sour and metallic tastes can be concealed (Rizzi, Boekley et al. 2004).

As in the case of pH, titratable acidity of all samples (see Table 7.2) decreased upon roasting. The biggest decrease was found for the sample Y which went from 50 units in the raw state to 5 units in the roasted state and from 92 units in raw state to 55 units in roasted state for the acidity titrated to pH 6 and pH 7, respectively. This large change in acidity values could be due to the low content of lipid in the sample Y that may have allowed heat to be in contact with the acids more directly during roasting, thus causing higher loss of acids through volatility than the samples with more lipids.

Table 7.2 Titratable acidity of raw and roasted Arabica, Robusta and sample Y titrated to pH 6 and 7.

Sample	pH (i)	pH (end)	Acidity*
Raw Arabica	5.52 a	6.03	12
		7.00	68
Raw Robusta	5.45 a	6.02	15
		7.00	66
Raw Y	5.10 b	5.99	50
		7.00	92
Roasted Arabica	5.62 A	6.00	10
		7.01	60
Roasted Robusta	6.51 B	-	-
		7.02	35
Roasted Y	5.90 A	6.04	5
		7.01	55

* Expressed as volume (ml) of the alkali solution (0.1 M NaOH) required to neutralize acidity of 100 g test sample to the points where pH equals 6 or 7
** Means with the same letter are not significantly different at 95% confidence level (p= 0.05). Raw and Roasted samples were statistically analyzed separately.

In general, although the acidity of sample Y in the raw state was much higher than those of Arabica and Robusta, roasting brought the acidity level of the sample Y down to become closer to those of the other two coffees. In this case, the acidity value obtained from the titration to pH 7 of roasted Y extract (55 units) was more comparable to that of the roasted Arabica extract (60 units) than to that of the roasted Robusta extract (35 units). For that reason, one may be able to expect that the perceived acidity (PA) or sourness level of the brew prepared from the roasted Y be more comparable to that of the brew prepared from the roasted Arabica than to that of the brew prepared the roasted Robusta.

7.2.3 Carbohydrate: Total Soluble Solid and Sugar Analyses

Carbohydrates are an essential class of compounds for the formation of aroma compounds, mainly by caramelization of the low-molecular-weight sugars and by Maillard reaction with the amino acids (Flament 2002a). Sucrose is the most abundant simple carbohydrate present in raw coffee. It acts as an aroma precursor during roasting, generating several classes of compounds such as furans, aldehydes and carboxylic acids that affect the flavour of the beverage (Perrone, Farah et al. 2008).

Since the taste in coffee is predominated by bitterness and astringency, the role of carbohydrate as taste compounds, hence, is trivial. However, its role in providing texture to coffee brews, i.e. viscosity and mouth-feel, is more apparent.

In this experiment, refractometry was used to measure the concentrations of total soluble solids (believed to be composed mostly of low-molecular-weight carbohydrates, i.e sugars) in the sample extracts (see section 2.5.2 for method). The results are expressed as degree Brix (°Brix) which represents % total soluble solids in the extracts. Determination of specific sugars, i.e. fructose, glucose, and sucrose, was also carried out to quantify concentrations of the target sugars individually. The analysis was done by HPLC (see 2.5.7 for method) and the results are shown in Tables 7.3 and 7.4

As seen in Table 7.3, the refractometer could only measure the contents of dissolved solids in the roasted samples. Possible explanation for the results could be that carbohydrates in raw coffee are mostly in the form of polysaccharides, i.e. arabinogalactan and galactomannan, which are not very extractable, and therefore lead to

low levels of dissolved solids in the extracts which were below the detection limit of the refractometer. The fact that dissolved solids were detected in the roasted sample extracts indicated that roasting improved the solubility and, thus, the extractability of the carbohydrates in the coffee samples.

Table 7.3 Total soluble solids (°Brix) in raw and roasted Arabica, Robusta and sample Y.

Sample	°Brix (of 10% w/v sample extract)	g Soluble Solid in 100 g Sample
Roasted Arabica	2.15 a	21.5 a
Roasted Robusta	2.20 a	22.0 a
Roasted Y	7.70 b	77.0 b
Raw Arabica	n.d	n.d
Raw Robusta	n.d	n.d.
Raw Y	n.d	n.d.

* Values are based on 2 replicates.

** Means with the same letter are not significantly different at 95% confidence level (p= 0.05).

*** Soluble solid contents in coffee samples were calculated based on % soluble solid in the 10% w/v coffee extracts converted to g soluble solids in the coffee samples.

Among the three roasted samples, sample Y contained the highest level of the total soluble solids (77% as opposed to 21.5% and 22% w/w in Arabica and Robusta, respectively). This high level of soluble solids in roasted Y could be due to the high content of MeOH extract fraction (70% d.b.) in it. As mentioned earlier, the MeOH extract fraction from Robusta coffee is believed to possess high concentration of sugars which are highly soluble in methanol/water and, thus, may be the cause for such a high soluble solid content in sample Y.

In general, the findings in this experiment support the results from the previous aroma analyses (in Chapters 5 and 6) that sample Y, upon roasting, produced high levels of furans, known as sugar degradation products, due to the high concentration of sugars

in the coffee system. In addition, the high level of sugar degradation products in the roasted Y sample may have been the cause for the marked sweet-related aroma notes detected by the judges during the sensory evaluation (see Table 6.4 in chapter 6).

Table 7.4 shows the results from sugar analysis by HPLC. As seen, the results disagree with those obtained from the refractometry in that higher levels of sugars were detected in the raw samples rather than in the roasted samples. The explanation for the contradiction could be due to the difference in extraction solvents used in the experiment (50% MeOH for HPLC sugar analysis vs 100% water for refractometry). Furthermore, given the fact that McCamey et al. (1990) proposed that bitterness is correlated with the total dissolved solids of a coffee, it is also possible that the total soluble solids detected in the roasted sample extracts were not only the combination of sugars, but also of the water-soluble bitter taste compounds presented in the samples after roasting, i.e. caffeine, trigonelline and chlorogenic acid derivatives, thus causing higher dissolved solids detected in the roasted sample extracts.

