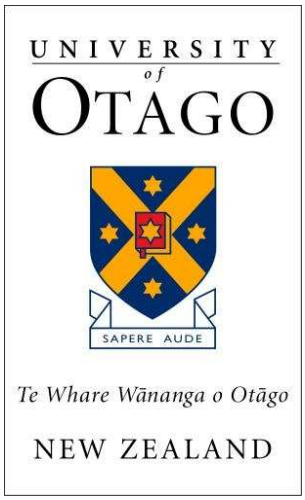

EFFECT OF PROCESSING ON THE FLAVOUR CHARACTER OF ARABICA NATURAL COFFEE

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Abstract

In the context of the current, flourishing specialty coffee industry, the processing of the coffee bean at the origin is increasingly becoming the main differentiation tool for specialty coffee producers around the world. The processing method of coffee at the origin may be one of three main types, depending on how the fleshy parts of the coffee fruit are removed and how the coffee bean is dried. The three main processing methods are often referred to as the washed process, pulped natural process and natural process.

The natural process consists of drying the whole coffee fruit like a prune prior to removing the pulp. It is the oldest processing method known and is currently gaining interest from the specialty coffee industry. The reasons for this are threefold – the 'characteristic' flavour of natural coffee is sought as a means for differentiation; natural coffee is a key ingredient of high quality espresso blends, and it is generally considered as more sustainable than other methods as it does not require water. The specialty coffee industry often describes the characteristic flavour of naturals as 'fruity' or 'winey', which is a profile sometimes called 'Mocha'. The flavour of natural coffee is thus currently in the focus of the specialty coffee industry. However, little is known of how the flavour is formed, the compounds responsible for it and how the process might be controlled so as to allow a reproducible expression of that flavour. Thus, the overall aim of this research was to understand the flavour of Arabica natural coffee as a product class and to understand the main formation pathway during the post-harvest drying process. This aim was achieved through a series of studies, to address 5 specific objectives.

The first objective was to develop a rapid flavour-profiling method based on the analysis of coffee cupping (coffee tasting) data. The second objective was to determine the effect of post-harvest processing methods (washed, natural and pulped natural) on the sensory profile of coffee. Both objectives were addressed in Chapter 3, where the effect of the main post-harvest processing methods on the sensory profile of coffee was investigated. Coffee tasting data from previous, unpublished research was analysed using a novel technique called Descriptive Cupping. The cupping data pertained to coffee cherries from 22 farms in the state of Guerrero, Mexico, which were processed using four post-harvest treatments: natural process, pulped-natural process and two variations of the washed process including fermentation under water

and dry fermentation. The samples were then hulled and assessed by licensed Q Graders (certified coffee tasters) using the Specialty Coffee Association of America (SCAA) cupping (coffee tasting) protocol. The qualitative descriptors provided by the cuppers were used to investigate differences in the sensory profile of the coffees produced from the four methods. Descriptor categories used by the cuppers (descriptor subgroups) were consolidated and integrated into a frequency table. Global chi-square (χ^2), χ^2 per-cell and non-symmetric correspondence analysis (NSCA) were performed on the frequency table.

The third objective was to characterise the sensory profiles of coffee processed using natural post-harvest methods. This objective was addressed in Chapter 4. Samples (22) of natural coffee, received from different origins, were assessed by a cupping panel trained at the University of Otago, and flavour profiles were generated using Descriptive Cupping. Chapter 4 also addressed the second objective by assessing washed coffee samples (9, coming from the same farms as 9 of the natural coffees) as points of reference.

The fourth objective was to identify flavour compounds responsible for key flavours of coffee processed using natural post-harvest methods. It was addressed in Chapter 5, where the same samples used for Chapter 4 were characterised using analytical methods. The headspace of the roasted coffee beans was analysed using Solid-Phase Extraction-Gas Chromatography coupled to Olfactometry and Mass Spectrometry (SPE-GC-MS/O) and Static Headspace- Gas Chromatography coupled to Olfactometry and Mass Spectrometry (SH-GC-O/MS). The headspace of the green coffee beans was analysed as supplementary data using Proton-Transfer-Reaction Mass Spectrometry (PTR-MS). The analytical results and the Descriptive Cupping results were analysed together using Multiple Factor Analysis (MFA).

The fifth objective was to investigate the effect of different natural post-harvest processing treatments (by favouring different fermentative populations) on flavour profiles and specific flavour compounds. This objective was addressed in Chapters 6 and 7. In Chapter 6, different natural coffee processing treatments (7) were compared, using the same raw material. The processing treatments were designed to vary the drying rate of different sections of the drying curve to favour fermentation by different kinds of microbial populations. A washed coffee sample was also produced as a witness from the same raw material. The resulting samples were characterised using Descriptive Cupping by a cupping panel from New Zealand and another cupping panel from Mexico. The results from both panels were compared using MFA.

In Chapter 7, these samples were characterised by the analytical methods (GC-MS/O and PTR-MS) used for the previous studies and both analytical and Descriptive Cupping data were integrated in an MFA.

An effect of the processing method on the flavour profile was found in Chapter 3, whereby the natural samples were characterised by tropical-fruity, dried-fruity, red-fruity or fermented flavour notes. Washed samples showed floral, spicy, or nutty flavour notes, while pulped-naturals tended to have a flavour profile that could range from natural to washed coffee. In the case of Chapter 4, the effect of the country of origin on coffee flavour was found to be more important than the effect of processing. This was linked to different geographically-concentrated factors, including the drying technology. Only when natural coffees showed a 'Mocha' character (fruitiness or wineyness) did they become clearly different from washed coffees. Moreover, the absence or presence of the 'Mocha' character was also found to be one of the main sources of variation within the natural coffees as a group.

In Chapter 5, thermal degradation products of sugars and Maillard reaction products were found to have an important role in the differentiation of samples. However, the main factors explaining the 'Mocha' character were associated with both amino acid catabolism (of valine, isoleucine and leucine) and ethanol fermentation. The esters produced by the ethyl esterification of 2- and 3-methylbutanoic acid were linked to a fresh, red-fruity character (strawberry, blueberry), which is the most common description of specialty natural coffees.

In Chapter 6 similar product spaces were obtained by the New Zealand and Mexican panels and coherence in the use of descriptors such as *fermented* was achieved. Overall, Descriptive Cupping was shown to be a rapid method for profiling coffee flavour with satisfactory levels of discriminating power and satisfactory panel-to-panel agreement.

In Chapters 6 and 7, the most distinct natural drying treatment was found to be the one termed 'honeying' treatment for the purpose of this thesis, which involved suspending the drying for the first two days. This resulted in an intense fermentation with a high production of ethanol, which confirmed ethanol plays a key role in the formation of fruity esters.

This research achieved its objectives by characterising the flavour of natural coffee from the sensory and analytical points of view. Findings from this research contribute to the field of

coffee science by providing the industry and future researchers with an explanation for the formation of characteristic fruity and winey flavours in natural coffee. Based on the findings of this research, coffee producers will be able to better control the flavour outcome of natural coffee. Further research is needed to characterise the role of non-volatile constituents of natural coffee, as well as to understand the role of specific fermentation types in the development of natural coffee flavour. Generally speaking, further research is needed to understand how to better control the coffee flavour outcome.

Resumen en español (abstract in Spanish) – efecto del procesamiento en el carácter del sabor del café natural

En el contexto de la floreciente industria actual de los cafés especiales, el procesamiento del grano de café en la región de origen se está convirtiendo cada vez más en la principal herramienta de diferenciación para los productores de cafés especiales alrededor del mundo. El método de procesamiento del café en la región de origen puede ser de tres tipos principales, dependiendo de la manera como se retiran las partes pulposas del fruto del café y la manera en que se seca el grano. A esos tres métodos de procesamiento principales se les llama a menudo proceso lavado, proceso natural y proceso despulpado natural.

El proceso natural consiste en secar el fruto entero del café como si fuera una ciruela pasa antes de retirar la pulpa. Es el más antiguo método de procesamiento conocido y en la actualidad está ganando interés por parte de la industria de cafés especiales. Las razones para ello son tres: el sabor 'característico' del café natural se busca como una manera de diferenciarse; el café natural es un componente clave en mezclas de 'espresso' de alta calidad, y generalmente se le considera más sustentable que otros métodos, ya que no requiere agua. La industria de cafés especiales a menudo describe al sabor característico de los naturales como 'afrutado' o 'vinoso', lo cual es un perfil que en algunas ocasiones se denomina 'Mocha'. Sin embargo, aunque el sabor de los cafés naturales es actualmente del interés de la industria de cafés especiales, se conoce poco sobre cómo se forma ese sabor, sobre los compuestos que son responsables de éste, y sobre cómo se podría controlar al proceso para permitir una expresión del sabor que sea reproducible. Por tanto, el objetivo general de esta investigación fue entender al sabor de los cafés naturales como categoría de productos y entender las principales vías de formación del sabor durante el proceso de secado pos-cosecha. Este objetivo se logró a través de una serie de estudios.

El primer objetivo fue desarrollar un método rápido de perfilación de sabor, basado en el análisis de datos de catación de café. El segundo objetivo fue determinar el efecto de los métodos de procesamiento pos-cosecha (lavado, natural y despulpado natural) en el sabor percibido del café. Se abordaron ambos objetivos en el Capítulo 3, en el que se investigó el efecto de los principales métodos de procesamiento pos-cosecha en el sabor percibido del café. Los datos de cataciones provenientes de anteriores estudios sin publicar se analizaron

utilizando una nueva técnica llamada Catación Descriptiva. Los datos de las cataciones se obtuvieron a partir de muestras de café cereza en 22 fincas del estado de Guerrero, México, que se procesaron por cuatro distintos métodos pos-cosecha: natural, despulpado natural y dos variantes del lavado (con fermentación sumergida y con fermentación en seco). Las muestras se trillaron y fueron evaluadas por Catadores Q certificados, utilizando el protocolo de cata de la Asociación de Cafés Especiales de América (SCAA). Los descriptores cualitativos generados por los catadores se utilizaron para investigar diferencias en el sabor percibido de los cafés producidos por los cuatro métodos. Los descriptores utilizados por los catadores se consolidaron por categoría y se integraron en una tabla de frecuencias. A la tabla de frecuencias se le aplicaron análisis de chi cuadrada (χ^2) global, χ^2 por celda y análisis de correspondencia no simétrico (NSCA).

El tercer objetivo fue caracterizar los perfiles sensoriales de café procesado por el método natural. Este objetivo se abordó en el Capítulo 4. Se evaluaron 22 muestras de café natural, recibidas de distintos países, por un panel de catación entrenado en la Universidad de Otago, y se generaron perfiles de sabor utilizando la metodología de Catación Descriptiva. El Capítulo 4 también abordó al segundo objetivo, al evaluar como puntos de referencia, 9 muestras de café lavado que procedieron de las mismas fincas que 9 de los cafés naturales.

El cuarto objetivo fue identificar compuestos responsables de notas de sabor relevantes en los cafés naturales. Este objetivo se abordó en el Capítulo 5, en el que se caracterizaron por métodos analíticos las mismas muestras utilizadas para el Capítulo 4. El espacio de cabeza de los granos tostados se analizó utilizando cromatografía de gases con extracción en fase sólida, acoplada a olfactometría y espectrometría de masas (SPE-GC-MS/O) y cromatografía de gases con muestreo del espacio de cabeza estático, acoplada a olfactometría y espectrometría de masas (SH-GC-O/MS). El espacio de cabeza de los granos de café verde se analizó como datos adicionales utilizando espectrometría de masas por reacción de transferencia de protones (PTR-MS). Los resultados analíticos y los resultados de Catación Descriptiva se analizaron conjuntamente utilizando análisis de factores múltiples (MFA).

El quinto objetivo fue investigar el efecto de diferentes tratamientos de proceso natural (que favorecen a distintas poblaciones fermentativas) en los perfiles de sabor y en compuestos de sabor específicos. Este objetivo se abordó en los Capítulos 6 y 7. En el Capítulo 6, se compararon diferentes tratamientos (7) de proceso natural, utilizando la misma materia prima.

Los tratamientos de proceso se diseñaron para variar la velocidad de secado en diferentes secciones de la curva de secado, a modo de favorecer la fermentación por distintos tipos de poblaciones microbianas. También se preparó un testigo de café lavado a partir de la misma materia prima. Las muestras resultantes se caracterizaron, utilizando Catación Descriptiva, por un panel de catación de Nueva Zelandia y otro panel de catación de México. Los resultados de ambos paneles se compararon utilizando MFA. En el Capítulo 7, las mismas muestras se caracterizaron por los métodos analíticos utilizados en los anteriores estudios (GC-MS/O y PTR-MS), y luego se integraron tanto los datos analíticos como los de Catación Descriptiva en un MFA.

En el Capítulo 3 se encontró que hay un efecto de los métodos de procesamiento en el sabor, ya que las muestras de café natural se caracterizaron por tener notas de frutas tropicales, frutas secas, frutas rojas o notas fermentadas. Las muestras lavadas mostraron notas florales, especiadas o de nueces, mientras que los despulpados naturales tendieron a tener un perfil de sabor que abarcaba desde el café natural hasta el lavado. En Capítulo 4, se encontró que el efecto del país de origen sobre el sabor del café fue más importante que el efecto del método de procesamiento. Esto se asoció con factores distribuidos geográficamente y que incluyen distintas tecnologías de secado. Los cafés naturales solamente pueden diferenciarse de manera clara de los cafés lavados cuando muestran un perfil tipo ‘Mocha’ (notas afrutadas o vinosas). Además, la ausencia o presencia del perfil ‘Mocha’ también resultó ser una de las principales fuentes de variación dentro de los mismos cafés naturales como grupo.

En el Capítulo 5, se encontró que los productos de la degradación de los azúcares y los productos de la reacción de Maillard tuvieron un papel importante en la diferenciación de las muestras. Sin embargo, los principales factores que explican al carácter tipo ‘Mocha’ se asociaron tanto con el catabolismo de aminoácidos (valina, isoleucina y leucina) como con la fermentación etílica. Los ésteres producidos mediante la esterificación de los ácidos 2- y 3-metilbutanoico se relacionaron con el carácter de fruta roja fresca (fresa, arándano agrio), que suele ser la descripción más común de los cafés naturales de especialidad.

Una comparación de los resultados de los dos paneles en el Capítulo 6 mostró una correlación satisfactoria entre ambos y una coherencia en el uso de descriptores tales como *fermentado*. En general, la Catación Descriptiva demostró ser un método para la perfilación rápida del

sabor del café con niveles satisfactorios de capacidad discriminativa y un nivel satisfactorio de consenso entre ambos paneles.

En los Capítulos 6 y 7, el tratamiento de proceso natural más diferente fue el llamado tratamiento ‘enmielado’ para propósitos de esta tesis, el cual involucró la suspensión del secado durante los primeros dos días. Esto resultó en una fermentación intensa, con alta producción de etanol, lo cual confirmó que el etanol juega un papel clave en la formación de los ésteres afrutados.

Esta investigación caracterizó al saber del café desde los puntos de vista sensorial y analítico. Los hallazgos de esta investigación contribuyen al campo de la cafeología al proveer a la industria y a los futuros investigadores de una explicación sobre la formación de los sabores afrutados y vinosos característicos del café natural. Basándose en los hallazgos de esta investigación, los productores de café podrán controlar mejor el sabor resultante de sus cafés naturales. Se requiere mayor investigación para caracterizar el papel de los componentes no-volátiles en el café natural, al igual que para entender el papel de tipos específicos de fermentación en el desarrollo del sabor de los cafés naturales. En general, se requiere mayor investigación para entender cómo controlar mejor el sabor resultante del café.

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Fernandez Alduenda, M.R., Lusk, K.A., Silcock P. & Birch, E.J. (2014). Descriptive Cupping: a rapid coffee flavour profiling method using the Specialty Coffee Association of America (SCAA) cupping protocol. Association for Science and Information on Coffee (ASIC) 2014 Conference. 8th – 13th of September, 2014, Armenia, Colombia. [Oral presentation].

Fernandez Alduenda, M.R., Silcock, P., Birch, E.J. & Lusk, K.A. (2014). Coffee fermentation volatiles: carry-over through roasting and their effect on brew flavour. 14th Weurman Flavour Research Symposium. 15th - 19th of September, 2014, Cambridge, UK. [Poster presentation].

Fernandez Alduenda, M.R., Birch, E.J., Silcock, P. and Lusk, K.A. (2013). Flavour formation in high-quality naturals: effect of fermentation in naturals flavour. 4th International Conference on Arabica Naturals, within the context of 8° Espaço Café Brasil – Semana Internacional do Café. 9th – 12th of September, 2013, Belo Horizonte, MG, Brazil. [Oral presentation].

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List of abbreviations and symbols

AEDA – Aroma Extract Dilution Analysis

ACE – Alliance for Coffee Excellence

ANOVA – Analysis of Variance

a_W – Water activity

CA – Correspondence Analysis

CAFECOL – Centro Agroecológico del Café A.C (Mexico)

cfu – Colony-forming units

CHARM – Combined Hedonic Aroma response Measurement

CQI – Coffee Quality Institute

DRBC – Dichloran Rose-Bengal Chloramphenicol

F1, F2, F3… - Factor 1, Factor 2, Factor 3…

FSCM – Finger Span Cross Modality

GC – Gas chromatography

GC-MS – Gas chromatography-mass spectrometry

GC-O – Gas chromatography-olfactometry

GC-MS/O – Gas chromatography-mass spectrometry/olfactometry

HS-SPE – Headspace-Solid Phase Extraction

ICAN – International Conference on Arabica Naturals

ICE – Intercontinental Exchange

ICO – International Coffee Organisation

KI – Kovats Retention Index

KIT – The Royal Tropical Institute (Netherlands)

MFA – Multiple Factor Analysis

m/z – mass/charge

NCA – National Coffee Association of USA

NSCA – Non-symmetric Correspondence Analysis

PCA – Principal Component Analysis

PTR-MS – Proton Transfer Reaction-Mass Spectrometry

PTR-ToF-MS – Proton Transfer Reaction-Time of Flight-Mass Spectrometry

RV – Regression Vector coefficient

SCAA – Specialty Coffee Association of America

SCAE – Specialty Coffee Association of Europe

SCAP – Specialty Coffee Association of Panama

SH-GC – Static headspace gas chromatography

SMEPS – Small Micro Enterprise Promotion Service (Republic of Yemen)

SNIF – Surface Nasal Impact Frequency

SPE – Solid Phase Extraction

SPE-GC-MS/O – Solid Phase Extraction- Gas chromatography- mass spectrometry-olfactometry

TDS – Temporal Dominance of Sensations

UFLA – Universidade Federal de Lavras (Brazil)

VOC – Volatile Organic Compounds

χ^2 – Chi square

1 Introduction

Coffee is one of the most important agricultural products in the international market. Each year, about 8 million metric tons of green coffee beans, worth around US $15 billion ($1.5 \cdot 10^{10}$) give rise to a retail coffee value of around US $150 billion ($1.5 \cdot 10^{11}$) worldwide (Schwan & Fleet, 2014). However, coffee is not all the same and coffee does not all have the same value. From the low-quality Robusta coffee that is the raw material for mainstream instant coffee at US$1.92 per kg (ICE, 2015) to the most expensive, award-winning, Panamanian natural geisha varietal at US$771.47 per kg (Stoneworks & SCAP, 2013), the value of the green coffee bean can vary by a factor of over 400. The reason for such disparity is the existence of a flourishing specialty coffee industry within the broader, generic coffee industry. The specialty coffee industry is growing fast, with consumers willing to pay a premium for high-quality coffees. According to the National Coffee Association of USA (NCA), the daily consumption of specialty coffee beverages increased from 9% of U.S. adults in 2000 to 34% in 2014, and the value of the specialty coffee share represented 51% of the total coffee market value in the USA in 2014 (NCA, 2014). This means the yearly retail value of specialty coffee is about US$16 billion ($1.6 \cdot 10^{10}$) in USA alone (SCAA, 2012b).

Yet what is specialty coffee? Opinions differ. For the purpose of the cited market value study from NCA (2014), specialty coffee was defined as a category including espresso-based beverages, iced/ice blended coffee and premium whole bean and ground varieties. For the Specialty Coffee Association of Europe, specialty coffee is defined as a *"crafted quality coffee-based beverage, which is judged by the consumer* […] *to have a unique quality, a distinct taste and personality different from, and superior to, the common coffee beverages offered. The beverage is based on beans that have been grown in an accurately defined area, and which meet the highest standards for green coffee and for its roasting, storage and brewing"* (SCAE, 2014). Similarly, for the Specialty Coffee Association of America (SCAA), specialty coffee is defined as *"the highest-quality green coffee beans roasted to their greatest flavor potential by true craftspeople and then properly brewed to well-established SCAA developed standards"* (SCAA, 2012a). In short, the specialty coffee industry pursues an ideal of excellence in coffee.

The specialty coffee industry has self-explained the history of coffee from a highly commoditised industry in the 20th Century to an increasingly differentiated industry today

using the concept of the 'Three Waves of Coffee History'. This concept was explained by Pulitzer Prize winning food critic Jonathan Gold as follows:

> *"The first wave of American coffee culture was probably the 19th-century surge that put Folgers on every table* [or Gregg's or Nescafé in other countries – the initial commoditised, mainstream stage], *and the second was the proliferation, starting in the 1960s at Peet's and moving smartly through the Starbucks grande decaf latte, of espresso drinks and regionally labeled coffee. We are now in the third wave of coffee connoisseurship, where beans are sourced from farms instead of countries, roasting is about bringing out rather than incinerating the unique characteristics of each bean, and the flavor is clean and hard and pure" (Gold, 2008).*

In other words, the specialty coffee industry is currently experiencing the rise of the 'Third Wave of Coffee', which implies *"letting the coffee speak for itself"* (Cho, 2005). In an analogous way to how the wine market evolved in the 20th Century, the specialty coffee market is experiencing a growing interest in the product's story: its country, region and even farm of origin, the way it was produced (organic, bird-friendly, shade-grown, etc.) and the processing it underwent at the origin (Cho, 2005).

But what does 'coffee processing at the origin' mean? Many consumers do not know that coffee undergoes a long process at the origin, before it is shipped to the consuming countries as green beans. The fresh coffee fruit, also called the 'coffee cherry' needs to be converted into the dry, green coffee beans. The fleshy parts of the fruit need to be removed and the beans need to be dried. That process can be done in a number of different ways, and the specialty coffee industry in general and the 'Third Wave' coffee industry in particular are aware that the coffee processing at origin can have a large impact on the final coffee flavour. The type of coffee processing at the origin is becoming increasingly important as a source of differentiation for specialty coffees at all levels of the coffee industry, from the farm to retail (Davids, 2014).

More and more coffee producers around the world are seeking to differentiate their products in the specialty market. Since it would be almost impossible for a farmer to move the farm to a different geographical area and it would be very costly to replace the current coffee varieties in a given farm, the main path left for producers to differentiate their products is through innovative processing. In the last ten years, innovative producers and the specialty coffee industry in general have been focusing more and more on the processing at the origin as a means to impact coffee flavour (Davids, 2014). The following quote illustrates the current

enthusiasm of the specialty coffee industry for the trend of focusing on processing at the origin:

> *"[…] as a younger generation of coffee producers, roasters and importers has discovered, variations in processing that are carefully and mindfully pursued can alter the sensory properties of coffee in amazing and exciting ways" (Davids, 2014).*

What are these 'processing methods'? The following chapters will discuss them extensively. Summarising, for the purpose of this introduction, in the fruit, the beans are covered by a fleshy tissue called 'mucilage' and surrounded by the fruit pulp and skin which need to be removed and the beans dried. One possible way to isolate and dry the beans is by drying the whole fruit like a prune and then removing the beans from the dried flesh – this is the so-called 'natural' process. An alternative pathway is by mechanically pulping the beans, next removing the mucilage and washing away the mucilage residues and lastly drying the washed beans – this method is called 'washed process'. A third way to remove and dry the beans is by pulping them to remove the fruit pulp and then drying them still covered by the mucilage prior to hulling, which is called 'pulped natural process' or, more recently, 'honey process' (Brando & Brando, 2014). In this thesis, the term 'honeying' treatment refers exclusively to a particular technique for processing natural coffees, and should not be confused with pulped natural coffee.

The natural process is particularly interesting for a number of reasons. Historically, it was the first process used and remained the only existing one until the 19th Century, when it began being displaced by the washed process. For the entire 20th Century, it was considered a low-quality, unreliable process. Yet in the last ten years, the specialty coffee industry has rediscovered the natural process, since it is attractive from the differentiation and sensory characteristics of the product points of view and from the sustainability perspective, (Diaz Pineda, 2008).

From the differentiation point of view, specialty natural coffees – also called 'New Naturals' so as to distinguish them from the mainstream natural coffee – are sought for their flavour. What is so distinctive about the flavour of natural coffees for the specialty coffee industry? A *"fruity flavour profile"* (Dupont, Törsäter, Engel, & Thomsen, 2007); a *"clean brandy-like fruit character, lightly fermented but free of molds. The sweet, lush aroma of blueberry in particular has become associated with these creatively fermented coffees"* (Davids, 2010); a

"distinct taste, which can be very exotic and complex; spicy, fruity, aromatic and may be slightly fermented" (Madsen, 2011). Overall, the high-end specialty coffee industry, rediscovering the natural coffees in the last few years, has called them *"funky (this can both be good and bad) and some could certainly be described as trendy"* (Madsen, 2011).

From the sensory point of view, natural coffee is a key ingredient in high-quality espresso blends (Brando & Brando, 2014; Illy & Viani, 2005; Violoni, 2010). From the sustainability point of view, the natural coffee has a very low water use compared to the washed process, where the organic matter-rich waste pollutes the waterways. In the last three decades, the level of water pollution due to coffee washing stations has raised increasing concern at origin countries (Illy & Viani, 2005). Since most natural coffee is sun-dried, the process does not involve CO_2 emissions, which are common in the case of machine-dried washed coffees.

However, science is a few years behind the pace of the coffee industry in this case. Since the natural coffees were undervalued for over one hundred years by the industry, they were also neglected by science. They were judged *"of poorer quality than washed coffee"* (Vincent, 1987), as *"mediocre grades, and there is always a serious risk of getting a very poor quality"* (Sivetz & Desrosier, 1963), and as a *"total reject"* (Puerta Quintero, 1999). In the last decade, though, natural coffees have begun to be the target of scientific research. There are currently two main research teams working with natural coffees from different points of view. The team at the Institute of Plant Biology of Technical University Braunschweig (Germany) has focused on the coffee bean metabolism during processing and how it affects the flavour precursors, whereas the team at the Universidade Federal de Lavras (Brazil) has looked at technological, microbiological, analytical and quality aspects of natural coffees.

Yet, to the best of our knowledge, many questions about the flavour of natural coffee remain unaddressed or only partially addressed by research. What are the differences in the flavour profiles of washed, pulped natural and natural coffees? What is the range of flavour profiles that can be obtained with natural coffees? Is there a characteristic flavour of natural coffees? Are there flavour profiles that can only be obtained with natural coffees? What are the compounds responsible for the characteristic natural coffee flavour? How are these compounds formed during the natural processing? How can the processing be controlled so as to obtain a particular flavour profile outcome?

The aim of this thesis was to begin answering those questions, in the understanding that science, like the specialty coffee industry, is only beginning to rediscover natural coffees. The approach to answer these questions focused, as Sunarharum, Williams, and Smyth (2014) suggest, in linking the components of coffee flavour to the sensory attributes of the cup. To achieve this, different sets of coffee samples were characterised by instrumental analysis of the coffee bean headspace, but the interpretation of the analytical results was based on the sensory characterisation of the samples, using a novel sensory method called 'Descriptive Cupping'. Descriptive Cupping involves the categorisation and statistical analysis (χ^2 and CA) of descriptive comments freely-elicited by expert tasters. Different natural process treatments were also investigated, in order to understand how the flavour is affected by processing parameters, as the drying rate of natural coffees can have a potential impact on the types of the microflora present and their growth. The aim and objectives of this research are further detailed in section 2.2.

While the present research has characterised the effect of processing on the flavour of natural coffees, further research is needed in a number of areas. The scope of this thesis did not involve understanding the role of non-volatile constituents of natural coffee or understanding the role of specific fermentation types in the development of natural coffee flavour. Generally speaking, further research is needed to understand how to better control the flavour outcome of natural coffees.

2 Literature review and objectives

2.1 Literature review

2.1.1 What is natural coffee?

2.1.1.1 Definition of natural coffee

Coffee is the name given to the product made from the seeds – called coffee beans – of the botanical genus *Coffea.* There are many ways to consume coffee, but almost all of them involve brewing a beverage from the roasted coffee beans (Belitz, Grosch, & Schieberle, 2009). The coffee beverage has become a very popular product. In 2013, 145,202,000 bags (60kg) of green coffee were produced in the world (ICO, 2014). Assuming an average of 6.6g of roasted coffee beans per coffee cup of 120mL, this production implies a world coffee consumption of approximately 3 billion cups ($3 \cdot 10^9$) every day.

Although there are several species of the genus *Coffea*, there are two species of commercial importance: *C. arabica* L. and *C. canephora* (Tarzia, Scholz, & Petkowicz, 2010). *Coffea arabica* or Arabica coffee is the most important species in terms of the volume produced and the value per kg (ICO, 2012). Even though beans from both species may be blended at the last step in production in consuming countries, consumers are usually not aware of what species they are drinking. In fact both species are too far apart in terms of genetics, production areas, trading markets, chemistry and flavour to be treated as a single product in a study. Therefore, unless specified otherwise, 'coffee' means Arabica coffee from here onwards.

The coffee tree bears a fruit called the coffee cherry. Coffee products are made from the beans inside that fruit. The different processing technologies are based on the anatomy of the coffee cherry and how the outer layers of the cherry are removed in order to obtain the coffee beans. Figure 2.1 shows a diagram of the coffee cherry. The outermost layer (exocarp) is essentially the fruit skin, which may be red or yellow when fully ripe, depending on the variety. The next layer (mesocarp) is composed by the fleshy pulp and mucilage. Inside the fruit, normally two seeds are found (the endosperm). Each of the seeds, called the coffee bean, contains an embryo, and each seed is covered by the spermoderm (called silverskin) and is surrounded by an endocarp called parchment (Avallone et al., 2000; Brando & Brando, 2014).

The process coffee undergoes between the harvest and the final product is long and involves a number of actors and facilities in both producing and consuming countries. The coffee is

shipped from the origin to the consuming countries in the form of 'green coffee'. This is the name given to the raw coffee bean, which needs to be roasted, ground and brewed – if not further industrialised – in order to prepare the coffee beverage. However, the process between harvest and the production of green coffee is itself long enough to be carried out usually in two separate facilities in most producing countries. The first stage of the process is usually carried out near the production site, while the second stage – called sometimes 'curing' – is usually performed at a more centralised location, prior to exporting and involves hulling and sorting the coffee beans (Vincent, 1987). From here onwards, unless specified otherwise, the words 'process' and 'post-harvest process' refer to the first stage of processing, prior to curing.

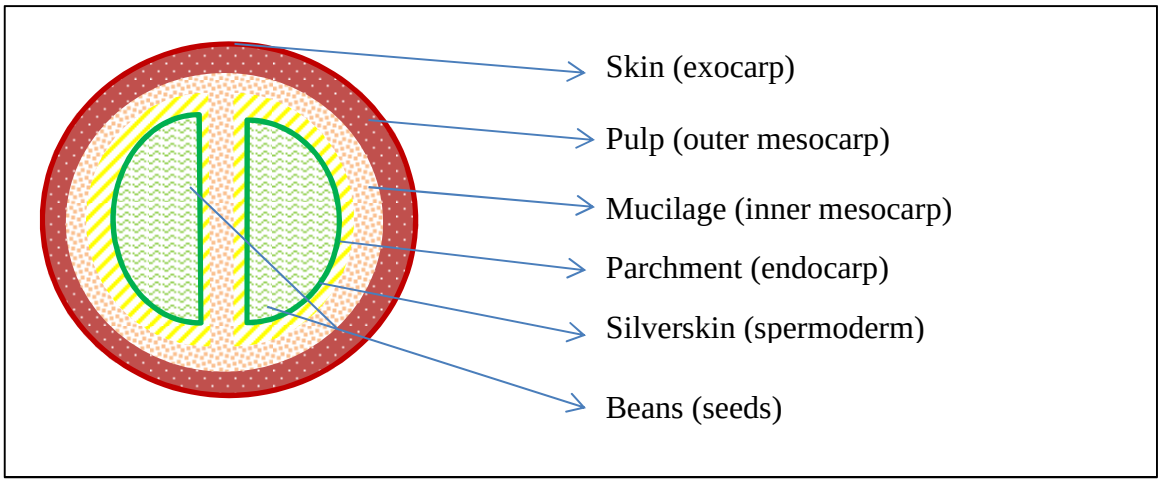


Figure 2.1. Anatomy of a coffee cherry. Diagram adapted from Avallone, Guiraud, Guyot, Olguin, and Brillouet (2000).

Until the 1980's, two different processing methods were used for the first post-harvest stage. One is called the dry method, while the other one is called the wet method. In the 'dry method', the whole coffee cherry is dried after being harvested until the beans attain around 12% moisture. The intermediate product shipped to the curing facility is the dried coffee cherry, which is then hulled to remove the dried outer layers of the fruit and to obtain the green beans. The dry method is carried out without the need of any water, as opposed to the 'wet method'. In the wet method, water is used for removing the skin, pulp and mucilage from the beans. The pulping operation removes the skin and pulp. Then the beans are either fermented or mechanically demucilised, and washed to remove the mucilage. The washed

beans are then dried to obtain parchment coffee. Parchment coffee is the intermediate product, which is hulled at the curing facility in order to remove the parchment and to obtain the green beans. A third method, increasingly popular since the 1990's, is the 'pulped natural' method. This process is half way between the dry and the wet methods, whereby the pulp is removed right after harvest and then the coffee is dried in contact with its mucilage (Brando & Bagersh, 2010; Brando & Brando, 2014; Tarzia et al., 2010).

An analogy to the grape wine processes can be made here to further become familiar with the differences between the coffee processing methods. For producing red wines, the product (the grape juice in this case) is left in contact with the grape skin to macerate; the skin imparts certain characters to the product, namely colour, astringency and body. For producing white wines, the skins and other plant parts are removed from the product and only the grape juice is processed – this allows for white wines to be made even from red grapes. Rosé wines are an intermediate product – the juice is left in contact with the skins only for a short period; therefore, their character is in between the two other processes. Rosé wines have a lighter body than red wines but are more astringent than white wines (Jackson, 2008; Ribereau-Gayon, Dubourdieu, Doneche, & Lonvaud, 2006). In an analogous way, in the processing of natural coffees, the product is left in contact with the mucilage, pulp and skin for a long time after the harvest; this imparts a specific character, namely a heavier body, to the resulting product (see the section about the flavour of the different processes in 2.1.2.2). In the process of the washed coffees, all the fruit layers are removed from the product as soon as possible, in order to avoid for their character to be imparted to the final flavour. The resulting washed coffees are lighter and 'brighter', so to speak, then their natural counterparts. The pulped natural coffees are an intermediate product, in which the skin and pulp are removed, but the product is left in contact with the mucilage. The character of pulped natural coffees is also between the natural and the washed coffees. Thus, natural coffees are analogous to red wines; washed coffees, to white wines, and pulped natural coffees, to rosé wines.

Natural coffee can now defined as the name given in many countries to coffee that was processed through the 'dry method' (Brando & Brando, 2014). The term 'natural' most likely comes from the fact that, in most cases, 'natural' drying is used – meaning sun drying, as opposed to machine drying (personal communication from Bruno Souza). The dry method is contrasted in Table 2.1 with the other two methods. From here onwards, the words 'natural',

'washed' and 'pulped natural' will be used preferably to other names, as these are the terms most used in the specialty coffee industry.

Table 2.1. Summary of the three main coffee processing methods.

Process name	**Dry process**	**Wet process**	**Pulped natural process**
Raw material	Fresh coffee cherry		
Main operations involved	Drying	Pulping, fermentation (or demucilation), washing, drying	Pulping, drying.
Process output	Dried cherry	Parchment coffee	Pulped natural parchment
Product name	Natural coffee	Washed coffee	Pulped natural coffee
Synonyms	Unwashed coffee Dry-process coffee Sundried coffee	Wet process coffee Full-washed coffee	Honey coffee Semi-washed coffee Semi-dry process coffee

2.1.1.2 Historical context of natural coffee

The history of natural coffees has moved through three stages. The first stage lasted from the discovery of coffee and its establishment as a crop to the invention of the washed process in the 19th Century. This was the stage when coffee was brought to Asian and American countries as a crop and the stage when coffee consumption in Western countries developed (Topik, 2000). The wet method was probably invented in Jamaica in the first half of the 19th Century, following the Industrial Revolution. The wet method became a significant innovation that quickly spread to many countries (Ukers, 1922). Until then, and since the discovery of coffee, the only method available was the dry process. La Roque – cited by Ukers (1922) – described this process in 1715 following a trip to Yemen. The main difference between the process described by La Roque and the patio sun-drying of natural coffee today is the fact that the coffee was not harvested in the ripe stage, but was allowed to shrivel on the tree prior to harvest and sun-drying. In that time, perceptions about flavour and quality were not linked to any processing method, but to the country of origin. The most famous and prized coffee 'brand' was Mocha. Mocha was the name of a port in Yemen, exporting almost all of the world's coffee production between the 15th and the 17th Centuries. The term 'Mocha' later became identified with the coffees showing that profile (Haggam, 2012).

The second stage, between the invention of the washed process in the 19th Century (Ukers, 1922) and the first recognition of naturals by the specialty coffee industry in the first ten years of the 21st Century, involved a displacement of natural coffees by washed coffees in most producing countries. The reasons for this were mainly cost and time efficiency, together with product consistency: the drying of the beans needs less energy if the outer layers of the cherry have been previously removed through pulping and washing (Ukers, 1922). However, as the washed process became the norm in most coffee producing countries, natural coffees became more and more marginalised. They were produced by farmers who could not afford drying technology or who did not perform selective picking. Most often, natural coffees were and still are produced from coffee cherries harvested by nonselective picking, implying different maturation stages are dried together without prior sorting, which results in a product of a poor and uneven quality (Brando & Brando, 2014). Furthermore, whole dried coffee cherries can also be a by-product of the wet method. This happens because in many wet mills (the processing stations for washed coffee), some of the cherries are rejected prior to pulping because they are hollow and some other cherries cannot be pulped using the machines they have because those cherries are too unripe or are already dry. The rejected cherries are then dried separately from the washed coffee and marketed as 'natural' coffee. The quality of these unwashed by-products of the wet mill is usually very poor, and this is one of the main reasons why many people in the industry regard naturals in general as a low quality product (Diaz Pineda, 2008). The poor quality of most naturals created a perception of the natural process as a 'poor quality process' during the whole 20th Century. The perception about natural coffees along the 20th Century is best illustrated by three quotes from three coffee industry authorities during that century:

> *"Generally speaking, washed coffees will always command a premium over coffees dried in the pulp" (Ukers, 1922)*
>
> *"[…] the best natural coffee is of a different character, but, by and large, about equal in quality to the best washed coffee grown under the same conditions. In general, it is harder to obtain the best quality of natural coffee. Too many conditions, difficult to control, tend to result in mediocre grades, and there is always a serious risk of getting a very poor quality". (Sivetz & Desrosier, 1963).*
>
> *"Under ideal conditions of dry weather, absence of green cherries in the picking, freedom from infection, sun drying for the first stages, and machine drying for the last stages, natural coffee may be of excellent quality, clean tasting and full bodied and, while different, fully as desirable as washed coffee. But the ideal conditions for drying*

> *natural coffee are nearly always difficult or impossible to realize, and most natural coffee is, to varying degrees, inferior to its washed coffee counterpart" (Vincent, 1987).*

These views implied that natural coffee may be of high quality but it requires a higher degree of control along the processing to achieve that quality. That level of control was difficult to achieve in the 20th Century because the specialty coffee industry only started developing in the last decade of the century, and only the specialty coffee industry can pay the premiums required to cover the cost of increased quality control. However, in the last few years, there has been a clear trend in the specialty coffee market favouring the now-called 'New Naturals'. The term 'New Naturals' first appeared around 2007 in trade journals and blogs, as a way to differentiate between the low-quality naturals that are oftentimes a by-product of the washed process and high-quality naturals that *"take as much care in the making of the natural coffee as they do with the washed coffee"* (Dupont et al., 2007). In 2008, the International Conference of Arabica Naturals (ICAN) was held for the first time in Acapulco, Mexico. The ICAN has had three more editions since the first one (Sana'a, Yemen, 2010; Addis Ababa, Ethiopia, 2012, and Belo Horizonte, Brazil, 2013), gathering stakeholders from the research institutions, government bodies, coffee producers and other members of the coffee industry, in order to understand natural coffees, improve their quality and create awareness about their importance. At the first ICAN, it was proposed to avoid applying the term *natural* to the low-quality by-products (Diaz Pineda, 2008).

Since that time, the growth of the New Naturals segment in the speciality coffee market has become a very clear trend. Producers have begun experimenting with naturals even in countries where they had been clearly banned, such as Colombia (natural Colombian coffee samples were part of the study in Chapter 4). Some reviewers are praising these naturals from regions that did not produce naturals traditionally and calling them 'New Naturals' (Davids, 2010). Although natural coffees have traditionally been seen as having a lower quality than washed coffees and thus usually fetch lower prices, some New Naturals are fetching very high prices. Moreover, even though the international prices for commodity coffee have been falling in the last few years and were around $2.64 USD per kg in 2013 (ICE, 2013), the New Naturals have been breaking price records every year (Fernandez Alduenda, 2012). In 2013, a natural from Panama (geisha varietal) fetched $771.48 USD per kg, probably the highest price ever paid to any green coffee (Stoneworks & SCAP, 2013).

2.1.1.3 The technology for producing natural coffee

Since the natural coffee process essentially implies just drying the whole coffee cherry, cherries of varying quality and ripeness can be processed as natural coffee. This is the main challenge for controlling and maintaining quality of natural coffees, as in many cases a poorly harvested raw material is just dried without any previous cleaning or sorting. The first critical operation in the processing of natural coffee is harvesting. The degree of ripeness of the raw material is key to obtaining a high quality cup flavour, as most flavour precursors, such as sugars and chlorogenic acids are dependent on the degree of ripeness (Amorim et al., 2009). There are three main methods of coffee harvesting: selective harvesting, which involves hand-picking the ripe cherries; stripping, which means pulling all the cherries from a branch, irrespective of their degree of ripeness, and mechanical harvest, which use handheld harvesters or large harvesters on wheels in a few regions in the world (Brando & Brando, 2014). Specialty natural coffees usually come from selectively-picked cherries, as the other two harvesting methods reduce the mean degree of ripeness of the raw material and may introduce foreign matter.

After harvesting, two optional operations (winnowing and separation of cherries) are helpful to improve the final product quality. Winnowing involves removing dust, sticks and other impurities by screening the cherries and is mostly carried out when selective picking has not been performed. Flotation is used to separate the partially dried and hollow cherries which float from the ripe and unripe cherries which sink in water. The final product becomes more uniform if the cherries are separated (Brando & Brando, 2014).

The technology used for drying the coffee cherry is of the lowest possible level. The most widely used method is simply to spread out the coffee cherries on the ground and dry them under the sun (Vincent, 1987). This is the reason why in some African countries naturals are also called ‘sundried’ coffees. The ground surface may be made of different materials: concrete, brick, stone and earth. The usual practice is to lay the coffee on the drying ground in the morning, stir it a few times along the day to even out drying and then to pick it up and bag it again in the evening, repeating this until the coffee attains around 12% moisture, which can take from under a week to four weeks, depending on a number of factors. In some places, raised beds are used for drying the coffee cherries, which avoids contact with the ground, allows the air to pass under the bed and simplifies manipulation. The materials used for raised

beds are usually vegetable fibre mats, plastic nets or mesh wire. The material used has an effect on the product quality (Berhanu, Ali, & Tesfaye, 2012). Another widely accepted innovation is the use of plastic greenhouses, drying huts or simply a rigid plastic cover over the raised beds. These facilities provide protection against the rain and drying huts can speed up the drying process by rising the ambient temperature and lowering the relative humidity (Oliveros-Tascón & Sanz-Uribe, 2011; VijayaVenkataRaman, Iniyan, & Goic, 2012), though sun drying is largely depending on the weather, even with drying huts. The main process parameter that can be controlled is the thickness of the coffee layer. Thinner layers increase the drying rate, as the average cherry is closer to the drying activity of the air and the sun rays, but the surface required for drying is largely increased. For example, a layer of 50-60mm of thickness takes 30-40kg of fresh cherries per m^2 of yard (Illy & Viani, 2005). A layer thickness of 30-40mm was suggested in the 1980's for avoiding over-fermentation (Vincent, 1987).

A common practice, for example, in Ethiopia is mixed drying (cherries from different days) with loads of around $40kg \cdot m^{-2}$. This has been found to result in poor quality (as assessed by SCAA cupping) when compared to thin-layer drying of cherries from a single harvesting day (Berhanu et al., 2012).

Illy and Viani (2005) recommend turning the cherries constantly during sun-drying (15-17 times per day), in order to remove the external moisture and avoid fermentation. They also recommend heaping the coffee every evening: up to 5-10cm height in the first few days, which can be increased until the coffee reaches 20-30% moisture. From this point onwards, they recommend heaping the coffee every evening and covering it with cotton or waxed fabric, in order to facilitate the moisture equilibration.

In some places – namely in Brazil – and especially under severe weather conditions mechanical drying is used for natural coffees. Washed coffees, on the other hand, are largely dried mechanically because the amount of energy required to dry the parchment is about half the energy required to dry the whole cherry. The most popular machine for drying coffee is a rotary drum dryer called 'Guardiola', after the person who invented it in Guatemala in the 19th Century, José Guardiola. Over the last 20 years, improvements have been made to the design of Guardiolas in order to increase efficiency and improve heat distribution (Brando & Brando, 2014; Vincent, 1987).

Once the whole cherry is dry, around 12% moisture, it can be hulled at the curing station in order to obtain the beans (Tarzia et al., 2010). However, in practice several months pass between drying and hulling and, in the case of Yemen, for instance, it is not uncommon to hull the cherries up to ten years after the harvest (SMEPS & KIT, 2009). It is preferred to store the beans as the dry cherry as they are believed to be more stable. In many cases, storage of up to one year in the dry cherry form is claimed to actually improve the flavour (Diaz Pineda, 2008). The main reason for storing the coffee is economic: either waiting for the prices to go up or as family cashable savings.

Hulling the dry cherries is not as easy as hulling the parchment coffee. The best-quality cherries are particularly tough to hull, because the husks are more hygroscopic and thus become a gummy paste inside the huller. Ironically, that is the reason some hulling (curing) stations actually prefer lower quality dried-cherries. Some stations prefer to dry the cherries for some hours prior to hulling, in order to make the skin a little bit more brittle (personal communication from H.M. Díaz Pineda).

2.1.1.4 Importance of natural coffee

About one third of the world's coffee is natural Arabica coffee (38.9 million 60kg-bags in 2008). Of the Arabica coffee, about 48% is natural (Diaz Pineda, 2008; ICO, 2008). Brazil, Ethiopia and Yemen are considered 'traditional' producing countries of Arabica natural coffees since a large proportion of their coffee output is processed as natural (Brando & Brando, 2014; Diaz Pineda, 2008). In the case of Yemen, for instance, 100% of its coffee output is Arabica natural coffee (Ezzi, 2008; Haggam, 2012). Nevertheless, by far the largest producer is Brazil, with about 84.2% of the world's Arabica natural coffee in 2008. Other countries that reported having produced Arabica natural coffee in 2008 to the International Coffee Organisation were Ethiopia (8.8%), Indonesia (1.9%), Ecuador (1.2%), Mexico (1.2%), Honduras (1.0%), India (0.8%) and Yemen (0.5%) (Diaz Pineda, 2008; ICO, 2008).

In recent years, natural coffees have received more attention from the industry and the specialty coffee industry in particular. There are three main reasons for that. The first reason is that the top-end specialty coffee industry is finding high quality naturals very interesting as a means to diversify the range of coffee flavours offered to consumers. A quote from Davids (2010), a specialty coffee reviewer and journalist, summarises this new trend:

> *"These 'new naturals' as we are calling them here, have become the darlings of many taste-leading small roasting companies and are the focus of informal but intense experimentation by some producers aimed at figuring out how to dry the fruit-encased coffee so that the fruit character is imparted to the beans with none of the nasty side effects (mold, excessive ferment) created by less-than-perfect drying and less-than perfectly ripe, healthy coffee fruit" (Davids, 2010).*

A second reason is that natural coffees are a key ingredient of espresso blends due to their body and sweetness. Since the espresso consumption in the world has been increasing, natural coffees enjoy an increasing demand, especially for high-quality blends (Brando & Brando, 2014; Illy & Viani, 2005; Violoni, 2010).

A third reason is sustainability: compared to the washed process, where the organic matter-rich waste pollutes the waterways, the natural process does not use water. Even if water is used for separating cherries by flotation, the pollution level is negligible, because the cherry does not transfer organic matter to the water (Illy & Viani, 2005). The dried coffee pulp produced as a by-product of natural coffees can be readily used in animal nutrition and biotechnological processes (Dias, Rodríguez-Valencia, Zambrano-Franco, & López-Núñez, 2014).

As an indication of the growing importance of naturals in the last years, the Alliance for Coffee Excellence (ACE), which organises the most important quality competitions and auctions of high-end coffee worldwide (The Cup of Excellence®), created the first auction for natural coffees in Brazil in 2012 (ACE, 2012). Another indication of the trend is the inclusion in the 'Q-Grader Exam' (the most important coffee taster certification program worldwide, organized by the Coffee Quality Institute – CQI) of a mandatory, natural coffee tasting test (CQI, 2014).

2.1.2 Coffee flavour and the main factors impacting it

Flavour remains the most important parameter for coffee consumers (Mori et al., 2008). However, coffee flavour is one of the most complex flavours known, which is why research efforts have been made to understand it for over a century (Sunarharum et al., 2014). More than 1000 volatiles have been identified in roasted coffee, of which at least 70 are considered potent odorants (Michishita et al., 2010). Coffee volatiles belong to many different chemical classes: hydrocarbons, alcohols, aldehydes, ketones, acids and anhydrides, esters, lactones,

phenols, furans and pyrans, thiophenes, pyrroles, oxazoles, thiazoles, pyridines, pyrazines, amines, miscellaneous nitrogen compounds, and miscellaneous sulphur compounds (Flament & Bessière-Thomas, 2002). Besides these volatile compounds affecting aroma and the olfactory component of flavour, other non-volatile compounds also influence the taste component of coffee flavour (Flament & Bessière-Thomas, 2002; Sunarharum et al., 2014). Important non-volatile compounds in roasted coffee that play a role in its organoleptic properties are trigonelline (together with nicotinic acid and methylnicotinamide), proteins and peptides that have not been degraded, polysaccharides, melanoidins, carboxylic acids, chlorogenic acids (including cinnamic, caffeic, ferulic, isoferulic, sinapic and quinic acids), lipids and minerals (including phosphoric acid) (Buffo & Cardelli-Freire, 2004).

Being so complex, coffee flavour is affected by many factors. Pre-harvest factors include genotype and phenotype of the coffee tree, geographical factors (soil, climate), farming practices, harvesting method and cherry maturity. Post-harvest factors include primary processing methods (which may include pulping, fermentation and drying – see Table 2.1), secondary processing methods (roasting, grinding) and brewing (Sunarharum et al., 2014).

Genetic differences in coffee begin at the species level. The two coffee species with commercial importance are *Coffea canephora* (known as 'Robusta') and *Coffea arabica* L. Both species show marked differences in flavour (Bicho, Lidon, Ramalho, & Leitao, 2013; Sanz, Maeztu, Zapelena, Bello, & Cid, 2002). The scope of the present research is limited to *C. arabica* only. Within the *C. arabica* species, there is a large number of varieties, and each variety has a different flavour expression (Chalfoun et al., 2013; Leroy et al., 2006; Pereira, Chalfoun, de Carvalho, & Savian, 2010).

The geographical factors (integrating the so-called 'terroir') and the farming practices also influence deeply the coffee flavour. This has been acknowledged by the coffee industry since the 18th Century, as the emphasis on sourcing coffee from specific origins demonstrates (Haggam, 2012; Ukers, 1922). The influence of terroir and farming practices on coffee flavour is very complex, but some of the main factors have been studied, such as climate (Bertrand et al., 2012; Joët et al., 2010) and shading (Bosselmann et al., 2009).

2.1.2.1 Effect of roasting on coffee flavour

Pre-harvest factors being equal, the single post-harvest operation that has a largest effect on coffee flavour is roasting. Roasting transforms the pea-like, vegetable odour of green coffee beans into the characteristic smell of coffee (Czerny & Grosch, 2000). The chemical reactions that occur during roasting have not been completely explained (Buffo & Cardelli-Freire, 2004). However, it is clear the main pathways are the Maillard reaction and the Strecker degradation, which have found to be the cause of at least 500 of coffee volatile compounds (Flament & Bessière-Thomas, 2002). Other reaction pathways occurring during roasting are breakdown of sulphur amino acids, breakdown of hydroxyl-amino acids, breakdown of proline and hydroxyproline that react with intermediate Maillard products, degradation of trigonelline, degradation of the quinic acid moiety, degradation of pigments and degradation of lipids (Buffo & Cardelli-Freire, 2004). The degree of roast, the roasting technology used and the roasting curve all influence the final coffee flavour. However, the roast degree that best expresses the region and process-related differences is the medium roast (Sunarharum et al., 2014).

2.1.2.2 Effect of processing method on coffee flavour

In coffee from the same species, the processing method of the coffee cherry (primary process) is second only to roasting as the main post-harvest factor influencing coffee flavour. Depending on the method used, coffee will have a different flavour. The difference in flavour between washed and natural coffees has been summarised by Selmar, Kleinwächter, and Bytof (2014), who say washed coffees are characterised by *"their full aroma and pleasant acidity"*, whereas the corresponding natural coffees present a *"so-called full body"*. This has been explained as a difference in the flavour precursors found in the bean after each process. The effect of the main processing coffee methods on the coffee components is summarised in Table 2.2. Reducing sugars and free amino acids are highest in natural coffees, while total sugars, chlorogenic acids and trigonelline are highest in washed coffees (Arruda et al., 2012; Knopp, Bytof, & Selmar, 2006). Actually, the free amino acid composition has proved to be different for each case, showing evidence of a different metabolism of the coffee bean during each kind of processing (Bytof, Knopp, Schieberle, Teutsch, & Selmar, 2005). A different amino acid and reducing sugar composition would influence the production of different Maillard derivatives. The yield of water-soluble polysaccharides and total extracted

polysaccharides in green coffee has also been observed to be different (highest in washed coffee, intermediate in pulped natural coffee, lowest in natural coffee). These differences in extracted polysaccharides continue in the coffee brew, where also the extracted protein content is highest for washed coffees. However, the perceived weaker body in the case of washed coffee brews could be due to a lower concentration of the mannan fraction in washed coffee brews (Tarzia et al., 2010). When comparing only washed versus pulped natural coffees, washed coffee showed higher levels of chlorogenic acids and trigonelline, but a lower concentration of sucrose (Duarte, Pereira, & Farah, 2010).

Table 2.2. Summary of the effects of the washed and natural processing methods on coffee components. Adapted from Selmar et al. (2014).

Component	Effect of processing method	References
Trigonelline	Slightly lower in washed.	Duarte et al. (2010)
Chlorogenic acids (CA)	Differences in total CA, individual CA and subgroups of CA.	Duarte et al. (2010); Balyaya and Clifford (1995); Joët et al. (2010).
Free amino acids	In naturals, glutamic acid is converted to γ-aminobutyric acid.	Bytof et al. (2005).
Bioactive amines	Differences in spermine, spermidine and putrescine.	Dias et al. (2012).
Sugars	In washed coffees, glucose and fructose are considerably reduced.	Knopp et al. (2006).
Polysaccharides	In washed coffees, polysaccharides are more easily extracted.	Joët et al. (2010); Tarzia et al. (2010).
Lipids	Increase during washed process.	Joët et al. (2010).

In all the primary processing methods (washed, natural and pulped natural), studies show there is some degree of fermentation of the layers surrounding the coffee bean (Avallone, Guyot, Brillouet, Olguin, & Guiraud, 2001; Schwan & Wheals, 2003; Silva, 2014; Silva, Batista, Abreu, Dias, & Schwan, 2008; Vilela, Pereira, Silva, Batista, & Schwan, 2010). These studies have focused on the identification of the microorganisms taking part in coffee fermentation. The effect of fermentation on the coffee flavour has mostly been studied for washed coffees (Alemán-Serra, 2008; Avallone, Brillouet, Guyot, Olguin, & Guiraud, 2002; Gonzalez-Rios et al., 2007a). In washed coffees, the effect of inoculation of lactic bacteria prior to fermentation has been studied, showing a larger concentration of lactic and acetic acids in the green coffee bean (Alemán-Serra, 2008) but no significant difference in the sensory attributes (Avallone et al., 2002). Other studies have compared the two main fermentation methods for washed coffees: fermentation under water vs. 'dry' fermentation (Gonzalez-Rios et al., 2007a, 2007b). Fermentation under water was characterised by higher

ester content, along with floral and caramel flavour notes. However, these last studies rely on the odour description of individual compounds found through GC-MS/O for the interpretation of their results, and do not study the flavour of the actual coffee matrix.

The effect of fermentation on natural coffee flavour has received little attention compared to the case of washed coffees. Previous, not peer-reviewed research, suggested fermentation affects the development of fruitiness and wineyness of naturals (Diaz Pineda & Fernandez Alduenda, 2007a). More recently, the effect of four different yeast strain inocula on natural coffee flavour was assessed in Lavras (Brazil), finding an influence of the yeast strain used on the resulting coffee flavour. The coffee inoculated with yeast showed higher flavour intensity than the control coffee. A strain of *Candida parapsilosis* (UFLA YCN448) and a strain of *Saccharomyces cerevisiae* (UFLA YCN727) showed the potential to produce caramelly, herby and fruity flavour notes. The study also suggested that washing the cherries prior to drying may have a positive effect on quality (Evangelista et al., 2014).

Drying is another operation that has been found to impact coffee flavour, regardless of the primary process method employed. However, the effect of drying is quite different in each case. Washed coffees have a very active bean metabolism on the first day of drying and that is the reason the monosaccharide levels of washed coffee beans are almost depleted, compared to natural coffees (Kleinwachter & Selmar, 2010). In washed coffee, using solar drying instead of mechanical drying produces a heavier body (due to higher lipid ester levels in the brew) and higher flavour intensity (due to a higher ester and aldehyde concentration in the brew). This has been explained as the effect of solar radiation on the pulp remnants on the surface of the bean (Lyman, Benck, & Merle, 2011). However, the effect of solar radiation has been contested recently by Selmar et al. (2014), who proposed the main cause for the difference between sun drying and mechanical drying is that during sun drying the temperature cycles daily from high daytime temperatures to lower night-time temperatures (Kleinwachter & Selmar, 2010). Many studies compare the effect of different drying temperatures on coffee beans from either processing method from different perspectives: mathematical simulation of washed coffee drying (Nilnont et al., 2011), quality indicators (Borem, Coradi, Saath, & Oliveira, 2008; Coradi, Borem, & Oliveira, 2008; Coradi, Borém, Saath, & Marques, 2007) or microscopic cell structure (Saath et al., 2010). Almost all of these studies agree that low-temperature drying produces better bean quality. Some studies have

looked at the effect of other drying parameters on quality scores: effect of rest periods of up to 30 days during machine drying, at different humidity levels of natural coffee (Isquierdo, Borém, Oliveira, Siqueira, & Alves, 2012) or the effect of location, sun drying methods, variety and cherry drying layer thickness on the quality scores of natural coffee (Berhanu et al., 2012).

2.1.3 The flavour of natural coffee compared to other processing methods

Natural coffees have several flavour features that make them different to washed coffees. However, until very recently, there seemed to be no agreement on the characteristic flavour of natural coffees. Moreover, the concepts of flavour character and coffee quality are often confused in the literature. The industry has always acknowledged the quality of naturals such as Yemeni Mocha and Ethiopian Harrar, though not always acknowledging them as natural coffees (Ukers, 1922). Nevertheless, those 'Mocha' flavours were acknowledged as coming from the dry process, which was considered 'inferior' (Ukers, 1922). As the second half of the 20th Century passed, some authors accepted natural coffees could be *"fully as desirable as washed coffee"*, even though this was *"nearly always difficult or impossible to realize"* (Sivetz & Desrosier, 1963; Vincent, 1987). In the late 1980's, descriptive analysis was used for the first time to compare green coffee profiles (Feria-Morales, 1989; ICO, 1991). Previous research from 1995 concluded that a high-quality natural from a traditional natural coffee producing region in Mexico was significantly different to high-quality washed Mexican coffees; it had more fruitiness, more nuttiness, more floral intensity and more wineyness than washed Mexican coffees, but less intensity of acidity (Fernandez Alduenda, 1995).

Unfortunately, an influential paper from those years concluded that natural coffee had a completely undesirable off-flavour, without having tested a representative natural coffee (Puerta Quintero, 1999). In that study, the 'cup quality' of different treatments (8) was assessed by a panel of expert cuppers, but none of the dry-method treatments were characteristic of a true natural: one of them employed unripe cherries only, while the other treatment was dried at 105°C. That study has been cited directly (Bytof et al., 2005; Gonzalez-Rios et al., 2007a, 2007b) and indirectly (Kleinwachter & Selmar, 2010; Kramer, Breitenstein, Kleinwachter, & Selmar, 2010; Tarzia et al., 2010) in order to support the statement that both natural and washed coffees differ in the cup quality, without going into detail over the sense of such a difference. Oftentimes, chemists, biochemists and

microbiologists have looked at the flavour of natural coffee (usually comparing it to washed coffee) from their own disciplines without linking their findings to any sensory study describing those flavour perceptions. One paper from 2010, for example, claims *"coffee produced by the wet method* [washed] *has less body and higher acidity; it is also more aromatic than coffee produced by the dry method* [natural]*, resulting in a higher acceptance by consumers"* without citing the source or justification for such statement (Tarzia et al., 2010). This statement contrasts with the results from previous, non peer-reviewed research, which involved cupping samples from different processing methods and which suggested the aroma of natural coffee is actually more intense than the aroma of washed coffee (Fernandez Alduenda, Diaz Pineda, Sierra Martinez, & Hernandez Martinez, 2010).

Selmar et al. (2014) explained that the previous, biased view of naturals as a low-quality product class may have been due to an unfair judgement of cup quality, since the natural coffees may have come from unripe raw material, while the washed coffees were harvested and prepared according to good practices. They propose that a fair comparison between washed and natural coffees can only be made if *"(1) comparable starting material is used; and (2) the conditions chosen are suitable to produce good-quality coffee with either way of processing"* (Selmar et al., 2014). Their research has extensively clarified the effect of the main processing methods on the flavour precursors of coffee (2.1.2). Nevertheless, the link between those differences in the contents of the coffee components and the actual differences in flavour has not been fully established by their research group, possibly due to a focus on the seed biochemistry as opposed to the product sensory characters.

Studies using sensory analysis, on the other hand, either overlook naturals (Bertrand et al., 2012; Bhumiratana, Adhikari, & Chambers IV, 2011; Oberthür et al., 2011) or do not report the processing method used for producing the coffees being analysed (Ribeiro, Ferreira, & Salva, 2011; Seo, Lee, & Hwang, 2009). Very few studies have focused on the characteristic flavour of naturals or the flavour differences between the main processes, using sensory techniques. A previous, non peer-reviewed research (Fernandez Alduenda et al., 2010) focused on the sensory differences, assessed by cupping, of samples processed by the three main methods and coming from the same farms. An analysis of the comments elicited by the cuppers suggested naturals showed a much broader range of aroma notes than washed and pulped natural coffees. Aroma notes specific to naturals were identified, belonging to the

following categories or 'subgroups': tropical fruits, berries, caramels, dried fruits, spices and taints. From the cupping scores point of view, data suggested natural coffees show better Fragrance/Aroma scores and better Body scores.

The researchers of coffee flavour usually have focused on instrumental analysis. Many coffee flavour studies describe the smell of isolated coffee odorants through olfactometry, without any sensory analysis on the coffee matrix (Akiyama et al., 2007; Akiyama et al., 2009; Gonzalez-Rios et al., 2007a). Even though numerous studies correlate the sensory attributes of coffee to specific compounds (Lindinger et al., 2008; Lindinger et al., 2006; Mayer, Czerny, & Grosch, 2000; Ribeiro, Augusto, Salva, Thomaziello, & Ferreira, 2009; Sanz et al., 2002; Violoni, 2010) or to other instrumental analysis not targeting specific compounds (Esteban-Díez, González-Sáiz, & Pizarro, 2004; Ribeiro et al., 2011), still more research is needed so as to link the characteristic sensory attributes of coffee to instrumental analyses and to the factors behind those characters. This gap was correctly identified by Sunarharum et al. (2014). Their insight needs to be fully quoted below:

> *"Knowledge on the chemical composition of coffee flavor is important, but reliable measurement and ranking of aroma components in coffee in the absence of good quality sensory information cannot effectively describe the importance, or the nature of contribution, of individual or groups of flavor components in coffee. Further, the coffee matrix itself interacts with volatiles and has a large impact on the perceived flavor assessed through a sensory study. Thus, matching or creating a comprehensive link on all components of coffee flavor and sensory quality will lead to a deeper understanding of coffee flavor. For example elucidating what compounds cause the nutty, cocoa, caramel, fruity, or 'coffee-type' flavor which can then be subsequently tracked back to individual processes involved in their formation" (Sunarharum et al., 2014).*

In the last few years, some coffee flavour studies have been approached in the way suggested by Sunarharum et al. (2014). Charles et al. (2015) combined sensory methods (Temporal Dominance of Sensations – TDS) and instrumental analysis (nosespace analysis via Proton Transfer Reaction-Time of Flight-Mass Spectrometry – PTR-ToF-MS) to investigate the impact of roasting degree and sugar addition on aroma release and perception in espresso coffee. In the case of natural coffees, the most relevant study in this respect investigated the effect on flavour of four yeast strains, inoculated on natural coffee prior to drying (Evangelista et al., 2014). The effect of the yeast strains was assessed through a combination of microbiological, analytical (organic acids and headspace analysis) and sensory methods

(SCAA cupping and temporal dominance of sensations – TDS). This last study is highly pertinent in the sense it is the first one to assess the effect of specific microbial populations on natural coffee flavour. Nevertheless, the design does not permit a comparison of the flavour of natural coffees versus the flavour of washed coffee or to describe the flavour of natural coffees as a product class.

Overall, there is a research gap in the characterisation of the natural coffee flavour from the sensory point of view, especially when considered independently to quality assessments. In which sense is the flavour of natural coffee different to the flavour of washed coffees? In a complex product as coffee, is there a 'natural coffee character' or is processing secondary to terroir as a character-generating factor in natural coffees? To the best of our knowledge, these questions have not been targeted before.

Furthermore, if there is such a thing as 'natural coffee character', how is it formed? Until recently, the most accepted hypothesis for the formation of the natural coffee flavour is the one proposed by Selmar, Bytof, and Knopp (2001): the flavour difference between different methods is due to *"metabolic reactions within the coffee seeds that differ markedly depending on the mode of post-harvest treatment"*. Yet evidence suggests that fermentation may also be playing an important role in the formation of natural coffee flavour (Diaz Pineda & Fernandez Alduenda, 2007a; Schwan & Wheals, 2003; Silva, 2014; Silva et al., 2008). Evangelista et al. (2014) have demonstrated different yeast strains inoculated on natural coffee will affect the brew flavour. However, for most of the natural coffee produced in the world, the final flavour just 'happens' – if a producer gets a particular desirable or undesirable flavour character one year, there is no guarantee the flavour will be achieved in the next year. This is due to the lack of knowledge of the basic flavour formation mechanisms in natural coffee. Therefore, there is another research gap in the identification of links between natural coffee processing treatments and flavour. How can specific target flavour profiles be achieved through processing? Or in other words, how do processing parameters such as drying rate affect the final cup flavour?

2.1.4 Methodologies for studying the flavour of coffee with focus on sensory interpretation

For the study of flavour in the cases of complex products such as coffee, the instrumental analysis needs to be interpreted using the human sensory perception as a guide. In this section, two approaches for gathering sensory data will be discussed. These two approaches are the sensory assessment of coffee and the olfactometry analysis, which is used for identifying odour-active compounds. Considerations about the headspace sampling methodology are also discussed.

2.1.4.1 Sensory approaches for studying coffee flavour

Descriptive sensory analysis has been the main method for studying coffee flavour from the sensory point of view. This approach involves the use of a trained sensory evaluation panel, the development of a descriptive vocabulary and the assessment of samples by rating the intensity of the sensory attributes. The International Coffee Organisation (ICO) started using descriptive sensory methods in the 1980's to develop a *"consumer orientated coffee descriptive vocabulary"* and influenced the use of this methodology in the following decades (Feria-Morales, 1989; Feria-Morales, 1993; Fernandez Alduenda, 1995; ICO, 1991). Other descriptive methodologies have been applied to coffee since the 1990's, such as Free Choice Profiling (Rubico & McDaniel, 1992) and similarity scaling (Williams & Arnold, 2008). Descriptive sensory methodologies are still used to study coffee flavour (Scholz, de Figueiredo, da Silva, & Kitzberger, 2013). However, as the cooling of the coffee brew is an important aspect of coffee consumption, other techniques have been applied in order to take into account the evolution of flavour perception over time. These techniques include Time Scanning Descriptive Analysis (modified descriptive analysis to assess an attribute every 10 seconds) (Seo, Lee, Jung, & Hwang, 2009), and Temporal Dominance of Sensations analysis (TDS)(Pineau et al., 2009), which requires the panellist to select the dominant sensation from a list at a given point in time every few seconds. TDS has been applied to coffee by Evangelista et al. (2014) and Charles et al. (2015).

Coffee is different to most generic food products and similar to other complex specialty products such as wine in the sense that its quality is usually assessed by expert tasters or quality graders. Coffee quality graders are called cuppers or cup tasters (Feria-Morales, 2002).

Even though the focus of cuppers is on quality grading, they still employ sensory assessment and are usually more experienced than descriptive analysis assessors. Therefore, coffee cuppers have been used to gather sensory data in numerous studies about coffee (Bertrand et al., 2012; Borém et al., 2013; Chalfoun et al., 2013; Evangelista et al., 2014; Feria-Morales, 1993; Oberthür et al., 2011; Ribeiro et al., 2011). However, the sensory data from coffee cuppers in the cited studies always refers to the perceived 'quality grade' of a coffee. Quality scores become a limitation when studying coffee flavour, as two coffees with very different flavour profiles can potentially have the same quality score. Therefore, the use of the quality score data from coffee cuppings as the only source of sensory information lacks the depth required for studying the complexity of coffee flavour and is potentially misleading.

However, this does not mean coffee cuppers cannot be used for acquiring reliable descriptive information. In the case of wine, for instance, expert tasters have been used to characterise the product using the free comments method. Lawrence et al. (2013) analysed the comments freely elicited by a panel of wine experts with a global Chi-square (χ^2), a χ^2 per cell test and a correspondence analysis (CA). Then they compared the results to classical profiling using multiple factor analysis (MFA) and found that both methods discriminated samples on a similar basis. It is pertinent to quote their conclusions below:

> *"The free comments method was advantageous for highlighting the specific characteristics of a number of products. This method is less time consuming and allows easy characterisation of wines. In conclusion, the free comments method may represent a convenient alternative to conventional descriptive analysis in a wine professional context or a convenient sensory mapping tool" (Lawrence et al., 2013).*

2.1.4.2 Headspace sampling

Flavour research has largely relied on the study of the volatile compounds in a food. The reason for this is that the sense of smell – which also plays a key role in flavour perception – is activated by volatile substances (Reineccius, 2005). Thus, for flavour and aroma analysis, headspace sampling has several advantages over the extraction of flavour compounds directly from the food matrix. The volatile compounds responsible for a product's odour are present in the headspace above the product and thus can be sampled from the headspace and analysed through gas chromatography (GC) without the need for purification.

The two main methods for headspace sampling are static headspace and dynamic headspace. In the static headspace technique, a food sample is placed in a vessel, which is closed with a Teflon®-lined inert septum. The vessel is incubated to allow the equilibration of VOCs between the food and the headspace. The headspace is then drawn into an air-tight syringe and injected into the GC. The main disadvantages of the static headspace technique are low sensitivity and difficulty for its application in quantitative studies (Reineccius, 2005). The advantage of the static headspace technique, it does not require the use of solvents, and thus the volatile compounds that elute during the GC solvent delay time can be analysed using this method. In coffee, up to 146 volatile compounds have been identified and semi-quantified using this technique (Sanz et al., 2002).

Dynamic headspace sampling methods involve stripping the headspace with a gas, followed by some type of concentration of the volatiles. In these methods, the sample headspace is purged with an inert gas (i.e. nitrogen or helium). The volatile compounds are then removed from the gas stream using different trapping techniques. Possible trapping systems include cryogenic methods, solid-phase microextraction (SPME) (Arthur & Pawliszyn, 1990), adsorptive polymers (i.e. Tenax®) and carbon-based molecular sieves (i.e. Carboxen® and charcoal). These systems favour the isolation of volatiles with a high vapour pressure, and the trapping method selected further influences the properties of the isolated compounds (Reineccius, 2005). An advantage of dynamic headspace sampling over static headspace sampling is that the adsorption of compounds is independent to equilibria between the food matrix and the headspace. Another advantage of these methods is that the volatile compounds can be directly injected in the GC by thermal desorption or can be transferred to a solvent. In coffee, for example, Michishita et al. (2010) purged the headspace of brewed espresso through a Tenax® and Carboxen® trap. Then they eluted the volatiles from the trap using diethyl ether and dichloromethane. The extracts were then concentrated prior to using them in GC-MS and GC-O analysis. The advantage of using solvent elution is that the resulting extracts can be stored and used for different, independent analyses (i.e. GC-O, GC-MS).

2.1.4.3 Gas chromatography-olfactometry (GC-O)

One of the most widely used techniques for the analysis of volatile compounds is GC. However, instrumental GC detectors do not provide information about the odour-activity or odour character of compounds. Furthermore, the human nose is more sensitive to some

compounds than even the most sensitive instrumental detector (Reineccius, 2005). These compounds can typically be termed odour-active compounds. GC coupled to olfactometry (GC-O) is a technique that allows the odour-active compounds to be identified through olfaction, as they elute through the GC column. An olfactometry port is a device which is coupled to the GC column and combines the eluted gas stream with humidified air, in order to increase the comfort of assessors and prevent their nostrils from desiccation (Acree, 1997). The assessors sniff the gas stream from the olfactometry port, which implies the assessor's nose is used as a GC detector. Part of the eluted flow can also be diverted to an instrumental detector, such as Mass Spectrometry (MS), in which case the technique is called GC-MS/O (Reineccius, 2005).

The output of GC-O is called an aromagram, which is analogous to a chromatogram. Aromagrams usually contain information about the GC retention time, the odour character of each odour-active peak and, depending on the GC-O technique used, information about the intensity of the peaks' odour or potency of the odour-active compounds (Reineccius, 2005). The main kinds of GC-O techniques used for analysing flavour volatiles are dilution to threshold, detection frequency and odour intensity techniques (Delahunty, Eyres, & Dufour, 2006).

The techniques based on dilution to perception threshold are useful for determining the threshold of the odour-active compounds. Two specific techniques of this kind are Aroma Extract Dilution Analysis (AEDA) and the Combined Hedonic Aroma response Measurement (CHARM). AEDA is a dilution analysis that requires the assessors to sniff the undiluted extract and a succession of diluted extracts until the targeted odour peak is no longer perceivable. The flavour dilution factor (the maximum dilution value detected) and the odour activity values (a measure of the odour potency) can be calculated (Acree, 1997). In coffee, AEDA has been used for screening potent odorants (Grosch, 1998). CHARM analysis, on the other hand, measures the duration of an odour perceived at each dilution. Therefore, CHARM values are the areas of those peaks in the aromagram (Acree, 1997). CHARM analysis has also been used in coffee to detect key odorants (Akiyama et al., 2008; Akiyama et al., 2007; Akiyama et al., 2009; Michishita et al., 2010). The main disadvantage of dilution techniques is that they require each assessor to run one sample at several dilutions, which usually makes these techniques more expensive and time consuming. The assessment of replicate samples is

thus usually not performed. Also, the number of assessors is limited, which implies a larger risk of underestimating an odour-active compound due to anosmia.

The detection frequency technique commonly used is the GC-Surface Nasal Impact Frequency (SNIF). This technique records the number of assessors who detect an odour-active compound at a specific retention time. It is used as a rapid method and an untrained panel can be used (Dussort et al., 2012), but it does not provide more information than the frequency of assessors giving a positive detection. The detection frequency can be correlated to intensity, but in cases of peaks with a 100% detection, intensity cannot be estimated (Delahunty et al., 2006).

Direct intensity techniques are based on the assessment of the intensity of an odour peak by assessors, who are required to rate the intensity using a scale. Intensity can be recorded as a single intensity for each peak or recorded continuously as a chromatography peak (Delahunty et al., 2006). Advantages of the odour intensity measurement techniques are that they include replicates, provide measures of intensity and provide odour character identification. The intensity of a peak may be measured using traditional ballot scales or through cross-modal devices, such as the Finger Span Cross Modality (FSCM) device. In FSCM, the assessors rate the intensity of each peak by sliding a bar using the breadth of their finger span. The use of the cross-modal assessment has the advantage that the estimation of intensity is instinctive, and thus less training is required to achieve reproducible intensity ratings (Delahunty et al., 2006; Niimi, Leus, Silcock, Hamid, & Bremer, 2010). In the University of Otago, a FSCM board was developed by SCL Ltd. (Dunedin, New Zealand). A rotating bar was incorporated, with intensity being related to the extent of axis rotation of the bar as the finger span increased (Niimi, 2009; Niimi et al., 2010).

2.2 Aim and objectives

2.2.1 Overall aim

The overall aim of this research was to understand the flavour of Arabica natural coffee as a product class, from the point of view of specialty coffee, and to relate that flavour to its main forming mechanisms during the dry post-harvest process. In order to achieve the overall aim, the following specific objectives have been identified to understand the effect of dry processing on the flavour generation of natural coffee.

2.2.2 Specific objectives

2.2.2.1 Objective 1 – To develop a rapid flavour-profiling method based on the analysis of coffee cupping (coffee tasting) data

In order to understand the sensory profile of coffee and to interpret the instrumental analysis results from the flavour point of view, a flavour profiling method was required. In line with the specialty coffee point of view of this thesis, a methodology (Descriptive Cupping) was developed for analysing the descriptive terms from the cuppers (coffee tasters), applying χ^2 tests and Correspondence Analysis on a descriptor frequency table. This method was applied in Chapters 3, 4 and 6. A reproducibility validation of the method using two independent panels was carried out in Chapter 6.

2.2.2.2 Objective 2 – To determine the effect of post-harvest processing methods (washed, natural and pulped natural) on the sensory profile of coffee

The effect of the main post-harvest processing methods on the flavour of coffee was investigated through two complementary approaches. In the first approach, described in Chapter 3, the region of origin was kept constant (the state of Guerrero, Mexico) and four different processing treatments (natural process, pulped natural process and two variations of the washed process) were applied on the same raw material, using farms (22) from the region for sampling. These samples were assessed in Mexico by a panel of cuppers (graders) accredited on the Specialty Coffee Association of America's (SCAA) cupping (tasting) protocol (SCAA, 2009a). The sensory data for this first study was collected from previous, unpublished research.

To further achieve Objective 2, Chapter 4 contrasted natural versus washed coffee samples coming from the same farm and harvest, from multiple coffee producing countries. The samples were received at Otago and assessed by a panel trained in the same cupping protocol (SCAA). The cupping panel was trained at the University of Otago and the international samples for the second study were cupped. The cupping data was analysed following the Descriptive Cupping methodology.

2.2.2.3 Objective 3 – To characterise the sensory profiles of coffee processed using natural post-harvest methods.

Going further into the specifics of the sensory profile of natural process, Objective 3 was intended to investigate the different flavour profiles shown by natural coffees from different origin countries. This was described in Chapter 4, Natural coffee samples received from different origins were assessed by the trained cupping panel at Otago, and resulting data analysed using the Descriptive Cupping methodology.

2.2.2.4 Objective 4 – To identify flavour compounds responsible for key flavours of coffee processed using natural post-harvest methods.

This objective is a necessary step between understanding the flavour of natural coffees from the sensory point of view and understanding the formation of that flavour during the dry post-harvest process. This was described in Chapter 5. The same samples profiled by Descriptive Cupping (Objective 3) were used, to link analytical and sensory results. Washed coffee samples were also included, in order to make a link with Objective 2. Headspace volatiles were analysed in the green and the roasted coffee. The headspace analysis methods used were gas chromatography coupled to mass spectrometry and olfactometry (GC-MS/O – from the roasted bean samples, using both static head-space and headspace extracts) and proton-transfer-reaction mass spectrometry (PTR-MS – from the green bean samples).

2.2.2.5 Objective 5 – To investigate the effect of different natural post-harvest processing treatments (by favouring different fermentative populations) on flavour profiles and specific flavour compounds.

Achieving this objective (Chapters 6 and 7) involved carrying out different fermentation and drying treatments on natural coffee in a producing region. Drying parameters (coffee layer width, drying medium) were controlled in order to influence the drying rate and thus the degree of wild fermentation of the coffee cherries at different process stages. The sizes of the main microbial populations (bacteria, yeasts, moulds) were monitored during drying in a separate experiment (Chapter 6). The samples produced by the different treatments were characterised using the same sensory and instrumental methods of the previous approaches: Descriptive Cupping (Chapter 6), GC-MS/O (roasted bean headspace) and PTR-MS (green bean headspace) (Chapter 7).

2.3 Synopsis of studies

Table 2.3 presents a synoptic view of the studies carried out in the present research. In order to address the 5 specific objectives (2.2), 3 sample sets were analysed using Descriptive Cupping (Chapters 3, 4 and 6). Instrumental analyses were carried out on 2 of the sample sets (Chapters 5 and 7). Chapter 8 presents a general discussion integrating all studies,

Table 2.3. Synoptic table of the studies carried out in the present research.

Sample set	Analyses carried out	Thesis chapter	Objectives addressed
56 samples - 22 natural - 19 washed - 15 pulped natural From Mexican state of Guerrero	Descriptive Cupping - Mexico (Guerrero state) Q-Grader panel	Chapter 3	Objective 1 Objective 2
32 samples - 22 natural - 9 washed - 1 pulped natural From 7 producing countries.	Descriptive Cupping - New Zealand trained panel	Chapter 4	Objective 2 Objective 3
	- PTR-MS - SPE-GC-MS/O - SH-GC-MS/O	Chapter 5	Objective 4
15 samples - 7 natural treatments in duplicate - 1 washed sample From a single raw material batch (Mexican farm).	Descriptive Cupping - New Zealand trained panel - Mexico (CAFECOL) Q-Grader panel	Chapter 6	Objective 1 Objective 5
	- PTR-MS - SPE-GC-MS/O - SH-GC-MS/O	Chapter 7	Objective 5

All the studies were conducted directly by the PhD candidate. The experimental designs, methodology selection and data analyses were carried out personally by the candidate, with guidance from his supervisors. The samples used for Chapter 3 were prepared by a team of technicians from the Guerrero Coffee Board, following the protocol designed by the candidate. The samples used for Chapter 6 were cupped independently by a laboratory in Mexico, in order to compare results with what was obtained in New Zealand. Asides from this, all experiments and tests were carried out by the candidate, with the help of the many people mentioned in the Acknowledgements section.

3 Effect of processing method on quality and sensory profile of coffee from a single Mexican state, using Descriptive Cupping by Q-Graders

3.1 Introduction

3.1.1 Expert cuppers as a descriptive tool

Conventional descriptive sensory analysis methods have been described as "*the most sophisticated tools in the arsenal of the sensory scientist"* (Lawless & Heymann, 2010). Unfortunately, training and maintenance of a descriptive sensory panel is costly. Similar to wine, coffee has the advantage of the expert tasters. There are currently three main coffee taster certificates operating internationally: the Intercontinental Exchange Inc. (ICE) 'Licensed Grader' (ICE, 2012), the Specialty Coffee Association of America (SCAA) Coffee Taster Certificate (SCAA, 2013) and the Coffee Quality Institute (CQI) 'Licensed Q-Grader' certificate (CQI, 2013a). The most relevant one in terms of number of graders certified and number of countries participating is the CQI's Licensed Q-Grader program. More than 2700 Q-Graders worldwide in 61 countries are certified (CQI, 2013b, 2013c). Licensed Q Graders evaluate coffee quality according to an industry standard but share some characteristics with selected and trained assessors due to the features of the training and certification procedures. Q-Graders are required to pass taste and olfactory acuity tests. They are trained in a common coffee descriptor vocabulary (CQI, 2013a). For example, fragrance and aroma descriptor training is based on the system by Lenoir and Guermonprez (1997), which uses 36 external references and classifies coffee bouquet notes in four groups of three subgroups each (Table 3.3). Evaluation of green coffee follows the SCAA Cupping Protocol (SCAA, 2009a) that is based on Lingle's cupping method (Lingle, 1986). The method requires Q Graders to base their scores on the attributes' perceived intensity and qualitative descriptions.

While cupping has been used to measure coffee quality, (Amorim et al., 2009; Barbosa et al., 2012; Berhanu et al., 2012; Evangelista et al., 2014; Marsh, Yusianto, & Mawardi, 2011; Ribeiro et al., 2011; Rodriguez, Frank, & Yamamoto, 1969), descriptive information generated by Q-Graders has not been used to describe the underlying differences in flavour among coffees.

The concept of 'specialty coffee', on the other hand, is relevant from the perspective of this research, since natural coffees are recently receiving more attention only because they are starting to be valued by the specialty coffee market (see Chapters 1 and 2). This specialty

coffee approach has also influenced the choice of SCAA cupping (SCAA, 2009a) as a sensory tool in this research.

Therefore, the first aim of the present chapter was to analyse and interpret data from previous, unpublished research, applying a cupping data analysis methodology ('Descriptive Cupping') developed during the current research (*Objective 1* – 2.2.2.1).

3.1.2 Coffee processing methods

Although the washed process is the most widely practiced for Arabica coffees throughout the world (Diaz Pineda, 2008), the natural process is currently receiving interest from the specialty coffee industry (Davids, 2010, 2013). The coffee industry has acknowledged for many years that coffee resulting from different processes will have a different flavour (Sivetz & Desrosier, 1963; Ukers, 1922; Vincent, 1987). However, there is limited agreement on the characteristic flavours in the cup that define natural, washed and pulped-natural coffees. Natural coffees from a traditional natural coffee producing region in Mexico were described as more fruity, nutty, winey and floral, and less acidic, compared to washed coffees (Fernandez Alduenda, 1995) and Brazilian naturals have been described as having intense body and aroma, mild acidity and sweet taste (Mori et al., 2008). Sometimes, the term 'Mocha' is used to describe the character of some naturals, implying the presence of desirable fermented, fruity flavour notes (Akiyama et al., 2008; Ukers, 1922). However, natural coffees have also been described as *"the worst treatment"* and having a *"reject"* quality (Puerta Quintero, 1999). Recent coffee studies investigating coffee flavour either do not include naturals in the sample set (Bertrand et al., 2012; Bhumiratana et al., 2011; Oberthür et al., 2011) or do not report the post-harvest processing method used to prepare the samples (Ribeiro et al., 2011; Seo, Lee, & Hwang, 2009). A better understanding of the effect that processing method has on coffee flavour is needed.

Therefore, the second aim of the present chapter was to determine the effect of post-harvest processing treatments on the sensory profile of coffee (*Objective 2* – 2.2.2.2). These treatments were natural process, pulped natural process and two variations of the washed process (fermentation under water and dry fermentation), which are abbreviated in this study as N, PN, Wh and Wd, respectively.

3.2 Materials and methods

3.2.1 Samples

3.2.1.1 Geographical origin

Coffee (*Coffea arabica*) cherries were harvested (2008-2009 crop) from farms (22) across four different regions of the state of Guerrero, Mexico: Zihuatanejo, Las Mesas, Atoyac and La Montaña (Figure 3.1). Sampling was stratified according to producing regions (four strata – one stratum per region) and the number of farms sampled in each stratum was proportional to each region's coffee output. Table 3.1 lists the samples (56) included in the present study (19 washed, 15 pulped natural, 22 natural).

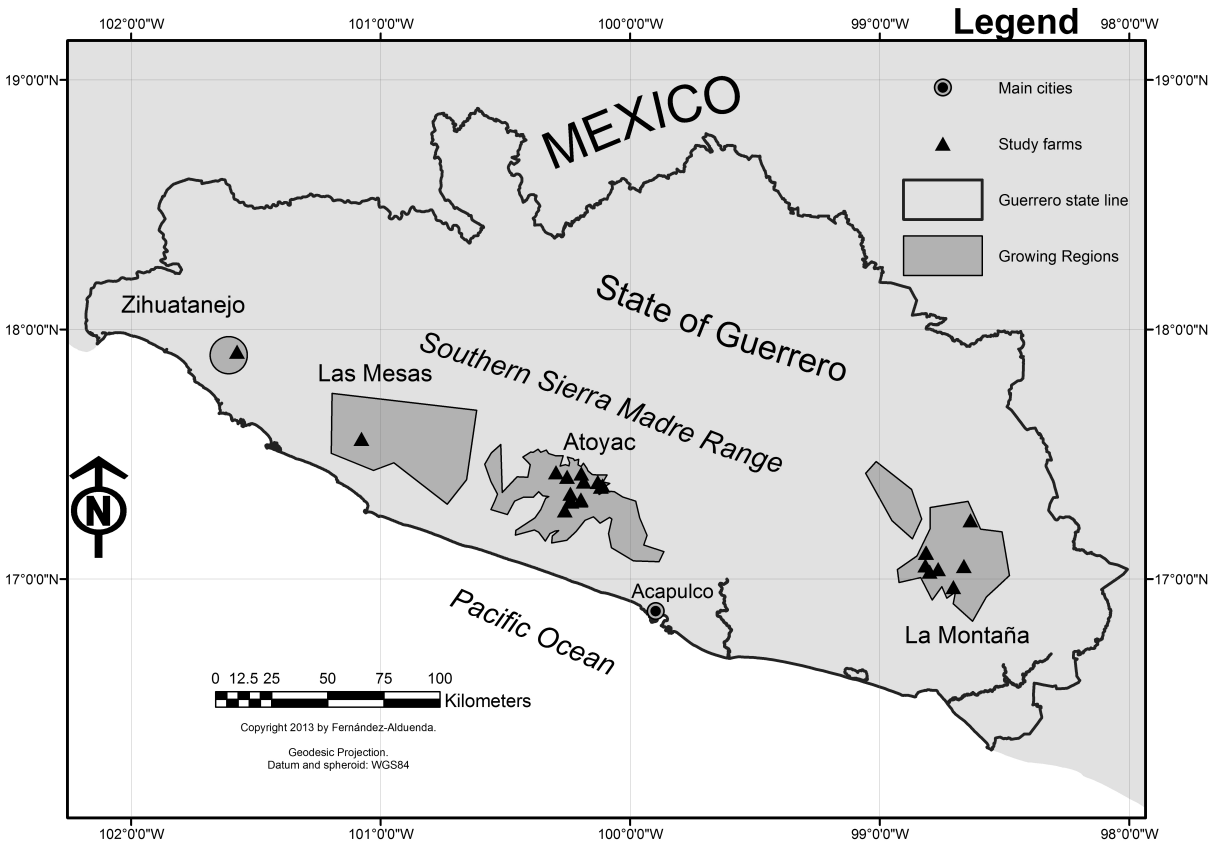


Figure 3.1. Coffee producing regions of the state of Guerrero, Mexico and location of sampled farms

Table 3.1. Sample identification by region, altitude, variety and processing method.

Sample ID (Farm code plus processing code) [a]	Region	Altitude (m)	Variety
2N	Atoyac	1583	Typica
2Wd		1583	Typica
2Wh		1583	Typica
3N		1683	Red Bourbon
3Wd		1683	Red Bourbon
3Wh		1683	Red Bourbon
5N		1393	Typica
5Wd		1393	Typica
12N		960	Yellow Bourbon
12PN		960	Yellow Bourbon
7N		986	Typica
7PN		986	Typica
7Wd		986	Typica
7Wh		986	Typica
9N		1450	Typica
9Wh		1450	Typica
15N		1265	Typica
15PN		1265	Typica
15Wh		1265	Typica
16N		1173	Typica
16Wh		1173	Typica
18N		1382	Typica
18Wh		1382	Typica
21N		1280	Typica
21PN		1280	Typica
21Wd		1280	Typica
21Wh		1280	Typica
24N		1121	Caturra
24PN		1121	Caturra
26N		1048	Typica
26Wd		1048	Typica
26Wh		1048	Typica
29N		941	Red Bourbon
29PN		941	Red Bourbon
36N	La Montaña	1366	Typica
36PN		1366	Typica
36Wh		1366	Typica

Sample ID (Farm code plus processing code) [a]	Region	Altitude (m)	Variety
51N		1980	Typica
51PN		1980	Typica
52N		1234	Typica
52PN		1234	Typica
53N		1198	Typica
53PN		1198	Typica
53Wd		1198	Typica
54N		1095	Mundo Novo
54PN		1095	Mundo Novo
55N		1297	Mundo Novo
55PN		1297	Mundo Novo
60N		1074	Caturra
60PN		1074	Caturra
57N	Las Mesas	1003	Typica
57PN		1003	Typica
57Wd		1003	Typica
49N	Zihuatanejo	1457	Garnica
49PN		1457	Garnica
49Wh		1457	Garnica

(a) Each sample ID is composed of the farm code and one or two letters indicating the processing method: Wh – washed with fermentation under water; Wd – washed with dry fermentation; PN – pulped-natural; N – natural.

3.2.1.2 Post-Harvest Processing

Coffee cherries from each farm were hand-picked. This was to ensure the cherries were from the same variety and a minimum of 90% of cherries were ripe. Harvested cherries were divided into four portions and each portion underwent one of the following four treatments.

Washed with fermentation under water (Wh). The coffee cherries were pulped, removing the skin (exocarp) and pulp (outer mesocarp) layers but leaving the bean covered by the parchment (endocarp) and the mucilage (inner mesocarp) layers (see Figure 2.1). The resulting beans were placed in a vat and submersed in water, and the low-density beans that float were removed. The denser beans were drained, submerged in a vat of fresh water and fermented until the mucilage loosened and was ready to be removed (estimated by feel). The fermented beans were washed until the mucilage was completely removed. The washed beans were sun dried in a thin layer (2-3 cm), which was turned up four times during the warmer

hours of the day (10:00-14:00). Sun drying continued for 6-10 days until beans attained 11% moisture.

Washed with dry fermentation (Wd). The same procedure as Wh was followed, except no water was added to the vats during fermentation. Instead, the beans were left to ferment in the vats without water until the mucilage was able to be removed and then washed.

Pulped-natural (PN). The same procedure as Wh was followed except the coffee was sun dried (4-12 days) directly after pulping with the mucilage intact.

Natural (N). Whole coffee cherries were placed in a vat, submersed in water and floating cherries discarded. The remaining cherries were sun dried for up to 14 days. For the first 2 days of drying, the cherries were stacked in 5cm layers and turned gently twice a day. From the third day, the layer was reduced to 1-2cm and turned four times per day, until the cherries attained 11 % moisture.

A total of 88 coffee samples were produced (22 farms x 4 post-harvest processes). However, for logistical reasons, some samples were not cupped or were not cupped by all cuppers. Therefore, only samples (56) tasted by all cuppers were included in this study.

3.2.1.3 Sample roasting

Samples were roasted and cupped in the cupping laboratory at the 'Universidad Autónoma de Guerrero', Chilpancingo (Guerrero), Mexico. Each sample was first hulled, then roasted according to the SCAA Cupping Protocols (SCAA, 2009a). Samples were roasted for 8-12 minutes to achieve a light to light-medium degree of roast using a FincaLab® coffee roaster (Cafés Sustentables de México S. de R.L. de C.V., Tepic, Mexico). All samples were roasted the day prior to being cupped.

3.2.1.4 Sample brewing

The SCAA Cupping Protocols (SCAA, 2009a) were followed for the grinding, brewing and serving of samples. Samples were ground so that 70-75% of the particles could pass through a 0.841mm sieve aperture (US standard size 20 mesh). Coffee to water ratio was kept at 8.25g of coffee per 150mL water. For each sample, five cups were used, which were ground and brewed individually. The solids contents in the brewing water were verified to meet the

protocol specification of 125-175ppm. Brewing started at a water temperature of 93°C, and the brew was left to steep for 3-5min prior to breaking the crust.