Fructose was not detectable in any of the samples, while glucose was found below 1% in Arabica (0.34 - 0.49% d.b.) and Robusta (0.31 - 0.48% d.b.). Sample Y, however, contained a little higher glucose than the other two samples (1.21 - 1.62% d.b.). Tressl et al. (1983) reported 0.45% (unit unspecified) total reducing sugar in raw Robusta which is comparable to the glucose content in raw Robusta found in this experiment (0.48% d.b.). Since glucose and fructose are the major reducing sugars in coffee and other reducing sugars are normally found in trace amounts (see Table 1.5), the total content of glucose and fructose in coffee should, therefore, represent for the most part the total reducing

sugar in coffee. Consequently, it might be reasonable to assume that the level of total reducing sugars in the raw Robusta is comparable to that reported by Tressl et al. (1983).

Table 7.4 Sugar contents in raw and roasted Arabica, Robusta and sample Y.

Sample	% Fructose (d.b.)	% Glucose (d.b.)	% Sucrose (d.b.)
Raw Arabica	n.d.	0.49 a	6.21 a
Raw Robusta	n.d.	0.48 a	3.77 b
Raw Y	n.d.	1.62 b	8.19 c
Roasted Arabica	n.d.	0.34 A	0.45 A
Roasted Robusta	n.d.	0.31 A	0.26 A
Roasted Y	n.d.	1.21 B	1.20 B

* Values are based at least on 2 replicates.
** Means with the same letter are not significant different at 95% confidence level (p= 0.05).
***Raw and Roasted samples were statistically analyzed separately.

Glucose content of raw Arabica (0.49 %. d.b.) was four times higher than the total reducing sugar content in raw Arabica reported by Tressl et al. (1983), 0.1% (unit unspecified). The high level of glucose detected in this experiment could be due to the hydrolysis of sucrose that may have occurred during processing and/or storage of the coffee, and, therefore, resulted in more reducing sugars in the sample. Other factors could be differences in sources of coffees and/or maturity stages of the beans.

As regards sample Y, the significantly higher content of glucose in raw Y (1.62% d.b.) than those of the other two coffees was most likely due to the high content of MeOH extract fraction (70% d.b.) in the sample as explained earlier.

Overall, the results for reducing sugars in raw coffees agreed with the finding of Kroplien (1973, 1974) in that green coffee contains higher level of glucose than fructose and that fructose can only be found occasionally. The content of fructose reported by him (if found) was trivial (0.02% d.b.).

With respect to sucrose, the sucrose levels in the raw samples (3.77% to 8.19% d.b.) were consistent with those reported in literatures (3.8% to 10.7% d.b. by Campa et al. (2004) and 3.4% to 6.1% d.b. by Trugo and Macrae (1983). The finding that raw Arabica contained more sucrose (6.21% d.b.) than raw Robusta (3.77% d.b.) is also in agreement with that reported by Trugo and Macrae (1983) who confirmed higher levels of sucrose in green Arabica than in Robusta (6.1 vs. 3.4% d.b.). Campa et al. (2004) proposed that the higher content of sucrose in Arabica raw beans could be the main explanation for consumers' preference for Arabica to Robusta coffee since sucrose is one of the key aroma precursors in coffee. Given this statement, it might be reasonable to assume that the higher preference by the judges in aroma quality of the brew prepared from roasted Y (compared to that prepared from roasted Robusta) in the sensory study (see Chapter 6) could also be due to the higher level of sucrose in the raw Y that was twice as much as that in the raw Robusta.

Upon roasting, approximately 90% of sucrose was lost in all samples (see Table 7.4). Glucose, however, did not seem to be affected as much (approx. 30% loss). The explanation for the considerable loss of sucrose is that, upon roasting, sucrose degrades into reducing sugars, i.e. glucose and fructose, which react with amino acids to form many coffee flavour components through Maillard reactions (as explained in section 1.1.7). More sucrose loss can also occur at higher roasting temperature through caramelization. A possible explanation for the lesser loss of glucose could be that while glucose is being used by Maillard reactions, the degradation of sucrose during roasting also produces more glucose at the same time, thus maintaining the glucose levels.

Wolfrom et al. (1960) found only trace amounts of sugar after roasting the coffee that contained 5.5% of sucrose prior to roasting. Hughes and Thorpe (1987) reported that sucrose contents in roasted coffee (blended varieties) varied from 0.228 % to 0.740 % depending on roasting conditions. These values reported by them are comparable to those found in this experiment, which are 0.45% d.b. and 0.26% d.b. for the roasted Arabica and the roasted Robusta, respectively.

Although the roasted Y sample contained up to three times higher the contents of sugars than the other two original coffees, this sugar level may still be too low to contribute noticeable sweetness and/or changes in viscosity/body/mouth-feel to the coffee, especially when the coffee is brewed and the sugar concentration is more diluted.

In conclusion, the total soluble solid content of roasted Y (77% w/w) was more than three times higher than those of the roasted Arabica (21.5 %w/w) and the roasted Robusta (22% w/w). The majority of the dissolved solids in all the coffee samples, however, were expected to be bitter taste compounds presented after roasting, i.e. caffeine and CGA derivatives, rather than sugars (according to the low level of sugars found in the sugar analysis). Therefore, it might be reasonable to assume that sample Y contains higher contents of bitter taste compounds than the other two original coffees, which, as a consequence, might lead to more bitter taste in a drink made from roasted Y than those made from the original coffees. The results from sugar analysis agreed with most of those reported in that sugar content is higher in Arabica than in Robusta. Sugar levels of sample Y were the highest among the three coffee samples both in the raw and roasted states. However, despite the high sugar content of the roasted Y, this sugar level

might still be too low to overcome the predominant bitterness and astringency in coffee, nor to contribute any changes in the texture of the coffee, i.e. viscosity, body and mouth-feel, especially when in the form of a coffee drink where the sugar concentration is more diluted. Therefore, the texture of the drink prepared from sample Y is not expected to alter too far from those prepared from Arabica or Robusta.