3.2.2 Cuppers

The cupping panel was composed of Licensed Q Graders (2 males, 1 female, mean age 38 years) from Mexico. The panel size is in accordance with the Q Coffee certification system, which states that a panel of three licensed Q Graders is needed to test and cup a coffee sample (CQI, 2013d). Similar numbers of cuppers have been reported by Oliveira et al. (2013) (2 cuppers), Berhanu et al. (2012) and Rodriguez et al. (1969) (3 cuppers), and Barbosa et al. (2012) (4 cuppers). Cuppers had an average of 13 years of experience in the coffee industry and all regularly cupped both natural and washed coffees from the state of Guerrero for at least 2 years prior to the study.

3.2.3 Flavour profiling of samples by Descriptive Cupping

3.2.3.1 Cupping procedure

Samples were cupped over a total of nine days during April, May and August, 2009. All samples were coded with three digit numbers and cupped in a random order. Samples were presented in cupping sessions with 6 samples per session. Up to 5 sessions were presented each evaluation day. At the beginning of each cupping period, cuppers 'calibrated' for scale use through a mock cupping, followed by a discussion.

Samples were assessed using the SCAA Cupping Protocol (SCAA, 2009a), in which 10 'attributes' are scored along three steps, using 5 cups per sample (Table 3.2): (1) assessment of fragrance of the dry coffee grounds, brewing of the cups and assessment of aroma of the brew; (2) tasting of the brew when its temperature is between 60°C and 70°C, and (3) tasting of the brew as it approaches room temperature.

Table 3.2. Attributes, definitions, step of assessment and type of scales rated as part of SCAA cupping protocol (SCAA, 2009a).

Attribute or concept rated	Definition	Cupping step when it is assessed	Type of scale	Other data produced
Fragrance/aroma	The aromatic (orthonasal) aspects of the coffee grounds and brew	1	6 to 10, with 0.25-point intervals.	Fragrance intensity (5-point scale), aroma intensity (5-point scale), fragrance and aroma qualities (descriptors).
Flavour	Combined impression of all the gustatory sensations and retro nasal perceptions	2		Flavour descriptors.
Aftertaste	Flavour emanating from the back of the palate and remaining after the coffee is expelled or swallowed			Aftertaste descriptors.
Acidity	Sourness of the brew			Acidity intensity (4-point scale), acidity quality (descriptors).
Body	Mouthfeel of the brew			Body intensity (4-point scale), body quality (descriptors).
Balance	How flavour, aftertaste, acidity and body of the sample work together and complement or contrast to each other			-
Uniformity	Consistency of flavour across the different cups of the sample tasted	3	0 to 10, with each of the 5 cups worth 2 points (if attribute present) or 0 (if absent).	-
Clean cup	Lack of interfering negative impressions from first ingestion to final aftertaste			-
Sweetness	Fullness of flavour as well as any obvious sweetness			-
Overall	Holistically integrated rating of the sample as perceived by the individual cupper		6 to 10, with 0.25-point intervals.	General remarks

Attribute or concept rated	Definition	Cupping step when it is assessed	Type of scale	Other data produced
Total score	The sum of individual scores for attributes above.	3	-	-
Defects	Negative or poor flavours that detract from the quality of the coffee		0 to -20. 2 or 4 negative points for each defective cup, depending on intensity.	Type of defect.
Final score	Total score minus defects		-	-

In the first step, within 15 minutes after samples were ground, cuppers sniffed the coffee grounds in each cup; the odour of the dry grounds is called 'fragrance'. Next, the coffee cups were brewed by pouring hot water to each one. This creates a 'dome' or 'crust' of wet coffee grounds on the surface of the cup. The smell of the brew is termed aroma and it was assessed at two time points: immediately after pouring water and 3-5 minutes after the cups were poured, when the crust was broken with the use of a spoon. The fragrance intensity, fragrance quality, aroma intensity and aroma quality are all taken into account to score the 'fragrance/aroma' attribute. This attribute is rated on a 6-10 point structured scale with 0.25 point intervals (Table 3.2).

In the second step, the foam is skimmed from the cup. When the brew cools to 60-70°C, it is slurped vigorously using a spoon. The brew is held in the mouth until the attributes are assessed and then expelled from the mouth. At this point flavour, aftertaste, acidity, body and balance are assessed. These attributes are also rated on a 6-10 point scale (Table 3.2).

In the third step, the brew is slurped and tasted once or twice more as it approaches 32°C. Uniformity, clean cup, sweetness, overall and defects are assessed at this point. Only overall is rated on a 6-10 point scale. The other attributes are rated depending on their presence or absence in each cup. Total score is the sum of all the attributes scores. Penalty points can be deducted if defects are found (Table 3.2).

All scoring was recorded on an official SCAA Cupping Form. Overall, SCAA Cupping produces three data sets: (a) quality scores for the cupping attributes of fragrance/aroma, flavour, aftertaste, acidity, body, balance, uniformity, clean cup, sweetness, overall, defects

and total score of the sample, (b) attribute intensity ratings for fragrance, aroma, acidity and body, and (c) freely-elicited descriptive terms about fragrance, aroma, flavour, aftertaste, acidity and body.

3.2.3.2 Descriptive terminology

Cuppers used qualitative descriptors to justify their attribute ratings. They were instructed to use the standard Q-Grader structured vocabulary for bouquet (Lenoir & Guermonprez, 1997). The structured bouquet vocabulary consists of 36 aroma references, grouped in 4 sets (enzymatic, sugar browning, dry distillation, aromatic taints and faults). Each set is divided in 3 subgroups. However, as the vocabulary was designed for describing the bouquet of washed coffees, cuppers were allowed to use their own descriptors for unwashed coffee flavours, when necessary. Where cuppers were not able to produce a specific flavour descriptor, they were asked to use a broader term from a standard library, to increase the level of consensus and simplify the creation of the standard lexicon. Cuppers also elicited their own descriptors for describing taste or mouthfeel perceptions.

3.2.4 Data analysis

3.2.4.1 Effect of processing method on quality scores and attribute intensities

The effects of individual farm and processing method on coffee quality scores and attribute intensities were investigated. Two-way analyses of variance (ANOVA) with farm and processing method in the model were conducted. Separate analyses were carried out on the scores for fragrance/aroma, flavour, aftertaste, acidity, body, balance and overall, as well as on intensity ratings for fragrance, aroma, acidity and body. A significance level of 5% was considered. Mean scores of significant attributes for farm and processing method effects were then compared using Tukey's *post-hoc* test. IBM SPSS software (version 21) was used to perform this analysis.

3.2.4.2 Categorisation of descriptive terms

A nested descriptor catalogue (Table 3.3) was created, based on Lenoir and Guermonprez (1997) and enhanced with the subgroups introduced to describe the bouquet of unwashed coffees as well as taste or mouthfeel perceptions. Descriptors used for the fragrance or aroma sections were analysed separately to the ones used for the flavour section. Descriptors were

grouped into categories, called descriptor subgroups. Standard Q-Grader training classifies bouquet descriptors into four bouquet 'groups' (enzymatic, sugar-browning, dry-distillation, and taints and faults), which encompass twelve subgroups: *vegetable*, *floral*, *fruity*, *caramelly*, *chocolaty*, *nutty*, *spicy*, *pyrolytic*, *resinous*, *fermented*, *phenolic* and *earthy*. Descriptor subgroups added to aid the description of unwashed coffees were *tropical-fruity*, *red-fruity* and *dried-fruity*. A *'hard cup'* subgroup was created to include bitterness, harshness and astringency, even though these descriptors do not belong to bouquet.

Table 3.3. Lexicon for the fragrance, aroma and flavour sections of the cuppings – categories used for aggregating descriptors into subgroups

Group	Subgroups (used in contingency table)	Descriptor
ENZYMATIC [a*]	*Floral*	*Tea-rose*
		Coffee blossom
		Honey
	Vegetable	*Potato*
		Sweet peas
		Cucumber
		Beans
		Mown lawn
	Fruity	*LEMON*, Citrus [b]
		Apricot, peach
		Apple
		Orange, grapefruit
		Lemon pie
	Tropical-fruity[+]	Mango
		Pineapple
		Banana
		Passion fruit
	Red-fruity[+]	Strawberry
		CHERRY, black cherry
		Berry
		Blueberry
		Cranberry
SUGAR BROWNING[*]	*Caramelly*, sweet-smelling	*Caramel*
		Fresh butter, ricotta
		Peanuts
		Molasses, sugarcane, raw sugar
	Chocolaty	*Toasted bread*
		Dark chocolate, cocoa
		Vanilla
		Cereal, biscuit
	Nutty	*Roasted almonds*
		Walnuts
		Roasted hazelnuts
	Dried-fruity[+]	Prunes
		Tamarind
		Pumpkin

Group	Subgroups (used in contingency table)	Descriptor
DRY DISTILLATION*	*Spicy*	*Coriander seed*
		Black pepper
		Cloves
		Cinnamon
		Aniseed
	Pyrolytic	*Malt*
		Roasted coffee
		Pipe tobacco
	Resinous	*Blackcurrant*
		Maple syrup
		Cedar, wood
		Mint
AROMATIC TAINTS*	*Fermented**	*Basmati rice*
		Coffee pulp
		Medicinal
	Phenolic	*Smoke*
		Rubber
		Boiled beef
	Earthy	*Earth*
		Straw
		Leather
		Past-croppish, cardboardy
		Mould
Other descriptors, not part of the bouquet	Hard cup	Bitter
		Dirty, harsh
		Astringent

(a) Descriptors in *italics* are part of Q Grader training using an external reference (Lenoir & Guermonprez, 1997).
(b) Descriptors in the same cell are considered synonyms.
(*) Descriptors marked with an asterisk were not mentioned by any cupper in this dataset, although they belong to the standard bouquet notes structure.
(+) Subgroups marked with a plus sign were created to better represent the flavour of unwashed coffees.

3.2.4.3 Effect of processing method on descriptor subgroups

In order to investigate the effect of processing method on coffee flavour, the descriptor categories ('descriptor subgroups' – Table 3.3) mentioned for each sample were analysed, using a methodology based on Lawrence et al. (2013) and termed 'Descriptive Cupping'. A contingency table was created with coffee sample cuppings as rows (56), descriptor subgroups for columns (31) and number of occurrences for cell counts (Appendix 1).

Independence between samples and subgroups was tested with the 'Monte Carlo' method (5000 simulations, $\alpha = 0.05$) (Metropolis & Ulam, 1949). This method is more suitable than the customary Chi-square test based on the Chi-square approximation, since to safely use the latter the theoretical counts should not be lower than 5. In order to identify significant subgroups, global χ^2 and χ^2 per-cell analyses were carried out on the contingency table (Symoneaux, Galmarini, & Mehinagic, 2012). Global χ^2 was used to identify significant subgroups across the whole data set, while χ^2 per-cell was used to identify them in individual samples.

Non-symmetrical correspondence analysis (NSCA – with subgroups depending on samples) was applied to the contingency table in order to visualise the relationship between samples and subgroups. NSCA was used instead of symmetrical correspondence analysis because, if a link can be proven between the variables, the inertia values tend to be higher. Both the χ^2 and the correspondence analysis were carried out using XLstat (Addinsoft SARL).

3.2.4.4 Correlation between subgroups and quality scores

The degree of correlation between flavour profiles and quality scores was investigated through the regression vector (RV) coefficient, to identify the link between both data sets and to analyse similarity between them (Robert & Escoufier, 1976). The RV coefficient can be used as a measure of similarity of two data sets, in an analogue way to the R coefficient in linear regressions. All the first factors of the NSCA that together accounted for more than 75% of total inertia were compared with the factors of the quality scores PCA that accounted for more than 75% of total inertia (Bécue-Bertaut & Pagès, 2008; Lawrence et al., 2013). The RV coefficient was calculated using XLstat (Addinsoft SARL).

3.3 Results and discussion

3.3.1 Effect of processing methods on coffee quality and attribute intensities

Results from analysis of variance showed there was a significant effect ($p<0.05$) of the processing method and farm for the seven quality scores, with the exception of processing method on the aftertaste score ($p=0.063$). There was a significant effect ($p<0.05$) of processing method on fragrance intensity and aroma intensity, and not on acidity and body intensities. No significant effect of farm was observed on the attribute intensities.

The processing methods were compared for significant quality scores and attribute intensities using Tukey's *post-hoc* test. Naturals had a significantly higher final score than washed coffees, thus indicating 'better' quality than washed coffees in Guerrero. These results are specific to this region but could also be true for other traditional natural coffee-producing regions.

Natural coffees had a significantly higher fragrance/aroma score (7.78) than the three other post-harvest processing methods (7.52-7.58) (Table 3.4). The higher score in fragrance/aroma may be explained by significantly higher fragrance intensity and aroma intensity coupled with the presence of fruity notes and more complexity than washed coffees. Naturals also had significantly higher body and balance scores than washed, although not different from pulped-natural coffee. The higher body score in naturals indicates they are perceived as thicker than washed. Pulped-natural coffees, on the other hand, had a significantly higher acidity score than washed. Washed treatments (Wh and Wd) were not significantly different to each other but in the case of overall score, Wh was significantly lower than natural.

Table 3.4. Mean quality scores of attributes significantly different for processing method.

Attribute*	Wh	Wd	PN	N
Fragrance intensity	2.2^{a}	2.3^{a}	2.5^{a}	3.2^{b}
Aroma intensity	2.0^{a}	2.0^{a}	2.6^{ab}	2.9^{b}
Fragrance/aroma	7.52^{a}	7.54^{a}	7.58^{a}	7.78^{b}
Acidity	7.27^{a}	7.25^{a}	7.45^{b}	7.39^{ab}
Body	7.30^{a}	7.30^{a}	7.45^{ab}	7.55^{b}
Balance	7.33^{ab}	7.27^{a}	7.48^{b}	7.48^{b}
Overall	7.27^{a}	7.35^{ab}	7.45^{ab}	7.52^{b}
FINAL SCORE	81.44^{a}	81.50^{a}	82.09^{ab}	82.80^{b}

*Means sharing the same letter within a row are not significantly different ($p>0.05$). Intensity was rated on a 5-point scale; quality scores were rated on a 6-10 scale.

Coffee is a very complex product. It is to be expected that all the factors leading from tree to cup will have an effect on final flavour and quality. In the case of farm, this is partly due to geographical factors, namely the altitude, which has traditionally been recognised as being linked to quality (Bertrand et al., 2012). However, human factors may also have played a key role in the coffee quality of some individual farms. Although efforts were made to carry out the same harvesting and processing methods in all farms, ultimately every grower prepared

his farm's samples individually. Handling practices before, during and after harvest may have affected quality scores in some farms.

3.3.2 Effect of processing method on flavour profiles

The 'Monte Carlo' test of independence on the contingency table shows there was a significant link between samples and subgroups. The observed value of χ^2=1946.5 implies a value of p<0.0001, with a critical χ^2 value of 1746.5 (df=55, p=0.05). The global χ^2 analysis showed that for a descriptor subgroup to be significant (p<0.05), the χ^2 of the subgroup needs to be above the critical value of χ^2=73.3 (df=55). The most discriminant subgroups in the global χ^2 analysis included: *pyrolytic aroma* (χ^2=137.2, df=55, p<0.001), *earthy flavour* (χ^2=117.4, df=55, p<0.001) and *earthy aroma* (χ^2=95.0, df=55, p=0.001). Pyrolytic and earthy flavour notes drew the attention of cuppers because they are considered undesirable. Other significant (p<0.05) subgroups include *vegetable aroma* (χ^2=92.8, df=55, p=0.001), *pyrolytic flavour* (χ^2=91.6, df=55, p=0.001), *dried-fruity flavour* (χ^2=90.7, df=55, p=0.002), *hard-cup* (χ^2=90.4, df=55, p=0.002), *vegetable flavour* (χ^2=76.4, df=55, p=0.030) and *floral flavour* (χ^2=74.1, df=55, p=0.044).

Descriptor subgroups which did not discriminate among samples as they had a value of p>0.95 (df=55, χ^2<39.0) include *chocolate aroma* (χ^2=22.1, df=55, p=1.000), *chocolate flavour* (χ^2=26.1, df=55, p=1.000) and *nutty aroma* (χ^2=36.0, df=55, p=0.978), suggesting they are typical components of Guerrero coffee flavour profile, regardless of its origin or processing method.

Samples with a descriptor subgroup significantly above the theoretical average frequency, using the χ^2 per-cell test, are summarised in Table 3.5. *Tropical-fruity* flavour notes and *dried-fruity aroma* were significant in five samples (3N, 12N, 15N, 26N, 51N), but only in natural coffees. *Earthy* flavour notes, *pyrolytic* flavour notes, *floral flavour* and *caramelly flavour* were significant subgroups in some samples, regardless of their processing method. Other significant subgroups were specific to one sample only (*resinous flavour*, *vegetable* flavour notes, *fermented aroma*, *dried-fruity flavour*, *floral aroma*, *fruity flavour*, *hard cup*).

Table 3.5. Descriptor subgroups with a frequency significantly higher than theoretical value ($\chi 2$ per-cell test).

Processing method	Sample	Significantly high descriptor subgroups (α = 0.05)
Natural	3N	Tropical fruit flavour [a]
	5N	Resinous flavour [b]
	7N	Earthy aroma, earthy flavour
	12N	Dried fruit aroma [a]
	15N	Tropical fruit aroma [b]
	26N	Dried fruit aroma [a]
	51N	Tropical fruit flavour [a]
	55N	Floral flavour
	57N	Pyrolytic aroma, pyrolytic flavour
Pulped-natural	12PN	Vegetable aroma [b], vegetable flavour [b]
	21PN	Fermented aroma [b]
	24PN	Pyrolytic aroma
	29PN	Dried fruit flavour [b]
	51PN	Floral flavour
	52PN	Caramels flavour
	57PN	Earthy aroma, earthy flavour
Washed	2Wd	Floral aroma [b]
	3Wh	Pyrolytic aroma
	3Wd	Fruity flavour [b]
	7Wh	Hard cup (flavour) [b]
	26Wh	Caramels flavour
	53Wd	Pyrolytic aroma

(a) Significant descriptor subgroups *specific to a processing method* and having more than one occurrence in that method.

(b) Significant descriptor subgroups specific to one sample.

NSCA shows that 76.4% of the total variation was represented in the first 9 factors. Inertia took values of 17.0% and 13.8% on Factor 1 (F1) and Factor 2 (F2), respectively. This low level of inertia from free comments analysis is similar to the one reported by Lawrence et al. (2013), who also analysed free comments data (for wine) with a global Chi-square, a Chi-square per cell test and a correspondence analysis. They suggest it may be due to the large number of terms used by assessors. However, this low level of inertia may also be due to the limitations of the cupping method employed. For example, the large amount of samples used did not allow replication, while the research budget limited the number of participants. Therefore, this low level of inertia may be due to noise in the data set. This does not reduce

the validity of the finding, as F1 and F2 are still the dominant effects. The results of NSCA for the first two factors on the samples and the subgroups are shown on Figures 3.2 and 3.3, respectively.

Pyrolytic aroma (contribution of 38.1% to F1) and *pyrolytic flavour* (23.8%) were positively loaded on F1, and characters like *spicy aroma* (contribution of 6.4%) and *fruity flavour* (6.3%) were negatively loaded. *Pyrolytic* flavour notes are characteristic of over-roasted coffees and may be due to a low bean density in the case of beans from warmer (lower altitude) areas or to some poor control during sample preparation. *Pyrolytic* flavour notes may be perceived as 'undesirable' and draw the attention of cuppers, hence their level of contribution. Samples from the three processing methods (3Wh, 24PN, 53Wd and 57N) were characterised as *pyrolytic* (Table 3.5) and were positively loaded in F1 (Figure 3.2).

Chocolate aroma (contribution of 21.6% to F2) and *earthy aroma* (16.1%) were positively loaded on F2, contrasting with *fruity aroma* (contribution of 12.8%) and *fermented aroma* (8.5%), which were negatively loaded. *Earthy aroma* in this case implies mostly a straw-like quality, which is an indicator of ageing (fading) of the green coffee (Rendón, de Jesus Garcia Salva, & Bragagnolo, 2014). For example, samples 7N and 57PN were characterised in Table 3.5 by *earthy* flavour notes and were highly loaded on F2 (Figure 3.2). Like the case of *pyrolytic*, *earthy* flavour notes can be shown by coffee regardless of its processing method. However, the subgroups negatively loaded on F2 (*fruity aroma* and *fermented aroma*) were characteristic of natural samples.

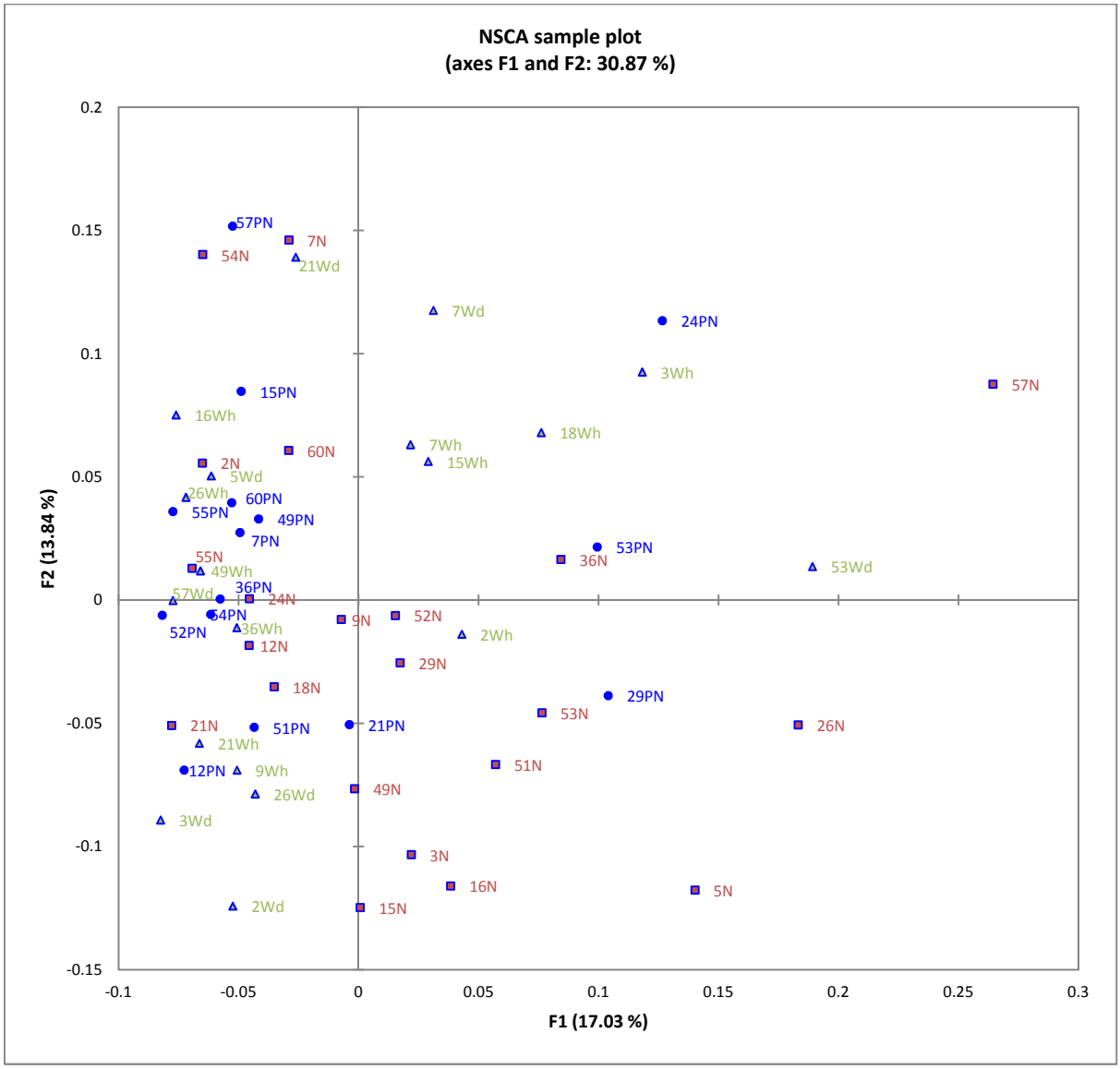


Figure 3.2. Non-symmetrical correspondence analysis (NSCA) map representing the projection on F1 and F2 of natural (square), pulped-natural (circle) or washed (triangle) coffees for 56 samples from the state of Guerrero, evaluated using the Descriptive Cupping method.

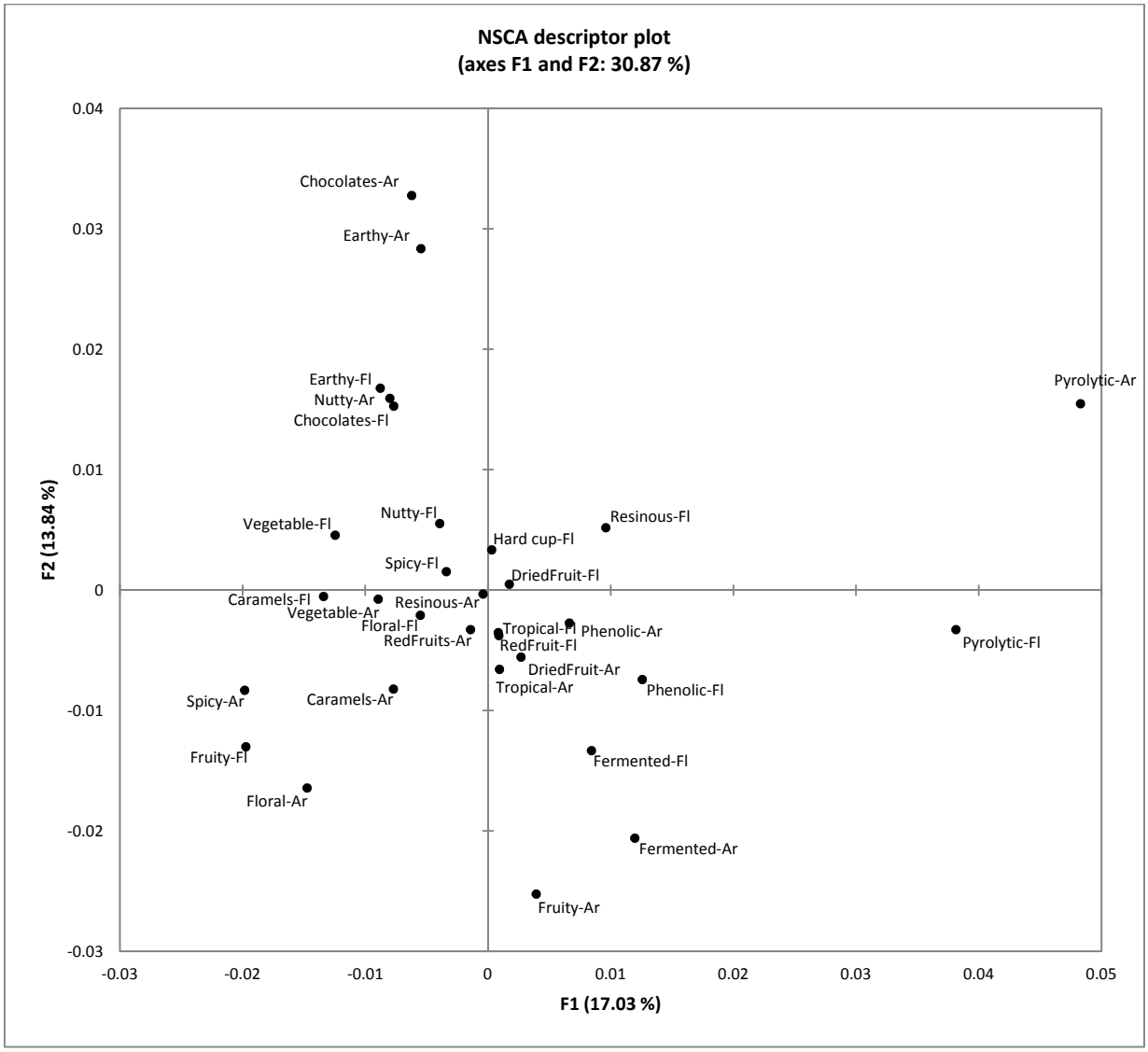


Figure 3.3. Non-symmetric correspondence analysis (NSCA) map representing the projection on F1 and F2 of descriptor subgroups for 56 coffee samples from the state of Guerrero, evaluated using the Descriptive Cupping method. ***Ar: Fragrance/Aroma; Fl: Flavour.***

An effect of the processing method on flavour is shown by the NSCA, in spite of the presence of subgroups like *pyrolytic* and *earthy* in a number of samples from different methods. Many samples presented the 'typical' profile expected for each processing method – *fermented*, *red-fruity* and *tropical-fruity* for classical, 'Mocha' naturals (Akiyama et al., 2008; Ukers, 1922); *spicy*, *floral* and *nutty* for washed coffees; pulped-naturals somewhere in between and with a wider distribution, as an intermediate method between naturals and washed. Figure 3.2 shows a region of the graph populated mostly by natural samples (lower-right quadrant). Samples characterised by *tropical fruit* in Table 3.5 (3N, 15N, 51N) or *dried fruit* (26N, 29PN) are

found in this area (the 'Mocha' character). Another region of the graph is populated mostly by washed samples (lower-left corner, samples 2Wd, 3Wd, 9Wh, 26Wd), characterised by *floral aroma*. Other parts of the graph (top-left quadrant) have a mixed population: *chocolaty aroma*, for instance, can characterise samples from any method, as it was shown to be a typical subgroup in the sample set by the global χ^2 analysis. Some natural and pulped-natural samples clearly had a 'washed coffee-like' profile. The opposite case – washed coffees having a *fermented* profile – did not occur. The former may be most easily explained by the process history of those samples: samples 55N, 54N, 60N and 21N, for example, were the only natural samples with a drying time under 8 days. This short drying time would not allow the *fruity*, *fermented* character to be developed in the samples.

The level of overlap between different treatment groups (for example, in the top-left quadrant of Figure 3.2) may also be due to a high level of noise, produced by the limitations of the cupping method (i.e. absence of replicas, low number of cuppers, etc.).

3.3.3 Correlation between subgroups and quality scores

The first nine factors of NSCA (76.4% total inertia) and the first factor of the quality scores PCA (79.2% of inertia) were used to calculate the RV coefficient between both tables. The RV coefficient was 0.165, showing there is little correlation between the subgroups and the quality scores. This implies an absence of bias towards a set of subgroups, meaning that cuppers do not base their scores on the presence of a given flavour note: high-score coffees, for example, can possess many different flavour profiles. However, this does not imply there is not a link between some subgroups and a lower or higher score. *Pyrolytic* and *earthy* flavour notes are two examples of highly detectable flavours that are considered undesirable and would affect a sample's score.

Descriptive Cupping has an application to provide the flavour profiles of coffee. The χ^2 tests and the meaningfulness of NSCA imply there was enough degree of agreement among cuppers to differentiate samples. The number of cuppers is enough to obtain significant results, implying the information in databases built using data from 3 cuppers (CQI, 2013e) could be analysed *a posteriori*. More work is needed to verify these results against traditional descriptive methodologies.

3.4 Conclusion

In this study, Q Graders were used to explore links between processing method, flavour and quality in coffees from the state of Guerrero, Mexico. In a complex product like coffee, many factors influence flavour, although the importance of each individual factor on final flavour is different for each case. In coffee from most of the farms studied, the effect of the processing method is evident: natural coffees tend to show *red-fruity*, *dried-fruity*, *tropical-fruity* and *fermented* flavours (Mocha profile); washed coffee tends to show more *floral*, *spicy* and *nutty* flavours; pulped-naturals, as an intermediate method between washed and natural, can be found approaching either profile. Some overlapping cases of natural coffees approaching the washed profile are also found. The relationship between processing and natural coffee flavour will be further researched in the following chapters.

4 Characterisation of the sensory flavour of natural coffee expanded to the international context, using Descriptive Cupping by a trained panel

4.1 Introduction

Chapters 1 and 2 presented natural coffee as a little-studied product class, oftentimes described in contradictory terms by both researchers and the industry. As a first approach to the study of natural coffee flavour, Chapter 3 used Descriptive Cupping to compare the effect of different processing methods on the flavour of coffee coming from one single producing region. However, the large effect of geographical factors and geographically-localised cultural practices on coffee flavour has been acknowledged for a long time (Ukers, 1922). Therefore, fixing the region of origin, as in Chapter 3, may be useful for understanding the effect of different processing methods, but a different approach is needed for understanding the flavour profiles of natural coffees as an international product class. Thus, this chapter focuses on a category appraisal of naturals coming from different producing countries in the context of specialty coffee. Although this is not an exhaustive survey of all natural-coffee producing countries, the use of coffees from a diverse set of countries ensures a wide variability among samples.

The aim of this study was to characterise the flavour of natural coffee from different producing countries using Descriptive Cupping. The study investigated the flavour of natural coffee in the context of the different processing methods, namely washed process, pulped natural process and natural process (a contribution to *Objective 2*, 2.2.2.2). In addition, the flavour variation among natural coffees as a group was also studied (*Objective 3*, 2.2.2.3). To complete Descriptive Cupping, a panel (n=7) was recruited and trained in the SCAA cupping protocol (SCAA, 2009a). Training of the cupping panel incorporated the introduction of natural-specific olfactory vocabulary in addition to the 'standard' SCAA cupping vocabulary.

4.2 Materials and methods

4.2.1 Samples

4.2.1.1 Origin of green beans

Green coffee samples (32) from the 2011-2012 harvest were supplied by exporters and farmers from 7 natural coffee producing countries (Table 4.1). 22 samples were processed using the dry method (natural coffee) and required between 4 and 21 days to achieve 12% moisture. The natural coffee samples were selected to represent variability within the flavour

spectrum of natural coffee. When possible, washed (9) or pulped natural (1) samples were sourced from the same farm as the natural sample. These samples were used to provide points of reference for the flavour of other processing methods.

Table 4.1. Origin, variety and processing information for 32 coffee samples.

Country	Region	Variety	Process	Drying time (days)[b]	Drying Method	Sample code
Brazil	Alta Mogiana, SP	Mondo Novo + Catuai	Natural	4	Concrete patio + dryer	BNO
		Catuai + Catucai	Natural	4	Concrete patio + dryer	BNI
		Catuai	Washed	4	Concrete patio + dryer	BWI
		Yellow Bourbon	Washed	4	Concrete patio + dryer	BWW
		Yellow Bourbon	Pulped natural	4	Concrete patio + dryer	BPW
	Sul de Minas, MG	- [a]	Natural	-	-	BNX
Colombia	Antioquia	-	Natural	15	Raised beds	CNX1
		-	Washed	7	Raised beds	CWX1
		-	Washed	7	Raised beds	CWX2
Dominican Republic	Azua	95% Typica; 5% Red Caturra	Natural	21	Concrete patio	DNT
		95% Typica; 5% Red Caturra	Washed	12	Raised beds + concrete patio	DWT
Ethiopia	Harrar	-	Natural	-	-	ENX
Mexico	Chiapas	Maragogype	Natural	-	-	MNM
		Maragogype	Washed	-	-	MWM
		Typica	Natural	-	-	MNT
		Typica	Washed	-	-	MWT
	Hidalgo	-	Natural	-	-	HNX
		-	Washed	-	-	HWX
	Veracruz	Geisha	Natural	11	Drying hut	VNG
		Geisha	Washed	-	Raised beds	VWG
Nicaragua	Nueva Segovia	Bourbon	Natural	12-15	Raised beds	NNB1
		Bourbon	Natural	12-15	Raised beds	NNB2
		Bourbon	Natural	12-15	Raised beds	NNB3
		Bourbon	Natural	12-15	Raised beds	NNB4
		Bourbon	Natural	12-15	Raised beds	NNB5
		Pacamara	Natural	12-15	Raised beds	NNP1

Country	Region	Variety	Process	Drying time (days)[b]	Drying Method	Sample code
Nicaragua	Nueva Segovia	Pacamara	Natural	12-15	Raised beds	NNP2
		Maracaturra	Natural	12-15	Raised beds	NNR
		H2 Hybrid	Natural	12-15	Raised beds	NNH
		Caturra	Natural	12-15	Raised beds	NNC
		Yellow Catuai	Natural	12-15	Raised beds	NNY
Panama	Chiriquí	Geisha	Natural	-	-	PNG

(a) (-) – Data not available.
(b) To achieve 12% moisture.

4.2.1.2 Characterisation of the green beans

Water activity, colour, tapped bulk density and defects of the green beans were measured. Water activity was measured using an 'Aqua Lab model CX3' dew point water activity meter (Decagon Devices Inc., Pullman, WA, USA). Colour was measured in the 'CIE L* a* b*' colour space (daylight colour, D65 illuminant, 10° field of view) using a Miniscan XE Plus colorimeter (HunterLab Inc., Reston, VA, USA). Water activity and colour were measured in triplicate. Bulk density was calculated from the weight of 500mL total volume, tapping the measuring cylinder on the bench, for the beans to settle.

Green bean defects were measured using the SCAA green grading protocol (SCAA, 2009b). A sample of 350g was inspected for category I and II defective beans. Category I defects include full black beans, full sour beans, dried cherry/pod, fungus damaged beans, severe insect damaged beans and foreign matter. Category II defects include partial black beans, partial sour beans, parchment/pergamino beans, floater beans, immature/unripe beans, withered beans, shell beans, broken/chipped/cut beans, hull/husk and slight insect damaged beans. The number of category I and category II defects is calculated based on a different weight (number of defective beans) for each defect type. The defects that have the largest negative impact on flavour, such as full black beans or full sour beans, require only one defective bean for one 'full defect' to be counted. Defects that do not have such a large impact on cup flavour require more defective beans (between 3 and 10 defective beans, depending on the case) for one 'full defect' to be counted (SCAA, 2004, 2009b).

4.2.1.3 Roasting of green beans

For roasting, samples were grouped in heterogeneous blocks of 6 samples. Samples were roasted in batches (2-4), depending on sample size (Table 4.2). The roasting order of samples for each batch was randomised within each 6-sample block.

Table 4.2. Examples of the number and size of batches used for roasting of green beans depending on sample size

Sample size (g)	Batch (g)			
	1	2	3	4
255	100	100	-	-
340	100	100	100	-
480	200	200	80	-
560	200	200	100	-
980	200	200	200	200
1080	200	200	200	200

Samples were roasted according to the SCAA roasting protocol (SCAA, 2009a), with the exception of roasting time. Over 7 days, all samples were roasted in a Gene Café CBR-101 roaster (Gene Café, Ansan City, Republic of Korea).

The roaster was first preheated above 100°C and cooled to 60°C at the beginning of each roasting day. The roasting protocol was adjusted depending on the sample's bulk density. For samples with a high bulk density (>700g·L^{-1}), the final air temperature was programmed at 250°C. At the onset of the first crack, the programmed temperature was lowered to 3°C above the first crack temperature. For samples with a low bulk density (<700g·L^{-1}) the temperature was set at 240°C and not modified.

For high moisture samples (a_W>0.73), a drying cycle at 140°C for 5 minutes was used, followed by roasting according to their density. The end of roast was approximately 2 minutes after the first crack, when the fume odour started to shift from pungent to vanilla-like or nutty. After each batch was cooled, weight loss was measured as a roast degree indicator. The target for weight loss for each batch was set at 16.0% ± 2.2%, in order to account for the variations in moisture content, bean size and bean density, which may lead to different weight loss levels for the same roast degree. Complying roasted batches were packed under vacuum (0.9atm

vacuum) and left overnight at room temperature for degassing. The next day they were frozen at -20°C.

Prior to use, all roast batches from the same sample were combined. After use, excess samples were refrozen.

Luminance was measured on the roasted and ground coffee as L* in the CIELAB colour space (see 4.2.1.2). The SCAA protocol (2009a) specifies a target roast colour of 55-60 on the standard Agtron scale. This target is approximately equivalent to an L* range of 17-29 in the CIELAB colour space (Azeredo, 2011; Jiménez Ariza, Diezma Iglesias, & Correa Hernando, 2011; Moura et al., 2007).

4.2.1.4 Sample brewing

The SCAA Cupping Protocols (SCAA, 2009a) were followed for the grinding, brewing and serving of samples. Samples were ground using a 'BarAroma^{TM}' coffee grinder (Breville Pty., Botany, NSW, Australia) in the 'Filter' grind setting. The grinder setting was chosen so that 70-75% of the particles could pass through a 0.841 mm sieve aperture (US standard size 20 mesh). Samples were brewed in 300mL mugs by adding hot water to the coffee grounds. The coffee to water ratio was kept at 8.25g of coffee per 150 mL water. For each sample, five mugs were used, for which beans were ground and brewed individually. Brewing started at a water temperature of 93°C, and the brew was left to steep for 3-5 min prior to breaking the crust.

4.2.2 Recruitment and training of coffee cupping panel

A cupping panel (5 males, 2 females) was recruited from the local coffee and hospitality industries in Dunedin, New Zealand. Over 9 sessions the panel was trained on the SCAA Cupping for the evaluation of natural coffee. Ethical approval for this study was obtained from the University of Otago Human Ethics Committee (reference number 12/163). The participants were offered no incentives; however, participants were highly motivated by the expectation of learning new skills for the coffee profession. Training was carried out in an open food grade laboratory with several benches and kitchens (Laboratory 1, Department of Food Science, University of Otago). The open laboratory layout was preferred over the sensory booth profile, in order to replicate the traditional cupping environment.

In session one, the overall SCAA Cupping Protocol (SCAA, 2009a), described in Chapter 3, was demonstrated to cuppers, through the cupping of a sample of Nicaraguan natural coffee. The panel leader explained and demonstrated each step of the cupping procedure, the use of the SCAA cupping form, the different attributes assessed and the general grading criteria, while the cuppers carried out the cupping procedure on their own. After the cupping, the results were discussed. After the session, each cupper was allowed to keep the cupping form as reference.

During each of the next seven session (sessions 2-8 – Appendix 2), cuppers were trained on one specific cupping attribute including: body, acidity, aftertaste, flavour, fragrance/aroma, balance and overall. In session nine, the cuppers were trained on the use of the Catador® iPad® App to record the cupping data.

Each training session was broken down into three sections. In section one, 10 blind olfactory reference mugs were given to cuppers. Each of the references detailed in Appendix 2 was placed in a ceramic mug (when concentrated aromas were used as reference, they were placed on a cotton ball inside the mug). The mug was covered with aluminium foil, in order to conceal the reference, but the foil was pierced several times using a knife in order to allow the odour to be released from the mug. Each mug was covered with a Petri dish, in order to prevent the odour from escaping the mug. Cuppers were asked to take a mug, remove the Petri dish, sniff the mug above the aluminium foil and then record the descriptor for the corresponding odour. Identification performance was monitored by the panel leader, by verifying that each cupper correctly named the olfactory references. The panel leader wrote on the whiteboard the descriptors provided by each cupper, facilitated reaching a consensus for the descriptor used, and provided the correct descriptor for each reference. Definitions for the attributes were not used, as in traditional descriptive analysis techniques. Cuppers were required to review any incorrect responses. Panel acuity in section one was measured by the number of correct descriptors produced by each cupper.

The references were used to train cuppers to identify the standard coffee aroma subgroups and corresponding descriptors used for coffee cupping Q by Grader training. These aroma subgroups included floral, vegetable, fruity, caramelly, chocolaty, nutty, spicy, pyrolytic, resinous, fermented, phenolic and earthy flavour notes (Chapter 3). Cuppers were also trained in four additional aroma descriptor subgroups specific to natural coffees. These subgroups

included sweet spices (cinnamon and cardamom), dried fruit (prunes, raisins, dates and dried figs), red fruit (açaí flavouring, redcurrant jelly) and tropical fruit (passion-fruit flavouring).

During section two, the attribute in focus was explained by the panel leader and the key concepts related to it were defined. References were provided as training aids in the case of body, acidity and flavour (see 11.2.1.8, 11.2.2.8, 11.2.2.9, 11.2.4.8 and 11.2.4.9). Cuppers sampled references for the attribute in focus and practiced the assessment of the attribute using the references.

During section three, two coffees were cupped for the attribute in focus during the training session, using the SCAA Cupping Protocol (SCAA, 2009a), described in Chapter 3, and recording results on the SCAA cupping form. The pair of coffees were selected for training based on their contrasting features (Appendix 2). The panel leader cupped the coffees together with the other cuppers, and led the discussion about how the attribute in focus was expressed in the coffees.

4.2.3 Flavour profiling of samples by Descriptive Cupping

4.2.3.1 Cupping procedure

All samples were cupped in duplicate over 6 evaluation sessions and using a random order. The cupping environment was the same used for training (4.2.2). During each session, 6 samples were assessed by the panel following the SCAA (2009a) protocol described in Chapter 3 (3.2.3.1). The 6 samples of each session were arranged on a bench, using 5 cups of coffee per sample. All the cuppers, including the panel leader, cupped the 6 samples at a time, following the protocol in 3.2.3.1. However, instead of using a paper ballot, evaluations were recorded using the Catador® App for iPad® (Compuservice, S.A., Guatemala City, Guatemala). The Catador® App is a special app designed for data collection on an iPad®, using the SCAA (2009a) protocol, which means the ballot and scales used are the same ones used for SCAA cupping. Each attribute was scored using the app and descriptors were written in a notes data field. After the cupping session, the Catador® session was saved and closed, and a discussion about the results was led by the panel leader.

As a result of the evaluations, three types of data were obtained: (a) quality scores for the cupping attributes of fragrance/aroma, flavour, aftertaste, acidity, body, balance, uniformity,

clean cup, sweetness, overall, defects and total score of the sample, (b) attribute intensity ratings for fragrance, aroma, acidity and body, and (c) freely-elicited descriptive terms about fragrance, aroma, flavour, aftertaste, acidity and body. However, for this chapter, the quality scores were only included as a supplementary table in the MFA (4.2.4.4), as the focus of the study was on flavour as opposed to perceived quality.

4.2.3.2 Descriptive terminology

As in Chapter 3, cuppers were instructed to use qualitative descriptors from their training (Table 3.3) to justify their attribute scores. When necessary, cuppers were allowed to use their own descriptive terms. Where cuppers were not able to produce a specific descriptor, they were asked to use a broader term of either a subgroup or group.

4.2.4 Data analysis

4.2.4.1 Analysis of data from green bean characterisation and roasting

The mean water activity of green beans was calculated as a decision-making parameter for roasting, as explained on 4.2.1.3. The mean colour parameters (mean values for L*, a* and b*), together with the bulk density of green beans were later included in the Multiple Factor Analysis (MFA) as supplementary variables (see 4.2.4.4). The number of defects was considered only as descriptive data in section 4.3.2.

The weight loss of the samples after roasting and the mean luminance of the roasted samples (L*) were compared against the target values (16.0% ± 2.2% weight loss; L* between 17-29) so as to insure consistency.

4.2.4.2 Categorisation of descriptive terms

The freely-elicited descriptive terms were grouped, based on the cupping section they were describing (Table 3.2), into separate lists for bouquet, taste, aftertaste duration, acidity, and mouthfeel. Bouquet consisted of fragrance, aroma, and the olfactory components of flavour and aftertaste terms. Within each list, terms with the same meaning were merged into one descriptor. For example, ‘chocolates’, ‘dark chocolate’ and ‘chocolaty’ were merged into the descriptor ‘chocolate’. Next, the descriptors were merged by category – here called ‘subgroups’. In addition to the subgroups for bouquet from ‘Le Nez de Café’ (Lenoir &

Guermonprez, 1997) mentioned in Chapter 3 and the four specific to naturals (from 4.2.2), supplementary descriptor subgroups were added based on cuppers' comments. Descriptors for taste, aftertaste duration, acidity, and mouthfeel were also listed and categorised.

The terms in this study were classified in a total of 33 subgroups (Table 4.3). The 'bouquet subgroups' included from the 'Le Nez du Café®' vocabulary (see Chapter 3) were *caramelly, chocolaty, citrus-like, earthy, fermented, floral, fruity, nutty, phenolic, pyrolytic, resinous, spicy, stone-fruity, toasty, woody* and *vegetable;* from the supplementary natural coffee vocabulary (see 4.2.3), *dried-fruity, red-fruity, tropical-fruity*, and from the freely-elicited subgroups, *acid-smell, fungal, complex, past-croppish* and *pungent.* As stated previously (4.2.4), additional descriptors to those of the standard and natural subgroups were included to account for supplementary cupper comments. Taste descriptors included *bitter* and *sweet.* Aftertaste duration consisted of the term *long-aftertaste.* The acidity group contained the subgroups *dry-acidity, medium-acidity* and *sweet-acidity.* Mouthfeel contained *rough-body, smooth-body* and *astringent.*

Table 4.3. Lexicon for the fragrance, aroma, flavour, aftertaste, acidity and body sections of the cuppings – categories used for aggregating descriptors into subgroups.

Group	**Subgroups (used in contingency table)**
Enzymatic	*Vegetable*
	Floral
	Fruity
	Citrus-like
	Stone-fruity
	Tropical-fruity
	Red-fruity
Sugar-browning	*Caramelly*
	Chocolaty
	Toasty
	Nutty
	Dried-fruity
Dry distillation	*Spicy*
	Pyrolytic
	Resinous
	Woody
Aromatic taints	*Fermented*
	Phenolic
	Earthy
Unclassified, freely-elicited terms	*Acid-smell*
	Pungent
	Fungal
	Complex
	Past-croppish
Taste	*Bitter*
	Sweet
Aftertaste duration	*Long aftertaste*
Acidity	*Dry acidity*
	Medium acidity
	Sweet acidity
Mouthfeel	*Rough body*
	Smooth body
	Astringent

4.2.4.3 Descriptive Cupping analysis

To study the flavour of natural coffee in the context of the different processing methods and the flavour variation among coffees processed through the dry method, Descriptive Cupping data were analysed using the same methodology employed in Chapter 3.

To study the flavour of natural coffee in the context of the different processing methods, the methodology was applied for all samples (n=32). To study the flavour variation among coffees processed through the dry method, the methodology was applied to only the natural samples (n=22). A contingency table was constructed for each dataset (Appendix 3 presents the 32-row contingency table), with the samples as rows (32 or 22), the descriptor subgroups as columns (33) and number of occurrences for cell counts.

Significance tests were run on the respective contingency tables for each dataset. Independence between samples and descriptors was tested with the 'Monte Carlo' method (5000 simulations, $\alpha = 0.05$) (Metropolis & Ulam, 1949). In order to identify significant descriptors, global χ^2 and χ^2 per-cell analyses were carried out on the contingency tables (Symoneaux et al., 2012). Global χ^2 was used to identify significant descriptors across the whole data set, while χ^2 per-cell was used to identify significant descriptors within individual samples ($\alpha = 0.05$). Duplicate samples were aggregated for Monte Carlo and χ^2 methods.

Non-symmetrical correspondence analysis (NSCA) was applied to the contingency tables in order to visualise the relationship between the samples and descriptors. For the NSCA, duplicates were not aggregated, but were used as individual cuppings. This was done to examine the reproducibility of the cuppers by observing the mapped distance between duplicates. All statistical analyses were carried out using XLstat (Addinsoft SARL).

4.2.4.4 Correlation between descriptors, quality scores and green bean variables

To further understand the links between the natural coffee flavour profile, attribute intensities, quality scores and the green coffee variables (from 4.2.1.2), the correlations between the descriptors and the other data sets (attribute intensities and scores, bean density and bean colour) were explored using multiple factor analysis (MFA)(Abdi, Williams, & Valentin, 2013; Lawrence et al., 2013).

Two sets of variables were kept as active tables in the MFA: the descriptor contingency table and the mean attribute intensities reported for fragrance, aroma, acidity and body. These two data tables were included as active tables because they are complementary (qualitative and quantitative) of the cuppers' descriptive perception of the coffees. The remaining variables were considered supplementary data and were organised into two tables: one for the density and the colour (L*, a* and b*) of the green beans and another for the mean quality scores (fragrance/aroma, flavour, aftertaste, acidity, body, balance and overall) from the samples' cuppings.

The degree of correlation between the tables was investigated through the regression vector (RV) coefficient, to identify the link between each pair of tables and to analyse similarity between tables (Robert & Escoufier, 1976). The MFA and the calculation of RV coefficients were carried out using XLstat (Addinsoft SARL).

4.3 Results and discussion

4.3.1 Descriptive Cupping method

The Descriptive Cupping method described in Chapter 3 and the present chapter is based on a coffee cupping protocol firmly established in the coffee industry – the SCAA Cupping Protocol (SCAA, 2009a). Two additions were made to the SCAA Cupping Protocol – (a) an emphasis in the use of descriptors, including enhanced training in descriptors for fruity character for the purpose of this thesis, and (b) a protocol for the analysis of the freely-elicited descriptors, based on Lawrence et al. (2013) - see 3.2.4.

Descriptive Cupping was developed as a rapid, low cost method which has a very specific application for the analysis of coffee flavour. It was out of the scope of this thesis to validate the performance of Descriptive Cupping against more traditional descriptive analysis techniques, however, this work was carried out in another study at the University of Otago (Wilson, Lusk, Fernandez Alduenda & Silcock, 2015). Wilson et al. (2015) trained a descriptive panel using the same coffee samples described in this chapter. The panel developed a vocabulary of 24 descriptors for coffee aroma (7 descriptors), taste (4 descriptors), flavour (7 descriptors) and aftertaste (6 descriptors). Coffee samples (12) used in this chapter were also analysed by the descriptive panel, and 13 descriptors were found to significantly ($p<0.05$) differentiate the coffees. An MFA was used by Wilson et al. (2015) to

compare the results obtained by the Descriptive Cupping panel in this chapter versus the results obtained by the traditional descriptive panel. The RV coefficient indicated that the two sensory methods had a significant correlation, and both sensory methods showed that country of origin and post-harvest processing methods were important in explaining the flavour of coffee.

Table 4.4 presents a comparison between the main features of Descriptive Cupping versus traditional descriptive analysis techniques. Overall, the main advantages of Descriptive Cupping are rapidness, low cost, its applicability in countries of origin and the possibility of reanalysing cupping data from previous studies. As trained cuppers and licensed Q Graders are already trained, are widely available in most countries of origin and have a tendency to volunteer for coffee flavour studies, the time and cost of the selection, training and assessment stages can be kept low. As the descriptors elicited in any cupping can be analysed using this methodology, historical data can be analysed retroactively, which may give a new meaning to a large volume of cupping data in different countries of the world.

Overall, the main limitations of Descriptive Cupping are its specificity to coffee (though the methodology can be applied to expert graders of any product provided they generate a descriptive account of each sample), the bias of most cuppers as coffee graders, the qualitative nature of the data collected and the time required for analysing the descriptors and constructing the contingency table. As coffee grading experts, cuppers tend to focus their attention on flavour features key to grading coffee quality, such as the presence of flavour defects or uncommon flavours, which can lead to these effects dominating the analysis by masking the effect of interest and increasing the amount of noise in the analysis. This will be further discussed in the next sections, as the results for the present chapter are presented.

Table 4.4. Comparison between Descriptive Cupping and traditional descriptive analysis techniques

Concept	**Descriptive cupping**	**Traditional descriptive analysis techniques** [a]
Application	Only coffee and coffee products	Foods, beverages, cosmetics, fabrics..
Assessors	Cuppers (usually licensed Q Graders – see Chapter 3)	Trained panelists
Lexicon	Based on cupping terminology, namely on Lenoir & Guermonprez, 1997.	Usually generated by the panel.
References	Based on coffee industry references, such as Le Nez du Café®	Developed for each case
Training and operation costs	When licensed Q Graders are available, little or no selection and training costs are involved. Volunteer cuppers are widely available.	Panel selection and training costs are involved. Usually panel members are paid for their services.
Type of descriptive data collected	Quantitative only for 'intensity of fragrance', 'intensity of aroma', 'intensity of acidity' and 'intensity of body'. See Table 3.2. Qualitative (frequency of mentions) for aroma and flavor descriptions.	Quantitative, based on a scale for each attribute.
Data analysis	Preparation of a contingency table, followed by Correspondence Analysis (CA). See 3.2.2.	Usually Analysis of Variance (ANOVA) to analyse the panel performance and to determine significant treatment effects and attributes. This may be followed by multivariate data analysis, usually Principal Component Analysis (PCA) to interpret dominant data trends.

(a) *Lawless & Heymann, 2010.*

4.3.2 Characterisation of the green beans

The coffee samples were characterised in green bean. Each green bean sample's water activity, colour parameters, bulk density and defect counts are presented in Table 4.5.

Table 4.5. Mean water activity, mean CIELAB colour parameters, bulk density, total defects and primary defects of green coffee samples used.

SAMPLE	Water activity [a]	Colour (CIELAB)[a]			Bulk density ($g \cdot L^{-1}$)	Defects (SCAA, 2009b)	
		L*	a*	b*		Total defects	Category I defects
BNI	0.486 ± 0.013	49.46 ± 0.28	1.17 ± 0.02	18.67 ± 0.09	711.6	9	0
BNO	0.473 ± 0.002	47.68 ± 0.08	1.59 ± 0.01	19.13 ± 0.13	710.8	11	2
BNX	0.529 ± 0.004	46.56 ± 0.48	1.36 ± 0.06	18.23 ± 0.14	717.6	3	1
BPW	0.502 ± 0.005	46.33 ± 0.16	1.19 ± 0.08	16.43 ± 0.16	710.0	3	1
BWI	0.515 ± 0.011	45.76 ± 0.32	1.24 ± 0.02	17.07 ± 0.16	721.0	5	1
BWW	0.475 ± 0.001	45.58 ± 0.24	0.72 ± 0.02	15.58 ± 0.14	711.8	2	0
CNX1	0.579 ± 0.002	36.74 ± 0.46	4.34 ± 0.15	18.25 ± 0.43	764.4	6	3
CWX1	0.483 ± 0.002	43.89 ± 0.93	2.71 ± 0.13	18.51 ± 0.14	761.0	3	1
CWX2	0.625 ± 0.003	48.75 ± 0.96	-0.20 ± 0.07	16.98 ± 0.26	707.4	4	0
DNT	0.486 ± 0.008	45.39 ± 1.24	2.79 ± 0.07	20.34 ± 0.15	740.9	8% [b]	N/A [b]
DWT	0.553 ± 0.004	46.55 ± 0.46	1.70 ± 0.03	16.44 ± 0.15	719.5	20	3
ENX	0.583 ± 0.005	49.94 ± 0.60	2.86 ± 0.06	21.41 ± 0.04	695.8	27	20
HNX	0.546 ± 0.002	41.91 ± 0.94	1.51 ± 0.02	16.35 ± 0.34	715.2	26	0
HWX	0.571 ± 0.014	45.59 ± 0.37	1.26 ± 0.02	16.16 ± 0.03	728.8	13	0
MNM	0.492 ± 0.003	47.97 ± 1.72	2.52 ± 0.03	20.56 ± 0.16	735.8	1	1
MNT	0.527 ± 0.008	46.16 ± 1.32	3.31 ± 0.03	22.07 ± 0.22	754.4	0	0
MWM	0.614 ± 0.006	47.69 ± 0.80	0.71 ± 0.02	16.50 ± 0.52	717.4	0	0
MWT	0.601 ± 0.001	46.15 ± 0.96	1.31 ± 0.08	15.81 ± 0.21	752.6	0	0
VNG	0.473 ± 0.014	41.63 ± 1.29	3.44 ± 0.16	20.55 ± 0.74	750.5	7	0
VWG	0.573 ± 0.019	42.57 ± 1.09	1.70 ± 0.32	15.68 ± 0.53	721.9	21	4
NNB1	0.593 ± 0.002	45.18 ± 0.45	3.99 ± 0.20	23.93 ± 0.03	760.8	0	0
NNB2	0.465 ± 0.016	43.00 ± 0.73	2.90 ± 0.04	20.71 ± 0.32	767.4	0	0
NNB3	0.578 ± 0.003	46.39 ± 1.48	2.21 ± 0.14	20.82 ± 0.20	762.8	4	4
NNB4	0.523 ± 0.006	49.23 ± 1.30	2.04 ± 0.10	20.83 ± 0.24	768.6	0	0
NNB5	0.575 ± 0.002	44.92 ± 0.58	2.44 ± 0.20	19.88 ± 0.01	759.6	3	3
NNC	0.618 ± 0.011	44.96 ± 0.18	2.89 ± 0.05	21.11 ± 0.20	756.2	2	2
NNH	0.612 ± 0.002	42.16 ± 0.63	2.00 ± 0.05	18.25 ± 0.40	758.6	0	0
NNP1	0.574 ± 0.002	46.90 ± 0.48	2.64 ± 0.03	21.72 ± 0.09	718.2	2	1
NNP2	0.599 ± 0.003	45.42 ± 0.55	2.08 ± 0.09	20.26 ± 0.18	721.8	0	0
NNR	0.557 ± 0.002	44.29 ± 0.93	1.87 ± 0.03	19.82 ± 0.15	731.8	2	0
NNY	0.566 ± 0.006	45.52 ± 1.57	2.86 ± 0.11	21.13 ± 0.09	754.0	0	0
PNG	0.556 ± 0.007	50.80 ± 0.19	3.83 ± 0.02	24.55 ± 0.10	708.5	1	0

(a) Plus/minus 1 standard deviation. n=3.

(b) Defects reported as percentage (w/w) when they are too many to be counted. N/A – Data not available.

The large variation in green bean parameters reflects the variation in origin, variety and processing method. The sample set was not intended to be an exhaustive survey of natural coffees; however, it was selected for its representation of the variation in specialty natural coffees. Water activity ranged between 0.44 and 0.63. In the case of colour, L* ranged between 37 and 51, a*, between -0.2 and 4.3, and b*, between 15.6 and 24.5. Bulk density ranged from 696 to 769g·L^{-1}. From the quality point of view, 14 samples comply with the defect specifications for specialty coffee, meaning that no more than 5 total defects and 0 primary defects were present in the sample (SCAA, 2009b). However, the rest of the samples do not comply and one sample (DNT) had too many defects to be counted – they were measured by weight. The abundance of samples that do not comply with the SCAA standard for Specialty Grade green bean reflects the actual market situation, as only about 10% of the world coffee production has the potential to be considered of specialty grade (personal communication from T. Lingle).

4.3.3 Degree of sample roasting

Green beans roasting times ranged between 11:40 and 16:00 minutes. Weight loss from the green beans ranged between 13.8% and 18.2% (s=0.8%). Therefore the target roast degree (16.0% ± 2.2%) was achieved for all samples.

Roasted samples were characterised by a luminance (L*) of 19.79 ± 2.99 (s=1.08). Luminance results are dependent on particle size (Mwithiga & Jindal, 2003) and the measurement instrument (Azeredo, 2011), while weight loss results depend on the initial moisture content. In this case, the luminance corresponds to the target range of 17-29, which was chosen to comply with the SCAA protocol (2009a) standard roast of 55-60 on the standard Agtron scale.

4.3.4 Flavour of naturals in the context of different processing methods

Flavour profiles for all cupped samples were generated from the freely-elicited terms using the sequential protocol for analysing Descriptive Cupping used in Chapter 3, in which the first step is the preparation of a contingency table (Appendix 3).

The dependence between descriptors and samples was significant ($p<0.001$), as detected by the Monte Carlo method applied on the frequency table (aggregated duplicates, 5000 simulations). The observed value of χ^2=1153.4 implies a value in the interval [0.000, 0.001]

with 99% confidence, with a critical χ^2 value of 1068.5 (df=31, p=0.05). This means there is an effect of sample on the descriptor subgroups used by cuppers.

The global χ^2 analysis showed that for a descriptor subgroup to be significant ($p<0.05$), the χ^2 of the subgroup needs to be above the critical value of $\chi^2=45.0$ (df=31). The subgroups (8) that were found to vary significantly among the coffees were *past-croppish* ($\chi^2=67.9$, df=31, p=0.001), *red-fruity* ($\chi^2=57.4$, df=31, p=0.003), *fungal* ($\chi^2=56.7$, df=31, p=0.003), *phenolic* ($\chi^2=53.7$, df=31, p=0.007), *tropical-fruity* ($\chi^2=53.7$, df=31, p=0.007), *rough body* ($\chi^2=52.7$, df=31, p=0.008), *stone-fruity* ($\chi^2=49.9$, df=31, p=0.017) and *woody* ($\chi^2=47.6$, df=31, p=0.029). 'Past-croppish' was the most discriminant attribute (p=0.001) and indicates the flavour of fading green coffee after some time of storage. The fading implies a general loss of flavour characters and the appearance of a 'cardboardy' or 'woody' flavour (Rendón et al., 2014). Fading is not linked to a particular origin or processing method as its occurrence is possible in any coffee.

The descriptor subgroups which did not discriminate among samples as they had a value of $p>0.95$ (df=31, $\chi^2<19.3$) were *chocolaty* ($\chi^2=16.3$, df=31, p=0.986) and *complex* ($\chi^2=18.7$, df=31, p=0.960). The non-discriminating subgroups would be typical of the profiled coffees and suggest they are common in these coffees regardless of origin or processing method. This confirms the finding of Chapter 3, where chocolaty flavour notes were also found to be the least discriminant ones, which implies chocolaty character is one of the main overall characteristic flavours of coffee at this level of roast.

The χ^2 per-cell applied to determine significant descriptor subgroups for individual samples found that, for 17 samples, one to three descriptor subgroups were significantly above the theoretical average (χ^2 test per-cell, $\alpha=0.05$). The samples with a distinct character are shown in Table 4.6. *Phenolic* was significant in three Brazilian samples (both washed and natural) and one Mexican sample. Fruity characters including *tropical-fruity, red-fruity* and *stone-fruity* were significant in four Nicaraguan natural samples. *Floral* character was significant in a washed Colombian sample. *Past-croppish*, an indicator of green bean fading, was observed in samples BNI, ENX, and VWG. Appearance of past-croppish in samples from different countries and processing methods is possible as the fading process of green beans is independent of country of origin and processing method (Rendón et al., 2014).

Table 4.6. Samples presenting significant characters (χ2 test per-cell, α=0.05).

Sample	Country of origin	Character
BNI	Brazil	Earthy, past-croppish, phenolic
BNO		Phenolic, vegetable
BNX		Pyrolytic
BWI		Caramelly, wood
BWW		Phenolic
CWX2	Colombia	Floral
ENX	Ethiopia	Past-croppish
HNX	Mexico	Phenolic
NNB1	Nicaragua	Sweet-acid
NNB2		Fermented
NNB3		Smooth body
NNB4		Tropical fruit
NNB5		Red-fruity
NNC		Red-fruity
NNP1		Fungal
NNR		Red-fruity, stone-fruity
VWG	Mexico	Past-croppish

Visualisation of the flavour profile obtained by NSCA on the frequency table (non-aggregated duplicates) shows a total inertia of 0.027 in 32 factors. The level of total inertia of the first 30 factors of the present study (also 0.027, as the last two factors had negligible inertia) can be compared to the level of inertia reported in Chapter 3, which presented 30 factors (0.036). Even though the inertia in Chapter 3 is 33% higher than in this study, both are still in the same order of magnitude (Greenacre, 2006), meaning the variability is similar in both cases. 77.0% of the total variation was explained in the first 10 factors. Factors 1 and 2 (F1 and F2) had inertia of 28.2% and 9.1%, respectively. As explained in 3.3.2, this low level of inertia may be due to the large number of terms used by assessors (Lawrence et al., 2013) or to limitations of the cupping method (noise in the dataset).

Red-fruity (29.7% contribution) and *tropical-fruity* (10.7% contribution) were the main contributing characters to F1 and were negatively loaded. *Phenolic* (9.0% contribution) and *caramelly* (7.4% contribution) were positively loaded on F1 (Figures 4.1 and 4.2).

All washed samples (except CWX1) and the pulped natural sample were positively loaded on F1. This would indicate that fruitiness is not characteristic of washed coffees. This finding

supports Chapter 3 and further suggests that fruitiness is a feature of some natural coffees and may contribute to the so-called 'Mocha' character (Akiyama et al., 2008). However, some natural samples were also positively loaded on F1, indicating that not all natural samples present fruitiness (Figure 4.1). This finding is similar to Chapter 3, where some natural samples from Guerrero (Mexico) did not have the fruity character common to naturals.

Along F1 samples tended to distribute based on their country of origin, more so than their processing method. In general, the Nicaraguan samples were negatively loaded, on the first dimension, while the Brazilian samples were positively loaded. The Mexican samples were located around the origin, between the Nicaraguan and Brazilian samples. The predominance of origin over processing methods and variety in the exhibited flavour variation may explain the traditional focus of the coffee industry on the beans' source, rather than on varietal or processing factors. The reasons for the large effect of the origin are not limited to geographical factors and may be attributed to cultural aspects, such as the farming system, the harvesting practices and the processing technologies used in different countries. For example, in the regions of Brazil where the samples were sourced, coffee is grown in highly intensive plantations where coffee is machine-harvested and machine dried at a fast rate (Cheney, 1999; Melenikiotis, 1998). Whereas more traditional farming practices are used in Nicaragua in which, coffee may be grown under shade, be hand-picked and sun-dried at a slow rate (Craipeau, 1992; Raichle, Sinclair, & Ferrell, 2012; Ruben & Zuniga, 2011). These practices in both countries are kept constant regardless of coffee variety or the processing method used.

Factor 2 (F2) was positively loaded with 'sugar browning' characteristics including *chocolaty* (18.4%) and *caramelly* (13.8%). F2 was negatively loaded with *floral* (12.9%) and *citrus-like* (8.0%) on the negative side. The separation between duplicate samples tends to be higher along F2 than along F1 (for example, NNB5, NNB4 and BNI are separated along F2). This implies the level of precision for F2 is lower and should be interpreted carefully.

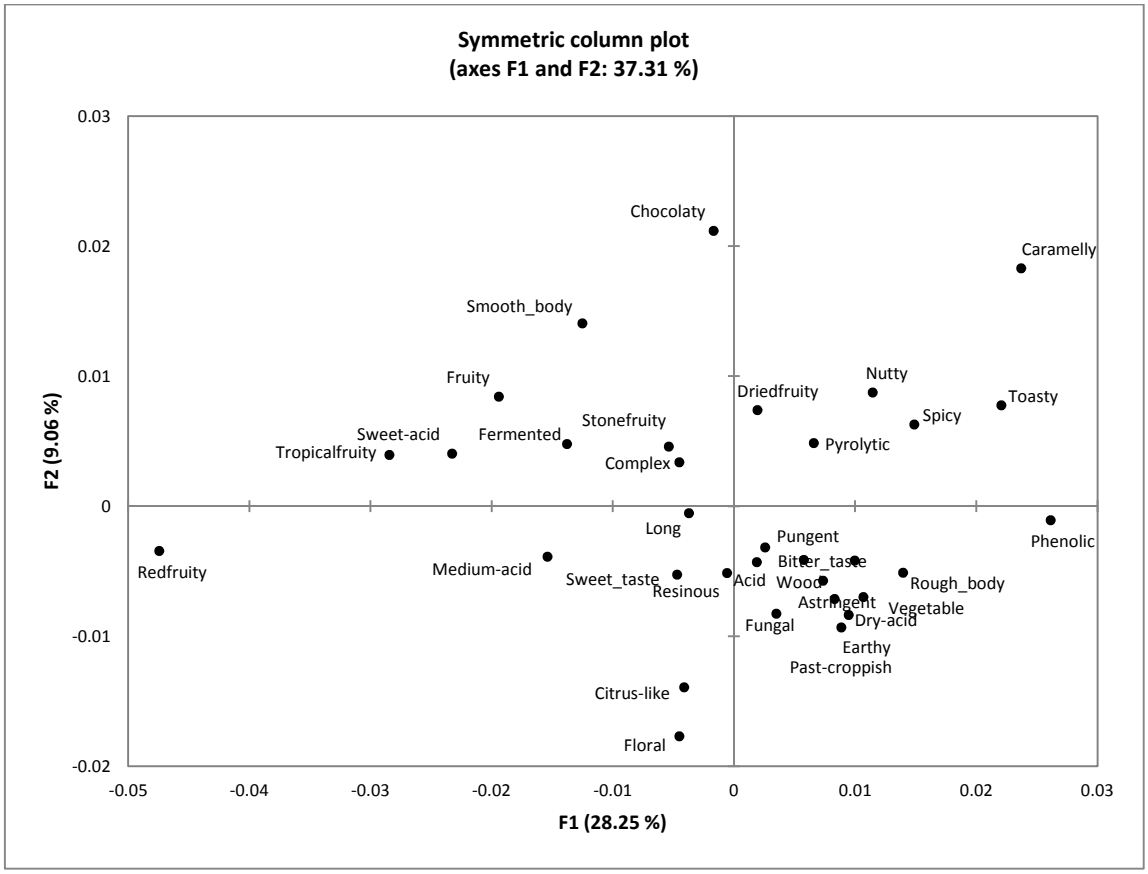


Figure 4.1. Non-symmetric correspondence analysis (NSCA) map representing the projection on F1 and F2 of descriptor subgroups for 32 coffee samples (16 in duplicate) of different processing methods, evaluated using the Descriptive Cupping method.

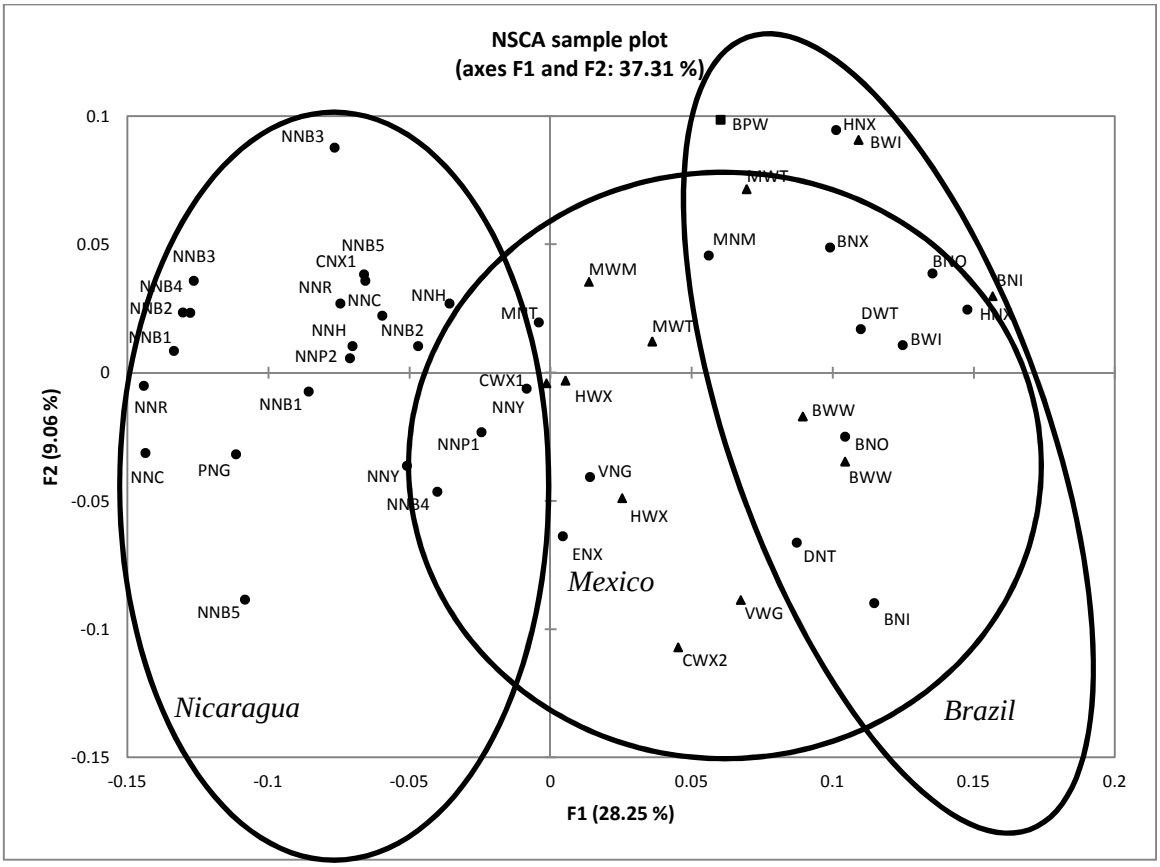


Figure 4.2. Non-symmetrical correspondence analysis (NSCA) map representing the projection on F1 and F2 of natural (circle), washed (triangle) and pulped-natural (square) coffees for 32 samples (16 in duplicate), evaluated using the Descriptive Cupping method. Samples with the same name are duplicates.

4.3.5 Flavour profiles of the natural coffee sample subset

The natural samples (22) were analysed as a subset of the total samples set to study flavour variation among natural coffees as a class. Significance was tested on the contingency table. The dependence between descriptors and samples was significant (p=0.003), as detected by the 'Monte Carlo' method applied on the frequency table (aggregated duplicates, 5000 simulations). The observed value of χ^2=787.9 implies a value in the interval [0.001, 0.004] with 99% confidence, with a critical χ^2 value of 733.4 (df=21, p=0.05). This means there is an effect of sample on the descriptor subgroups used by cuppers.

The global χ^2 analysis showed that for a descriptor subgroup to be significant (p<0.05), the χ^2 of the subgroup needs to be above the critical value of χ^2=32.7 (df=21). The 7 attributes found to vary significantly among the coffees included *past-croppish* (χ^2=52.7, df=21, p<0.001), *phenolic* (χ^2=48.1, df=21, p=0.001), *fungal* (χ^2=46.2, df=21, p=0.001), *stone-fruity* (χ^2=41.5,

df=21, p=0.005), *rough body* (χ^2= 40.0, df=21, p=0.007), *pungent* (χ^2= 35.5, df=21, p=0.025) and *woody* (χ^2=33.3, df=21, p=0.043). Fruitiness did not discriminate among the natural coffees as was the case between processing methods (4.3.4). This would imply fruitiness may be a more typical character of naturals than a unique feature of some samples.

The descriptor subgroups which did not discriminate among samples as they had a value of p>0.95 (df=21, χ^2<11.6) were *chocolaty* (χ^2=10.0, df=21, p=0.979), *complex* (χ^2=10.4, df=21, p=0.973) and *medium-acidity* (χ^2=11.5, df=21, p=0.952). The least discriminant subgroups would be typical of natural coffee profile findings, as least discriminant would suggest that they are common in naturals regardless of origin. Chocolaty has again to be found the least-discriminating subgroup, as in 4.3.4 and Chapter 3, which means chocolate may be the most characteristic descriptor of any kind of coffee, at least when roasted to the roast degree used in this study.

Application of χ^2 per-cell analysis found that, for eight samples, one or two descriptor subgroups were significantly above the theoretical average. These subgroups were *earthy, fermented, fungal, past-croppish, phenolic, pyrolytic, woody* and *vegetable* (Table 4.7, χ2 test per-cell, α = 0.05). This would indicate that these eight samples had distinct character. The number of samples presenting a significant character dropped to less than half the number found for the complete set analysis.

Table 4.7. Natural samples presenting significant characters (χ2 test per-cell, α=0.05).

Sample	Character
BNI	Earthy, past-croppish, phenolic
BNO	Phenolic, vegetable
BNX	Pyrolytic
ENX	Past-croppish, wood
HNX	Phenolic
NNB2	Fermented
NNP1	Fungal

NSCA on the frequency table (non-aggregated duplicates, 34 rows including 12 duplicates, 33 columns) shows a total inertia of 0.026 in 32 factors (the number of factors equals the number of columns minus one). This variability is only 4% smaller (Greenacre, 2006) than the one of the table with all samples in 4.3.4 (0.027). 75.3% of the total variation was represented in the

first 8 factors. This implies variability of the natural samples can be explained using fewer factors than for all samples together (77.0% of total variation in the first 10 factors). The reason for this is the lower number of samples (rows of the contingency table), which implies there is a smaller amount of information to be represented. Inertia took values of 31.3% and 9.0% on F1 and F2, respectively. Refer to 3.3.2 for a discussion about possible causes for the low inertia level in the dataset (large number of descriptors and limitations of the cupping method).

Samples positively loaded on F1 (Figures 4.3 and 4.4) were described as *red-fruity* (26.4% of contribution) and *tropical-fruity* (8.5%). Those negatively loaded on F1 were described as *phenolic* (9.9%). This corresponds to the two commonly recognised natural coffee profiles identified in Chapter 3. The first is the so-called 'Mocha' character, characterised by its fruitiness. The second is a profile closer to that of washed coffee with little or no fruitiness. These natural coffee samples that lack fruitiness (HNX, BNX, BNO, BNI) are plotted near the washed coffees in Figure 4.2. These two profiles appear to be due to a process factor having a geographical concentration. The Nicaraguan samples (enclosed in the ellipse, Figure 4.4) are negatively loaded on F1, and described by the Mocha-like descriptor subgroups. The Brazilian samples (enclosed in the ellipse) are positively loaded and described as more *phenolic* and *caramelly*. The Mexican samples (enclosed in the ellipse) sit between Nicaraguan and Mexican samples, near the origin. The reasons for the geographical concentration of process factors were explained in section 4.3.4.

Factor 2 (F2) is positively loaded with *fungal* (10.4%), and negatively loaded with *chocolaty* (21.6%) and *pyrolytic* (10.0%). However, the distance between duplicates along this factor is larger than along F1; for example, the placement of both duplicates for BNO, BNI, and NNB5 are on both sides of the axis. Furthermore, chocolaty was found to be the 'least-discriminating' character in the χ^2 test. This implies F2 should be interpreted carefully. At any rate, the characters mostly represented by F2 do not depend on the origin or the presence of other characters: chocolaty character, for example, may or may not be combined with fruitiness.

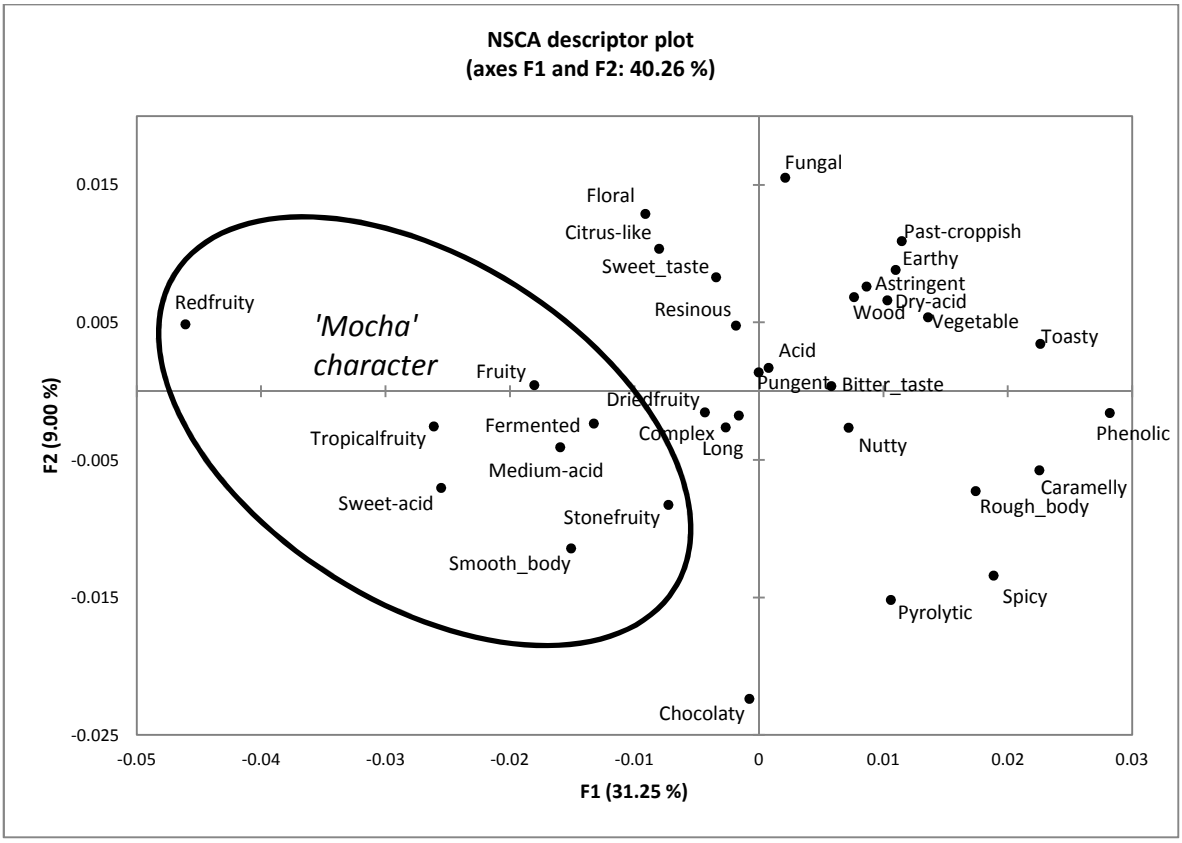


Figure 4.3 Non-symmetric correspondence analysis (NSCA) map representing the projection on F1 and F2 of descriptor subgroups for 22 natural coffee samples (12 in duplicate), evaluated using the Descriptive Cupping method.

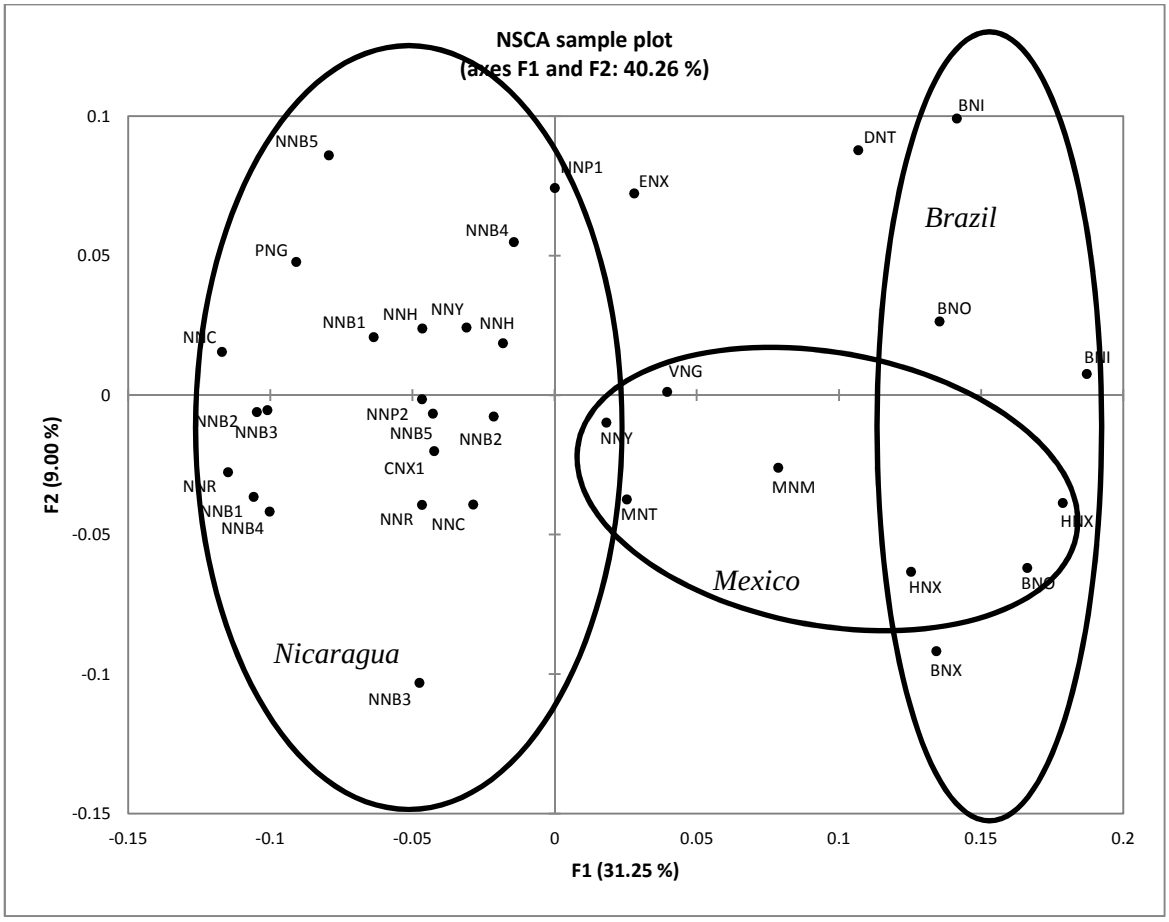


Figure 4.4 Non-symmetrical correspondence analysis (NSCA) map representing the projection on F1 and F2 of natural coffees for 22 samples (12 in duplicate), evaluated using the Descriptive Cupping method. Samples with the same name are duplicates.

4.3.6 Correlations between flavour profile and other variables

This study also sought to understand the links between the flavour profiles of natural coffees, their quality evaluations and the characteristics of the coffee in green bean. Correlations between the descriptor subgroups and the attribute intensities, quality scores obtained by cupping, along with the green bean variables (4.2.4.4) were explored using MFA.

Table 4.8 shows the RV coefficient (indicative of correlation between the variable groups) between the active tables of the MFA (flavour descriptors and attribute intensities) and the other variable groups. RV coefficients show there is correlation between the descriptors and the other types of data. The correlation of both active tables with the MFA model is above 0.850, which implies both active tables are well represented by the model. There is also a correlation (RV=0.598) between both active tables, which means some descriptor subgroups

are associated with the perceived intensity of attributes. The correlation between the green coffee characterisation and the active tables (RV=0.439 for the flavour descriptors table and RV=0.371 for the attribute intensities table) is lower than for the other groups of variables but it can still be considered for interpretation. In this case, as opposed to Chapter 3, the quality scores are correlated with the flavour descriptors table (RV=0.639) and with the attribute intensities table (RV=0.761), implying some of the subgroups in this study were associated with the perception of quality.

Table 4.8. RV coefficient between active tables (flavour descriptors and attribute intensities) and other groups of variables for 22 natural coffee samples, calculated within an MFA.

Variable group	RV coefficient	
	With flavour descriptors	With attribute intensities
MFA	0.922	0.861
Flavour descriptors	1.000	0.598
Attribute intensities (fragrance, aroma, acidity and body)	0.598	1.000
Green coffee characterisation (see 4.3.2)	0.439	0.371
Quality scores (Fragrance/Aroma, Flavour, Aftertaste, Acidity, Body, Balance and Overall)	0.639	0.761

The MFA resulted in a total of 32 factors (sum of eigenvalues=5.912). 75.7% of the total variability was represented in the first 9 factors. Factor 1 (F1) explains 31.8% of the total variability, while factor 2 (F2) explains 8.3%. These low levels of explained variability in F1 and F2 are due to the low variability explained by F1 and F2 in the descriptive dataset, as discussed in 4.3.5 (large number of descriptors and limitations of the cupping method – see also 3.3.2).

The contribution of the flavour descriptor table to F1 and F2 is 49.8% and 79.7%, respectively, while the contribution of the attribute intensities is 50.2% and 20.3% to F1 and F2, respectively. The squared cosines of the flavour descriptor table on F1 and F2 are 0.518 and 0.090, respectively, while the square cosines of the attribute intensities are 0.973 and 0.011 on F1 and F2, respectively. The squared cosines of the supplementary tables are also

highest on F1: 0.736 for the green bean characterisation and 0.995 for the quality scores. As the square cosine of a table for a given factor is a measure of how well the table is being represented in that factor, the fact that the square cosines of all tables are highest for F1 means that the MFA interpretation can be based on F1.

Figure 4.5 shows the MFA map for the variable groups studied, and Figure 4.6 shows the MFA map for the 22 natural coffee samples. Attribute intensities were all positively loaded on F1. Attribute intensities were directly correlated with a natural coffee's 'Mocha' character (*red-fruity*, *tropical-fruity*, *fruity*, *fermented*) and sweetness (*sweet-acid*), and inversely correlated with *phenolic*, *dry acid* and woody-vegetable characters (*wood*, *vegetable*, *past-croppish*) on F1. The distribution of origins along F1 is similar to the one shown by the NSCA (4.3.5) with Brazilian samples negatively loaded, Nicaraguan samples positively loaded, and Mexican samples located around the origin. In the case of the MFA, this indicates the attribute intensities for fragrance, aroma, acidity and body are weakest in the Brazilian samples (samples beginning with 'B' in Figure 4.6) and strongest in the Nicaraguan samples (samples beginning with 'N' in Figure 4.6).

When the supplementary tables are examined, in this study, general fruitiness and sweetness were in turn correlated with higher quality scores, a higher raw bean density and a darker, redder/yellower raw bean colour (Figure 4.5). These characteristics were present mostly in Nicaraguan samples (samples beginning with 'N' in Figure 4.6). This colour in the raw natural beans may be due to a slower drying rate, which allows for more intense pulp fermentation.

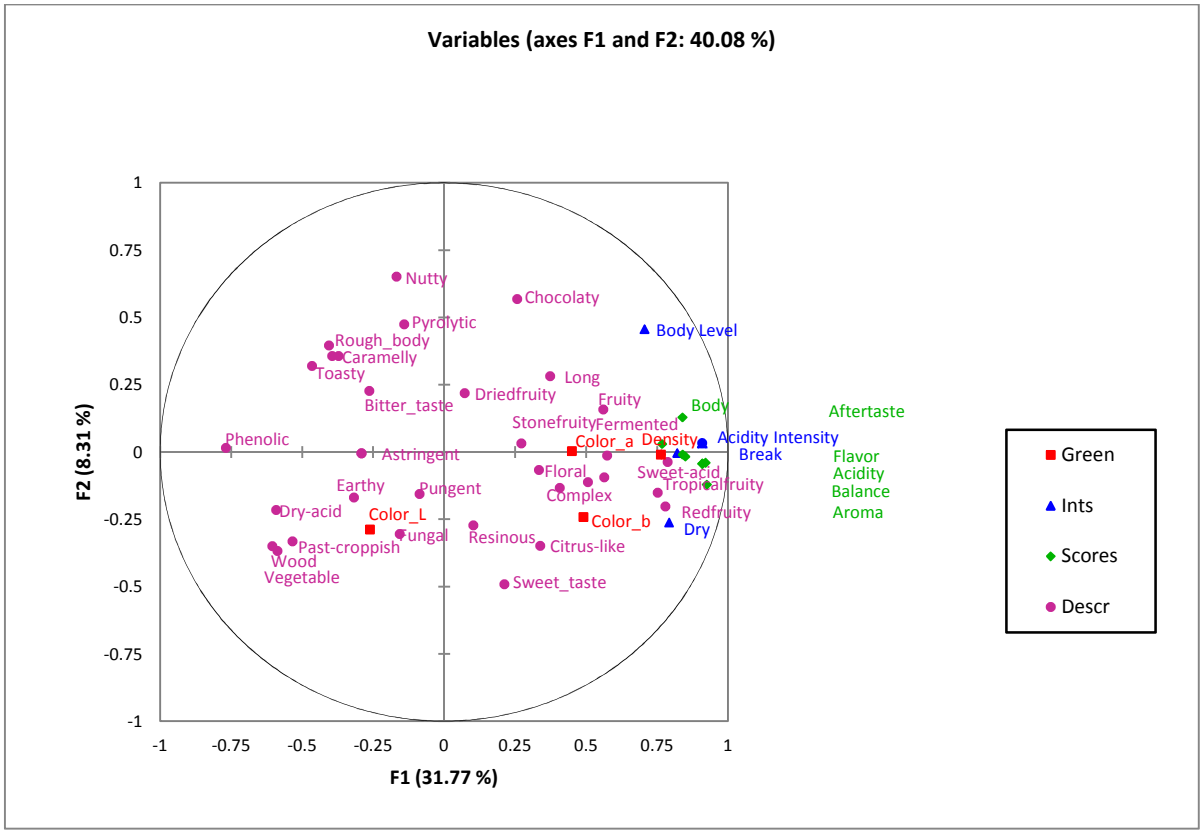


Figure 4.5 Multiple factor analysis (MFA) map representing the projection on F1 and F2 of descriptor subgroups ('Descr' – circle), attribute intensities ('Ints' – triangle), quality scores ('Scores' – diamond) and green bean parameters ('Green' – square) for 22 coffee samples, evaluated using the Descriptive Cupping method.