7.2.4 Analysis of Chlorogenic Acids (CGA)

Chlorogenic acids (CGA) are ubiquitous in the plant kingdom. They are a family of esters of quinic acid with several hydroxycinnamic acids, particularly caffeic, ferulic and p-coumaric acids. In coffee they are essentially mono- and diesters and by far the prevailing acids (Maier 1993) both in the raw and roasted states (Clifford 1985).

Chlorogenic acids (CGA) are involved in the bitterness of coffee due to their decomposition into the phenolic compounds during roasting (Kühnl, Koch et al. 1987). Therefore, their composition in coffee is very important in determining coffee cup quality (Moreira, Trugo et al. 2001).

The three main classes that represent around 98% of the total CGA in coffee are caffeoylquinic acids (CQA), feruloylquinic acids (FQA), and dicaffeoylquinic acids (diCQA) (Clifford and Staniforth 1977). Clifford et al. (1985) found a taste threshold of 50 ppm for the 5-CQA in distilled water, this being a bitter recognition threshold. A study by Kellard et al. (Kellard, Clifford et al. 1988) found a bitter threshold of 3-CQA to be similar to that of 5-CQA. At higher concentration (500-1000 ppm), a metallic note, sourness and other notes can also appear.

Since CQA, FQA and diCQA are the major classes of CGA in coffee, they were investigated in this study. The analysis was carried out using High Performance Liquid Chromatography (HPLC) employing a method modified from that of Moon et al. (2009) (see section 2.5.6 for detail regarding the method).

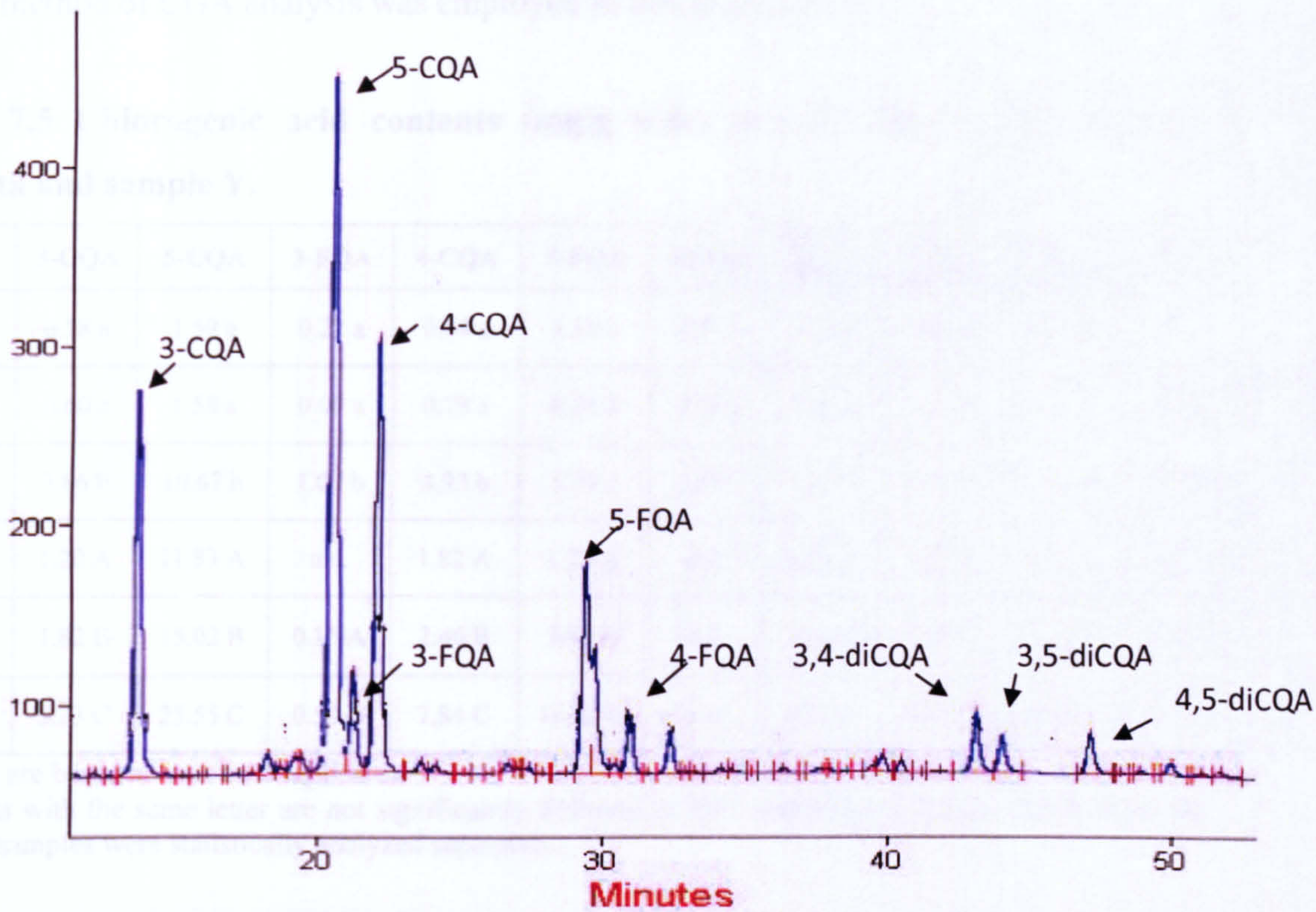


Figure 7.2 Typical HPLC chromatogram from the CGA analysis. CQA, FQA, and diCQA represent caffeoylquinic acids, feruloylquinic acids and dicaffeoylquinic acids, respectively.

As demonstrated in Figure 7.2, the HPLC separated the CGA derivatives satisfactorily. Quantification of all the CGA derivatives was based on a standard curve of 5-caffeoylquinic acid (5-CQA) as it was the only CGA standard available from the chemical supplier. Hence, it should be noted that the values obtained were expressed as relative concentrations which were intended to be used for comparison purposes among

the samples in this experiment. Identification of 5-CQA was based on comparison between the retention time of the compound and that of its standard (Sigma Aldrich®). Identification of the other CGA derivatives was done by comparing their retention times to those of the same compounds in the chromatogram reported by Moon et al. (2009) whose method of CGA analysis was employed in this experiment.