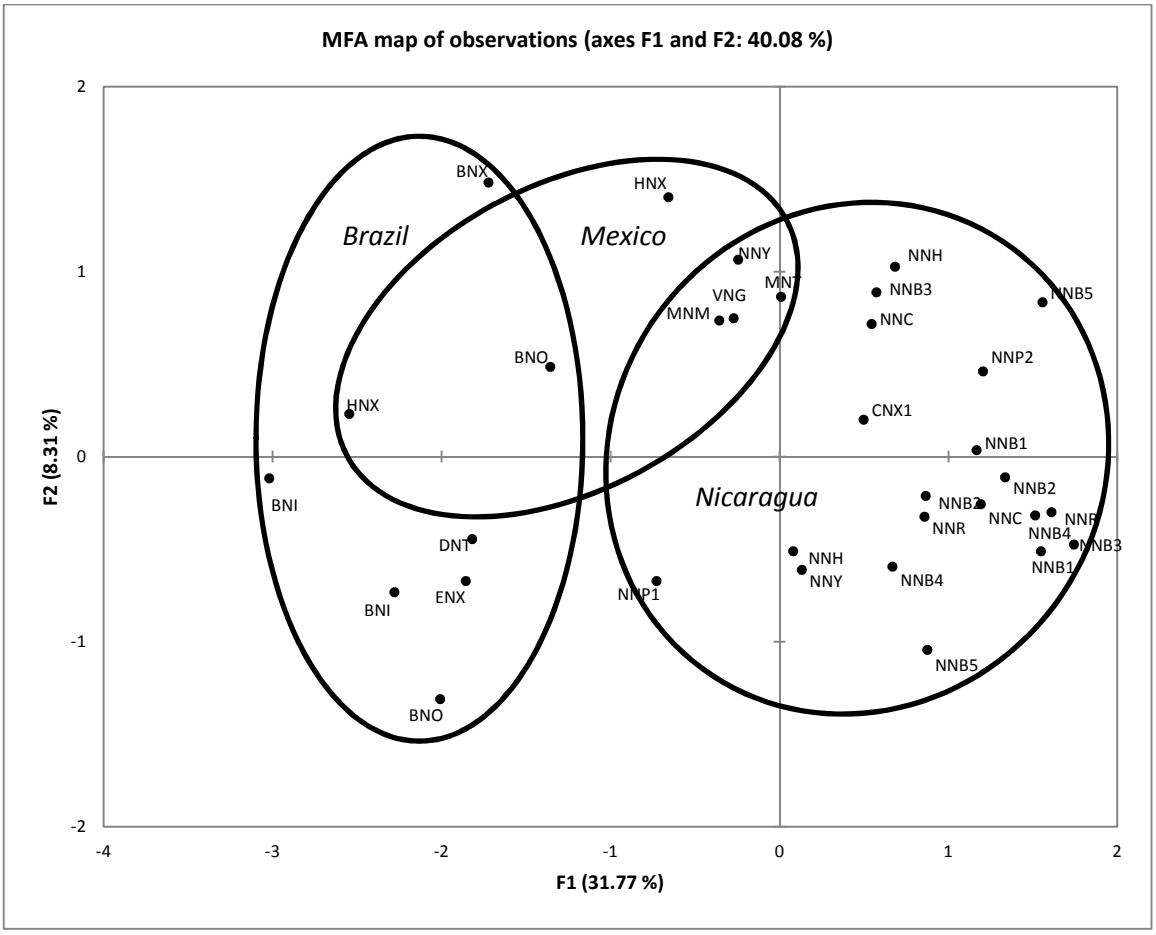


Figure 4.6. Multiple factor analysis (MFA) map representing the projection on F1 and F2 of natural coffee samples (22), evaluated using the Descriptive Cupping method.

4.4 Conclusion

This chapter aimed at understanding the flavour variation of natural coffees as a product class within the context of specialty coffee as a whole, employing Descriptive Cupping as a technique to analyse coffee flavour profiles and compare the flavour of natural coffees to the one of samples from other processing methods. In order to achieve this, natural coffee samples from different countries of origin were procured. Samples from the same farms made through other processing methods were used as points of reference.

A wide variability among coffees was achieved, as can be seen in the green coffee samples' characterisation (4.3.2). This heterogeneous sample set resulted in a wide range of flavour profiles, as revealed by the low inertia levels of the first NSCA factors.

Chapter 3 revealed a combined effect of the farm of origin and the processing method on the flavour profile, even within a single coffee producing region, such as Guerrero. However, in the present chapter, since the producing regions are so varied, the effect of the origin becomes predominant. Nevertheless, it must be noted that the origin implies, together with the merely geographical factors like soil and climate, different farming systems, as well as different harvesting and processing technologies. These cultural factors may be playing a role at least as important as the physical environment where coffee is grown.

Only when natural coffees show a 'Mocha' character do they become clearly different from washed coffees. The absence or presence of the 'Mocha' character may be more important for flavour variability as a whole than origin itself. Moreover, its absence or presence is also one of the main sources of variation within the natural coffees as a group. In this chapter, natural coffees presenting a 'Mocha' character received higher quality scores than non-fruity natural coffees. The understanding of the 'Mocha' character – what it is and how it is produced – seems to be a relevant question in the study of natural coffee. The following chapters will deal with the answers to this question.

5 Volatile organic compounds as related to the fruity fragrance of natural coffee from different origins

5.1 Introduction

The previous chapters have shown that natural coffees are most easily differentiated from other processing methods when they present fruity or winey flavour notes – a profile which has been called 'Mocha' character (Akiyama et al., 2008; Ukers, 1922). The compounds causing the Mocha character have not been fully characterised. Akiyama et al. (2008) relate the Mocha character with a country of origin (Ethiopia or Yemen), with no mention of a processing method effect, and suggest it may be linked to the 'raspberry ketone' (4-(4'-hydroxyphenyl)-2-butanone). T. Hofmann, in a personal communication cited by Folmer (2014) (without reporting methods), links the fruitier character of a natural sample from Peru to the significant presence of ethyl 3-methylbutanoate, ethyl 2-methylbutanoate and ethyl methylpropanoate, when compared to a washed sample and an untreated sample from the same raw material. However, as shown in the previous chapters, the expression of fruitiness in naturals ranges from little or no fruitiness to a fruity character that may be expressed as dried-fruit, tropical-fruit, red-fruit or berry, overripe-fruit or even winey flavour notes. The compounds or ratios between compounds related to these nuances are not known.

The aim of this chapter was to investigate the compounds that give rise to the fruity and sometimes winey character termed Mocha that most easily differentiates natural coffee from other methods (*Objective 4*, 2.2.2.4). This was done by looking at the aspect of coffee where this character is most easily perceived: the fragrance of the dry coffee grounds, which in cupping terms means the headspace above roasted, ground beans. Gas chromatography coupled to olfactometry and mass spectrometry (GC-MS/O) was the main tool used for characterising the ground coffee headspace. A non-destructive method (proton-transfer-reaction mass spectrometry – PTR-MS) was used to investigate the green bean headspace prior to roasting, as a supplementary source of data. The correlations between the chemical profiles and the flavour profiles from Chapter 4 were then investigated in order to find the compounds related to the Mocha profile.

5.2 Materials and methods

5.2.1 Samples

The sample set studied was the same used for Chapter 4 (4.2.1), plus two additional samples (Table 5.1). The sample set includes 24 natural coffee samples, 1 pulped natural coffee sample and 9 washed coffee samples from 7 countries (Brazil, Colombia, Dominican Republic, Ethiopia, Mexico, Nicaragua and Panama). Prior to roasting headspace analysis of the green coffee was performed by PTR-MS (5.2.2). The roasted samples for the subsequent analyses were taken from the same batches used for cupping in Chapter 4. Therefore, the roasting method is the same one described in 4.2.1.3.

Table 5.1. Additional samples for instrumental analyses.

Country	Region	Variety	Process	Drying time (days)[b]	Drying Method	Sample code
Colombia	Antioquia	- [a]	Natural	15	Raised beds	CNX2
Mexico	Veracruz	Garena	Natural	-	Concrete patio	VNE

(a) (-) – Data not available.
(b) To achieve 12% moisture.

5.2.2 Headspace analysis of green coffee by PTR-MS

The composition of the volatile compounds in the headspace of the green coffee beans was determined by high sensitivity PTR-MS. PTR-MS was chosen for headspace analysis of the green beans, as it is highly sensitive and non-destructive. This means the beans could later be roasted.

For each sample, green coffee beans (30g) were placed in duplicate 500mL glass bottles (Schott Duran, Germany) capped with an inlet and an outlet on the top, and using glass fittings inserted into Teflon rings to create an air tight system. Two polytetrafluoroethylene (PTFE) tubes were fitted into the glass fittings as inlet and outlet. The closed bottle was held at 23 ± 1°C for 30min. At this point, the bottle headspace was immediately analysed using the PTR-MS (Ionicon Analytik GmbH, Innsbruck, Austria). The bottle headspace was replaced using instrument grade synthetic air, flushed through an active charcoal filter (Supelcarb®, Supelco, Bellefonte, PA, USA) and drawn into the PTR-MS. The PTR-MS inlet consisted of a ~1m long 1/16″ outer diameter Silcosteel™ capillary (Restek Co., Bellefonte, PA, USA)

heated to 110°C. Headspace air was sampled at a continuous flow rate of 50 ± 1cm^{3}·min^{-1} over a mass/charge (m/z) range of m/z 20-180 under drift tube conditions of 600V, drift pressure of 2.2mbar, drift-tube temperature of 80°C, and E/N value of 140Td (Td = Townsend; 10-17cm^{-2}·V^{-1}·s^{-1}). Each measurement consisted of 8 cycles with a dwell time of 200ms per m/z (32s per cycle), taking 4.2min for one sample. This was preceded by the measurement of a blank (a bottle with no sample) under the same conditions, to enable the background to be subtracted.

5.2.3 Headspace analysis of roasted and ground coffee using headspace-solid-phase extraction (HS-SPE)

For the roasted coffees, the method development prioritised the presence of fruity and fermented notes in the extracts. A modified version of the sample preparation method reported by Michishita et al. (2010) was used. The headspace of the roasted and ground beans was captured by purge-and-trap, headspace-solid-phase extraction (HS-SPE), eluted with a solvent and concentrated. Samples were roasted and ground, as this was similar to the evaluation by the trained cuppers for the dry 'fragrance' (Chapters 3 and 4).

Liquid headspace extracts permitted the analysis of the same extract by gas chromatography-olfactometry (GC-O), gas chromatography coupled with mass spectrometry (GC-MS) and the combination of the two (GC-MS/O). The compounds that eluted prior to the end of the solvent peak in GC-MS were analysed separately, through static headspace gas chromatography-mass spectrometry (SH-GC-MS – 5.2.6).

For each sample, roasted coffee beans (15g) were ground in duplicate using a 'BarAroma^{TM}' coffee grinder (Breville Pty., Botany, NSW, Australia) in the 'Filter' grind setting. The resulting ground coffee was placed in a 250mL glass bottle (Schott Duran, Germany) capped with an inlet and an outlet on the top, and using glass fittings inserted into Teflon rings to create an air tight system. Two PTFE tubes were fitted into the glass fittings as inlet and outlet. The sample headspace VOCs were displaced using nitrogen (50 ± 1cm^{3}·min^{-1}; BOC® 'oxygen free', 99.99% purity: water vapour≤25ppm; oxygen≤10ppm; BOC New Zealand, Auckland, New, Zealand), which was passed into the bottle through the inlet using PTFE tubing and stainless steel connections and valves (Figure 5.1) for 120min, with the bottle shaken every 30min. The coffee headspace gases passed from the bottle outlet through a glass column packed with Carboxen® 569 (400 ± 9mg; 20-45 mesh; Sigma-Aldrich Co. LLC., St.

Louis. MO, USA), previously washed under vacuum with dichloromethane (25mL; LiChrosolv®, liquid chromatography grade, 99.9% purity, Merck KGaA, Darmstadt, Germany). The column was then washed with dichloromethane (7mL) and the eluate retained. The eluate was kept at -20°C in glass vials (two, 4mL), capped with PTFE-lined lids, for 1-5 weeks, until it was concentrated.

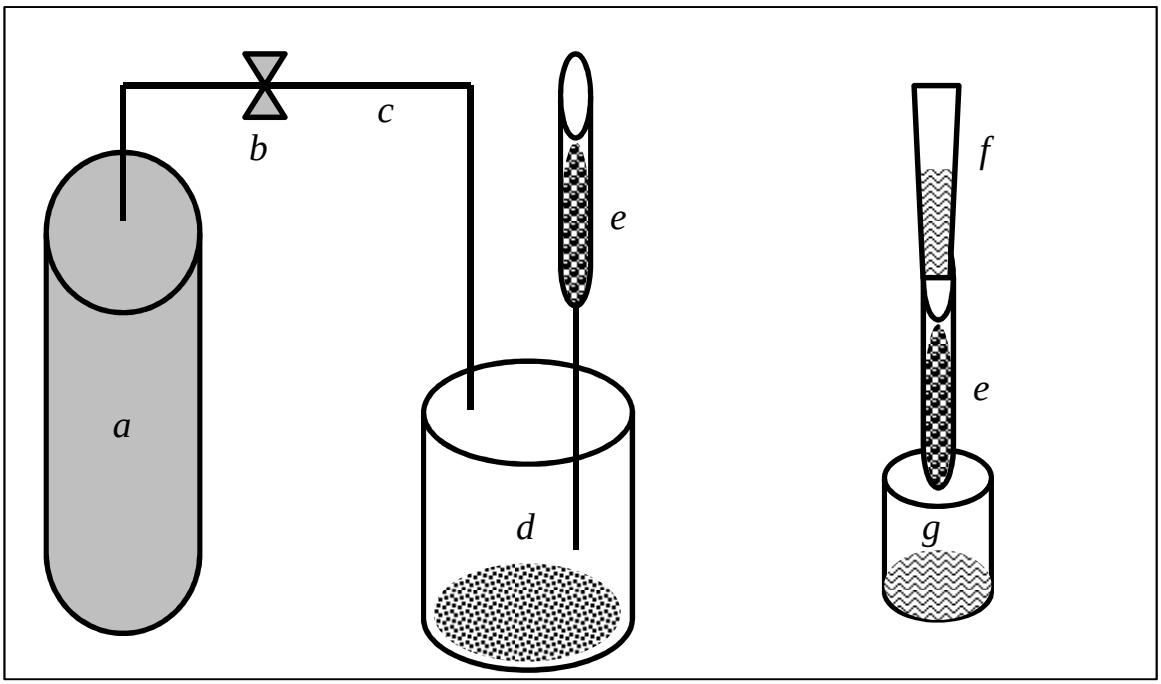


Figure 5.1. Diagram of HS-SPE apparatus. Key: (a) Nitrogen cylinder, (b) needle valve for regulating flow; (c) PTFE tubing; (d) Schott bottle with ground coffee sample; (e) glass column packed with Carboxen 569; (f) dichloromethane being funnelled into column after extraction; (g) vial where eluted extract is collected.

An internal standard (125µL, 60 ± 1ppm, (1R)-endo-(+)-Fenchyl alcohol; CAS Number 2217-02-9; 96% purity; part No 196444; Sigma-Aldrich Co. LLC., St. Louis. MO, USA) was added to the eluate prior to concentration. The eluates were concentrated to 0.50mL using a Micro Kuderna-Danish Sample Concentrator apparatus (Supelco, Bellefonte, PA, USA) with a 2mL receiving vessel. Nitrogen (BOC® 'oxygen free'; BOC New Zealand, Auckland, New, Zealand) was gently blown (flow of 60 ± 5$cm^{-3} \cdot min^{-1}$) over the surface of the eluate for 3-4 hours until the concentrate volume was 0.5mL. An aliquot of the concentrate for further GC injections (125µL) was placed in a 2mL vial with a 200µL insert and capped with a PTFE-

lined lid. The remaining concentrate was placed in a 2mL vial with a PTFE-lined lid. Both vials were stored at -20°C.

5.2.4 Identification of odour-active compounds of extracts through GC-MS/O

GC-O was used to filter the compounds for odour-active compounds and to link the aroma sensory characters of coffee samples (profiled through Descriptive Cupping – Chapter 4) to the analytical identification of the compounds responsible for such characters (through GC-MS – 5.2.5). This involved the selection of a set of dissimilar samples, the training of a GC-O panel and the assessment of the selected samples using GC-O coupled to GC-MS (GC-MS/O). Ethical approval 08-083 was revised on 25-7-2014 and obtained for this study.

5.2.4.1 Sample selection for GC-O analysis

Samples (17) were pre-selected from characterization studies (12 from the international appraisal set, Chapter 4, 4 from the field experiment set, Chapter 6 and an extract from a freshly-roasted, Guatemalan natural coffee), based on the sensory profile of all samples as well as their green-bean headspace PTR-MS profiles. These 17 samples were sniffed in duplicate by an expert assessor, using an Agilent 5890 gas chromatograph (Agilent Technologies Inc., Wilmington, DE, USA). A ZB-WAXplus capillary column (Zebron 17538 column; 30m·0.32mm·0.5µm film thickness; Phenomenex Inc., Torrance, CA, USA) was used. The injection of 1µL of each HS-SPE concentrate was done using the warm-needle injection technique, with the GC inlet in split-less mode. The GC oven was held at 50°C for 2min, programmed to 150°C at 3°C·min^{-1} up to 150°C, programmed to 220°C at 10° C·min^{-1} and then held for 20min. The sniffing time was limited to the first 40min. The GC-O data were recorded through handwritten note-taking of the elution time, the odour descriptor and the intensity (in a 1-10 scale) of each odour peak. From the results, five samples were selected for further GC-O analyses, using the number of unique odours present in each sample as criteria for covering the widest range of odorants.

5.2.4.2 GC-O panel recruitment and training

GC-O assessors (5; 2 female, 3 male; age range 30-43) were recruited from the members of the coffee cupping panel (Chapter 4). The assessors were trained in 2 sessions. In the first session, the whole group was trained in an odour descriptor tree (Table 5.2), which is the

tiered descriptor structure providing groups and subgroups of aroma, based on the extended cupping descriptor catalogues used for naturals in Chapter 4 and specific GC-O peaks from sample selection runs. The purpose of GC-O and its working principle was explained to the assessors, describing the instrument they would be using and what they would do. The descriptor tree was discussed with assessors and then practiced by having each assessor use the tree as a tool to describe 10 concealed aroma mugs. As a practice for the GC-O sniffing conditions, each assessor was presented with a concealed aroma mug for 10 seconds and was asked to describe it verbally to the group, using descriptors from the tree.

Table 5.2. Odour descriptor tree used in the training of the GC-O assessors

First level - GROUP	Second level - SUBGROUP	Third level – FLAVOUR NOTE EXAMPLES
ENZYMATIC	Herby	Potato
		Sweet peas
		Cucumber
	Floral	Tea rose
		Coffee blossom
		Honey
	Generic fruity	Lemon
		Apricot
		Apple
	Red-fruity	Blueberry
		Strawberry
		Grapes
	Tropical-fruity	Passion-fruit
		Mango
		Pineapple
	Dried-fruity	Sultanas
		Prunes
		Dried figs
SUGAR-BROWNING	Nutty	Roasted almonds
		Roasted hazelnuts
		Walnuts
	Caramelly	Fresh butter
		Caramel
		Roasted peanuts

First level - GROUP	Second level - SUBGROUP	Third level – FLAVOUR NOTE EXAMPLES
SUGAR-BROWNING	Chocolaty	Vanilla
		Toasted bread
		Dark chocolate
DRY-DISTILLATION	Spicy	Cloves
		Black pepper
		Coriander seed
	Resinous	Cedar
		Blackcurrant
		Maple syrup
	Pyrolytic	Malt
		Pipe tobacco
		Roasted coffee
AROMATIC TAINTS AND FAULTS	Earthy	Earth
		Straw
		Leather
		Musty
	Fermented	Coffee pulp
		Basmati rice
		Medicinal
		Alcohol
	Phenolic	Boiled beef
		Smoke
		Rubber
OTHER	Pungent	Chili pepper
		Vinegar

On the second training session, each assessor was individually trained to assess the odours using the GC-MS/O olfactometry port, including the intensity of each odour-active peak using a finger span cross modality matching (FSCM) device and verbally recording the character of each odour-active peak (Niimi et al., 2010). In this session, each assessor went through 3 GC-O measurements. For the first two assessments, a mix of 13 miscellaneous compounds was assessed using a short program (15 min). The first training measurement involved focusing on the assessor's breathing rate and the use of the FSCM. The second measurement focused on verbally recording the odour peak descriptors and simultaneously rating the peak intensity

using the FSCM. The third measurement simulated an actual measurement using a coffee extract under the same GC conditions that were later used for assessment (5.2.4.3).

5.2.4.3 GC-MS/O assessment of selected samples by the trained panel

The 5 selected coffee HS-SPE concentrates were assessed by GC-O assessors (in duplicate and using a random order). Each panellist assessed two samples in a one-and-a-half hour session. The HS-SPE concentrate (1µL) was injected into the gas chromatograph (Agilent® 6890, Agilent Technologies Inc., Wilmington, DE, USA), using the instrument's auto-sampler. An HP-Wax capillary column (Agilent 19091X-216 column; 60m·0.32mm·0.5µm film thickness; Agilent Technologies Inc., Wilmington, DE, USA) was used for the chromatographic analyses. A split-less injection mode was used, in order to maximise the odour intensity. The injector temperature of the GC was set at 220°C and the carrier gas was helium, with a nominal column flow rate of 2.7mL·min^{-1}. The oven temperature was held at 50° \C for 2min, increased to 130°C at 3°C·min^{-1} and then immediately to 220°C at 10°C·min^{-1}. The analysis time per sample was 31 minutes. The assessors rated the intensity of each odour-active peak using an FSCM device. At the same time, they verbally elicited a descriptor for the character of each peak. The verbal description was recorded using a microphone attached to the sniffing port.

Simultaneously, mass spectrometry detection was carried out, after a solvent delay of 7.70min, using a 'Y' connector at the end of the GC column to divert approximately half of the flow into a mass selective detector (Agilent® Technologies model 5975B VL MSD; Agilent Technologies Inc., Wilmington, DE, USA). The mass spectrometer operated in the electron impact ionization mode (70eV), with a scan range of 30 to 300m/z. The ion source temperature was set at 230°C.

5.2.5 Semi-quantification of odour-active compounds in HS-SPE concentrates through GC-MS

Each of the HS-SPE concentrates produced in duplicate in 5.2.3 was injected (1µL) into the gas chromatograph (Agilent® 6890, Agilent Technologies Inc., Wilmington, DE, USA). HP-Wax capillary column (Agilent 19091X-216 column; 60m·0.32mm·0.5µm film thickness; Agilent Technologies Inc., Wilmington, DE, USA) was used for the chromatographic analyses. The injector temperature of the GC was set at 230°C and helium was the carrier gas

with a column flow rate of 1mL·min^{-1} and a split flow rate of 10mL·min^{-1}. The oven temperature was held at 50°C for 2min, programmed to 220°C at 3°C·min^{-1} and held for 20min.

Mass spectrometry analysis was carried out with no diversion of the flow to the olfactometry port, after a solvent delay of 7.30min, using a mass selective detector (Agilent® Technologies model 5975B VL MSD; Agilent Technologies Inc., Wilmington, DE, USA) coupled to the gas chromatograph. The mass spectrometer was operated in electron impact ionization mode (70eV), with a scan range of 35 to 300m/z. The ion source temperature was set at 230°C.

5.2.6 Semi-quantification of complementary compounds using static headspace gas chromatography-mass spectrometry (SH-GC–MS)

To enable the semi-quantification of those VOC with a retention index smaller than the solvent delay time, VOC composition of roasted and ground coffee headspace was analysed for the 34 samples using static headspace gas chromatography-mass spectrometry (SH-GC–MS).

For each sample, roasted coffee beans (4.5g) were ground in duplicate using a 'BarAroma^{TM}' coffee grinder (Breville Pty., Botany, NSW, Australia) in the 'Filter' grind setting. The ground coffee (4g) was placed in headspace vials (22mL), which were immediately sealed with silicone rubber Teflon caps. Each vial was sequentially incubated, one at a time, at 60°C for 15min in a headspace sampler (HP® 7694, Agilent Technologies Inc., Wilmington, DE, USA).

The vials were then pressurized to 3.8PSI with carrier gas (He) for 18s, the sample loop was filled for 3s and the loop was equilibrated for 1.2s. The headspace was injected for 30s from the sampler loop, through a transfer line at 70°C, into the GC (Agilent® 6890, Agilent Technologies Inc., Wilmington, DE, USA).

A HP-Wax capillary column (Agilent 19091X-216 column; 60m·0.32mm·0.5µm film thickness; Agilent Technologies Inc., Wilmington, DE, USA) was used for the chromatographic analyses. The injector temperature of the GC was set at 230°C and helium was the carrier gas with a column flow rate of 1mL·min^{-1} and a split flow rate of 10mL·min^{-1}. The oven temperature was held at 40°C for 6min, programmed to 150°C at 3°C·min^{-1} and

then to 190°C at 10°C·min^{-1}. The mass spectrometry analysis was carried under the same conditions of 5.2.5.

5.2.7 Identification of odour-active compounds with a retention index smaller than the solvent delay time using SH-GC-MS/O

Odour-active peaks in the first 11 minutes of the SH-GC chromatogram were identified through olfactometry in two of the 34 samples, using a 'Y' connector at the end of the GC column to divert approximately half of the flow into a sniff port. The analyses were carried out as per 5.2.4.1 by one assessor. The two samples used in this analysis (NNB2 and 7B) were selected from the 5 samples used for GC-O analysis in section 5.2.4.3. These two samples were selected because they presented a larger number of peaks and higher peak intensities with a retention index smaller than the solvent delay time.

5.2.8 Data analysis

5.2.8.1 PTR-MS data from green coffee headspace

The compounds in the headspace of green coffee samples were detected as m/z by the PTR-MS. The net m/z concentrations were calculated by subtracting the mean reading of the background air from cycles 2-8 from the sample's mean scanning profiles from cycles 2-8. The resulting dataset of 160 mass ions was depurated following a sequential process. First, the instrument-related mass ions were eliminated (21 – H_3O^+; 30 – NO^+; 32 – O_2; 37, 38 and 39 – CO_2+H_2O clusters). Then, the m/z with an average below 1 count per second (arbitrary threshold) were eliminated. Next, obvious isotopologues, clusters with a water molecule and fragments of alcohols minus a water molecule were eliminated (34, 35, 51 – methanol; 27, 48 and 49 – ethanol; 43, 44, 62 – propanol; 46 – acetaldehyde; 74, 76 – isotope of 75; 88 – isotope of 87, and 90 – isotope of 89).

The significant m/z were found using analyses of variance (ANOVA) for every m/z with 'sample' as fixed factor in the model. A significance level of 5% was considered. Mean concentrations of significant m/z were then compared using Tukey's *post-hoc* test. IBM SPSS software (version 21) was used to perform this analysis. The significant m/z ratios that could be not separated in more than one group with the Tukey test were not considered for further analyses. The concentration of m/z 20 and 21 were also kept out of further analysis because

they represent hydronium ions. The values for each m/z of the remaining significant data were normalised ($(X - m)\cdot s^{-1}$, where m is the mean and s is the standard deviation) and a principal component analysis (PCA) was carried out on the normalised data, to map the variation of the headspace composition in the green coffee sample set.

5.2.8.2 Identification of odour-active compounds from GC-MS/O data

Aromagrams were produced from the FSCM data generated from the 5 judges, for each of the 5 duplicate samples. The aromagrams were aligned using SpecAlign (version 2.4.1; Cartwright Group, PTCL, University of Oxford, UK). The audio recordings of all samples were also aligned using Audacity (version 2.0.5; a free, open-source, cross-platform software for recording and editing sounds). A table of odour-active peaks was produced, including the onset and end retention times, the intensity rated by each assessor in each sample duplicate (or 0 if no peak was found) and the odour character mentioned by each assessor. The table was summarised by representing the relevance of each peak by the number of assessors rating it for each sample, the sum of intensities and the average intensity for each sample, and a representative descriptor.

The identity of each GC-O peak was investigated in the corresponding data from the GC-MS/O or from the SH-GC-MS data (5.2.6) in the case of peaks eluting before the solvent delay time. The baseline of the GC-MS/O chromatograms was corrected using metAlign software (Rikilt Institute of Food Safety, Wagenigen UR, Netherlands). The compounds were then tentatively identified based on mass spectra matching with the standard NIST-08 MS library (National Institute of Standards, Gaithersburg, MD, USA). The Kovats Index (KI) of every peak was calculated from the retention times of an alkane mix under the same GC conditions. The tentative identity of the peak was confirmed by comparing to the literature-reported KI and odour character of each compound (Akiyama et al., 2003; Flament & Bessière-Thomas, 2002; Gonzalez-Rios et al., 2007a; Sanz et al., 2002). Some characteristic odour-active peaks were not matched to any MS spectrum but were nevertheless tentatively identified from their KI and characteristic odour.

5.2.8.3 Semi-quantification of odour-active compounds in HE-SPE concentrates and static headspace of coffee grounds from GC-MS data

The odour-active peaks tentatively identified using the MS spectrum were quantified using a single ion for each compound, in order to reduce the error from co-elution with other compounds. A characteristic m/z ion was chosen for each compound. The compounds were integrated for all the samples (5.2.1) in duplicate using the GC-MS data from 5.2.5. The peak area of each compound was adjusted based on the concentration of the internal standard (fenchyl alcohol). The compounds eluting before the solvent delay time were integrated from the SH-GC-MS data without any adjustment.

ANOVA, with 'sample' as fixed factor in the model, was used as a selection tool for PCA, and as such, a value of $p<0.15$ was used. Mean peak areas of significant compounds were compared using Tukey's *post-hoc* test (IBM SPSS software, version 21). The values for each compound of the remaining significant data were normalised ($(X-m)\cdot s^{-1}$) and a principal component analysis (PCA) was carried out on the normalised data, to map the variation of the headspace compounds in the roasted and ground coffee sample set.

5.2.8.4 Multiple factor analysis (MFA) including the Descriptive Cupping dataset

In order to understand the links between the natural coffee flavour profile and the VOC profiles in the headspace of green and roasted coffee, the correlations between the descriptor contingency table (from Chapter 4), the odour-active compounds in the roasted bean headspace and the mass ions detected in the green bean headspace were explored using multiple factor analysis (MFA) (Abdi et al., 2013; Lawrence et al., 2013).

Two sets of variables were kept as active tables in the MFA: the descriptor contingency table (Chapter 4) and the semi-quantification of the odour-active compounds (5.2.8.3). These two data tables were included as active tables because they pertain to the same samples (the roasted and ground coffees). The PTR-MS data of the significant mass ions were considered supplementary data because they are not directly related to the roasted coffee flavour.

The degree of correlation between the tables was investigated through the regression vector (RV) coefficient, to identify the link between each pair of tables and to analyse similarity

between any two tables (Robert & Escoufier, 1976). The MFA and the calculation of RV coefficients were carried out using XLstat (Addinsoft SARL).

5.3 Results and discussion

5.3.1 PTR-MS data from green coffee headspace

PTR-MS is a non-destructive VOC analysis technique based on the 'soft' ionisation of VOCs using hydronium ions. The resulting m/z are then measured through mass spectrometry. PTR-MS was used to analyse the headspace of green beans, as the non-destructive nature of this technique enabled further roasting and analysis of the coffee samples. The main objective of analysing the headspace of green coffee was to see if the composition of that headspace correlated with the composition of the headspace of roasted beans or with the sensory characteristics of roasted coffee (see 5.3.4 and specifically Figure 5.9).

In the PTR-MS technique, most compounds are measured as their molecular mass +1, but some compounds form clusters with water (mass +18) while other compounds are fragmented, i.e. losing an H_2O unit (Yeretzian, Jordan, Badoud, & Lindinger, 2002). The correlations between some mass ions enable the identification of clusters, fragments or isotopes. However, this implies the number of significant m/z in the study of a sample set may be larger than the number of significant compounds in the headspace. In this study, a preliminary ANOVA of the PTR-MS data showed a high number of significant peaks: 86 mass ions were found significant at a level of $\alpha=0.05$. However, after the dataset depuration (5.3.1), only 22 mass ions were found relevant for use in the PCA. A second ANOVA using only these mass ions showed all 22 resulting mass ions had a significance level of $p < 0.0005$

Table 5.3 summarises the relevant mass ions found after depuration. Most of them have been tentatively identified, based on Yeretzian et al. (2002) and Romano et al. (2014). Table 5.3 also shows the number of sample groups found by the Tukey post-hoc test. Some of the mass ions are capable of separating samples in ten groups or more. Key examples of known, highly discriminant mass ions are methanol (m/z 33), isobutanal (m/z 73), 3-methylbutanal (m/z 87), 2- and 3- methylbutanol (m/z 89), 3-methylbutenoic acid (m/z 101) and 3-methylbutanoic acid (m/z 103). Most of these compounds have been reported as products of catabolic activity (Roze et al., 2010; Thonning Olesen & Stahnke, 2004). Once the database had been

depurated, the only high correlation (r>0.95) between two mass ions was between m/z 45 (ethanol) and m/z 71 (3-methylbutanol acetate).

Table 5.3. Relevant mass ions in green coffee headspace of 34 international coffee samples analysed using PTR-MS – tentative identification and number of Tukey groups.

m/z ratio	Tentative identification	Number of groups of samples using Tukey post-hoc analysis
29	Unidentified, fragment of ethanol [d]	5
31	Formaldehyde fragment [d]	15
33	Methanol [a,b]	13
41	Unidentified	12
42	Acetonitrile [d]	7
45	Acetaldehyde [a]	2
47	Ethanol [a]	7
55	Butanal [c]	5
57	Alcohol fragment [b]	6
61	Propanol [a], acetic acid, methyl formate [b], acetate fragment [d]	1
65	Ethanol(H_2O) [a]	9
69	Isoprene [a], octanal fragment, 1-octen-3-ol fragment [d]	3
71	Fragment of 3-methylbutanol acetate [e]	6
73	Isobutanal, Butanal, Butanone [a]	10
75	Propanoic acid, Butanol, Isobutanol [a], fragment of short-chain fatty acid (odd carbons) [d]	6
81	Pyrazine [d]	4
85	Methyl butenal [b], alcohol (1-hexenol, nonanol), ester fragment [d]	9
87	3-Methyl-2-buten-1-ol, 2*E*-butenoic acid, 3-methyl butanal, 2,3-butanedione [a]	10
89	Isopentanol, pentanol, isobutanoic acid, 2-methyl-butanol, 3-methylbutanol [a]	12
97	2*E*,4*E*-Hexadienal [a]	11
99	2*E*-Hexenal [a]	8
101	Hexanal, 2,3-pentanedione, 3-methyl butenoic acid [a]	10
103	3-Methyl butanoic acid, pentanoic acid, 1-hexanol [a]	14

(a) Identification reported by Yeretzian et al. (2002).
(b) Identification reported by Romano et al. (2014)
(c) Identification reported by Soukoulis et al. (2013)
(d) Personal communication – P. Silcock.
(e) Identification reported by Ezra et al. (2004)

The PCA of the significant PTR-MS data explained the variability in a total of 22 factors. However, 83.6% of the variability was explained by the first three factors (F1 to F3). Factors 1 and 2 (F1 and F2) represented 59.7% and 14.9% of the variability. Figure 5.2 presents the projection of the significant mass ions on F1 and F2. 19 of the mass ions were best represented by F1, as explained by the fact that their square cosines were largest for F1 than for any other factor. It seems F1 is best described by some products of amino acid catabolism (Roze et al., 2010; Thonning Olesen & Stahnke, 2004), as the main contributing mass ions to F1 were m/z 87 (3-methyl-2-buten-1-ol, 2*E*-butenoic acid, 3-methyl butanal or 2,3-butanedione – 6.79%), m/z 85 (methyl butenal, 1-hexenol, nonanol), ester fragment – 6.56%), m/z 69 (isoprene, octanal, 1-octen-3-ol – 6.38%) and m/z 71 (3-methylbutanol acetate – 6.16%). F2, on the other hand, is best described by methanol, as the main contributing mass ions to F2 were m/z 33 (methanol – 18.76%) and m/z 31 (formaldehyde – 16.56%).

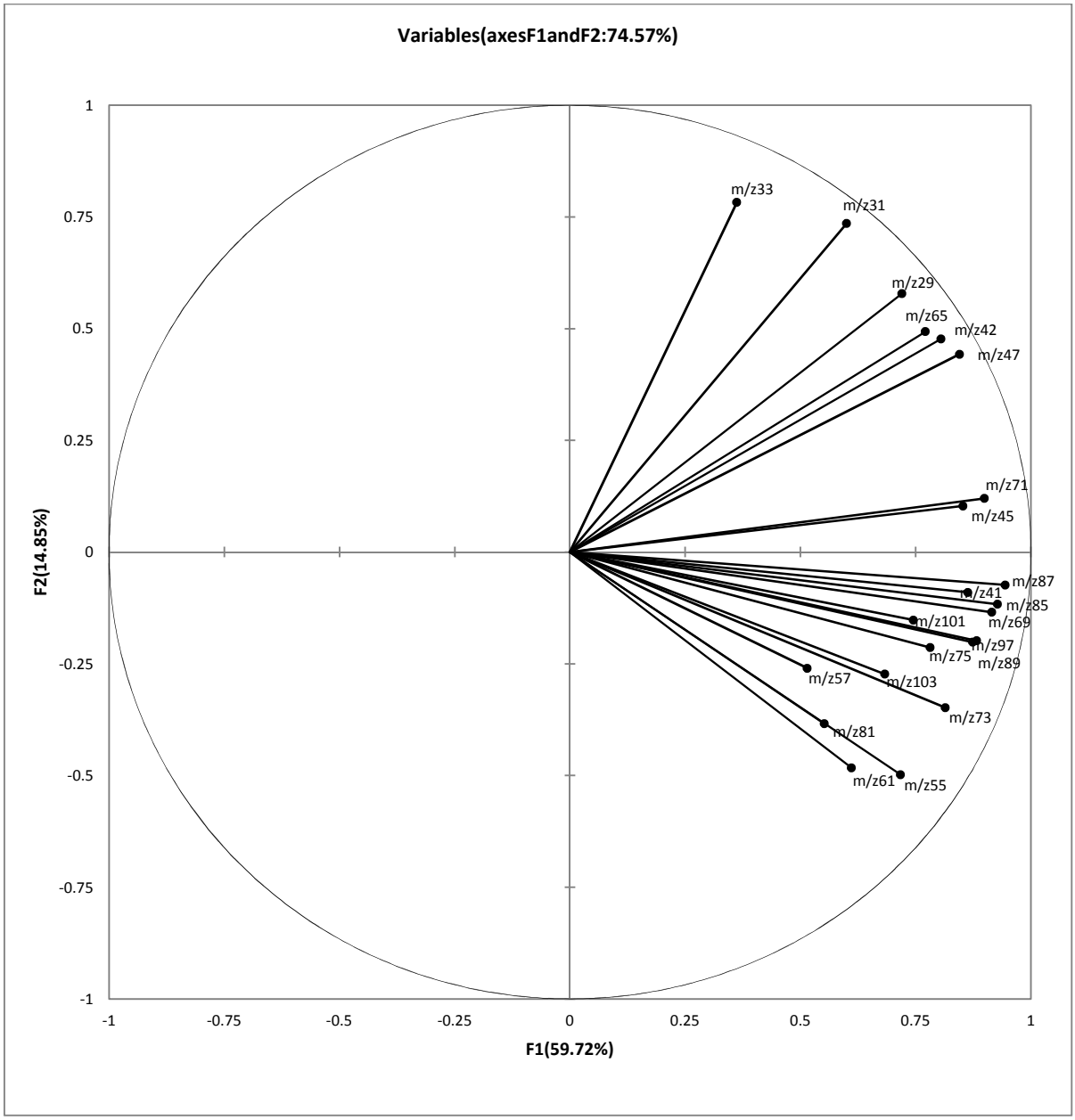


Figure 5.2. Principal component analysis (PCA) map representing the projection on F1 and F2 of the significant ($p<0.05$) mass ion (m/z) values for 34 green coffee samples, evaluated using PTR-MS.

Figure 5.3 presents the projection of samples on F1 and F2 of the PCA of significant PTR-MS data. The placement of samples along F1 (59.7% of variability) suggests an effect of both geographically localised factors and processing factors on the green bean headspace VOCs. From the point of view of the effect of origin, at the negative end of F1, samples HNX (natural) and HWX (washed) can be found – both coming from the state of Hidalgo, Mexico. Next to the right, all Brazilian (starting with a 'B' – enclosed in the ellipse) and Dominican

Republic (starting with ‘D’) samples from all methods are clustered on the negative side of F1. However, for some regions, the effect of processing seems to be larger than that of the geographical factors: washed samples from the state of Chiapas, Mexico (starting with ‘MW’) are located on the negative side of F1, while the natural ones (starting with ‘MN’) are on the positive side, with MNT next to the positive end. That is the same case with Colombian (starting with ‘CW’ for washed and ‘CN’ for natural) samples and also samples from the state of Veracruz, Mexico (starting with ‘VW’ for washed and ‘VN’ for naturals). In fact, all washed samples are located in the leftmost two-thirds of the negative side of F1. The natural samples accompanying the washed samples on the negative side of F1 (HNX, BNO, BNI, BNX, DNT, ENX) have a headspace profile similar to the washed and may have presented little catabolic activity compared to other naturals. The reason for this may be a shorter fermentation time due to a faster drying rate – this will be further explored in Chapter 6. On the other hand, all samples on the positive side of F1 are natural, suggesting a processing effect in the increase of most significant m/z, probably due to catabolism of the coffee pulp compounds. All Nicaraguan samples (starting with an ‘N’ – enclosed in the ellipse) are found in the positive side of F1.

The variation along F2 was not as clear as along F1. F2 would suggest a geographical effect, rather than a processing effect, on the concentration of methanol. All the Chiapas samples were highest along F2, regardless of their processing method. On the other hand, Colombian, Veracruz and some Nicaraguan samples tended to be on the negative side of F2.

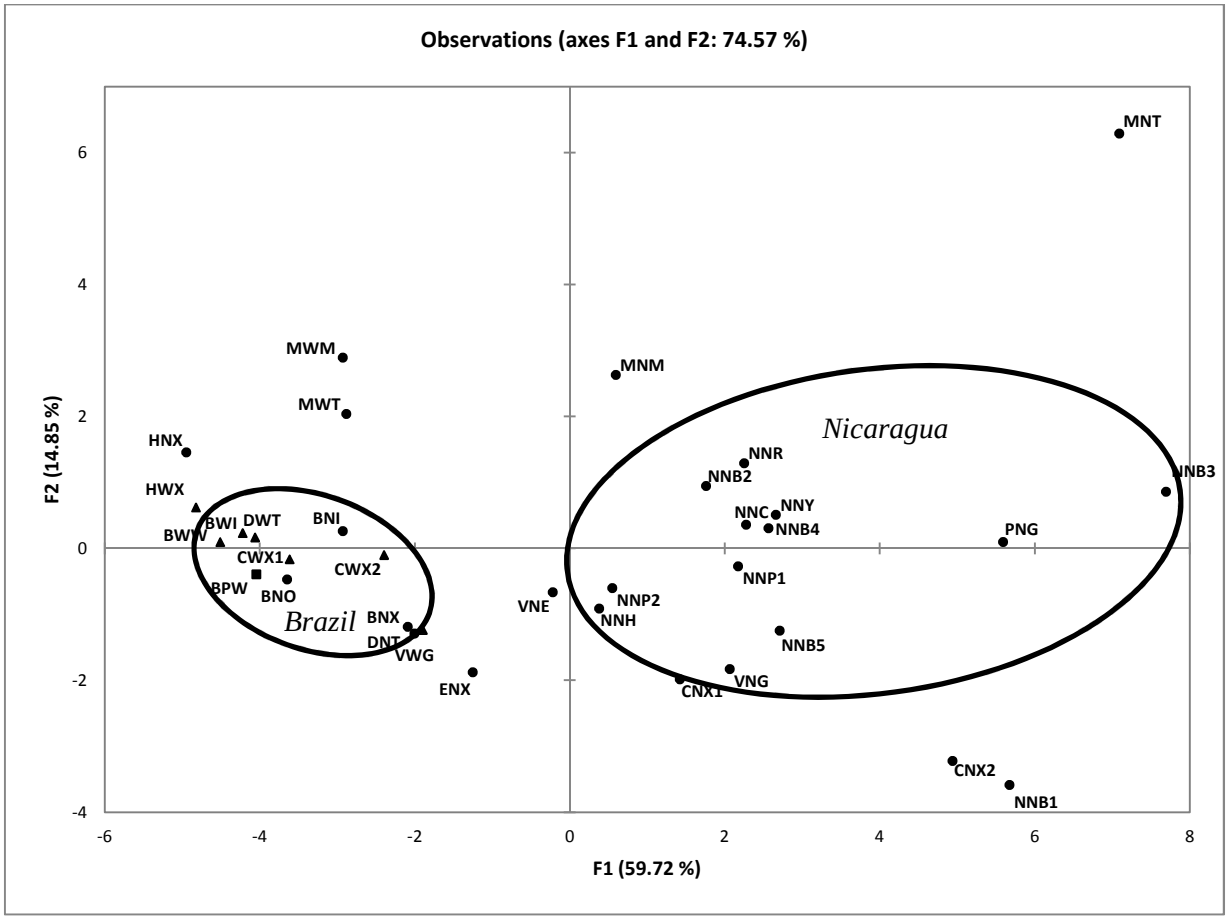


Figure 5.3. Principal component analysis (PCA) map representing the projection on F1 and F2 of the headspace from natural (circle), washed (triangle) and pulped-natural (square) coffees for 34 green bean samples, evaluated using PTR-MS.

5.3.2 Identification of odour-active compounds from GC-MS/O data

The analysis of GC-MS/O aromagrams of 5 SPE roasted coffee headspace concentrates from 5 assessors revealed 60 odour-active peaks (Table 5.4). Table 5.4 indicates the retention times and Kovats retention index (KI) for each odour-active peak, the frequency of assessors detecting it and the sum of intensities rated by the assessors. The sum of intensities was preferred over the intensity average as it reflects the number of assessors detecting the peak. The representative character descriptor of each peak was selected from the descriptors elicited by the assessors.

The samples were selected from the set assessed by the expert (5.2.4.1) with the aim of covering the widest range of odour-active peaks. The 5 samples selected include a natural 'Bourbon' varietal from Nicaragua (NNB2), a washed coffee from Hidalgo, Mexico

(unknown varietal – HWX), a natural 'Typica' varietal from Chiapas, Mexico (MNT), a natural 'Yellow Bourbon' from Guatemala which was roasted months after the previous samples (GNW – only used for odour identification and not included in the other analyses) and a sample from the drying rate study (Chapter 6), which is a natural 'Typica' varietal from Veracruz, Mexico (7B). Most of the peaks were shown by all samples, although intensities were different. Some peaks were absent from one or more samples: the peak between 7.8 and 8min (apple) was not detected in the washed coffee. Also, not present in the washed coffee were peak 23 (resinous), 25 (watermelon), 32 (earthy), 37 (rancid butter), 48 (dusty/tobacco). Other peaks were absent from one or more of the natural samples: 14 (potato), 16 (stinky), 19 (resinous), 22 (mushroom to cinnamon), 34 (flatulence), 38 (roses), 39 (cucumber), 43 (oniony or spicy), 44 (cabbage), 45 (earthy chocolate), 47 (rancid butter), 51 (fermented, tropical fruit), 52 (sweet/tobacco), 59 (tea-rose/dusty) and 60 (pipe tobacco). Due to the small number of samples, no conclusions can be made from these differences in the olfactory profile; however, this situation reflects the complexity of coffee as a product and the diversity of the overall sample set.

The first 7 peaks in Table 5.4, prior to 8 min of retention time, appeared before the solvent delay time, and thus this region was investigated using SH-GC-MS/O (5.2.6). Table 5.5 shows the result of SH-GC-MS/O for the region equivalent to the solvent delay time in the SPE-GC-MS/O. In this region, 10 odour-active peaks were detected using SH-GC-MS/O, 7 of which were identified and 5 of which were able to be matched with the SPE-GC-MS/O peaks from the same region. The known, odour-active peaks identified by SH/GC-MS/O were integrated for all samples and later added to the dataset of known, odour-active peaks from SPE-GC-MS/O.

Table 5.4. Odour active peaks of 5 roasted coffee samples measured through SPE-GC-O using 5 assessors – for each peak, peak number, onset retention time (RT), Kovats index (KI), main character, frequency of assessors detecting it (*f* – includes duplicates) and sum of intensities rated (Σi).

Peak No	Onset RT (min)	End RT (min)	KI	Representative character	Sample NNB2		Sample HWX		Sample MNT		Sample GNW		Sample 7B	
					f	Σi	*f*	Σi	*f*	Σi	*f*	Σi	*f*	Σi
[a]	4.8	5	857	Solvent	3	172	4	159	3	90	4	152	3	93
[a]	5.4	6	881	Peanut	6	112	5	187	5	307	5	230	5	156
[a]	6	6.2	916	Toasted bread	5	219	6	285	6	248	5	254	4	121
[a]	6.7	6.9	930	Ethanol/Fruity	5	250	4	120	4	187	6	223	4	142
[a]	6.9	7	945	Ethanol/Fruity	3	152	3	116	5	122	6	217	2	22
[a]	7.1	7.5	959	Peanut to fruity	9	451	8	490	10	498	10	507	10	523
[a]	7.8	8	972	Apple	5	225	0	0	3	183	7	290	3	140
8	8	8.3	993	Butter (sometimes to fruity)	6	382	8	517	8	501	8	466	8	480
9	8.8	9.1	1027	Apple	2	54	3	69	6	212	6	167	2	89
10	9.7	10	1061	Strawberry	8	301	3	40	9	440	10	437	9	366
11	10	10.2	1069	Butter	6	305	9	334	10	491	8	447	7	334
12	10.2	10.4	1075	Blueberry	7	416	1	33	7	358	8	486	6	299
13	11.7	11.9	1131	Blueberry	6	283	6	344	4	220	5	287	6	329
14	13	13.1	1177	Potato	0	0	1	15	2	73	0	0	0	0
15	13.7	13.8	1205	Caramel	3	129	1	74	0	0	0	0	1	86
16	14.2	14.3	1213	Stinky	2	108	0	0	3	161	0	0	0	0
17	15.2	15.3	1242	Berry	4	116	3	144	2	108	4	57	2	32
18	15.5	15.6	1251	Caramel	1	90	1	18	6	186	2	26	0	0
19	16.7	16.8	1287	Resinous	2	89	3	139	0	0	2	155	0	0
20	17	17.1	1298	Ethanol/Winey	1	87	2	27	8	291	1	4	4	56
21	17.2	17.4	1302	Musty cloth	2	113	2	98	3	188	5	195	3	199
22	17.6	17.9	1314	Mushroom to cinnamon	2	133	1	58	3	118	3	205	0	0
23	18.3	18.4	1333	Resinous	1	53	0	0	1	25	3	146	1	45
24	18.6	18.7	1345	Ethanol to Fruity/Caramel	7	240	2	71	3	56	7	153	3	70
25	20.1	20.4	1393	Watermelon	4	162	0	0	1	23	6	201	3	77
26	20.5	20.7	1404	Ethanol / Caramel-fruity	2	25	1	11	6	239	5	134	4	92
27	21.1	21.3	1425	Rubber	2	109	1	61	4	280	4	181	4	159

Peak No	Onset RT (min)	End RT (min)	KI	Representative character	Sample NNB2		Sample HWX		Sample MNT		Sample GNW		Sample 7B	
					f	Σi	*f*	Σi	*f*	Σi	*f*	Σi	*f*	Σi
28	21.3	21.5	1429	Earthy/Chocolaty	4	137	1	74	5	287	4	229	4	119
29	21.7	21.9	1450	Potato	5	295	7	401	6	392	6	384	6	348
30	21.9	22.1	1456	Earthy/Chocolaty to Fruity	8	390	7	386	9	456	9	535	6	283
31	22.2	22.4	1463	Vinegar	4	268	2	80	5	281	8	476	3	194
32	22.4	22.6	1465	Earthy	5	161	0	0	5	91	1	60	4	164
33	22.8	23.1	1478	Earthy/Chocolaty	8	507	7	339	8	416	8	362	8	436
34	23.4	23.6	1502	Flatulence	1	14	1	83	1	71	1	32	0	0
35	23.6	23.8	1510	Earthy/Chocolaty and Fragrant	8	322	9	399	10	479	7	373	10	398
36	24.3	24.5	1536	Fragrant to ‘Poblano’ chilli	9	480	8	466	9	499	9	453	10	542
37	24.5	24.6	1549	Rancid butter	2	73	0	0	2	17	3	84	1	52
38	24.6	24.7	1548	Roses	0	0	1	54	2	133	0	0	1	22
39	24.8	24.9	1555	Cucumber	2	99	1	70	2	11	4	265	0	0
40	25	25.2	1576	Tropical fruit	2	79	1	24	1	42	4	250	1	11
41	25.4	25.6	1588	Earthy/Chocolaty	1	10	9	284	9	421	1	79	8	310
42	25.7	25.8	1593	Fruity/Spicy	1	78	1	14	1	40	1	19	2	59
43	25.8	26	1599	Oniony or spicy	3	129	1	5	0	0	2	108	2	59
44	26	26.1	1614	Cabbage	0	0	6	233	7	212	1	51	3	199
45	26.2	26.3	1613	Earthy/chocolaty	0	0	2	77	2	132	3	207	0	0
46	26.3	26.5	1614	LPG	3	132	1	20	2	106	4	239	1	38
47	26.5	26.7	1637	Rancid butter	0	0	1	17	0	0	2	81	1	36
48	26.7	27	1643	Dusty/Tobacco	1	89	0	0	1	39	2	95	1	39
49	26.9	27	1645	Mushroom	3	163	2	30	5	219	6	361	4	245
50	27	27.2	1657	Basmati rice	10	633	9	483	10	596	10	698	10	497
51	27.2	27.4	1677	Fermented/Tropical fruit	3	141	2	177	0	0	6	246	1	19
52	27.4	27.5	1683	Sweet/Tobacco	0	0	1	72	2	78	1	93	0	0
53	27.8	28	1687	Boiled beef	4	153	4	147	4	142	5	220	2	18
54	28.3	28.5	1705	Tobacco	3	110	3	125	3	154	3	189	3	70
55	28.6	29.1	1749	Tobacco	4	252	3	203	2	143	5	316	4	156
56	29	29.1	1766	Pipe tobacco	3	207	1	12	3	152	1	62	3	207
57	29.2	29.4	1786	Skunky	1	4	1	6	1	43	5	190	3	61
58	29.4	29.5	1800	Caramel	1	34	2	104	1	37	3	93	1	48
59	29.7	29.9	1809	Tea-rose/Dusty	0	0	9	367	7	304	3	71	3	138
60	29.9	30	1853	Apple/Pipe	0	0	3	137	3	205	1	97	0	0

Peak No	Onset RT (min)	End RT (min)	KI	Representative character	Sample NNB2		Sample HWX		Sample MNT		Sample GNW		Sample 7B	
					f	Σi	*f*	Σi	*f*	Σi	*f*	Σi	*f*	Σi
				tobacco										
61	30.1	30.5	1880	Walnuts	3	164	3	164	2	90	6	276	4	169
62	30.5	30.8	1892	Walnuts	6	266	4	194	5	322	6	294	4	215
63	30.8	31	1926	Burnt	3	51	3	122	3	204	4	199	2	75

(a) The first 7 peaks were detected during the MS solvent delay time. This region was later covered using data from SH-GC-MS.

Table 5.5. Odour active peaks in the first region of the SH-GC-MS/O chromatogram (corresponding to the solvent delay region of SPE-GC-MS) – peak number, representative character, identified compound and possible equivalence to SPE-GC-O. (-) – no data.

Peak No	Descriptor from SH-GC-MS/O	Identification	Possible matching descriptor from SPE-GC-O
1	Flatulence	Methanethiol	Solvent
2	Honeyed/Peanuty	Acetaldehyde	Peanut
3	Burnt	Dimethyl sulphide	-
4	Lemony	Methyl formate	-
5	Nutty/Ricey, Cucumber	Furan	-
6	Peanuty/Fruity/Chocolate	Propanal, 2-methyl	Toasted bread
- [a]	Skunky/Resinous	Unidentified	-
-	-	Unidentified	Ethanol/Fruity
-	-	Unidentified	Ethanol/Fruity
7a	Toasted bread/Peanut/Fruit	Butanal, 2-methyl	Peanut to fruity
7b	-	Butanal, 3-methyl	-
-	Apple	Unidentified	Apple
8	Fresh butter	2,3-Butanedione	Butter (sometimes to fruity)

(a) Unidentified peaks were not assigned a number.

The odour-active peaks of Table 5.4 were investigated in order to identify them (5.2.8.2). Table 5.6 presents all the odour-active peaks that were able to be identified, based on their mass spectra profile, retention index and odour character. Peaks 1 to 7b were incorporated from the SH-GC-MS/O results. A letter was added to the peak number to indicate when a co-elution of two or more compounds was detected – i.e. both 2-methylbutanal (peak 7a) and 3-methylbutanal (peak 7b) may be responsible for the toasted bread/peanut/fruit character reported. In some cases, like peaks 10, 16, 17, 25 and 60, a co-elution was found, but one of

the co-eluting compounds was not likely to be responsible for the odour activity and was thus left out of the table. Table 5.6 also includes the CAS registry numbers of the identified peaks and reports the mass ion used for single-ion integration of each peak. Overall, excluding the internal standard (fenchyl alcohol – peak 42), 46 odour-active compounds were identified, belonging to the following classes: alcohols, aldehydes, ketones, acids, esters, lactones, furans, pyrazines and sulphur compounds (Flament & Bessière-Thomas, 2002).

Table 5.6. Identified odour-active compounds used for semi-quantification from GC-MS data - peak number,olfactory character, compound name, CAS number and ion used for peak integration.

Peak No	GC-O Character	Identified compound name	CAS No	Integration ion (m/z)
1 [a]	Flatulence	Methanethiol	74-93-1	47
2 [a]	Honeyed/Peanuty	Acetaldehyde	75-07-0	44
3 [a]	Burnt	Dimethyl sulphide	75-18-3	47
4 [a]	Lemony	Methyl formate	107-31-3	60
5 [a]	Nutty/Ricey, Cucumber	Furan	110-00-9	39
6 [a]	Peanuty/Fruity/Chocolate	2-Methylpropanal	78-84-2	72
7a [a]	Toasted bread/Peanut/Fruit	2-Methylbutanal	96-17-3	57
7b [a]	Toasted bread/Peanut/Fruit	3-Methylbutanal	590-86-3	44
8	Butter (sometimes to fruity)	2,3-Butanedione	431-03-8	43
10b	Strawberry	Ethyl 2-methylbutanoate	7452-79-1	102
11	Butter	2,3-Pentanedione	600-14-6	100
12	Blueberry	Ethyl 3-methylbutanoate	108-64-5	88
16b	Stinky	3-Methylbutan-1-ol	123-51-3	42
17a	Berry	Pyrazine	290-37-9	53
18	Caramel	Furfuryl methyl ether	13679-46-4	81
19a	Resinous	3(2H)-Furanone, dihydro-2-methyl-	3188-00-9	43
19b	Resinous	2-Methylpyrazine	109-08-0	53
21	Musty cloth	2-Butanone, 3-hydroxy-	513-86-0	43
22a	Mushroom	1-octen-3-one	4312-99-6	55
22b	Cinnamon	2-Propanone, 1-hydroxy-	116-09-6	74
24a	Ethanol to Fruity/Caramel	2,5-Dimethylpyrazine	123-32-0	42
24b	Ethanol to Fruity/Caramel	2,6-Dimethylpyrazine	108-50-9	39
24c	Ethanol to Fruity/Caramel	2-Ethylpyrazine	13925-00-3	53
25a	Watermelon	1-Hydroxy-2-butanone	5077--67-8	88
26a	Ethanol/Caramel-fruity	2-Ethyl-6-methylpyrazine	13925-03-6	121
26b	Ethanol/Caramel-fruity	2-Ethyl-5-methylpyrazine	13360-54-0	121
28	Earthy/Chocolaty	Pyrazine, trimethyl-	14667-55-1	42

Peak No	GC-O Character	Identified compound name	CAS No	Integration ion (m/z)
31	Vinegar	Acetic acid	64-19-7	60
32	Earthy	Pyrazine, 3-ethyl-2,5-dimethyl-	13360-65-1	135
33a	Earthy/Chocolaty	2-Propanone, 1-(acetyloxy)-	592-20-1	43
33b	Earthy/Chocolaty	Furfural	98-01-1	39
35	Earthy/Chocolaty and Fragrant	Furfuryl formate (co-eluted)	13493-97-5	81
36	Fragrant to Poblano chilli	Pyrazine, 2-methoxy-3-(2-methylpropyl)-	24683-00-9	124
37	Rancid butter	Propanoic acid	79-09-4	74
38	Roses	2-Furanmethanol, acetate	623-17-6	81
40	Tropical fruit	2-Methylpropanoic acid	79-31-2	43
42	Fruity/Spicy	*Fenchyl alcohol (internal standard)*	1632-73-1	81
43	Oniony or spicy	5-Methylfurfural	620-02-0	53
45	Earthy/chocolaty	Dimethyl sulfoxide	67-68-5	63
47	Rancid butter	Butanoic acid	107-92-6	60
50	Basmati rice	γ-Butyrolactone	96-48-0	42
51a	Fermented/Tropical fruit	3-Methylbutanoic acid	503-74-2	60
51b	Fermented/Tropical fruit	2-Methylbutanoic acid	116-53-0	74
56	Pipe tobacco	2,5-Furandione, 3,4-dimethyl	766-39-2	54
57	Skunky	2(5H)-Furanone	497-23-4	55
60b	Apple/Pipe tobacco	Hexanoic acid	142-62-1	60
63	Burnt	Dimethyl sulfone	67-71-0	79

(a) Peaks 1 to 7b were incorporated from SH-GC-MS/O data (5.2.6). The rest of the peaks were taken from SPE-GC-MS/O data (5.3.2).

5.3.3 Semi-quantification of odour-active compounds in HE-SPE concentrates and static headspace of coffee grounds from GC-MS data

The known, odour-active compounds of Table 5.6 were integrated and the resulting dataset was investigated using ANOVA (5.2.8.3). The 10 compounds that were found to be significant at a level of $p<0.05$ were acetaldehyde (peak 2), dimethyl sulphide (peak 3), methyl formate (peak 4), ethyl 2-methylbutanoate (peak 10b), ethyl 3-methylbutanoate (peak 12), 3-Methylbutan-1-ol (peak 16b), 2-ethyl-6-methylpyrazine (peak 26a), 3-ethyl-2,5-dimethyl-pyrazine (peak 32), furfural (peak 33b) and γ-butyrolactone (peak 50). Another 11 compounds were found to discriminate samples at a p value between 0.05 and 0.15: methanethiol (peak 1), 2-methylpropanal (peak 6), 2-methylbutanal (peak 7a), pyrazine (peak 17a), 3-hydroxy-2-butanone (peak 21), 1-hydroxy-2-propanone (peak 22b), 2-ethylpyrazine (peak 24c), 2-ethyl-5-methylpyrazine (peak 26b), 2-furanmethanol acetate (peak 38), 5-methylfurfural (peak 43) and dimethyl sulfoxide (peak 45).

The semi-quantified data for all 21 compounds significant at values of $p<0.15$ (Appendix 4) were investigated using a PCA. High correlations ($r>0.90$, Pearson (n-1) coefficient) were found between 2-methylpropanal and 2-methylbutanal, between 2-ethyl-5-methylpyrazine and 3-ethyl-2,5-dimethyl-pyrazine, between acetaldehyde and methyl formate, between 1-hydroxy-2-propanone and furfural, and between 2-ethylpyrazine and 2-ethyl-5-methylpyrazine.

The PCA of the significant GC-MS/O data explains the variability in a total of 21 factors. However, 81.2% of the variability is explained by the first four factors (F1 to F4). Factors 1 and 2 (F1 and F2) represent 29.4% and 24.0% of the variability. Figure 5.4 presents the projection of the significant odour-active compounds on F1 and F2. F1 is the factor that best represents 9 of the compounds, as explained by the fact that their square cosines are largest for F1 than for any other factor. The main contributing compounds to F1 were methyl formate (peak 4 – 9.83%), 1-hydroxy-2-propanone (peak 22b – 9.75%), acetaldehyde (peak 2 – 9.50%) and furfural (peak 33b– 9.41%). Methyl formate is found in green coffee but its concentration increases during roasting, possibly due to the esterification of formic acid (Flament & Bessière-Thomas, 2002), which in turn is formed by the degradation of sucrose during roasting (Ginz, Balzer, Bradbury, & Maier, 2000). In this study, methyl formate was highly correlated with acetaldehyde, which in coffee is mainly produced by sugar pyrolysis during roasting (Flament & Bessière-Thomas, 2002). 1-hydroxy-2-propanone has been linked to the base-catalysed degradation of fructose (Flament & Bessière-Thomas, 2002). 1-hydroxy-2-propanone was highly correlated to furfural, which is also produced from the thermal degradation of single sugars (pentoses and glucose) (Flament & Bessière-Thomas, 2002). Therefore, F1 seems to be best described as ‘sugar degradation products’.

For the case of F2, on the other hand, 5 of the compounds are best represented by this factor, based on their square cosines. The main contributing compounds to F2 were 2-ethylpyrazine (peak 24c – 17.31%), 2-ethyl-5-methylpyrazine (peak 26b – 15.63%) and 2-ethyl-6-methylpyrazine (peak 26a – 14.52%). Thus, F2 is best described by ‘alkylpyrazines’. Pyrazines result from the Maillard reaction, which involves the interaction of free amino acid and simple sugars in a heated system. 2-ethyl-6-methylpyrazine, for example, has been found in a heated cysteine/glucose model system (Flament & Bessière-Thomas, 2002). Thus, the

main difference between F2 and F1, at the precursor level, would be the presence of free amino acids in the case of F2.

Figure 5.5 presents the projection of samples on F1 and F2 of the PCA of significant, tentatively identified, odour-active compounds ($p<0.15$). The placement of samples along F1 (29.4% of variability) suggests mostly an effect of geographically localised factors. At the negative end of F1, all Brazilian samples (starting with 'B') can be found. Next to the right, they are followed by samples from Dominican Republic (starting with 'D') and the state of Hidalgo, Mexico (starting with 'H'). Colombian samples (starting with 'C') and samples from Chiapas, Mexico (staring with 'M') tend to be centred on the origin, and most Nicaraguan samples (starting with 'N') are found in the positive end of F1. However, there are some exceptions: samples NNH and NNY are on the negative side of F1 – their main difference with the other Nicaraguan samples is the variety, as NNH is a sample of an H2 Hybrid, while NNY is a 'Yellow Catuaí' varietal. F1 does not seem to reflect an effect of processing, as samples of the same origin seem to be clustered regardless of their process. Since F1 is related to sugar-degradation compounds, it is likely the origin would have an impact on the amount of sugars in the beans. Not only do cooler climates favour a higher sugar concentration (Joët et al., 2010), but also the harvesting practices (Amorim et al., 2009), which are geographically localised; hence a regional impact could be observed on the sugar concentration of the coffee beans.

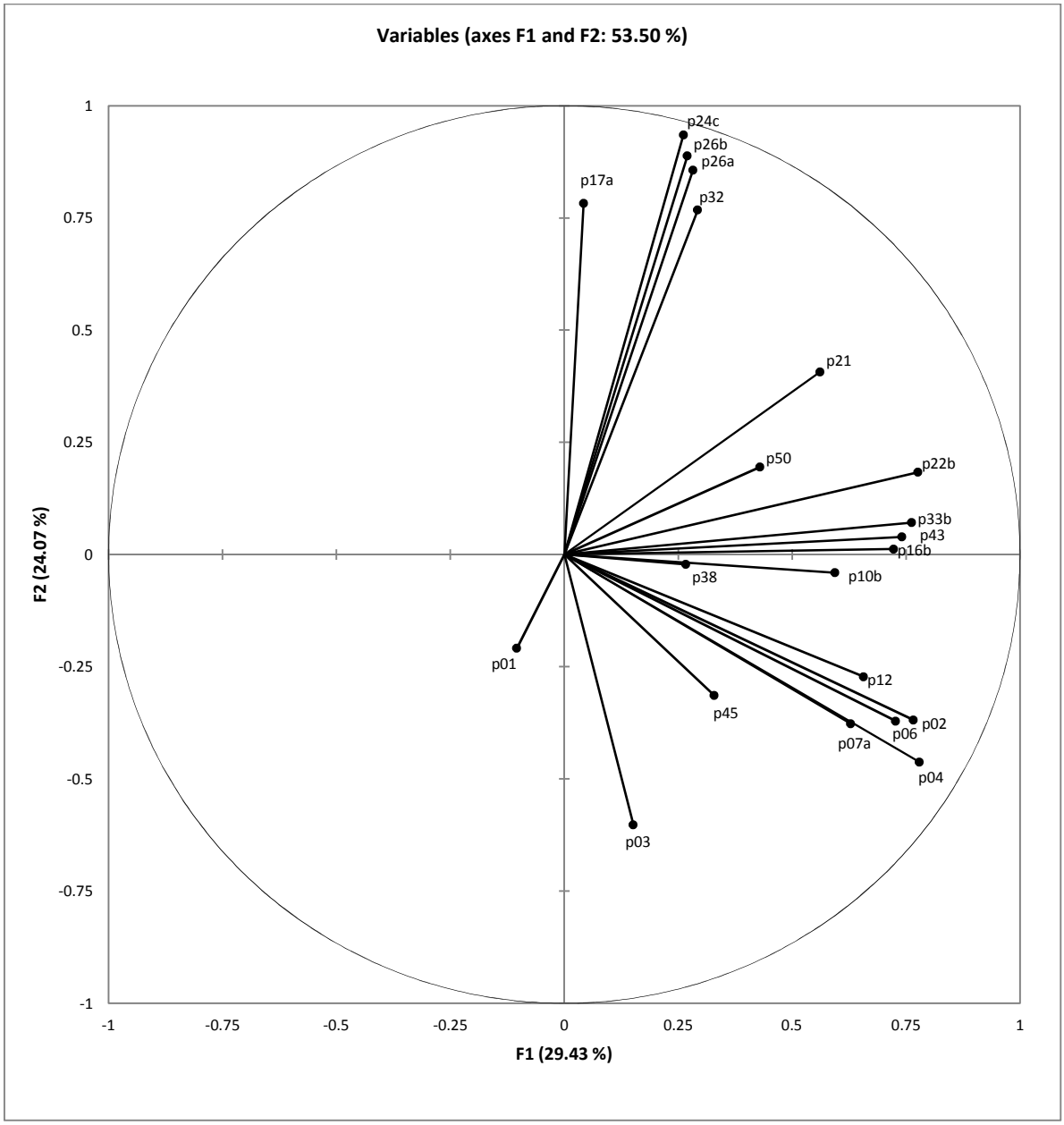


Figure 5.4. Principal component analysis (PCA) map representing the projection on F1 and F2 of significant odour-active compounds (p<0.15) for 34 roasted coffee samples, evaluated using GC-MS. Peak legend: p01, methanethiol; p02, acetaldehyde; p03, dimethyl sulphide; p04, methyl formate; p06, 2-methylpropanal; p07a, 2-methylbutanal; p10b, ethyl 2-methylbutanoate; p12, ethyl 3-methylbutanoate; p16b, 3-methylbutan-1-ol ; p17a, pyrazine; p21, 3-hydroxy-2-butanone; p22b, 1-hydroxy-2-propanone; p24c, 2-ethylpyrazine; p26a, 2-ethyl-6-methylpyrazine; p26b, 2-ethyl-5-methylpyrazine; p32, 3-ethyl-2,5-dimethyl-pyrazine; p33b, furfural; p38, 2-furanmethanol acetate; p43, 5-methylfurfural; p45, dimethyl sulfoxide; p50, γ-butyrolactone.

The placement of samples along F2 (24.1% of variability) again suggests mostly an effect of geographically localised factors. Colombian samples are located on the negative side of F2, while Brazilian and Dominican Republic samples are found on the positive side. The reason for the placement of the other origins is not as clear, though. For example, in the case of the Chiapas samples, the ones of the 'Maragogype' variety (MNM and MWM) are located on the negative side of F2, while the samples of the 'Typica' variety (MNT and MWT) are located on the positive side. Thus, an effect of coffee genotype may also be playing a role along F2.

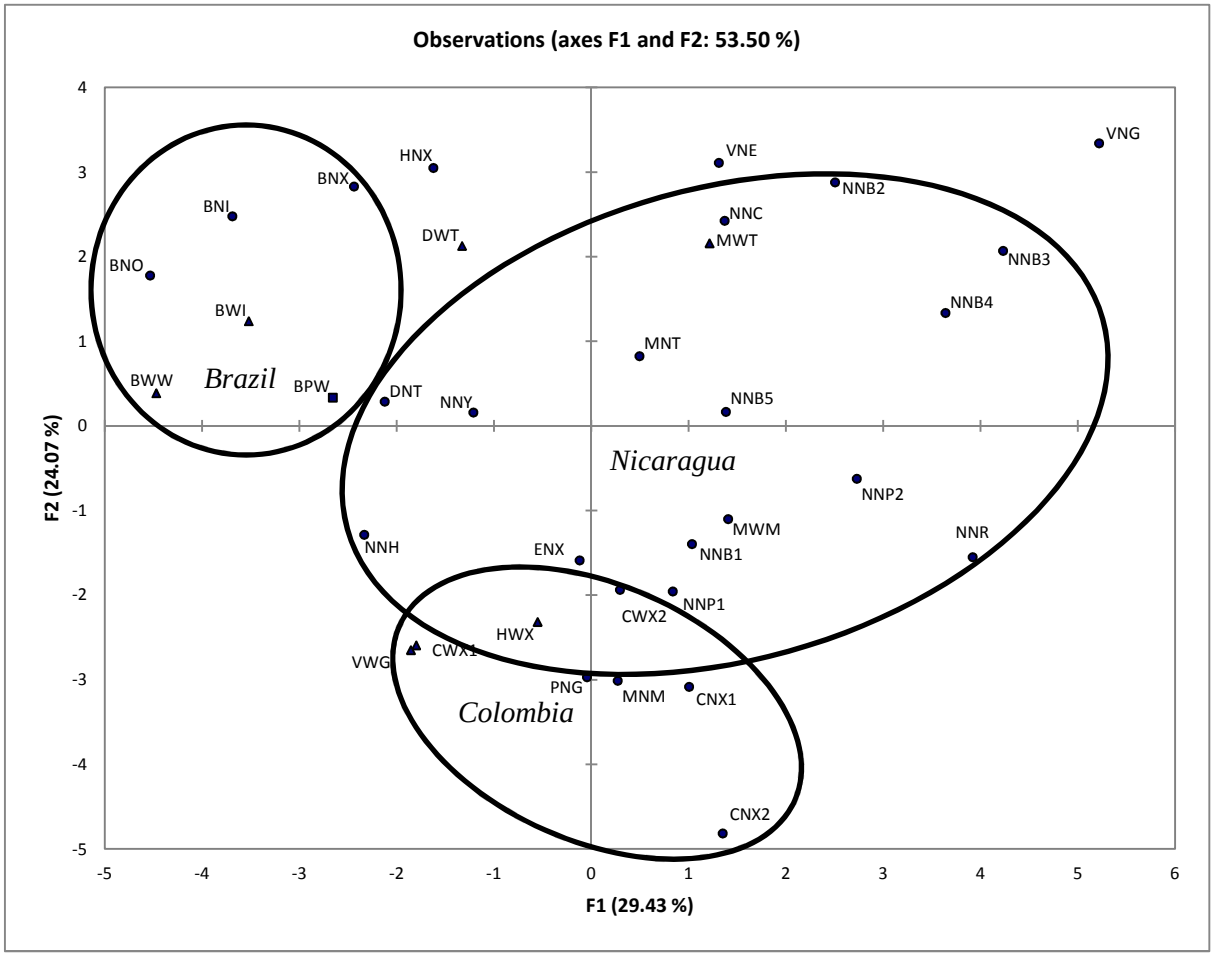


Figure 5.5. Principal component analysis (PCA) map representing the projection on F1 and F2 of natural (circle), washed (triangle) and pulped-natural (square) coffees for 34 samples, evaluated using GC-MS (known, odour-active compounds significant at a level of $p < 0.15$).

A better separation based on the processing methods seems to be given by F3, which represents 16% of variability. Figure 5.6 shows the projection of the significant odour-active compounds on F1 and F3. The reason for an explained variability lower than 50% is the choice of factors used for this chart (F1 and F3, as opposed to F1 and F2). At any rate, the

explained variability is still above 45% and four of the PCA variables are best represented by F3, based on their square cosines, which are the reasons why this factor is explored here. The main contributing compounds to F3 are dimethyl sulfoxide (peak 45, earthy/chocolaty character – 15.8%) on the positive side and ethyl 2-methylbutanoate (peak 10b, strawberry character – 12.1%) on the negative side of F3. The ethyl 2-methylbutanoate is a fruity ester with a lower threshold than its methyl counterpart (Flament & Bessière-Thomas, 2002) and results from the esterification of 2-methylbutanoic acid, which in turn is a catabolic degradation product of isoleucine (Roze et al., 2010; Thonning Olesen & Stahnke, 2004).

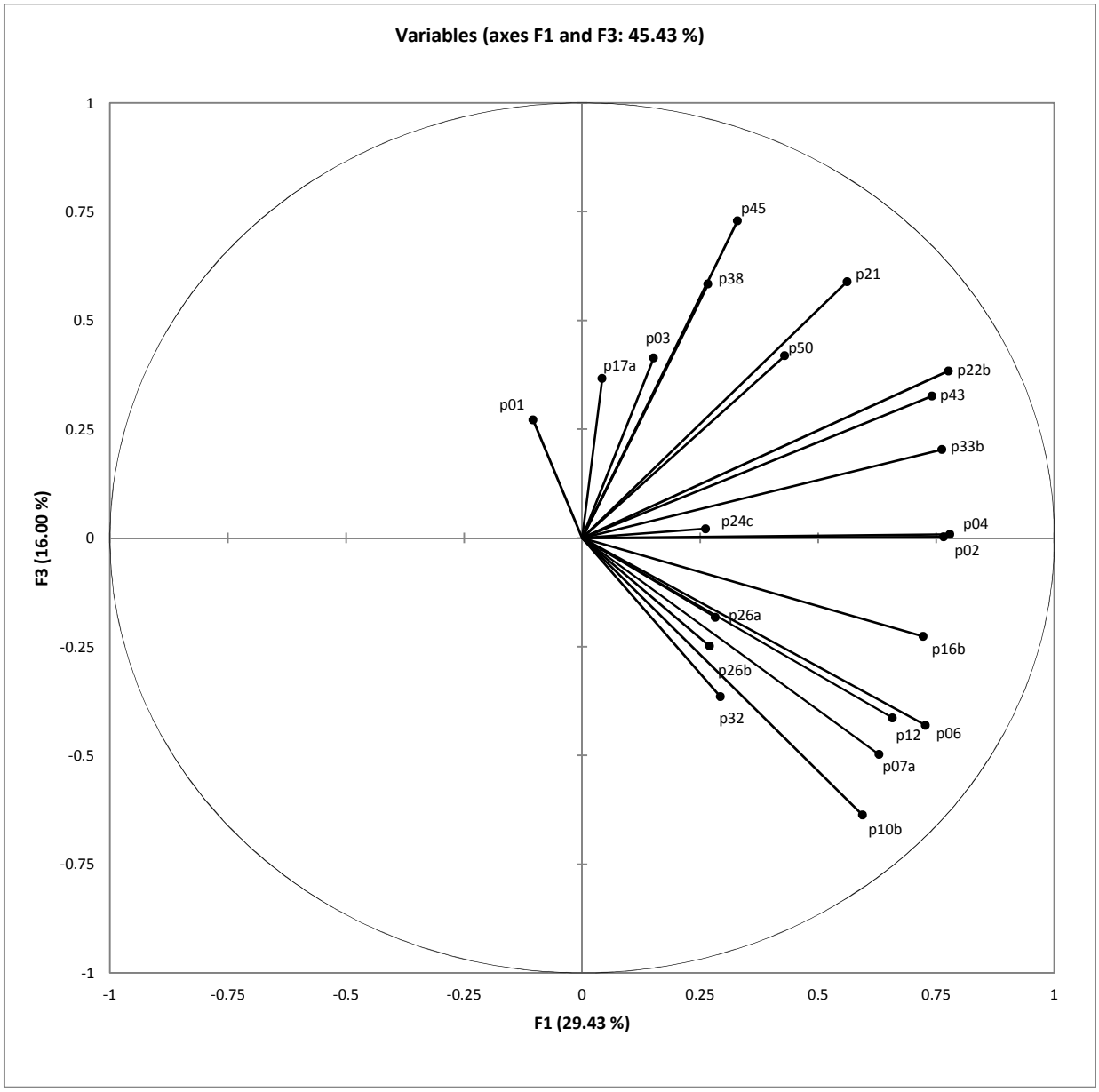


Figure 5.6. Principal component analysis (PCA) map representing the projection on F1 and F3 of significant odour-active compounds ($p < 0.15$) for 34 roasted coffee samples, evaluated using GC-MS. Peak legend: p01, methanethiol; p02, acetaldehyde; p03, dimethyl sulphide; p04, methyl formate; p06, 2-methylpropanal; p07a, 2-methylbutanal; p10b, ethyl 2-methylbutanoate; p12, ethyl 3-methylbutanoate; p16b, 3-methylbutan-1-ol ; p17a, pyrazine; p21, 3-hydroxy-2-butanone; p22b, 1-hydroxy-2-propanone; p24c, 2-ethylpyrazine; p26a, 2-ethyl-6-methylpyrazine; p26b, 2-ethyl-5-methylpyrazine; p32, 3-ethyl-2,5-dimethyl-pyrazine; p33b, furfural; p38, 2-furanmethanol acetate; p43, 5-methylfurfural; p45, dimethyl sulfoxide; p50, γ-butyrolactone.

Figure 5.7 shows the projection of samples on F1 and F3. In this case, the effect of processing is clearer than in the previous figures using F1 and F2. Most washed samples are on the positive side of F3. The two washed samples on the negative side are next to the axis. Washed samples tend to appear displaced to the positive side of F3, relative to their natural counterpart, which is the case for DWT vs. DNT; HWX vs. HNX; CWX2 vs. CNX2; MWT vs. MNT, and MWM vs MNM. There are some exceptions to this, though: BWI vs. BNI, and VWG vs. VNG. This separation of the processing methods based on ethyl 2-methylbutanoate may be due to a fermentation of amino acids in the coffee-cherry pulp during drying. On the other hand, the formation path of dimethyl sulfoxide and the reason why it is higher in some samples need to be further researched.

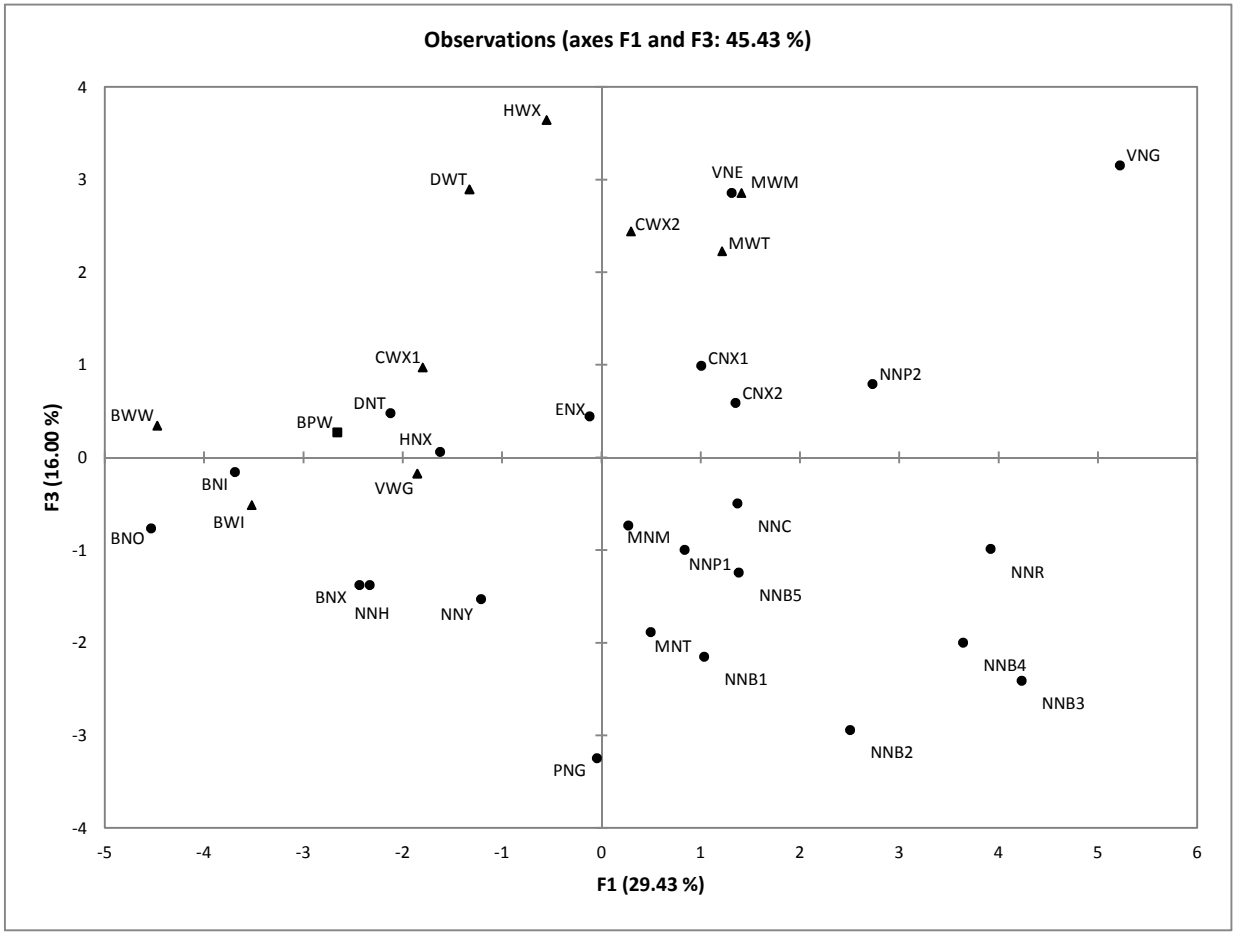


Figure 5.7. Principal component analysis (PCA) map representing the projection on F1 and F3 of natural (circle), washed (triangle) and pulped-natural (square) coffees for 34 samples, evaluated using GC-MS (known, odour-active compounds significant at a level of p<0.15).

Going back to the ANOVA of the known, odour-active compounds (5.3.3), the 3 compounds that presented a value of $p>0.70$ (and thus did not discriminate) were 1-octen-3-one (peak 22a, mushroom, $p=0.896$), trimethylpyrazine (peak 28, earthy/chocolaty, $p=0.728$) and 2(5H)-furanone (peak 57, skunky, $p=0.712$). These compounds could contribute to the generic odour of coffee. For example, 1-Octen-3-one is formed during autoxidation of linoleic acid and methyl-linoleate (Flament & Bessière-Thomas, 2002), and is in the list of potent odorants of green arabica coffee (Czerny & Grosch, 2000). Trimethylpyrazine may be formed by condensation of pyruvaldehyde with biacetyl, involving the Strecker degradation of an amino acid, and it has the lowest odour threshold among mono-, di-, tri- and tetramethylpyrazines (Flament & Bessière-Thomas, 2002).

5.3.4 MFA including the Descriptive Cupping dataset

The links between the coffee flavour profile and the VOC profiles in the coffee headspace were explored using multiple factor analysis (MFA – 5.2.8.4). Two tables were kept active in the MFA: the Descriptive Cupping descriptors from Chapter 4 and the GC-MS/O significant ($p<0.15$) odour-active compounds (5.3.3). The active tables have an RV coefficient (see Chapters 3 and 4) with the MFA of 0.874 and 0.877 respectively, indicating both are well represented by the MFA. Both active tables have an RV coefficient between them of 0.532, indicating there is some correlation between the descriptors and the GC-MS/O compounds. The MFA resulted in a total of 31 factors (sum of eigenvalues = 7.316). 76.06% of the total variability was represented in the first 8 factors.

Figure 5.8 shows the projection of both active tables on factor 1 (F1) and factor 2 (F2) of the MFA. The combined explained variability of F1 and F2 is 39.10%, due to the low variability of the descriptor dataset, which was discussed in 4.3.5 and 3.3.2 (large number of descriptors and possible limitations in the cupping method).

F1 explains 24.23% of the variability. Factor 1 of the descriptive table and factor 1 of the GC-MS table are correlated at a level of 0.689, implying the main source of variability for both tables can be explained by F1. The contribution of the flavour descriptor table to F1 is 50.7% while the contribution of the GC-MS/O compounds is 49.3%, implying both tables are contributing equally to F1. The squared cosines of both tables are highest for F1 (0.524 and 0.484, respectively for the descriptors and the GC-MS/O compounds), which means F1 is the

factor best representing both tables. Overall, F1 is a key factor for the interpretation of the MFA.

The main contributing flavour notes to F1 were *tropical-fruity* (4.63%), *red-fruity* (4.40%) and *sweet-acid* (4.12%), all of them contributing to the positive side of F1. On the negative side, *phenolic* (3.60%) and *dry acid* (2.51%) were the main contributing variables. This is in line with the NSCA of descriptors (4.3.4) and again implies that fruitiness is the main discriminating character between the samples. On the other hand, the main contributing odour-active compounds to F1 were 2-methylpropanal (peak 6, peanuty/fruity/chocolate – 5.40%), ethyl 3-methylbutanoate (peak 12, blueberry – 5.25%) and ethyl 2-methylbutanoate (peak 10b, strawberry – 5.03%), all of them contributing to the positive side of F1; also, all of these are products of catabolic degradation of amino acids and compounds with a fruity character. It seems clear that fruitiness – or Mocha character – which is the main discriminating character among these samples is largely correlated to the ethyl esters of branched-chain fatty acids and other catabolic degradation products of valine, isoleucine and leucine. Flament and Bessière-Thomas (2002) report the following character notes for these compounds: overripe-fruit for 2-methylpropanal, blueberry and fruity for ethyl 3-methylbutanoate, and apricot, apple and strawberry for ethyl 2-methylbutanoate, confirming the character found in this study by the GC-O panel. These findings also confirm the personal communication of T. Hofmann, cited by Folmer (2014), relating the fruity character of a natural coffee from Peru to the ethyl esters of branched-chain fatty acids. These branched-chain fatty acids do imply the initial presence of free amino acids, but more importantly, the presence of catabolic metabolism during processing.

Few compounds contribute to the negative side of F1: the main one is methanethiol (peak 1, flatulence) with 1.69% of contribution to F1. According to Flament and Bessière-Thomas (2002), methanethiol is the pyrolysis product of methionine, may also be produced in reactions involving cysteine and sugars, and presents a *"very objectionable, rotten-cabbage odour"*. In the MFA, methanethiol is most closely correlated to the *fungal* and *pungent* descriptors, all in the same quadrant. For methanethiol to be produced, amino acids need to be available intact (i.e. no degradation by catabolic reactions).

F2 of the MFA explains 14.9% of variability. The main contributing descriptors to F2 are *pyrolytic* (6.77%), *chocolaty* (5.29%), *toasty* (4.86%) and *caramelly* (4.59%), all on the

positive side of F2. On the other hand, the main contributing odour-active compounds to F2 are 2-ethylpyrazine (peak 24c, ethanol to fruity/caramel – 9.62%), 2-ethyl-6-methylpyrazine (peak 26a, ethanol/caramel-fruity – 9.21%) and 2-ethyl-5-methylpyrazine (peak 26b, ethanol/caramel-fruity – 8.05%). The formation of alkylpyrazines as Maillard reaction products was discussed in 5.3.3. Flament and Bessière-Thomas (2002) report a nutty, roasted, green, sweet, buttery and rum character for 2-ethylpyrazine; a hazelnut-like character for 2-ethyl-6-methylpyrazine, and a sugar-syrup character for 2-Ethyl-5-methylpyrazine. F2 is thus clearly a factor associated to the Maillard-products and their corresponding caramelly and toasty flavour notes. From the point of view of precursors, F2 implies the presence of free, non-degraded amino acids, which are possibly formed by the induction of germination during processing (Bytof et al., 2005).

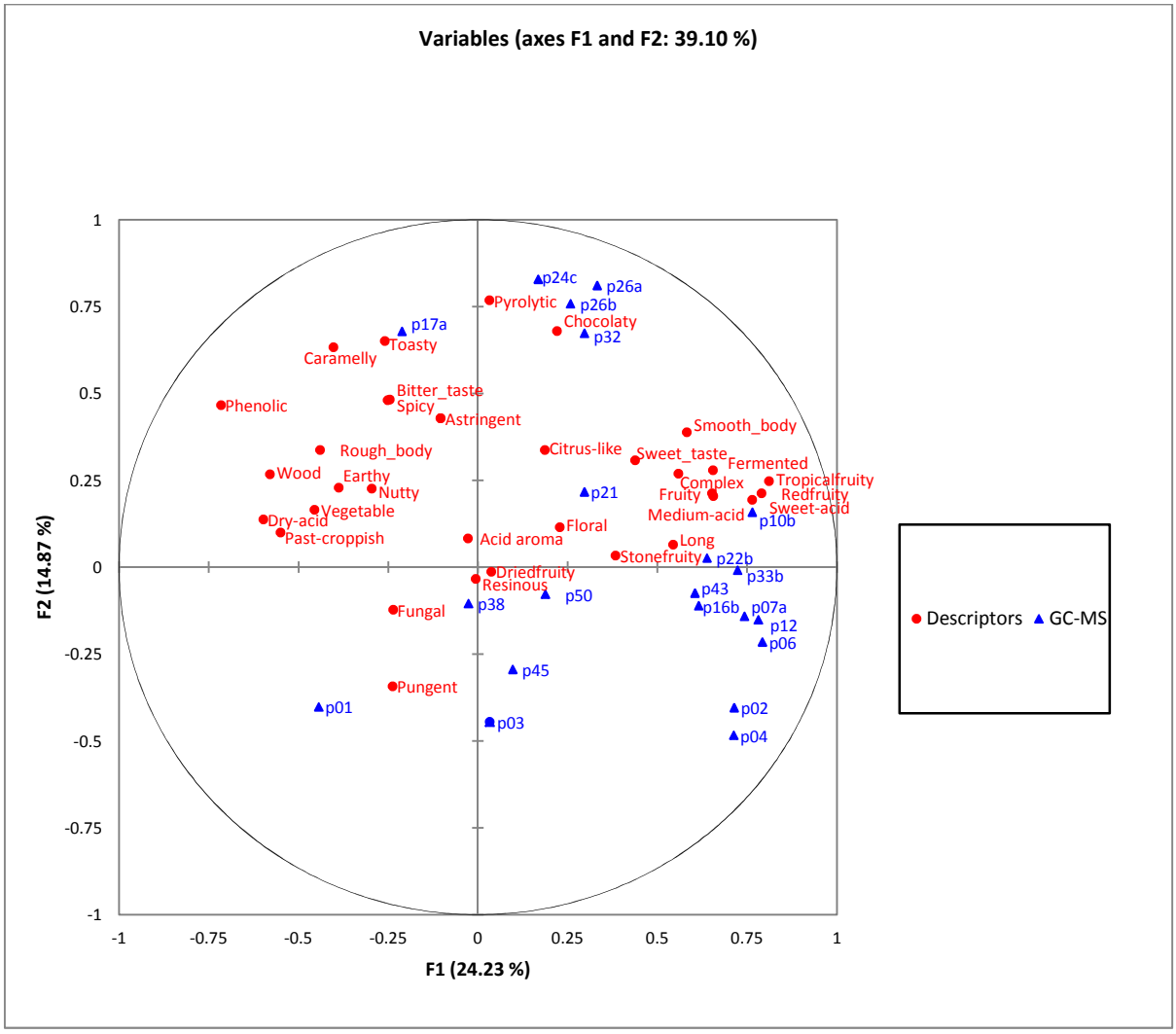


Figure 5.8. Multiple factor analysis (MFA) map representing the projection on F1 and F2 of descriptor subgroups (circle) and significant (p<0.15) odour-active compounds (triangle, see peak legend in Table 5.5).

Figure 5.9 presents the projection of the significant PTR-MS m/z, from the green bean headspace, as a supplementary table on the previous MFA. As it is a supplementary table, the degree of correlation of the PTR-MS data is not optimal but it still shows some correlation: the RV coefficient of PTR-MS data with the MFA is 0.517, while its RV coefficients with the descriptor table and the GC-MS/O table are 0.444 and 0.462 respectively. The square cosine of the PTR-MS table for F1 is 0.831, meaning the PTR-MS data are mostly represented by F1. The main contributing PTR-MS mass ions to F1 are m/z 45 (acetaldehyde, 0.80%), m/z 71 (3-methylbutanol acetate, 0.79%), m/z 87 (3-Methyl-2-buten-1-ol, 2*E*-butenoic acid, 3-methyl butanal, 2,3-butanedione 0.78%) and m/z 97 (2*E*,4*E*-Hexadienal, 0.73%) – see Table 5.3.