Table 7.5 Chlorogenic acid contents (mg/g w.b.) in raw and roasted Arabica, Robusta and sample Y.

Sample	3-CQA	5-CQA	3-FQA	4-CQA	5-FQA	4-FQA	3,4-diCQA	3,5-diCQA	4,5-diCQA	Total
Roasted Arabica	0.58 a	1.59 a	0.22 a	0.84 a	1.10 a	0.51 a	0.15 a	0.08 a	0.19 a	5.26 a
Roasted Robusta	0.60 a	1.58 a	0.09 a	0.78 a	0.48 b	0.50 a	0.07 a	0.05 a	0.11 a	4.26 b
Roasted Y	3.86 b	10.67 b	1.09 b	4.93 b	5.73 c	1.27 b	1.15 b	0.73 b	1.16 b	30.59 c
Raw Arabica	1.20 A	11.83 A	n.d.	1.82 A	1.24 A	n.d.	0.58 A	1.20 A	1.19 A	19.06 A
Raw Robusta	1.82 B	15.02 B	0.15 A	2.46 B	3.86 B	n.d.	1.85 B	1.67 B	2.12 B	28.95 B
Raw Y	5.73 C	25.55 C	0.50 B	7.84 C	10.82 C	n.d.	6.13 C	5.35 C	6.95 C	68.87 C

* Values are based at least on 2 replicates.
** Means with the same letter are not significantly different at 95% confidence level (p= 0.05). Raw and Roasted samples were statistically analyzed separately.

Table 7.5 shows that, in general, the levels of most CGAs were higher in raw coffees than in their corresponding roasted coffees, especially in Robusta where the raw coffee contained approx. 7 times higher the amount of CGA (total) found in its roasted version. The results indicate the loss of CGA upon roasting. Van der Stegen and Van Duijn (1981) reported that CGA degradation rate is faster in Robusta than in Arabica which is consistent with the finding in this experiment (see Figure 7.3 for % loss of each CGA after roasting). Among the three classes of CGA investigated, diCQAs underwent the highest loss (almost 100% in Robusta; see Figure 7.3). The findings of Van der

Stegen and Van Duijn (1981) are in agreement with this result as they also found diCQA disappeared in several of their coffees after roasting.

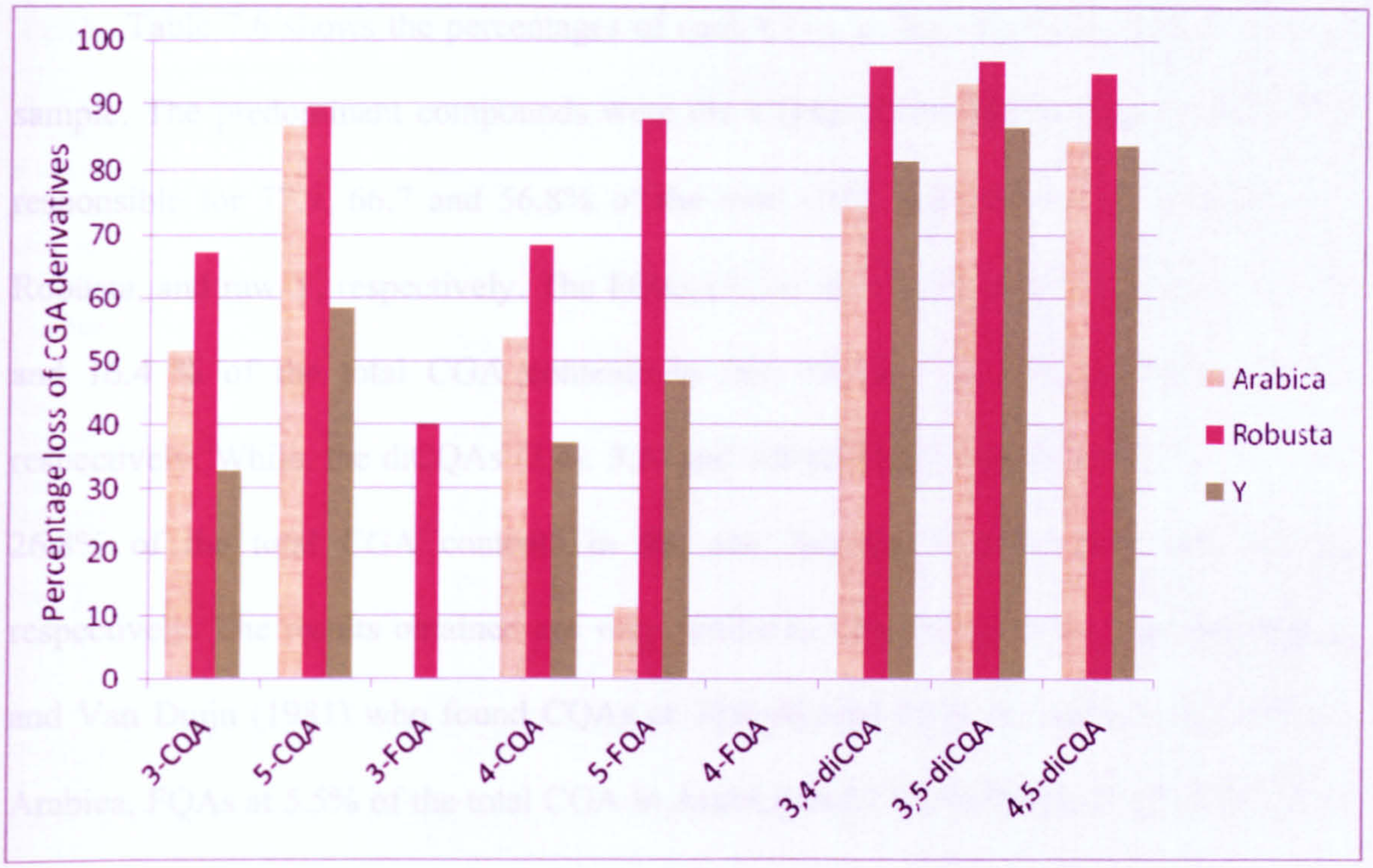


Figure 7.3 Percentage loss of the CGAs in Arabica, Robusta and sample Y after being roasted.