Most of these compounds have been reported as fermentation products (Rodriguez et al., 1969; Roze et al., 2010; Thonning Olesen & Stahnke, 2004). The acetaldehyde, together with ethanol – which in fact have a correlation of 0.869 with each other in the green beans (Pearson (n-1) coefficient – see 5.3.1) – may be related to the ethyl part of the fruity ethyl esters found by GC-MS/O. On the other hand, m/z 87 is related to 3-methylbutanal, which may be taking part in the formation of 3-methylbutanoic acid. These correlations suggest that the analysis of the green bean headspace can help understand the formation of flavour compounds from the precursors in the green bean. Fermentation products, specifically, seem to vary significantly in the green bean headspace and to be correlated with some specific flavour compounds in the roasted bean.

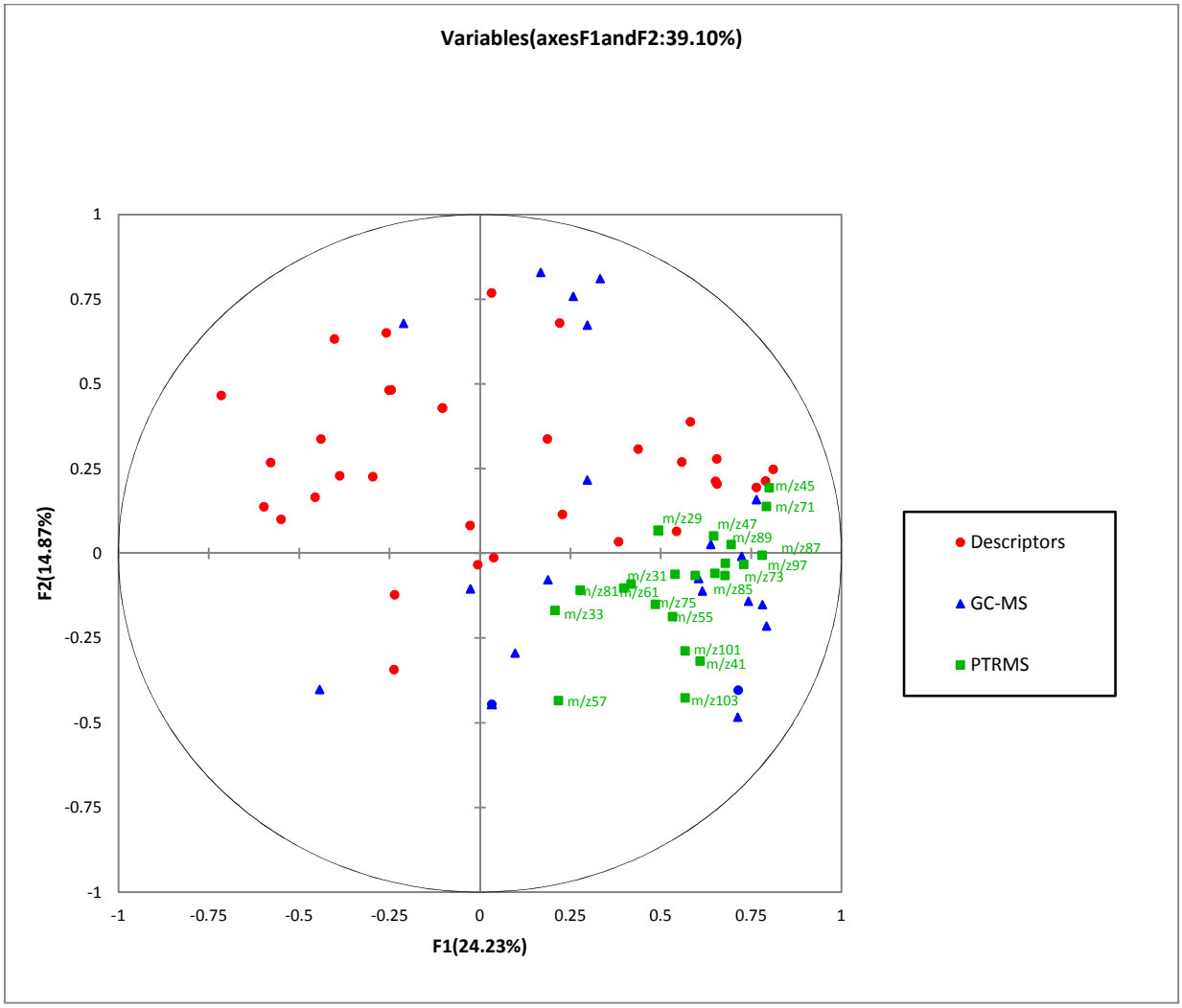


Figure 5.9. Multiple factor analysis (MFA) map representing the projection on F1 and F2 of descriptor subgroups (circle) and significant ($p<0.15$) odour-active compounds (triangle) as active tables, and significant ($p<0.05$) PTR-MS m/z values (squares) as supplementary table. Only key labels are shown, to improve readability. See the other labels in Figure 5.8.

Figure 5.10 maps the samples on F1 and F2 of the MFA. A similar configuration to the one previously produced by the CA of the descriptors (Figure 4.1) is found here. Following F1 from left to right, Brazilian samples are located near the negative end, followed by the Dominican Republic and Hidalgo (Mexico) samples, then by Colombian and Chiapas (Mexico) samples, and Nicaraguan samples tend to be located near the right end of F1. The effect of geographically localised factors seems more important than the effect of processing. Those geographically localised factors, once again, are not limited to the strictly environmental factors such as climate and soil. They include the choice of coffee varieties

used in different regions, the farming practices, harvesting practices and processing technologies. All other things being equal, a single factor such as the harvesting practices still could be potentially responsible for many of the differences found here: in regions where the harvest is done using machinery, like Brazil (Melenikiotis, 1998), the average ripeness of coffee cherries tends to be lower than when selective picking is used, like in Nicaragua (Craipeau, 1992). The average ripeness of the coffee cherries impacts directly the contents of sugars, amino acids and other precursors.

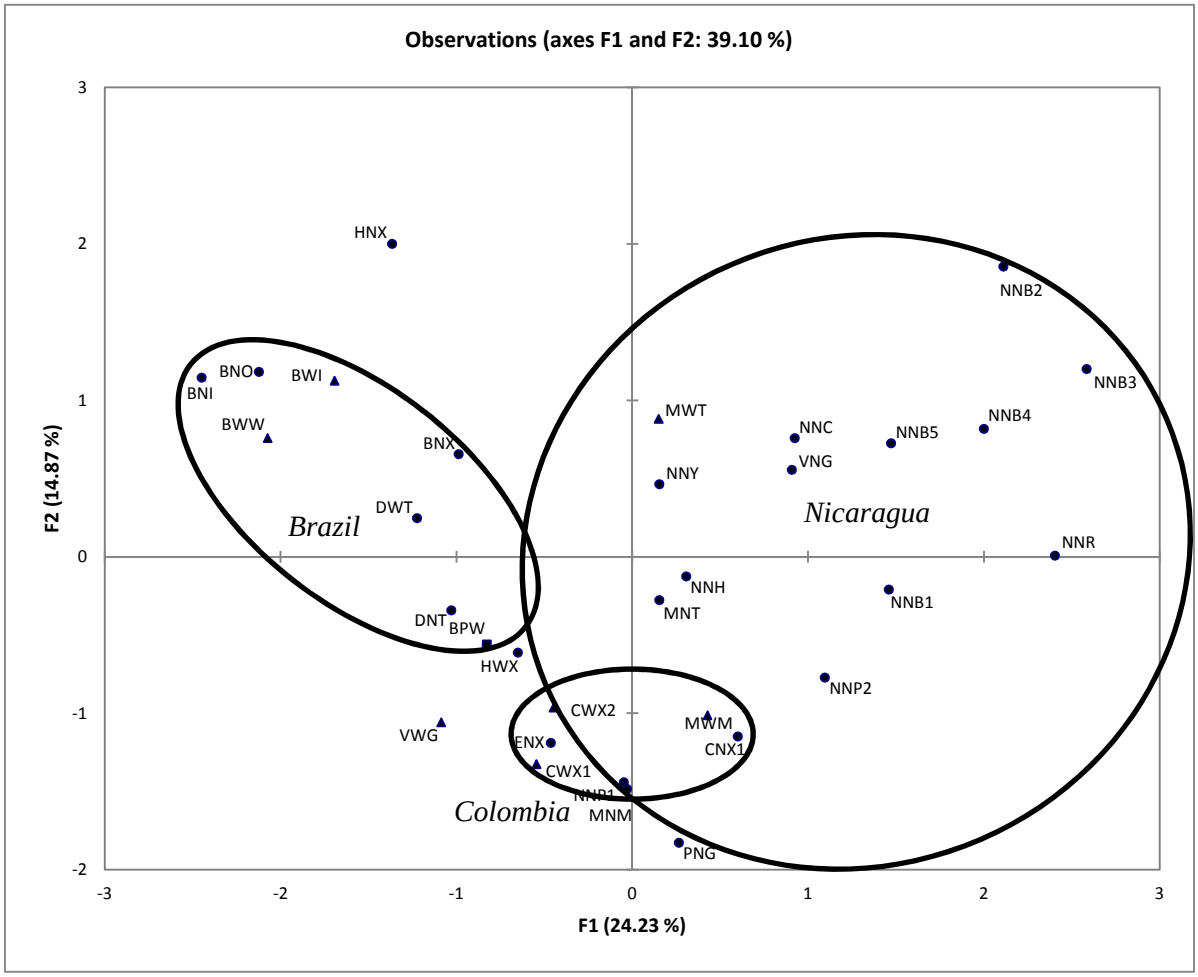


Figure 5.10. Multiple factor analysis (MFA) map representing the projection on F1 and F2 of natural (circle), washed (triangle) and pulped-natural (square) coffees for 32 samples, evaluated using the Descriptive Cupping method and GC-MS.

The thermal reactions during roasting still cannot fully explain fruitiness – or the Mocha character – which is the main character differentiating some specialty naturals, as was found in Chapter 4. The most likely explanation for fruitiness is the presence of ethyl 2-

methylbutanoate and ethyl 3-ethylbutanoate (in addition to 2-methylpropanal). For these two esters to be formed, a sufficient amount of ethanol must be available for esterification with 2- and 3-methylbutanoic acid. Selmar et al. (2001) claim that the differences between the processing methods are due to "*metabolic reactions within the coffee seeds that differ markedly depending on the mode of post-harvest treatment*". Granting that 2- and 3-methylbutanoic acid could be produced by the coffee cherry metabolism (even though they are also typical fermentation products), the presence of ethanol is most easily explained by a microbial fermentation. This line will be further explored in the next chapter.

5.4 Conclusion

This complex study focused on investigating the headspace volatiles of the coffee samples that were analysed through Descriptive Cupping in Chapter 4, in order to understand the link between the volatile compounds and the sensory profile of naturals. The main focus was kept on the odour-active volatiles of roasted coffee, while the headspace of the green beans was studied as complementary data using PTR-MS. The number of volatiles found in both the roasted and green bean headspace reflects the complexity and richness of coffee aroma. Thus, the data interpretation relied mostly on multivariate and multiple factor analyses.

Important effects on the headspace profile were found from thermal degradation products of sugars and Maillard reaction products (involving amino acids and sugars). However, the main factors explaining fruitiness in some naturals – the famous Mocha character and the main discriminant feature among naturals – seem to be related to both amino acid catabolism (of valine, isoleucine and leucine) and ethanol fermentation. The esters produced by the ethyl esterification of 2- and 3-methylbutanoic acid have a lower sensory threshold than their methyl counterparts and present a fresh, red-fruity character (strawberry, blueberry), which is the most common description of specialty natural coffees. The conditions for the formation of the fruity esters in natural coffee will be further explored in Chapter 6.

6 Effect of drying rate on the sensory flavour profile of natural coffee, as assessed by two independent cupping panels

6.1 Introduction

The previous chapters demonstrated the importance of the Mocha character, which can be described as the fruity and winey character present in some naturals. The Mocha character was found to be the main characteristic that differentiated natural coffees and other processing methods. It also differentiated within the group of natural coffees, as some naturals lack this feature. The coffee industry has identified the Mocha character as a desirable characteristic in naturals, as long as it is not accompanied by undesirable features, such as *"salty astringency"* (Davids, 2010).

Previous research (Diaz Pineda & Fernandez Alduenda, 2007a, 2007b; Fernandez Alduenda, Diaz Pineda, & Sierra Martinez, 2008) suggests the Mocha character may be linked to the drying rate during the 'natural' process. Furthermore, a variation of the natural process called 'enmielado' in Mexico (literally, 'honeying' or 'syruping', which should not be confused with the term 'honey coffee' that is sometimes applied to pulped-naturals) is used by some producers in Mexico, Ethiopia and Yemen. During 'honeying', drying is suspended during the first 8-48 hours after the harvest by piling up the coffee cherries. The cherries are piled until their surface becomes sticky, as if covered in syrup, hence the reference to 'honeying'. While producers using this 'honeying' process claim this results in a fruitier flavour and a better acidity (personal communication from H. Diaz Pineda), there is a lack of supporting scientific evidence.

Therefore the first aim of this study was to investigate the effect of the drying rates and the fermentation of the coffee cherries on the sensory properties of the resulting roasted coffee as determined by the Descriptive Cupping method (*Objective 5*, 2.2.2.5). The second aim of this study was to determine the reproducibility of the Descriptive Cupping methodology (*Objective 1*, 2.2.2.1) by the comparison of the sensory results from two, independent cupping panels.

In order to achieve those aims, seven different drying treatments were studied, including the 'honeying' process. A washed process witness (washed coffee from the same batch of raw material) was also included in the study. Raw material heterogeneity was controlled by subsampling the different treatments from one batch of coffee cherries. The drying rates were

controlled using low-technology techniques which would be available to the typical coffee producer.

Samples from the different treatments were roasted and cupped by two independent cupping panels – one in New Zealand and the other one in Mexico. The data from both panels was analysed and compared using the Descriptive Cupping approach employed in Chapters 3 and 4.

6.2 Materials and methods

6.2.1 Samples and treatments

6.2.1.1 Raw material

Coffea arabica L var. *typica* was chosen as the variety used in this study. This variety was selected as it is a parent variety of most coffee cultivars (Anthony et al., 2002), it is the most traditional variety in the Western Hemisphere and is widely used in the Mexican regions that produce natural coffee.

Ripe (completely red) coffee cherries (200kg) were hand-picked on the same morning (26th of January, 2013 – day 0) from a single farm at La Laguna village, Xico municipality, state of Veracruz, Mexico, (19.405638°N, 96.949814°W, 1050m above sea level, owned by Mr Conrado Arenas). The coffee cherries were immersed in fresh water to remove the floating cherries (underdeveloped or hollow beans), to lower their temperature and to wash the cherry surface so as to reduce the initial microbial load. The coffee cherries were then transported in burlap bags to the site where the drying treatments were carried out. The drying site was about one hour drive away, at 1250m above sea level.

6.2.1.2 Design of sample treatments

The seven different treatments studied in this chapter were designed based on the hypothetical microflora that might grow at each region of the drying curve (Figure 6.1). Figure 6.1 shows the weight loss and drop in water activity predicted for a 10kg batch of coffee cherries during drying at 25°C. The figure has been adapted from Corrêa, Goneli, Júnior, De Oliveira, and Valente (2010). According to Silva et al. (2008), a succession of microbial populations take part in the fermentation of the coffee cherry during drying, depending mainly on the water

activity of the coffee cherry. Bacteria, yeasts and moulds are the main microbial populations taking part in the fermentation. Each population is suggested to be predominant in one of the regions marked as A (bacteria), B (yeasts) or C (moulds) in Figure 6.1. In Region A bacteria may predominate until a water activity of 0.91 is reached. In Region B, yeasts are active between water activities of 0.91 and 0.85. Below a water activity of 0.85, the growth of mould is favoured (Silva, 2014).

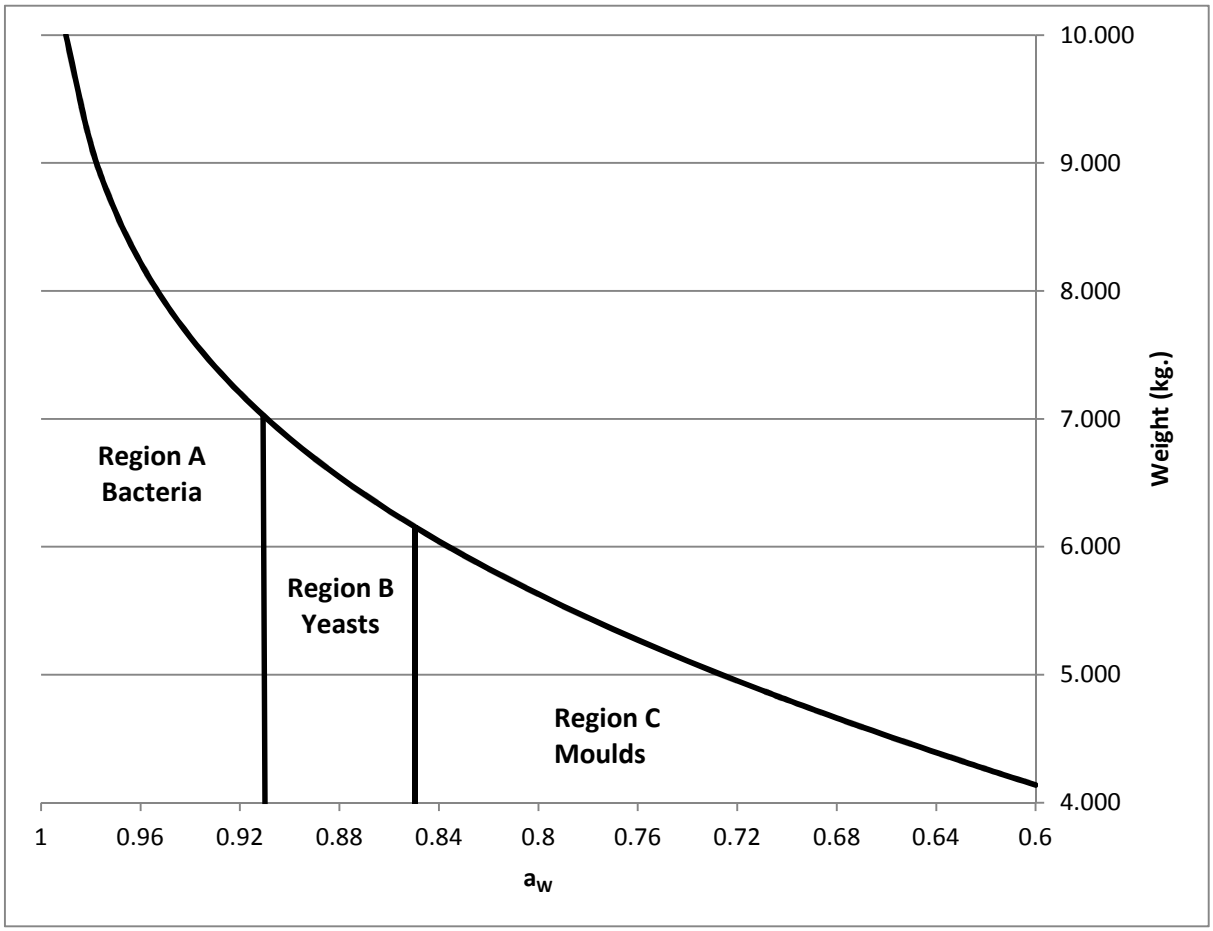


Figure 6.1. Weight of a batch of 10kg of coffee cherries as water activity (a_w) drops at 25°C. Isotherm created using the Modified Henderson equation from Corrêa et al. (2010). Regions A, B and C show the main microbial populations (Silva et al., 2008).

The experimental design of the 7 treatments is shown in Table 6.1. In each treatment, different microbial populations (bacteria, yeasts, moulds) were targeted for a longer (+) or shorter (-) period of fermentation by manipulating the rate of drying, to achieve decreased or increased time in each of the three regions (A, B or C), favouring the growth of different microbial populations. In addition to the 7 natural process treatments, washed coffee from the

same batch of raw material was produced as a witness, in order to have a point of reference in an analogous way to washed samples in Chapter 4. The cherries for the witness were selected from the same batch of cherries as used for the treatments, processed as washed coffee, and dried at a slow rate in order to have a final drying time similar to the natural treatment times. All treatments were dried to a common moisture level (12%).

Table 6.1. Design for drying treatments of natural coffee.

Treatment [a]	Region A [b]	Region B [c]	Region C [d]	Remarks
1	- [e]	-	-	As fast as possible.
2	0	0	0	Normal but steady speed.
3	++	-	-	'Honeying' process.
4	-	+	-	Yeasts favoured.
5	-	-	+	Moulds favoured.
6	+	+	-	Bacterial and yeast fermentation.
7	+	-	-	Bacteria favoured

(a) Washed coffee was added to these 7 treatments as a witness.

(b) Region A – Above $0.91a_W$.

(c) Region B – Between 0.85 and $0.91a_W$.

(d) Region C – between 0.60 and $0.85a_W$.

(e) Legend: (-) – Fast drying during this region for a short fermentation time; (0) – Implies a normal drying speed; (+) – Slower drying during this region for a longer fermentation time; (++) – Halted drying and piling up of coffee cherries in order to maximise fermentation during this region.

To shorten or lengthen the duration of fermentation in each of the three regions (A, B, C), the rate of drying was manipulated by changing the drying surface, drying environment, layer thickness and turning frequency. The vast majority of natural coffee producers outside Brazil still use solar drying. One of the main reasons why the drying protocol was controlled using only low-technology methods such as layer thickness and turning frequency was because those are the only control variables accessible to most natural coffee producers.

The *drying surface* was either a concrete patio or raised beds. The concrete patio achieved a higher temperature on sunny days, while the raised beds are more efficient on cloudy days as the air can circulate underneath the coffee layer. The drying *environments* were either inside a drying hut or outdoors. The drying hut achieves a higher ambient temperature but outdoors drying allows a direct exposure of the coffee cherries to solar irradiation on sunny days. The *layer thickness* ranged between 10 and $48 kg \cdot m^{-2}$, taken as fresh cherries. Thinner layers allow a more efficient and even drying. The *turning frequency* ranged between 0 and 4 times per

day. Higher cherry turning frequencies allow a faster and more even drying. These variables were fine-tuned on site depending on the actual progress of each treatment along the drying curve, aiming at shortening or lengthening the drying time during the different regions shorter or longer. As an example, this means that if one region was meant to be longer, when water activity reached the appropriate range, the drying process was slowed down (taking coffee out of the drying hut, decreasing the surface area, increasing cherry layer thickness, decreasing cherry turning frequency, and sometimes even bagging the coffee), in order for coffee to remain for a much longer time within the required water activity range.

A total of 15 samples were produced. The 7 treatments (Table 6.1) were produced in duplicate, along with the washed witness. On the day of harvest, a portion (24kg) of the raw material was separated from the batch so as to prepare the washed witness. Meanwhile, the remaining cherries were spread in the drying hut on raised mesh tables overnight.

6.2.1.3 Washed witness

The washed witness was coffee obtained from the same raw material used for the other treatments. The washed process employed mimicked the traditional practice and was equivalent to treatment Wd in Chapter 3. Fresh cherries (24kg) were pulped in a disc pulper, then immersed in water to remove the floating beans (usually underdeveloped or attacked by insects), drained and fermented without water for 36 hours in a plastic bowl, until the mucilage disintegrated. The parchment beans were then washed using fresh water and carried to the same site where the other treatments were being carried out in order to be dried. The parchment coffee was dried outside on a raised table ($1.6m^2$) for 14 days and brought indoors during the nights and rainy days. After drying to a moisture content of 12%, the parchment coffee was left to stand for 10 days and then hulled in a sample huller. The resulting green coffee was packed in foil bags and brought to New Zealand with the other samples.

6.2.1.4 Drying treatments

On the morning of day 1 (27^{th} of January), the batch of coffee cherries were divided to fourteen 12kg portions. Seven different drying treatments were applied (Table 6.1) in duplicate until a final moisture level of 12% was achieved. The drying period of the treatments varied from 17 to 24 days.

Treatment 1. Coffee cherries were left in the drying hut for the whole of the drying process. For the first two days, the 12kg of coffee cherries were spread on $1m^2$ black plastic mats. From the 3rd day onwards, the coffee was spread on the concrete floor with the aid of four $0.25m^2$ wooden frames. This coffee received a high turning frequency (about hourly between 11 a.m. and 4 p.m.). Treatment 1B was ready on day 17, while 1A was ready one day later.

Treatment 2. Coffee cherries for this treatment were spread (12kg of fresh cherries over $0.81m^2$) on a mesh raised table for the whole drying period. The batch received a high turning frequency and was left outdoors as long as possible. It was brought indoors in the evenings and on rainy days. This treatment was ready on day 24. On two very rainy days towards the end of the drying it had to be kept in a bag indoors to prevent absorbing moisture.

Treatment 3. This is what in Mexico is traditionally called the 'honeying' natural process. From day 1 until day 3, the coffee was piled inside wooden frames (12kg of fresh cherry over $0.25m^2$) outside on the concrete patio and covered at night with plastic film. From day 3, the cherries were spread on a mesh raised bed outdoors ($0.81m^2$) and received a high turning frequency. The batch was brought indoors in the evenings and on one rainy day. It was ready by day 18.

Treatment 4. This treatment went through three stages: (a) days 1 through 3, in the drying hut, on a $1m^2$ plastic mat with a high turning frequency; (b) day 4, in the drying hut, in a $0.5m^2$ pile with low turning frequency (once a day), and (c) from day 4 until the batch was ready on day 24, outdoors on $0.81m^2$ mesh raised table with a high turning frequency. This treatment was bagged for 2 rainy days towards the end of the drying, to prevent it from gaining moisture.

Treatment 5. This treatment went through four different stages: (a) days 1 and 2, in the drying hut, on a $1m^2$ black plastic mat and with a high turning frequency; (b) day 3 and 4, outdoors, on $0.81m^2$ of mesh raised bed and with a high turning frequency; (c) on days 5 through 10, the batch was kept in a plastic bag in the drying hut until a mould outbreak was evident; (d) From day 11 until day 24, it was dried outside on $0.81m^2$ of mesh raised table and with a high turning frequency (except on rainy days). One of the replicates (5B) was spread on day 10 by mistake on a raised table instead of being kept in the bag. To compensate, the batch was kept in the bag longer (on days 11 and 12) until it developed mould.

Treatment 6. This treatment also went through four different stages: (a) days 1 through 3, outside on a mesh raised table, piled on 0.41m^2 with low turning frequency; (b) days 4 and 5, in the drying hut, on a raised table, piled in 0.25m^2 wooden frames, with no turning; (c) day 6, in a plastic bag in the drying hut, and (d) day 7 through 24, outside on 0.81m^2 of mesh raised table, with high turning frequency (except when rainy). The batch was bagged for two rainy days at the end of the drying to prevent it from gaining moisture.

Treatment 7. On days 1 through 3, the cherries were piled on 0.41m^2 of a mesh raised table with a low turning frequency (once a day). From day 4 until the batch was ready on day 18, it was kept on 0.50m^2 of black plastic mat, in the drying hut, with a low turning frequency.

When the different treatments and replicates were finished, the resulting dried cherry samples were hulled and green coffee samples were brought to the University of Otago for further study. They were labelled with their treatment number and a letter 'A' or 'B' indicating the duplicate. The washed witness was labelled with the letter 'W'.

6.2.2 Variables monitored during drying

Every day, the drying parameters and the changes to the coffee cherries were monitored, either as independent variables that helped better fine-tune the drying process or as dependant variables. Table 6.2 summarises the independent and dependant variables monitored:

Table 6.2. Summary of variables monitored during drying.

Independent variables	Dependant variables
Ambient temperature (several measures/day)	Cherry aspect (photo)
Relative humidity (several measures/day)	Sample relative weight loss (daily)
Sample temperature (two measures/day)	Sample water activity
	Sample pH (every few days)

6.2.2.1 Ambient temperature and relative humidity

A maximum/minimum, digital hygro-thermometer (TFA Dostmann/Wertheim®, Denmark, catalogue number 30.5000.02) was used inside the drying hut to measure ambient temperature and relative humidity. An analogue hygro-thermometer (Brixco, China) was placed outside in

the shade. Temperature and relative humidity were read twice a day outside (12p.m. and 5p.m.) and thrice a day in the drying hut (7a.m., 12p.m. and 5p.m.) Daily maximum temperatures were also recorded.

6.2.2.2 Sample temperature

An infrared gun thermometer (Kintrex® brand, model IRT0424, Kintrex, Vienna, VA, USA) was used to measure the temperature of the coffee cherries. Temperature was measured twice daily (12p.m. and 5p.m.).

6.2.2.3 Relative weight loss of sample

The treatments were weighed daily (7a.m.) using a 50kg scale (Torino brand, Industrias Oken, S.A. de C.V., Morelia, Mexico).

6.2.2.4 Cherry aspect

Visual changes were recorded using daily photographs of the treatments, and a logbook was kept for recording observations such as stickiness, shininess, presence of moulds, etc.

6.2.2.5 Sample water activity (a_W)

The water activity was measured using an AquaLab® Water Activity Meter model CX3 (Decagon Devices Inc., Pullman, WA). The 0.760a_W Decagon Inc. verification standards were used for calibration. Every evening, the pulp (exocarp and outer mesocarp) of two cherries were placed in the instrument's sample cups. Two a_W readings were averaged for every sample.

6.2.2.6 pH

The pH was measured on days 0, 6 and 10. Ten cherries of each treatment were weighed; then an equal mass of purified water was added to them and they were crushed using a mortar and pestle. The pH of the resulting slurry was then measured using a portable pH meter (Hanna Instruments®, Padua, Italy).

6.2.3 Microbial counts during drying

6.2.3.1 Samples and treatments

In order to link the main drying treatments to microbial activity, a smaller scale experiment was carried out, to determine the change in the numbers of the three main microbial populations: bacteria, yeasts and moulds. In this experiment, 3 treatments with two replicates each (4kg of fresh cherry per replicate) were applied as follows:

a) Treatment 21 (the three treatments of the microbiology experiment were coded 21 through 23 to differentiate them from the main experiment). Normal rate drying (similar to treatment 2 on Table 6.1) – outdoors, on mesh raised table on a thin layer with regular turning frequency.
b) Treatment 22. Slow rate drying (similar to treatment 7 on Table 6.1) – outdoors, on mesh raised table, on a thick layer with limited turning of cherries.
c) Treatment 23. 'Honeying' process (similar to treatment 3 on Table 6.1) – two days piled in a bucket, followed by normal drying rate, outdoors on a raised table, with a regular turning frequency.

6.2.3.2 Microbiological methods

The samples (6.2.3.1) were analysed using microbiological methods on days 0, 1, 2, 4 and 7 of drying. Two different microbiological counts were performed on samples from the different treatments: bacteria count on a bacteria-specific WL Differential agar (Difco®, Becton, Dickinson and Company, Franklin Lakes, NJ, USA), and yeast and mould count on Dichloran Rose-Bengal Chloramphenicol agar (DRBC – Difco®, Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Every two days, at the end of the afternoon, ten cherries were sampled from every treatment replicate, placed into sterile bags and taken to the laboratory. Peptone solution (100 mL; 1% BD peptone, Becton Dickinson and Co., Franklin Lakes, NJ, USA) was added to each sterile bag and the bag was pressed by hand for around two minutes. This suspension was then serially diluted to the levels 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} by transferring 1mL of the preceding dilution to a vial with 9mL peptone solution.

Two WL Differential Medium plates and two DRBC Medium plates were inoculated at three different levels to give 10^{-3}, 10^{-5} and 10^{-6} plates. The resulting 12 plates for each sample were then incubated at room temperature (25-30°C). Bacteria plates were counted after three days;

yeast and mould plates, after seven days. Colonies presenting hyphae to the unaided eye on the seventh day were counted as moulds. This was verified in a few cases using a microscope (40 x 40 / 0.65; 160/0.17 objective).

6.2.4 Flavour profiling of samples by Descriptive Cupping at the University of Otago (New Zealand)

6.2.4.1 Sample roasting

For roasting, samples were grouped in blocks of 6 samples. Samples were roasted in batches (4 batches of 200g green coffee). The roasting order of samples for each batch was randomised within each 6-sample block. Roasting was carried out using the same protocol, equipment and control parameters stated in 4.2.1.3 for the international appraisal sample set, using a Gene Café CBR-101 roaster (Gene Café, Ansan City, Republic of Korea). The end of roast was approximately 2 minutes after the first crack, when the fume odour started to shift from pungent to vanilla-like or nutty. After each batch was cooled to room temperature, weight loss was measured as a roast degree indicator. The target weight loss for each batch was set at 16.0% ± 2.2%. Luminance was measured on the roasted and ground coffee as L* in the CIELAB colour space (see 4.2.1.2).

6.2.4.2 Cupping

The roasted samples were cupped in duplicate by the same panel used for the study in Chapter 4 and using the same methodology. The brewing procedure is described in 4.2.1.4 and the cupping procedure is reported in 4.2.3. The order of sample presentation was randomised, and the samples were presented blind, in sessions of 6 samples.

6.2.5 Flavour profiling of samples by Descriptive Cupping at the Agro-ecological Centre for Coffee (CAFECOL, Mexico) panel

In order to compare the Descriptive Cupping results from two different panels, in two different countries and with different backgrounds, subsamples of all the treatments (in duplicate) were left in Mexico to be roasted and cupped by a second panel. The cupping was carried out by the Agro-Ecological Centre for Coffee (CAFECOL, Centro Agroecológico del Café A.C., Avenida Orizaba #18, Xalapa 91020, Veracruz, Mexico) within the context of a

state-wide coffee quality competition ('3er Premio CAFECOL 2013 a la Calidad del Café Veracruzano', held in Huatusco, Veracruz, Mexico on the 28th March, 2013).

The samples were roasted, brewed and cupped following the SCAA cupping protocol (SCAA, 2009a). The cuppers (4 male, 3 female; age range 30-45) were all Q Graders licensed by the Coffee Quality Institute (CQI, 2013b). Six of the cuppers in this panel had little experience cupping naturals, but had at least 5 years of experience cupping washed coffee. The remaining cupper had 7 years of experience cupping both natural and washed coffees.

6.2.6 Data analysis

6.2.6.1 Terminology from the University of Otago (New Zealand) panel

The terms used by the cuppers were categorised using the same methodology reported in 4.2.4.2. The terms in this study were classified in a total of 31 'subgroups'. Bouquet subgroups included from the 'Le Nez du Café®' vocabulary (see Table 3.3) were *caramelly, chocolaty, citrus-like, earthy, fermented, floral, fruity, nutty, phenolic, pyrolytic, resinous, spicy, stone-fruity, toasty, woody* and *vegetable;* from the supplementary natural coffee vocabulary (see 4.2.3), *dried-fruity, red-fruity, tropical-fruity,* and from the freely-elicited subgroups, *fungal, plain bouquet* and *other.* Freely-elicited taste descriptors included *bitter, salty* and *sweet.* The acidity category contained the subgroups *dry-acidity, medium-acidity* and *sweet-acidity.* Mouthfeel contained the subgroups *rough-body, smooth-body* and *astringent.*

6.2.6.2 Terminology from the Agro-ecological Centre for Coffee (CAFECOL, Mexico) panel

The terminology used by the cuppers followed the standard bouquet terminology used by Q Graders (Lenoir & Guermonprez, 1997), including 12 bouquet 'subgroups': *floral, fruity, vegetable, nutty, caramelly, chocolaty, spicy, resinous, pyrolytic, earthy, fermented* and *phenolic*. The presence of notes belonging to these subgroups was reported for the four bouquet sections: fragrance, aroma, flavour and aftertaste. See 3.2.3 for a thorough description of SCAA cupping.

6.2.6.3 Descriptive Cupping analysis

Descriptive Cupping data from both panels were analysed using the same methodology detailed in Chapter 3 and 4.2.4.3. A contingency table was constructed for each dataset, with the samples as rows (15), the descriptor 'subgroups' as columns (31 for the Otago panel or 12 for the Mexico panel) and number of occurrences for cell counts. Significance tests were run on the respective contingency tables for each dataset. Independence between samples and subgroups was tested with the 'Monte Carlo' method (5000 simulations, α=0.05) (Metropolis & Ulam, 1949). In order to identify significant subgroups, global χ^2 and χ^2 per-cell analyses were carried out on the contingency tables (Symoneaux et al., 2012). Global χ^2 was then used to identify significant subgroups across the whole data set, while χ^2 per-cell was used to identify significant subgroups within individual samples (α=0.05). Duplicate samples and duplicate treatments were aggregated for Monte Carlo and χ^2 methods.

Non-symmetrical correspondence analysis (NSCA) was applied to the contingency tables in order to visualise the relationship between the samples and subgroups. For the NSCA, duplicates were not aggregated, but were used as individual cuppings. This was done to examine the reproducibility of the cuppers by observing the mapped distance between duplicates. In case the whole contingency table was not significant, only significant attributes, based on their χ^2 (χ^2 above the critical value for p=0.05) were kept for the NSCA. All statistical analyses were carried out using XLstat (Addinsoft SARL).

6.2.6.4 Comparison of both panels using MFA

The results of both panels were compared using multiple factor analysis (MFA) (Abdi et al., 2013; Lawrence et al., 2013). The degree of correlation between the tables was investigated through the regression vector (RV) coefficient, to identify the link between each pair of tables and to analyse similarity between any two tables (Robert & Escoufier, 1976). The MFA and the calculation of RV coefficients were carried out using XLstat (Addinsoft SARL).

6.3 Results and discussion

6.3.1 Treatment variables

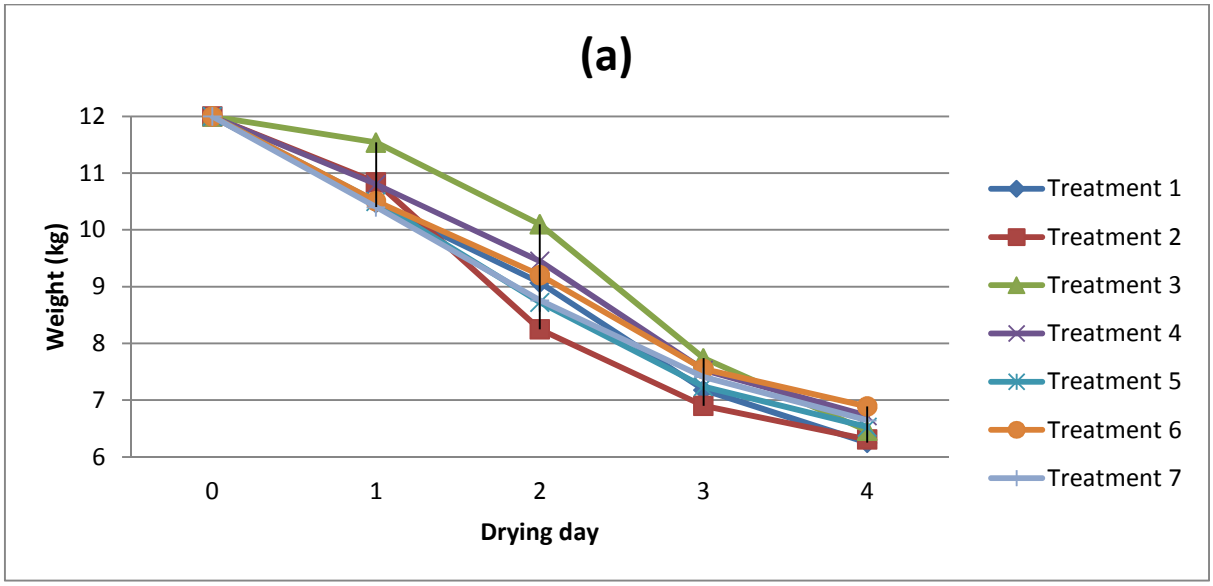
6.3.1.1 Weather conditions

Overall, the weather conditions were warm and dry during the drying process. During warm, sunny days, maximum temperatures of 28-34°C and minimum relative humidity of 30-45% were recorded outdoors. The maximum temperatures in the plastic hut usually reached 9°C higher than outdoors, while the minimum relative humidity usually was 25% lower than outdoors. On day 7 and days 19-22, cold front weather resulted in a temperature drop (below 15°C) and higher relative humidity (above 80%), sometimes with drizzle.

6.3.1.2 Drying curve and water activity

Figures 6.2(a) and 6.2(b) show the weight of each treatment along the drying process during days 0-4 and days 4-24, respectively. Figures 6.3(a) and 6.3(b) present the water activity of the cherry pulp for each treatment during days 0-4 and days 4-15, respectively (the a_W graph for days 16-24 is not presented, as a_W decreases slowly to values of 0.550-0.600).

During the first three days of drying, treatment 3 presented the slowest drying rate as it went through ‘honeying’, while treatment 2 presented the fastest drying rate. On day 3, the water activity measurements (Figure 6.3(a)) presented four groups of treatments: treatment 3, with the highest a_W, followed by a group formed by treatments 4, 6 and 7, next a group formed by treatments 1 and 5, and last, treatment 2, with the lowest a_W.



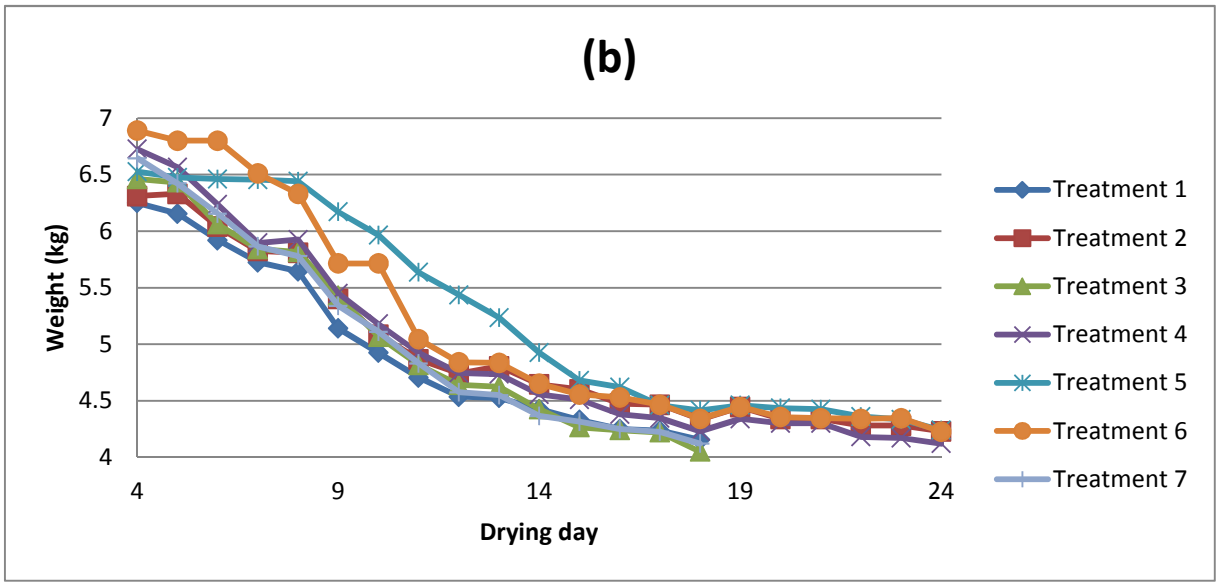


Figure 6.2. Sample weight on (a) days 0-4 and (b) days 4-24 of drying. Averaged by treatment.

Between days 4-14, treatment 1 was the treatment with the lowest daily weight. After day 15, treatment 3 (the honeying treatment) was the treatment with the lowest daily weight. After the 'honeying' was over, treatment 3 became the treatment with the highest drying rate, which suggests a loss of water-binding molecules due to a bacterial fermentation during 'honeying' and has been reported on washed coffee fermentation (Avallone et al., 2002; Avallone et al., 2001). Figure 6.3(a) shows an increase in water activity for treatment 3 during 'honeying', from day 1 to day 2, which also suggests water-binding molecules were degraded during this period.

After day 4 (Figure 6.3(b)), the drying of two treatments was interrupted by the procedure of bagging the coffee cherries. The drying rate of treatment 6 was slowed on days 4 and 5 (through piling of cherries) and bagged on day 6, to keep it at a higher moisture content than the other treatments. Treatment 5 was bagged from day 5 until day 11 and thus it remained the treatment with highest moisture from day 8 onwards. The pulp water activity of these bagged coffees increased as the moisture from the inside of the fruit tended to equilibrate with the exocarp moisture.

An increase in the water activity was also observed during humid days. Figure 6.3(b) shows a a_W increase for treatments 1, 2 and 3 on day 5 due to the equilibration of moisture from inside the bean, as the exocarp did not dry further on that day due to humid weather (however, the a_W increase of treatments 5 and 6 was due to bagging). This was also the case on day 13 for all the treatments located outdoors. The relative humidity outdoors was enough for the driest treatments to stop drying. However, inside the drying hut the relative humidity was still low enough to continue drying. This is the reason why drying huts are useful in regions with some humid days during the harvest season; even though the drying rate might be lower than outside on sunny days, the overall drying process was 6-7 days shorter in the drying hut.

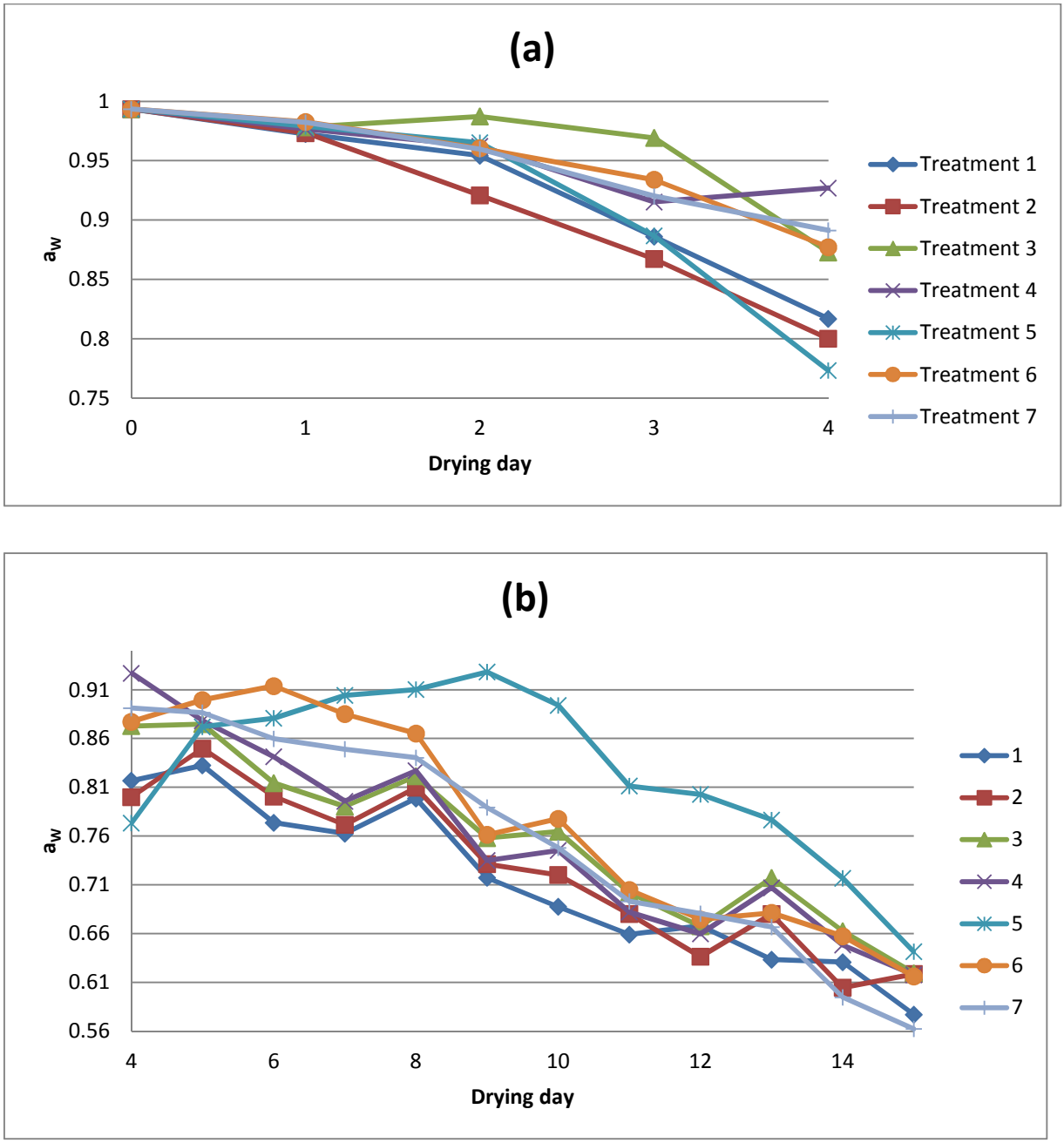


Figure 6.3. Evolution of water activity (a_w) during (a) days 0-4 and (b) days 4-15 of drying. Averaged by treatment.