Comparing the contents of CGAs among the samples, sample Y contained significantly higher amounts of all the CGAs investigated than the other two coffees both in the raw and roasted states. This finding supports the assumption made in the previous total soluble solid experiment (see section 7.2.3) in that the high total soluble solid content in sample Y could be due to the high level of bitter taste compounds (including CGA) in it.

The total CGA content of the raw Robusta was higher than the raw Arabica (see Table 7.5) which is in agreement with the finding of Clifford et al. (1985a). Clifford's

group also proposed that the higher total content of CGA in Robusta could be responsible for the inferior quality of brews made with it when compared to those made with Arabica.

Table 7.6 shows the percentages of each CGA in the total CGA content in each sample. The predominant compounds were the CQAs (3-,4- and 5-CQAs) which were responsible for 77.9, 66.7 and 56.8% of the total CGA contents in raw Arabica, raw Robusta, and raw Y, respectively. The FQAs (3-, 4- and 5-FQAs) represented 6.5, 13.9, and 16.4 % of the total CGA contents in raw Arabica, raw Robusta and raw Y, respectively. While, the diCQAs (3,4-. 3,5- and 4,5-diCQAs) represented 15.6, 19.5 and 26.8% of the total CGA contents in the raw Arabica, raw Robusta and raw Y, respectively. The results obtained are very similar to those reported by Van der Stegen and Van Duijn (1981) who found CQAs at 70% of total CGA in Robusta and 80% in Arabica, FQAs at 5.5% of the total CGA in Arabica and 12% in Robusta, and diCQAs at 15% of the total CGA in Arabica and 20% in Robusta.

According to Table 7.5, the total CGA content of the roasted Y was around 6 to 7 times higher than those in the original coffees (30.59 mg/g as opposed to 4.26 - 5.26 mg/g for roasted Robusta and roasted Arabica, respectively. Since CGA is known as a bitter taste compound (with a bitter taste threshold of 50 ppm in water; Clifford et al. (1985a) and its thermal degradation products, i.e. quinic acid lactones:3,4-O-dicaffeoyl-γ-quinide, 3,5-O-dicaffeoyl-epi-δ-quinide, and 4-5-O-dicaffeoyl-muco-γ-quinide, are also considered strongly bitter-tasting compounds in roasted coffee (Frank et al. 2006), the high levels of CGA in sample Y (both in raw and roasted states), therefore, suggested the

tendency that the brews made with the roasted Y could be more bitter than those made with Arabica or Robusta.

Table 7.6 Percentages of the CGAs in the total CGA content of each sample.

Sample	3-CQA	5-CQA	3-FQA	4-CQA	5-FQA	4-FQA	3,4-diCQA	3,5-diCQA	4,5-diCQA
Roasted Arabica	11.0	30.2	4.2	16.0	20.9	9.7	2.9	1.5	3.6
Roasted Robusta	14.1	37.1	2.1	18.3	11.3	11.7	1.6	1.2	2.6
Roasted Y	12.6	34.9	3.6	16.1	18.7	4.2	3.8	2.4	3.8
Raw Arabica	6.3	62.1	n.d.	9.5	6.5	n.d.	3.0	6.3	6.2
Raw Robusta	6.3	51.9	0.5	8.5	13.3	n.d.	6.4	5.8	7.3
Raw Y	8.3	37.1	0.7	11.4	15.7	n.d.	8.9	7.8	10.1

All things considered, further sensory analysis is needed to determine whether such a high increase in CGA content of the reconstituted Robusta (sample Y) could produce noticeable changes in the coffee bitterness and/or the overall sensory quality of the coffee, as well as whether the effects are positive or negative.

In addition, as CGA is recognized as the main contributor to antioxidant activity in coffee (Charurin et al. 2002), the fact that sample Y contains high CGA content could be beneficial to human health regardless of their effects on the coffee taste.

7.2.5 Analysis of Trigonelline and Caffeine

Caffeine and trigonelline are known to contribute around 10-30 and 1% to the total bitterness in a coffee beverage, respectively (Chen 1979; Voilley 1980).

According to Macrae (1985) and many other authors, trigonelline has little direct influence on the quality of coffee brews, having a bitter taste approximately a quarter the

strength of that of caffeine. However, its thermal degradation products have sensory and nutritional importance (Macrae 1985). Flament (2002b) and Ky et al. (2001) reported that, upon roasting, trigonelline acts as one of the key coffee flavour precursors contributing to the formation of many important aroma constituents such as furans, pyrazines, alkyl-pyridines, pyrroles, and methyl nicotinate.

The bitter taste thresholds of caffeine have been reported by many authors: 136–350 mg/l by Robinson et al. (2004), 75-155 mg/l by McCamey et al. (1990), and 60-200 mg/l by Clarke (1987). Trigonelline, however, was reported to be perceived as bitter at a much higher concentration (2500 ppm; Ordynsky 1965).

In addition, Azam et al. (2003) proposed that caffeine is a potential antioxidant that may contribute antioxidant activity to coffee. Other effects of caffeine on health include increasing mental alertness, cognitive functions, wakefulness, as well as reducing the risk of Parkinson's and Alzheimer's disease, kidney stones, gallstones, depression and suicide (Roger and James 2007).

In this experiment, caffeine and trigonelline were analyzed simultaneously using High Performance Liquid Chromatography (HPLC). Both raw and roasted ground samples (0.5 g each) were extracted with water (10 ml) at 50 °C, and filtered prior to HPLC injection. The method employed was modified from that of Casal et al. (1998). Quantification was based on the standard curves made from caffeine and trigonelline standard compounds purchased from Sigma Aldrich® (see section 2.5.8 for method).

Table 7.7 Trigonelline and caffeine contents in raw and roasted Arabica, Robusta and sample Y.

Sample	Trigonelline (g/kg sample w.b.)	Caffeine (g/kg sample w.b.)
Raw Arabica	5.91 a	3.96 a
Raw Robusta	5.04 a	9.32 b
Raw Y	12.78 b	28.13 c
Roasted Arabica	4.33 A	4.07 A
Roasted Robusta	3.50 A	7.22 B
Roasted Y	11.24 B	26.71 C

* Values are based at least on 2 replicates.

** Means with the same letter are not significanlyt different at 95% confidence level (p= 0.05). Raw and Roasted samples were statistically analyzed separately.

As shown in Table 7.7, the losses of caffeine and trigonelline during roasting are relatively modest compared to those of CGA in the previous experiment (see section 7.2.4). In Arabica, the level of caffeine even seemed to increase. Macrae (1985) pointed out that the actual percentage of caffeine present may increase by up to 10% on dry roasted basis, taking into account the weight loss of the beans. He also proposed that the loss of caffeine in coffee is limited by the increase of the sublimation point of caffeine during roasting (by increase of the pressure within the bean) and by a poor rate of diffusion through the outer layers. However, since the coffee samples used in this study were in ground form as opposed to whole beans, those limitations mentioned by Macrae (1985) may not apply to the samples in this experiment. Taking this fact into consideration, the minor losses of caffeine upon roasting could just be due to the intrinsic property of the compound itself that it might be resistant to the roasting conditions normally used for coffee regardless of the form/shape of the coffee.

The levels of caffeine in Robusta were significantly higher than those in Arabica both in the raw and roasted states (see Table 7.7). Sample Y contained around 3-7 times more caffeine than Arabica and Robusta. Casal et al. (2000a) reported that Robusta coffee beans contained higher concentrations of caffeine when compared to Arabica which is in agreement with the results from this study. However, their finding regarding trigonelline does not agree with the results obtained in this experiment in that, while they found trigonelline to be richer in Arabica, the results in this experiment showed no significant differences between trigonelline contents of Arabica and Robusta both in raw and roasted states. The causes for the disagreement could be due to many factors, i.e. differences in coffee sources and/or coffee process conditions. As in the case of caffeine, the levels of trigonelline in sample Y were the highest among the three coffee types, both in raw and roasted states. The findings from this experiment, therefore, also support the assumption made in the previous total soluble solid experiment (see section 7.2.3) in that the high total soluble solid content in sample Y could be due to the high level of bitter taste compounds, which include caffeine and trigonelline, in it.

Casal et al. (2000b) and Ky et al. (2001) proposed that the higher trigonelline contents in Arabica raw bean could partially explain its better cup quality when compared to Robusta. Taking this statement into account, the high level of trigonelline in sample Y could be one of the factors that helped improve the aroma quality of the reconstituted Robusta coffee (sample Y) from that of the original Robusta (as confirmed in the sensory study in Chapter 6).

With regards to caffeine, the high content of caffeine in the sample Y may help increase antioxidant activity in coffee. However, such a high level of caffeine (approx. 300% higher than that in original Robusta) could also contribute intense bitterness, and, consequently, affect the overall taste quality of the brews made with sample Y.

All things considered, the high level of trigonelline in the sample Y could be beneficial to its aroma quality due to the fact that trigonelline is one of the key coffee aroma precursors that contribute to the formation of many important aroma compounds upon roasting. More caffeine may also be beneficial to the coffee with regards to health aspect, e.g. due to its antioxidant potential. However, the bitterness from both compounds could also give a negative effect on the overall cup quality. Taking all these into consideration, further sensory studies are needed to evaluate whether the increase in bitterness caused by the high contents of caffeine and trigonelline in the sample Y are deemed acceptable, as well as to determine if it gives positive or negative effects on the overall flavour quality of the brews prepared from it.

7.2.6 Protein Analysis

Protein plays an important role as one of the key precursors for flavour development through Maillard reactions. During heating, most protein undergo some degree of hydrolysis, forming various peptides that are recognized as important flavour compounds and precursors (Izzo, Yu et al. 1992). Ginz and Engelhardt (1999) proposed that water-soluble proteins of raw coffee can be a source of bitterness as, after roasting, they could identify diketopiperazine derivatives, which are known as bitter principles in food stuffs.

Rizzi (1989) reported that Maillard reactions of peptides with fructose generated Strecker aldehydes. Moreover, Izzo et al. (1992) proposed that different peptides of varying sequences could serve as precursors for the production of a variety of food aromas.

In this experiment, the total protein analysis was carried out using the Bicinchonic acid protein assay (BCA) (Smith 1985). With this approach, the total proteins were measured based on the reactions between the peptide bonds in protein and Cu^{2+} ions from the Sodium Dodecyl Sulfate solution (SDS). The reduced Cu^{2+} ions are then reacted with bicinchoninic acids forming a purple-colored product that strongly absorbs light at a wavelength of 562 nm. Therefore, the total protein concentration was indicated by a colour change of the sample extract from green to purple in proportion to protein concentration, which was then measured by spectrophotometry (see section 2.5.5 for detail regarding the method). It should also be noted that the purpose of the analysis was to study protein as a potential aroma precursor. Thus, only raw coffee samples were analyzed in this experiment.

Table 7.8 Protein contents in raw Arabica, Robusta and Sample Y.

Sample	% Protein (d.b.)
Raw Arabica	3.3 a
Raw Robusta	4.7 b
Raw Y	7.9 c

* Values based on two replicates.

** Means with the same letter are not significantly different at 95% confidence level (p= 0.05).

Table 7.8 shows that the concentration of total protein in sample Y was significantly higher than those in Arabica and Robusta. The levels of total protein found in Arabica (3.3 % d.b.) and Robusta (4.7 % d.b.) were lower than those reported in the

literature (8.7 – 9.7 % d.b.; Thaler and Gaigl (1963). The disagreement could be due to the differences in the determination methods as well as the sources of the coffees.

Since peptides and proteins are also aroma precursors (besides free amino acids) (Ludwig et al. 1977), the higher content of protein in sample Y should, therefore, increase the potential for the sample Y to develop more aromas that are associated with interaction of amino acids, i.e. Strecker aldehydes, pyrazines, in coffee.

In conclusion, the high content of total protein in the sample Y suggests the potential for the sample Y to develop more Strecker aldehydes, and N-containing aroma compounds, i.e. pyrazines, during roasting. Furthermore, as protein was reported as a potential source for bitterness compounds after roasting (Ginz and Engelhardt 2001), the high content of protein in sample Y may also affect the bitterness level in the brews prepared from the sample Y as well.

7.2.7 Lipid Analysis

According to a review by Clifford (1985b), the major part of the crude lipid in coffee is typical seed oil, with triglycerides of fatty acids, some other esters and unsaponifiable matter.

In coffee brews, lipids play an important role in aroma retention since many important coffee aromas are oil soluble (Rizzi et al. 2004).

The effect of lipids in the Maillard reaction has been studied by many authors who cooked or roasted mixture of amino acids and reducing sugars in various vegetable

oils. The thermal oxidative degradation of lipids generates smaller molecules, i.e. aldehydes, that contribute to the formation of heterocyclic volatile compounds (Flament 2002a). Meynier and Mottram (1992) proposed that aldehydes formed by the oxidation of linoleic acid are probably involved in the reduction of the concentration of sulfur-containing compounds by reacting with one of their precursors namely hydrogen sulfide.

Keller and Kinsella (1973) reported that lipids, especially phospholipids caused rancid and warm-over-flavour in meat. Moreover, phospholipids are able to interact in the Maillard reaction to reduce the quantities of some heterocyclic compounds (Whitfield, Mottram et al. 1988; Farmer, Mottram et al. 1989).

The lipid analysis in this experiment was carried out using a gravimetric approach modified from that of Murphy and Cummins (1989) (see section 2.5.4 for method).

Since lipids are claimed to remain virtually unchanged during roasting process (Petracco 2005), only raw coffee samples were analyzed in this experiment. The results are shown in Table 7.9.

As expected, sample Y contained the lowest lipid content (4.8 % d.b.) among the three samples as there was no dichloromethane (DCM) extract fraction, which contained most of the lipid from the raw beans, in the make-up of sample Y (see Table 2.11 for sample Y composition). The lipid content in Arabica (22.6 % d.b.) was significantly higher than that in Robusta (16.9 % d.b.) which agreed with the findings reported by many authors in a review by Clifford (1985b). However, the lipid contents found in this experiment were higher than the ranges reported in literature, 22.6% and 16.9% as

opposed to 15-17% and 10-11.5% reported by Illy (2004), for Arabica and Robusta, respectively. The disagreement could be due to the variation in coffee sources as well as in the methods of analysis used.

Table 7.9 Lipid contents in raw Arabica, Robusta, and Sample Y.

Sample	% Fat (d.b.)
Raw Arabica	21.6 a
Raw Robusta	16.9 b
Raw Y	4.8 c

* Values based on two replicates.
** Means with the same letter are not significantly different at 95% confidence level (p= 0.05).

As mentioned earlier, lipid can lead to the formation of off flavours (rancid) in coffee during storage as well as interacting in the Maillard reaction to reduce the quantities of some heterocyclic compounds (Whitfield, Mottram et al. 1988; Farmer, Mottram et al. 1989). The low content of lipid in sample Y could, therefore, be beneficial to the coffee with regards to its shelf-life and its overall aroma quality. However, the fact that many important coffee aromas are oil-soluble could as well give negative effect to the coffee containing insufficient lipid content.

All in all, samples with low lipid content may be less likely to develop undesired aromas during storage. On the other hand, lipid content also affects retention of coffee aroma. As a consequence, the lower the lipid content the less the aromas are retained, and thus the aromas retained in the coffee will decrease faster during shelf-life.

7.3 CONCLUSION

The composition of non-volatile compounds in the reconstituted Robusta coffee, sample Y, was very different from those of the original Arabica and Robusta. Sample Y

contained significantly higher amounts of key Maillard-reaction precursors than the original coffees, i.e. sugars, protein, and trigonelline, which are advantageous to the coffee with respect to coffee aroma development. Nevertheless, the significant increases in bitter and astringent taste compounds in the sample Y, i.e. CGA, caffeine and trigonelline, could also deliver a negative impact on its taste. Sourness from total acidity, desired to some extent in coffee, is probably dominated by the intense bitterness caused by the high concentrations of caffeine and CGA as well.

The increased levels of CGA and caffeine, on the other hand, could contribute additional antioxidative activity to coffee although the balance between astringency/bitterness and health benefits is always a compromise.

All things considered, having more flavour precursors and more nutritional value may seem to benefit sample Y. However, sensory evaluation that involves tasting of the brew prepared from sample Y is needed to determine whether all of these chemical alteration on Robusta coffee give positive or negative effect on its overall sensory quality because, ultimately, the fine taste and aroma of coffee are what defines the quality of a coffee drink.

CHAPTER 8: CONCLUSION AND SUGGESTIONS FOR FUTURE STUDIES

8.1 CONCLUSION

The attempt to make use of a coffee process by-product, silver skin, as a source for natural coffee aroma for aroma improvement of coffee products with poorer quality, e.g. instant coffee, was not achieved in this study. However, the attempt to improve aroma quality of a poorer quality coffee specie, i.e. Robusta, by the use of refractionation and reconstitution approaches was more successful.

The stimulation of Maillard reactions in the silver skin by heat treatment along with enzyme treatment or the addition of reducing sugar, glucose, did not generate coffee aroma with quantity and quality resembling that of the original coffee. The fact that silver skin is a partially roasted material from the beginning (silver skin is the part of coffee beans that comes off during coffee roasting process) could be one of the reasons preventing it from generating more coffee aroma. Moreover, the conditions used to generate Maillard reactions in this experiment might, as well, have not been the best possible for the silver skin matrix.

Fractionation and reconstitution techniques facilitated chemical modification of raw Robusta coffee, providing a new reconstituted coffee (sample Y) with new aroma profile (as determined by GC-MS analysis) and improved aroma quality (as evaluated by a sensory study). Nevertheless, despite the improvement in aroma quality of the sample

Y, the significantly higher contents of bitter/astringent taste compounds, i.e. CGA, trigonelline and caffeine, as compared to those in the original coffees, could also affect the overall flavour quality of the final coffee product negatively. As a consequence, it is considered necessary that further sensory analysis that involves tasting/drinking of the brews prepared from roasted Y be carried out prior to making a final conclusion regarding the overall sensory quality of this new reconstituted Robusta coffee (sample Y).

In general, this project employed chemometric approach to manipulate chemical composition of Robusta coffee with the aim to create a new reconstituted Robusta with an improved sensory quality. The hypothesis was that chemical analysis of volatile fractions of the coffee samples can be used to measure the success. Experimental design as well as statistical analysis (Principal Component Analysis; PCA) was used as guidance for the reconstitution experiments. While, sensory evaluation was carried out to determine whether the changes in volatile compositions (as analysed instrumentally) resulted in the improvement of Robusta coffee aroma quality, as well as whether the quality of this new reconstituted coffee was comparable to that of Arabica.

It should be noted that the limitation in the sensory study (to sniffing only) was due to the fact that the materials used during the refractionation-reconstitution procedure were not food grade. The glassware and the facilities used throughout the project were also those for general lab use that could be contaminated by random substances and, therefore, may not be safe for the judges to consume.

With respect to industrial application, an evaluation of industrial feasibility is needed prior to putting these fractionation and reconstitution techniques into industrial practice. The aspects to be considered are not only those related to scale adjustment, i.e. from laboratory scale to industrial scale, but also cost-effective, waste management as well as consumer safety aspects (due to the use of solvents).

In order to produce the reconstituted coffee, there are four main steps that need to be added in extra to the conventional coffee making process. They are 1. Grinding of raw coffee beans, 2. Serial solvent extraction of the ground raw beans (fractionation), 3. Removal of the solvents and 4. Mixing of the fractions (reconstitution). In order to apply these steps to an industry, some modifications on the processes may be needed. Some considerations regarding how these processes/steps should be handled in an industry are as follow.

In an industry, the use of supercritical carbon dioxide (SC-CO_2) during raw coffee bean disintegration may not be needed. In the laboratory, SC-CO_2 was used to facilitate the grinding process to help create fine and more uniform particle size of the ground raw coffee which was for experimental control purposes, i.e. to avoid experimental error that could be caused by the ununiformity of the particle size. In industrial application, however, the need for a fine/uniform particle size may not be as important. Given that the handling of SC-CO_2 can be difficult and costly, it may be sensible to roughly grind the raw beans and omit the use of SC-CO_2 when applying this step into industrial practice. As regards the use of solvents, the use of solvents during the extraction/fractionation step not only adds cost to the process, but also creates chemical waste. Therefore, it might be

inevitable for an industry to incorporate an evaporator and distillation apparatus to the process (as done in the experiment). With the apparatuses installed, the solvents can be reused, hence reducing cost as well as chemical waste. With regard safety aspect, it is a responsibility of an industry to make sure that there is no DCM or MeOH residue left in the coffee fractions prior to the mixing (reconstitution) process. DCM can cause carbon monoxide poisoning if ingested (Fagin, Bradley et al. 1980). It is also considered potential carcinogenic compound (USDHHS 2000). Ingestion of small amount of MeOH (10 ml) can cause blindness (Vale 2007). As a consequence, it is necessary that an industry pay attention to this step strictly. If a food safety management system, i.e. HACCP (Hazard Analysis Critical Control Point), is available, it is recommended that the solvent removal step be set as a critical control point.

The use of reverse-osmosis filtered water at the reconstitution process (as done in the experiment) can also add a great deal of cost to the process. In the experiment, the use of the filtered water (Purelite®) was intended to control unnecessary experimental error that may be caused by contamination from tab water. For industrial practice, however, it may be worth to investigate if the use of filtered water can be replaced by tab/industry quality water as it would help save much of the process cost. Regarding roasting process, since the physical property of the reconstituted coffee is different from that of the conventional coffee, conventional roasting method/apparatus may not apply. Development of a temperature profile specific for roasting the reconstituted coffee, hence, is needed. It is, however, expected that lower temperature and/or less time would be required for the roasting due to the smaller particle size of the reconstituted coffee (as

compared to the size of whole coffee beans), which should hopefully not incur as much energy cost for the roasting process. More energy save is also expected at the final grinding step (of roasted coffee) as less time should also be required due to the smaller particle size of the reconstituted coffee.

All in all, the extra steps required for manufacturing this reconstituted coffee may incur a great deal of extra expense at the beginning of the investment. Nevertheless, if careful action on process cost management is taken, it is expected that reasonable process cost should be maintainable in the long run, allowing an industry to produce the coffee with affordable price that, hopefully, will make this new reconstituted coffee an alternative choice for Arabica coffee lovers.

8.2 SUGGESTIONS FOR FUTURE STUDIES

- Determination for an optimum condition for silver skin to generate more coffee aroma via Maillard reactions, e.g. by adjusting moisture content and pH of the silver skin, adding more sugar, as well as finding a suitable roasting temperature and time.
- Process adjustment for the production of sample Y to increase the feasibility for industrial manufacturing, i.e. to facilitate industrial machinery as well as to reduce processing cost which could involve

  - The use of coarsely disintegrated raw coffee for the fractionation procedure instead of finely ground raw coffee that requires the use of liquid nitrogen which is not only expensive but also unsafe

- The use of ethanol instead of methanol for the fractionation for consumer safety
- The optimization of roasting condition that should require lower temperature and/or less time as the size of the raw material are reduced (disintegrated vs. whole beans)

- Sensory evaluation looking at the overall organoleptic quality of the brew made with sample Y. As sample Y was described to possess more sweet aroma notes than the original coffees, it might also be interesting to look at the interaction between aroma and taste to see if the increase in the sweet aromas in sample Y can help lessen the perceived intense bitterness caused by the increase in bitter/astringent taste compounds in the sample Y.

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