6.3.1.3 Sample temperatures during drying

Figure 6.4 presents the daily average maximum temperatures of the treatments during drying. There is a temperature difference between the treatments in the drying hut and the treatments outdoors. The magnitude of that difference was weather-dependant. On sunny days, the outdoor treatments became hotter than the indoor treatments due to direct solar radiation on

their surface. This occurred despite the ambient temperature being warmer in the drying hut than outdoors. The maximum temperature attained by the coffee cherries remained lower inside the drying hut, which may prevent thermal damage to the bean.

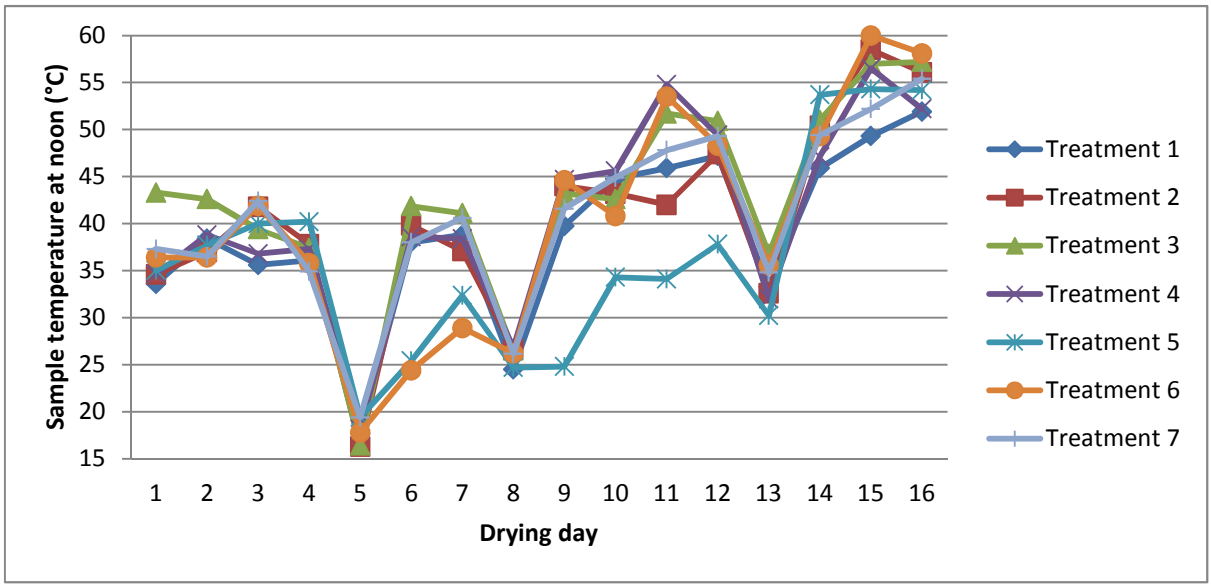


Figure 6.4. Sample daily maximum temperature (°C) on days 1-16. Measured at noon. Averaged by treatment.

Treatment 3 ('honeying' process) had a clearly warmer temperature while it went through 'honeying' on days 1 and 2. This fact suggests a large amount of heat was being generated due to microbial growth in treatment 3 and is also a common observation during the fermentation of washed coffees (Avallone et al., 2001). On the other hand, the cooler temperature of treatment 6 (days 6 and 7) and treatment 5 (days 6 thru 13) is due to bagging during those days, as bagged treatments were not exposed to solar radiation.

The daily maximum temperatures of all treatments tended to increase as the drying process advanced. This is explained by the decrease in the evaporative cooling effect as the coffee cherries lost moisture.

6.3.1.4 Evolution of pH during drying

An average drop of 1.2 pH units was observed between day 0 and day 10 (Figure 6.5). However, the pH drop varied between treatments; as shown in Figure 6.5, the pH drop of treatment 3 ('honeyed' treatment) is the largest. In contrast, the pH drop of the fastest-drying

treatments (1 and 2) was the smallest. This is explained by higher fermentation intensity in the 'honeying' process and the slow-drying processes, as confirmed during the microbiological experiment (6.3.2). A pH drop during the fermentation of washed coffee has been reported (Avallone et al., 2001). This has been linked to a raise in the total organic acid content in the green bean and consequently in a *"positive"* change in aroma and body (Gonzalez-Rios, 2008).

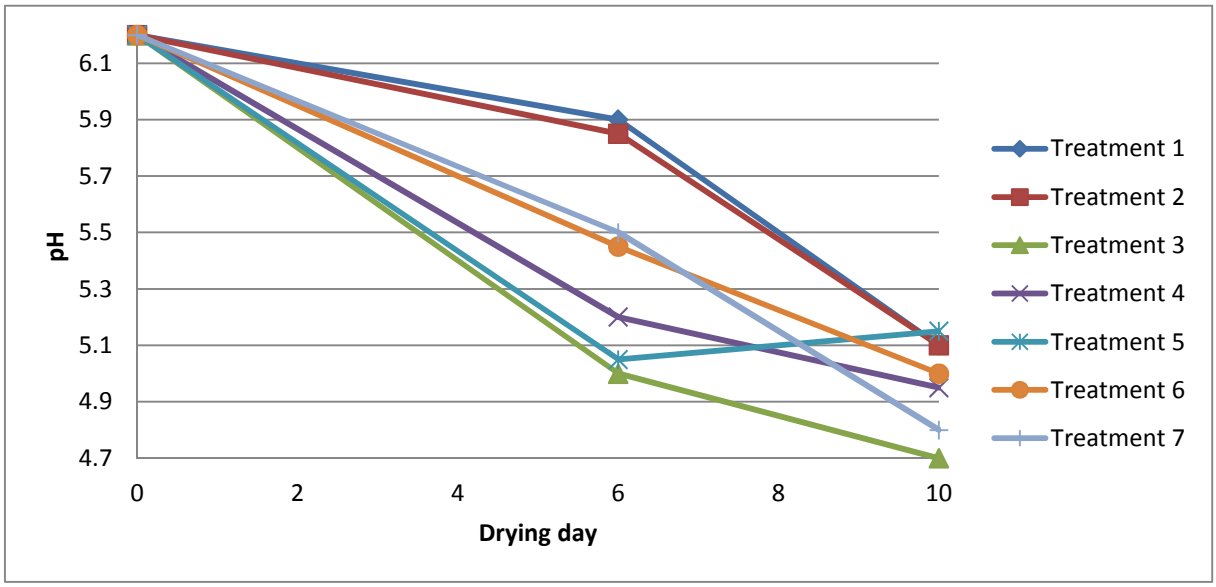


Figure 6.5. Evolution of pH during drying. Averaged by treatment.

6.3.1.5 Aspect of coffee cherries during drying

Differences were observed among the treatments from the aspect point of view. The treatments with the most uneven appearance were the ones that were dried using a thick layer, namely treatment 7, but also treatment 6 and 4, which is explained by the larger degree of exposure to sunshine of the cherries on the top layer. This unevenness has been reported to affect the sensory quality (Berhanu et al., 2012).

The colour of the cherries is another observation that implies a larger degree of fermentative activity was occurring in some treatments. Faster drying treatments, where fermentation is less likely to occur, resulted in an even raisin-like, very dark purple colour after a few days. This suggests non-enzymatic browning in the same way a sultana raisin gets its dark colour (Karathanos, Karanikolas, Kostaropoulos, & Saravacos, 1995). Treatment 3 ('honeyed' treatment) did not present browning: even at the end of drying, the cherries remained with a

brick-red colour. This suggests most of the sugars required for non-enzymatic browning were degraded by fermentation. In the case of treatments with an uneven appearance (treatments 4, 6 and 7), the unevenness is precisely noted for a different degree of browning between cherries, as the cherries on the top, sunshine-exposed layer became darker than the ones in the inner layers.

6.3.2 Microbiological experiment

The microbiology experiment was started on day 16 of the main experiment. Table 6.4 summarises the three treatments used (coded 21, 22 and 23).

Table 6.3. Treatments used in the microbiology experiment, and analogies to main experiment (6.4.1).

Code	Treatment description	Analogue to
21	Outdoors. Mesh raised table. Thin layer. Frequent turning of cherries.	Treatment 2, main experiment
22	Outdoors. Mesh raised table. Thick layer. Limited turning of cherries.	Closest one is treatment 7 of the main experiment
23	'Honeying' in a bucket outdoors for two days. From the third day, mesh raised table, thin layer with frequent turning of cherries.	Treatment 3, main experiment

The weight loss, water activity and pH curves of the 3 treatments in the microbiology experiment were analogous to the main experiment treatments as indicated by Table 6.4. Therefore, these data are not shown here, but can be found in Appendix 5 (section 11.5.2). Figure 11.3 and Figure 11.4 in Appendix 5 show the drying curves of treatments 21, 22 and 23, which correlate in general to the curves of treatments 2, 7 and 3, respectively, of the samples used for sensory analysis. The main differences between the drying curves of the samples used for sensory analysis and the drying curves of the samples used for the microbiological experiment are due to rainy weather conditions on days 3 through 6 of the microbiological experiment (see Appendix 5). These rainy conditions caused the drying rate to be lower during those days in the microbiological experiment than in the main experiment. Nevertheless, the targeted characteristics of each treatment (high drying rate for treatment 21, medium drying rate for treatment 22 and 'honeying' process for treatment 23) were maintained.

Figures 6.6 to 6.8 summarise the results for the microbiological experiment: bacteria count (Figure 6.6), yeast count (Figure 6.7) and mould count (Figure 6.8).

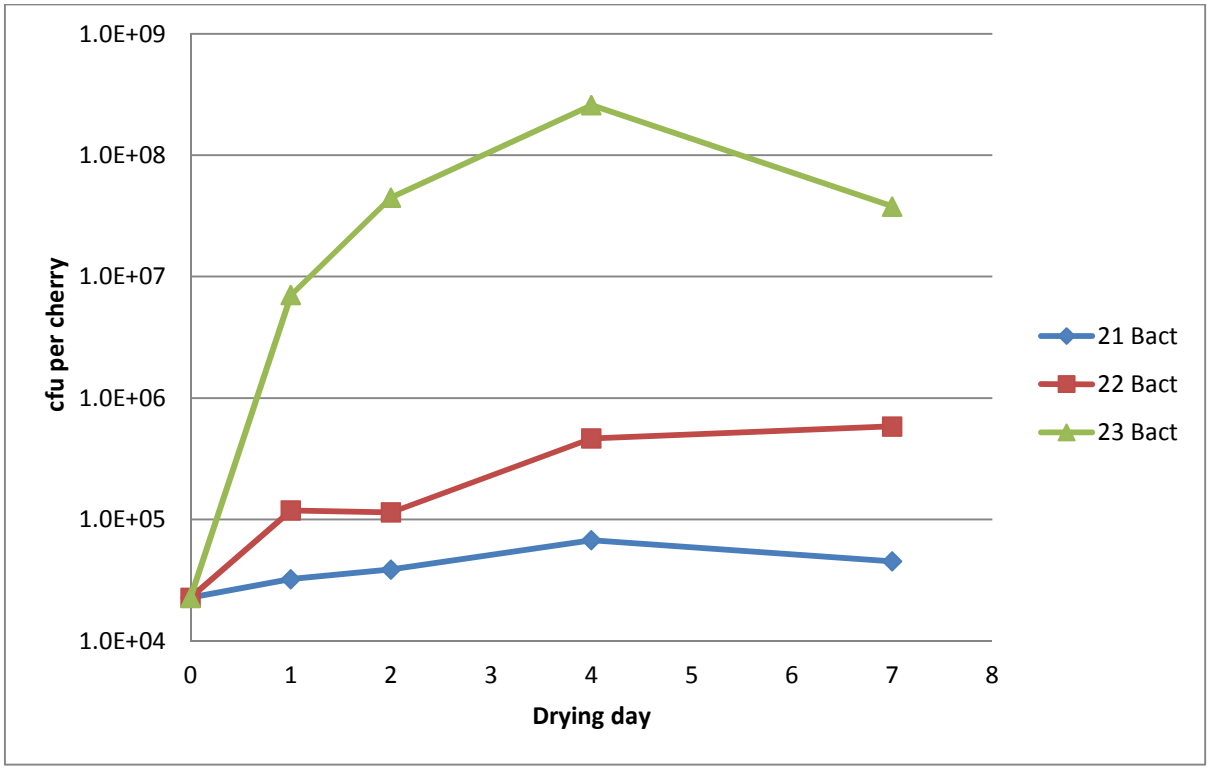


Figure 6.6. Bacteria count per coffee cherry on WL Differential agar after 3 days at 25-30°C. Averaged by treatment. Logarithmic scale.

The evolution of the three microbial counts (bacteria, yeasts and moulds) in the microbiological experiment is consistent with what is inferred from other observations like colour change and pH drop: there was a large difference in the microbial growth between the treatments. The traditional 'honeying' process, notably, seems to involve a fermentation of a much larger scale than the other treatments. The bacteria count of treatment 23 was 200 times larger than that of treatment 21 on day 1 and 1100 times larger on day 3. The yeast growth on treatment 23 was equally large compared to the other two treatments.

The initial bacteria population was $2.35 \cdot 10^4$cfu per cherry, increasing to $1.2 \cdot 10^5$cfu per cherry in treatment 22. Van Pee and Castelein (1972) reported a bacteria population on fresh coffee cherries (in Robusta coffee in the Congo) of $2.5 \cdot 10^5$ cfu$\cdot g^{-1}$, increasing to $1.5 \cdot 10^8$ cfu$\cdot g^{-1}$ during the first 24h and then decreasing to $3.5 \cdot 10^7$ cfu$\cdot g^{-1}$ by 72 h. Bacterial populations between 10^6 and 10^9cfu$\cdot g^{-1}$ were found by Silva et al. (2008) during the drying of Arabica coffee cherries in Brazil. The main possible reason for the lower average counts in this study is the washing of the cherries after the harvest, which reduces the initial microbial load and lowers the cherry temperature.

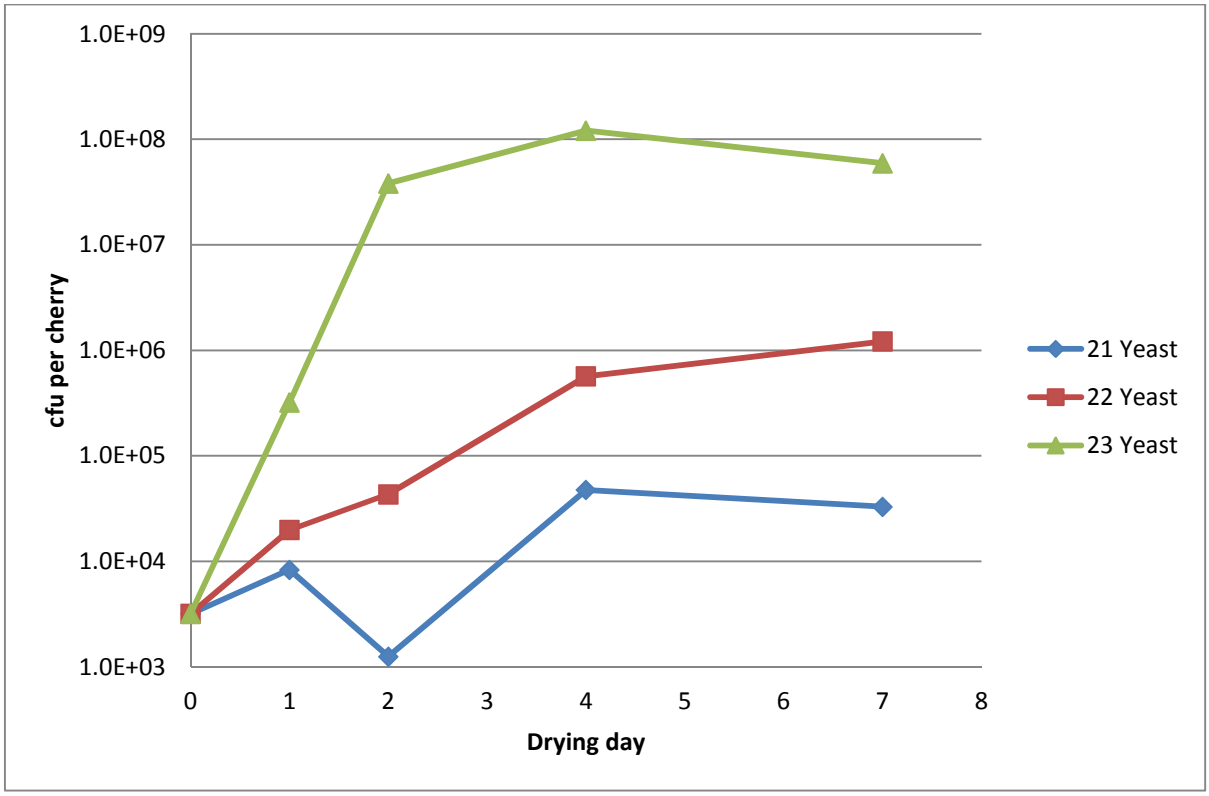


Figure 6.7. Yeast count per coffee cherry on DRBC, after 7 days at 25-30°C. Averaged by treatment. Logarithmic scale.

Silva et al. (2008) also found a maximum yeast population of 10^6cfu·g^{-1}, which is consistent to what was found here for treatment 21 and treatment 22. However, the 'honeying' treatment (treatment 23) showed a maximum yeast population of $1.2 \cdot 10^8$ cfu per cherry on day 4. On day 4, the average cherry weight was around 1g, meaning the maximum yeast population of the 'honeying' treatment was around 100 times higher than a conventional natural coffee treatment.

After day 4, a decline in the numbers of bacteria and yeasts in treatments 21 and 23 can be observed. However, a large increase in the number of moulds can be observed on day 6 for treatments 21 and 22 and on day 4 for treatment 23. A decline in the number of yeasts due to the decrease of water activity favour the growth of moulds (Silva, 2014). The maximum number of moulds is about 10^6cfu per cherry, which is the same reported by Silva (2014). The overall growth behaviour of the bacteria, yeasts and moulds is also consistent with the microbial succession reported by Silva et al. (2008).

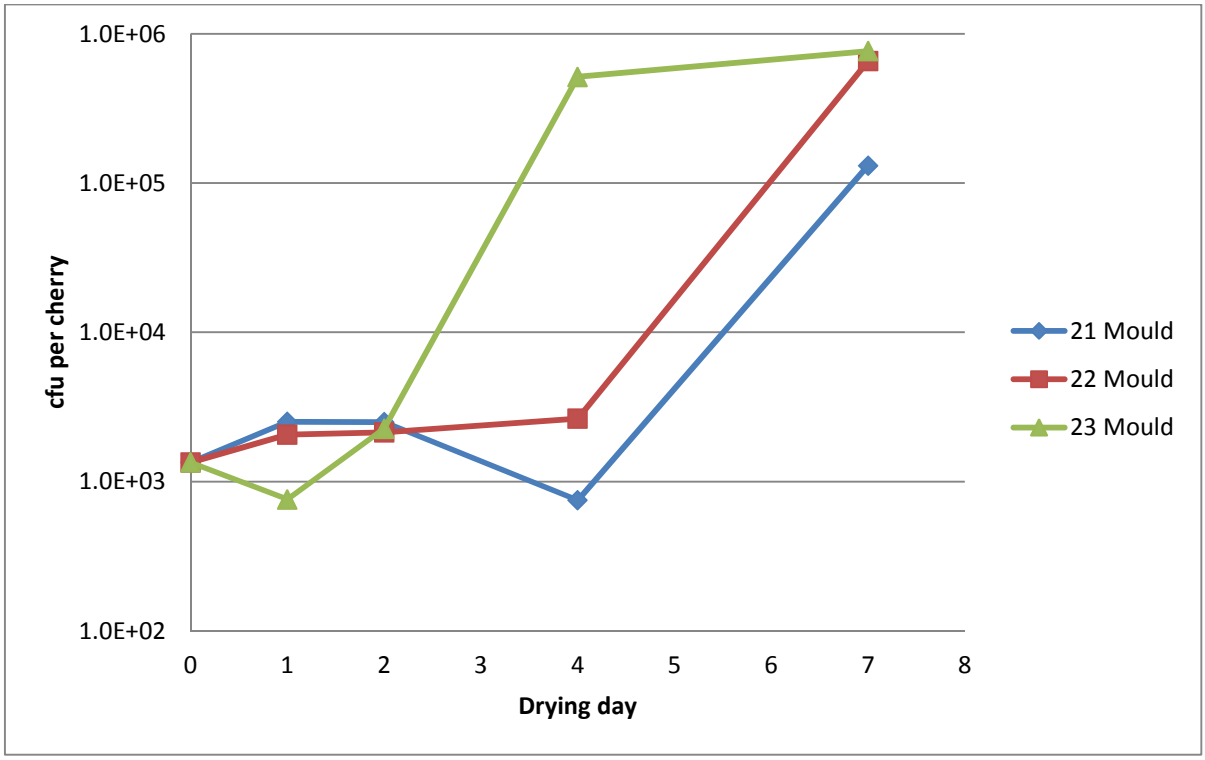


Figure 6.8. Mould count (colonies presenting hyphae) per coffee cherry on DRBC, after 7 days at 25-30°C. Averaged by treatment. Logarithmic scale.

6.3.3 Discussion about the effectivity of the treatment control

The treatment control variables used for this study (drying surface, drying environment, layer thickness and turning frequency – see 6.2.1.2) were chosen in order to mimic the drying control technology available to coffee producers. The following analysis of the results from sections 6.3.1 and 6.3.2 shows the control of these variables only partially achieves the targets established in Table 6.1.

Treatment 1 was targeted to be dried 'as fast as possible', while treatment 2 was targeted to follow a 'normal but steady speed'. Treatment 1 actually was the fastest drying treatment, as it achieved the final moisture earlier than other treatments. However, during the first three days, treatment 2 dried at a higher rate than treatment 1, as treatment 2 was exposed to the direct sunshine and the first drying days were clear and sunny. The drying environment (outdoors versus plastic hut) seems to have a different effect on the drying rate depending on the weather conditions. During clear, sunny days, the plastic cover of the plastic hut shields the coffee from the direct solar radiation and, even though the ambient temperature inside the

plastic hut rises over the outdoors temperature, the coffee inside the hut remains at a lower temperature than the coffee treatments located outdoors. On the other hand, during cloudy days, the treatments inside the drying hut reach a higher temperature than the treatments located outdoors. For future work, if the highest drying rate is required, the coffee should be moved between a drying hut and the outdoors patio, depending on the weather conditions.

Treatment 2 was an analogue of treatment 21 in the microbiological experiment (same protocol). Bacteria growth on treatment 21 increased by less than a factor of 10 (Figure 6.6). This implies the treatment effectively to limits bacterial growth due to the a_W reaching 0.90-0.92 after the first two days. In treatment 21, yeast growth only increased by a factor of 10 (from a count of $3.2 \cdot 10^3$ cfu per cherry to a count of $4.8 \cdot 10^4$ cfu per cherry after four days – Figure 6.7). When compared to the yeast growth in treatment 23, this implies a fast-drying treatment, such as treatment 21, can also effectively limit yeast growth probably due to the relatively fast decrease in a_W to 0.77-0.86 after the first four days. The growth of moulds, however (Figure 6.8), does not seem to be as limited by fast drying (treatment 21) as the growth of bacteria and yeasts, when compared to the other two treatments (treatments 22 and 23).

Treatment 3 is the termed ‘honeying’ process. A fermentation period with the a_W above 0.91 until the aspect of the cherry surface seemed ‘honeyed’ was targeted. This was achieved over two days (days 2 and 3), during which a_W remained above 0.95 (Figure 6.3). From day 4 onwards, the drying rate was increased and a_W decreased at a similar rate to the one of the faster-drying treatments (such as treatments 1 and 2). This implies the targeted drying curve for treatment 3 was achieved.

Treatment 23 in the microbiological experiment was an analogue of treatment 3 (same protocol). As discussed in section 6.3.2, the growth of bacteria and yeasts in the ‘honeying’ process is clearly at least two orders of magnitude higher than in the other treatments. This, again, was the objective of treatment 3.

In treatment 4, the growth of yeasts in the region of a_W between 0.85 and 0.91 was targeted, while the drying rate outside of this region was meant to be as high as possible. This was attempted by using a thicker layer of cherries and a slower turning rate during day 4, when a_W was in the region of interest. This caused a_W to reach 0.927 by the end of day 4 (Figure 6.3),

slightly higher than the target. After the results were analysed, it seems a second day of slow-drying conditions would have been needed for an adequate differentiation of treatment 4 from other treatments. Treatment 4 did not have an analogue treatment in the microbiological experiment and therefore the rate of microbial growth cannot be analysed.

In treatment 5, the growth of moulds in the region of a_W below 0.85 was targeted, while the drying rate above this region was meant to be as high as possible. This was attempted by keeping the coffee in a plastic bag on days 5 through 10, until a mould outbreak was evident. Figure 6.3 shows bagging was effective to suspend drying, even though the a_W during days 5 through 10 was actually above the desired range. Mould growth did happen, as shown by the mould outbreak observed on the surface of the coffee cherries; however, water activity during those days was high enough to allow yeast growth as well. Bagging the coffee cherries at a later stage along drying, instead of during days 5 through 10, would probably have ensured water activity was kept below 0.85 during bagging. Treatment 5 did not have an analogue treatment in the microbiological experiment and therefore the rate of microbial growth cannot be analysed.

In treatment 6, it target was to favour the growth of bacteria and yeasts by slow drying in the region of a_W above 0.85, followed by a fast drying below that point. This was attempted by using a thick layer of cherries and a reduced turning frequency from the beginning, followed by bagging the cherries on day 6. These measures were effective at keeping a_W above 0.85 until day 8. From day 9 onwards, fast drying conditions were employed, in order to bring the coffee to the desired level of moisture. Treatment 6 did not have an analogue treatment in the microbiological experiment and therefore the rate of microbial growth cannot be analysed.

In treatment 7, the growth of bacteria in the region of a_W above 0.91, followed by a medium drying rate period below that point, was targeted. This was attempted by using a thick layer of cherries and a low turning frequency, outdoors on a raised drying table, during the first three days. From the fourth day onwards, it was dried indoors, also using a thick layer of cherries and a low turning frequency. The objective was partially achieved, as a_W was kept above 0.91 only for the first three days. However, after analysing the overall results, treatment 7 was still differentiated from other treatments, as it followed a medium drying rate along the whole drying period.

The analogue to treatment 7 in the microbiological experiment (similar medium drying-rate protocol) was treatment 22. Treatment 22 shows a higher growth of bacteria and yeasts than the high drying rate treatment (treatment 21). Therefore, decreasing the drying rate by using a thick coffee cherry layer and a low turning frequency can differentiate a treatment by promoting bacterial and yeast growth.

Overall, most treatments were aligned with the target drying regimes. The main exceptions were treatments 4, 5 and 7, which were not aligned with the targeted regimes. However, these three treatments can still be differentiated from each other and from the other treatments. For example, treatment 5 clearly has the highest water activity of all treatments during the second week of drying, while treatment 7 clearly shows a medium drying rate along the whole drying period, when compared to the high drying rate and the low drying rate treatments. The drying control parameters proved to be sufficient for achieving differentiated treatments, even though the control was far from being ideal. Ideally, a dryer that could have enabled controlled drying conditions would have allowed the treatments to more strictly match the target regimes. In these sets of experiments this was not practical but to clarify some aspects of volatile organic compound generation future research should include an experiment using fully controlled drying conditions.

6.3.4 Descriptive Cupping of the sample set by two different panels

6.3.4.1 University of Otago (New Zealand) panel

Flavour profiles for the 7 treatments and the washed witness (15 samples) were generated from the freely-elicited terms using the sequential protocol for analysing Descriptive Cupping described in Chapter 3, in which the first step is the preparation of a contingency table (Appendix 6).

An initial table of 8 treatments (rows) and 31 descriptor subgroups (columns) was prepared by aggregating the mentions of descriptors by treatment and subgroup. The subgroups with most mentions were *caramelly* (88), *chocolaty* (86), *citrus-like* (62), *fruity* (59) and *red-fruity* (58). These five subgroups summarise the general characteristics of the sample set.

The dependence between subgroups and treatments was not significant (p=0.823), as detected by the Monte Carlo method applied on the frequency table (aggregated treatments, 5000

simulations). The observed value of χ^2 was 192.1, while the critical value of χ^2 was 243.5 (df=7, p = 0.05). This implies no link between the samples and the subgroups can be found with the current contingency table ($p<0.05$), and it also means a lower variability level, compared to the studies in Chapters 3 and 4. Therefore, the least discriminant subgroups ($\chi^2<7.0$, computed by dividing the critical value by the number of subgroups) were removed from the table. The five least discriminant subgroups were *smooth body* ($\chi^2=0.5$), *dried-fruity* ($\chi^2=2.0$), *spicy* ($\chi^2=2.0$), *medium acid* ($\chi^2=2.2$) and *fruity* ($\chi^2=2.7$). Other non-significant subgroups ($\chi^2<7.0$ – *astringent, chocolaty, citrus-like, earthy, fungal, nutty, other, phenolic, red-fruity, resinous, rough-body, sweet-acid, toasty, vegetable, woody*) were also removed and a second contingency table was prepared.

The second contingency table was produced with the 8 treatments as rows and 11 subgroups ($\chi^2>7.0$) as columns. The subgroups kept for this second table were *salty taste* ($\chi^2=13.6$), *caramelly* ($\chi^2=10.5$), *plain bouquet* ($\chi^2=10.5$), *tropical-fruity* ($\chi^2=10.3$), *stone-fruity* ($\chi^2=10.2$), *dry-acid* ($\chi^2=8.7$) *pyrolytic* ($\chi^2=8.5$), *bitter* ($\chi^2=8.3$), *floral* ($\chi^2=8.1$), *sweet taste* ($\chi^2=7.8$) and *fermented* ($\chi^2=7.8$). A second Monte Carlo test was done on this table (aggregated treatments, 5000 simulations). The observed value of $\chi^2=98.8$ implies a value of p=0.01, with a critical value of $\chi^2=89.8$ (df=7, p=0.05).

The χ^2 per-cell test (see Chapter 3) showed three treatments had a significantly higher frequency than expected for one character. *Caramelly* was the significant character for the washed witness; *salty taste*, for treatment 3 (the 'honeying' treatment), and *bitter*, for treatment 6. However, the χ^2 per-cell test can also tell which frequencies are significantly lower than expected. From that point of view, the washed witness had a significantly lower number of mentions than any natural treatment for *bitter*, *dry-acid*, *fermented*, *sweet taste* and *tropical-fruity*. This confirms the traditional views about the character of natural coffees when compared to the washed: more fruitiness, wineyness and sweetness than the washed, but a rougher acidity at the same time. The washed coffees, on the other hand, are traditionally perceived as more caramelly and floral, although the latter was not the case in this study. Then again, the notion that salty taste is associated with specific fruity naturals has also been held by the industry (Davids, 2010). In this case, saltiness was not associated with fruitiness in general but specifically with the 'honeying' treatment.

Visualisation of the flavour profile obtained by NSCA on the frequency table with the aforementioned 11 significant subgroups (non-aggregated treatments – 15 samples) shows a total inertia of 0.041 in 10 factors. A 77.7% of the total variation was explained in the first 3 factors. Factor 1 (F1) and factor 2 (F2) had inertia of 39.2% and 20.4%, respectively.

Figure 6.9 shows the projection on F1 and F2 of the 11 subgroups considered. The subgroups with the largest contribution on the positive side of F1 are *caramelly* (31.1%) and *pyrolytic* (15.1%). The subgroups with the largest contribution to the negative side of F1 are *floral* (20.8%), *tropical fruity* (18.6%) and *fermented* (10.8%). The subgroups with the largest contribution to F2 are *pyrolytic* (32.4%) and *bitter* (20.1%), both on the negative side.

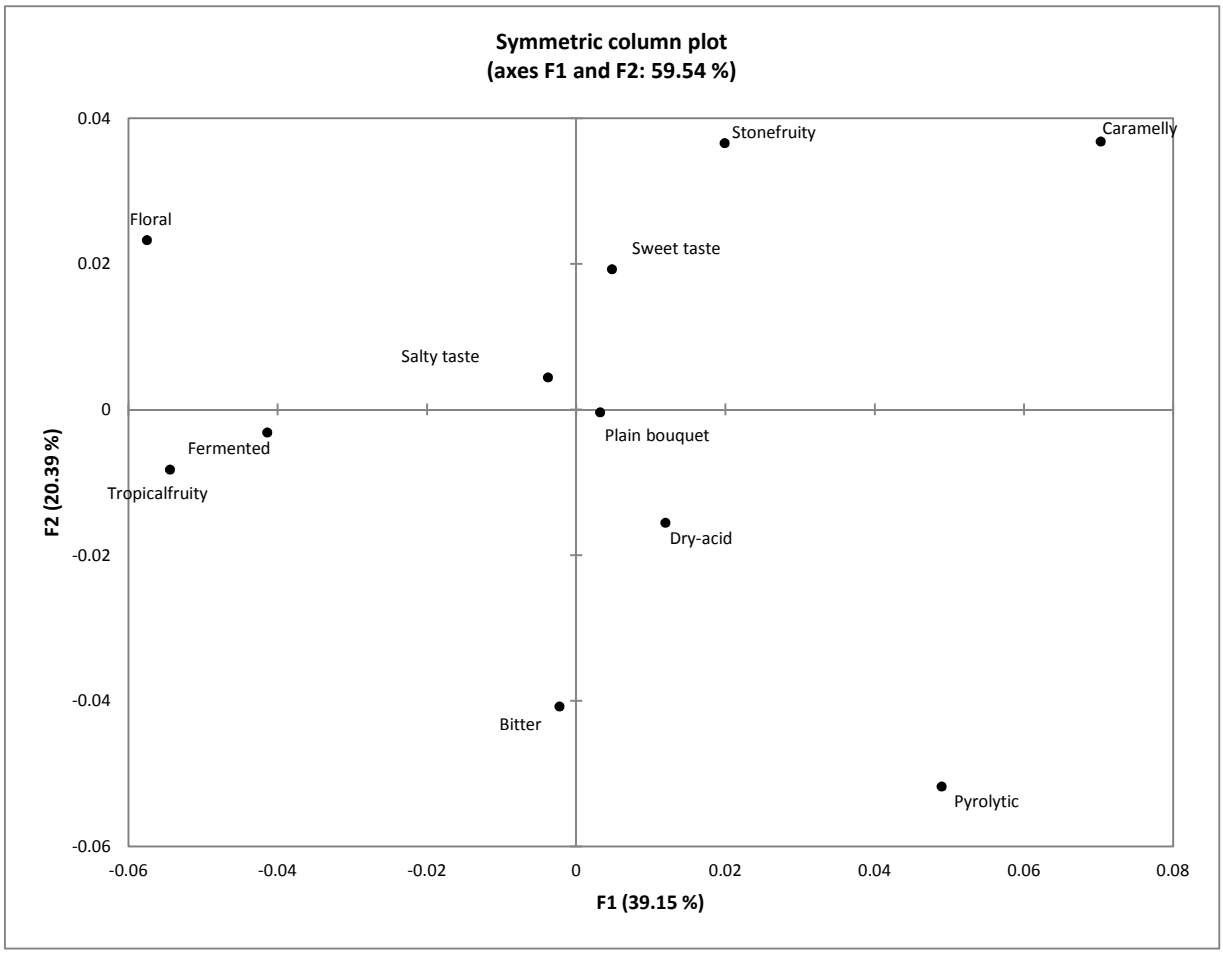


Figure 6.9. Non-symmetric correspondence analysis (NSCA) map representing the projection on F1 and F2 of 11 significant descriptor subgroups (χ^2>7) for 15 coffee samples of seven different natural coffee treatments and a washed witness, evaluated using the Descriptive Cupping method by a panel at the University of Otago, New Zealand.

The placement of the samples on F1 and F2 of the NSCA (Figure 6.10) shows most treatment duplicates have been located near each other (from left to right, 1B and 1A, 5A and 5B, 3B and 3A, 4A and 4B, 6B and 6A, 2B and 2A). The only treatment in which both duplicates are not closely located is treatment 7, (see 7.3.2 for the discussion of why treatment 7 might be different). This placement of duplicates close to each other suggests the cuppers' descriptors are not random: the washed witness (W) is clearly separate to the natural samples (showing a *caramelly* character) and treatment 2 (one of the fastest drying ones) is close to the washed. However, the placement of treatment 3 in this case does not reflect its nature as the most distinct treatment. Possibly, subgroups like *tropical-fruity* and *floral* attracted the attention of cuppers.

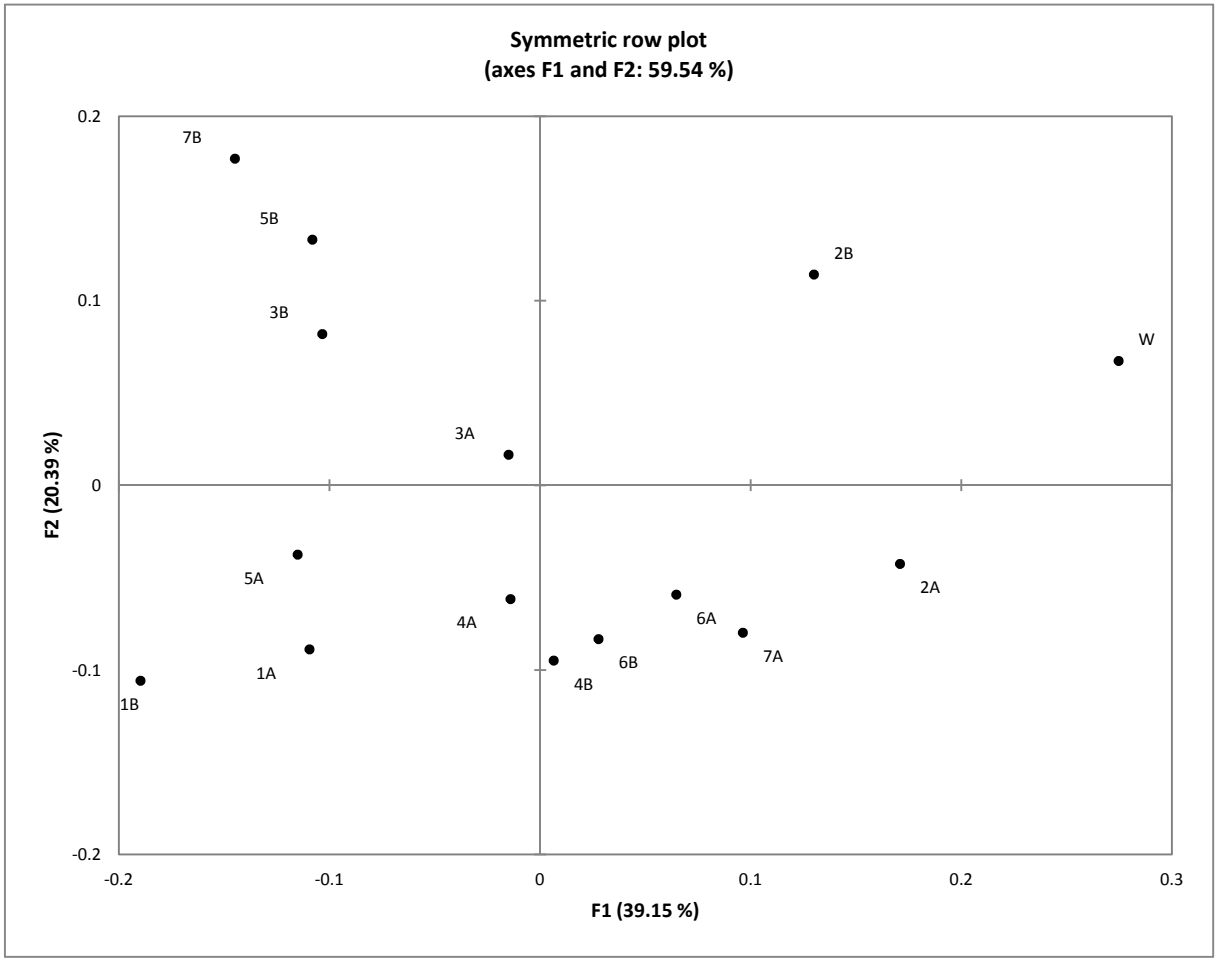


Figure 6.10. Non-symmetric correspondence analysis (NSCA) map representing the projection on F1 and F2 of 7 natural coffee treatments (1-7, with duplicates A and B) and washed coffee (W, assessed in duplicate), evaluated using the Descriptive Cupping method by a panel at the University of Otago, New Zealand.

6.3.4.2 Agro-ecological Centre for Coffee (CAFECOL, Mexico) panel

The samples (15) were also cupped by a panel assembled by CAFECOL in Mexico, in order to obtain data from an independent panel with a different background. Since the CAFECOL panel was formed by Q Graders with experience in cupping washed coffees, the descriptors used belonged to the bouquet descriptor tree used in the Q Grader training (Lenoir & Guermonprez, 1997), consisting of four aroma groups with three subgroups each: enzymatic (*floral*, *fruity*, *vegetable*), sugar-browning (*nutty*, *caramelly*, *chocolaty*), dry-distillation (*spicy*, *resinous*, *pyrolytic*) and aromatic taints (*earthy*, *fermented* and *phenolic*) – see Chapter 3. The contingency table (Appendix 6) was built with 8 treatments as rows (seven natural coffee treatments plus a washed witness) and the 12 subgroups as columns. The column for *phenolic* was removed because it was not mentioned. The subgroups with most mentions were *fruity*, *chocolaty* and *caramelly*, implying these are the most common characters found in the sample set. This is consistent with the assessment by the New Zealand panel (6.3.4.1).

The Monte Carlo test on the contingency table (aggregated treatments, 5000 simulations) resulted on an observed value of χ^2=266.8, much higher than the critical value of χ^2=91.0 (df=7, p=0.05), which implies there is a link between samples and subgroups with a confidence of p<0.001. The subgroups with the lowest values of χ^2 were *floral* (χ^2=6.3) and *resinous* (χ^2=9.4), implying these subgroups are less likely to discriminate among samples. The subgroups with the highest values of χ^2 were *vegetable* (χ^2=61.4), *nutty* (χ^2=46.5) and *fermented* (χ^2=33.2), meaning these subgroups were the most discriminant in the contingency table.

The χ^2 per-cell showed some treatments had a significantly higher number of mentions than expected for one or more subgroups. Treatment 3 (the 'honeying' treatment) had a significantly higher frequency of mentions for *pyrolytic*, *earthy* and *fermented*; treatment 4 was significantly more *fruity*, *caramelly* and *spicy*; treatment 7 was significantly more *caramelly* and *chocolaty*, and the washed witness was significantly more *vegetable* and *nutty*, but it also was significantly less *fruity* and *chocolaty* than any other treatment (in fact, it did not have any mention for *fruity*).

Visualisation of the flavour profile obtained by NSCA on the frequency table with the aforementioned 11 bouquet subgroups (non-aggregated treatments – 15 samples) showed a

total inertia of 0.043 in 10 factors (almost the same as the New Zealand panel using the significant subgroups). 83.5% of the total variation was explained in the first 3 factors, about 6% more than the New Zealand panel. Factors 1 and 2 (F1 and F2) had inertia of 45.9% and 25.5%, respectively.

Figure 6.11 shows the projection on F1 and F2 of the 11 subgroups. The subgroup most contributing on the positive side of F1 was *fermented* (33.5%). The subgroups contributing the most on the negative side of F1 were *caramelly* (25.5%) and *chocolaty* (14.6%). The most contributing subgroups to F2 were *vegetable* (18.1%) on the positive side, and *fruity* (50.2%) and *fermented* (15.9%) on the negative side.

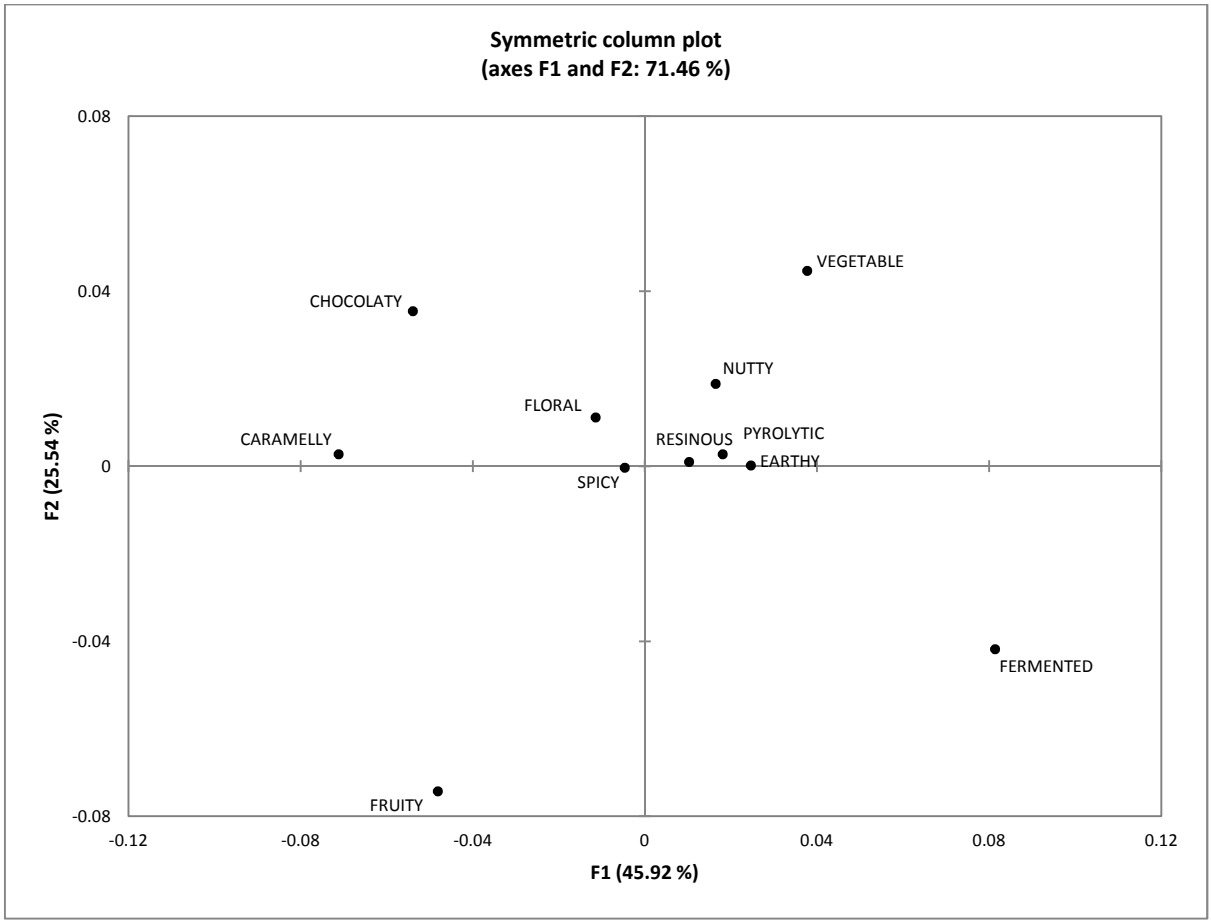


Figure 6.11. Non-symmetric correspondence analysis (NSCA) map representing the projection on F1 and F2 of 11 descriptor subgroups for 15 coffee samples of seven different natural coffee treatments and a washed witness, evaluated using the Descriptive Cupping method by a panel at the Agro-ecological Centre for Coffee, Mexico.

The placement of the samples on F1 and F2 of the NSCA (Figure 6.12) again shows the treatment duplicates close to each other, especially along F1, except for treatment 6, with both duplicates far from each other. This placement suggests a coherent description by the cuppers. The washed witness (W) is located near the positive end, as with the New Zealand panel, but in this case, the general placement of the natural samples along F1 is more in line with what would be expected from the different intensities of fermentations: the 'honeying' treatment near one end, the bulk of the natural treatments on the other end and treatment 5 in between the two.

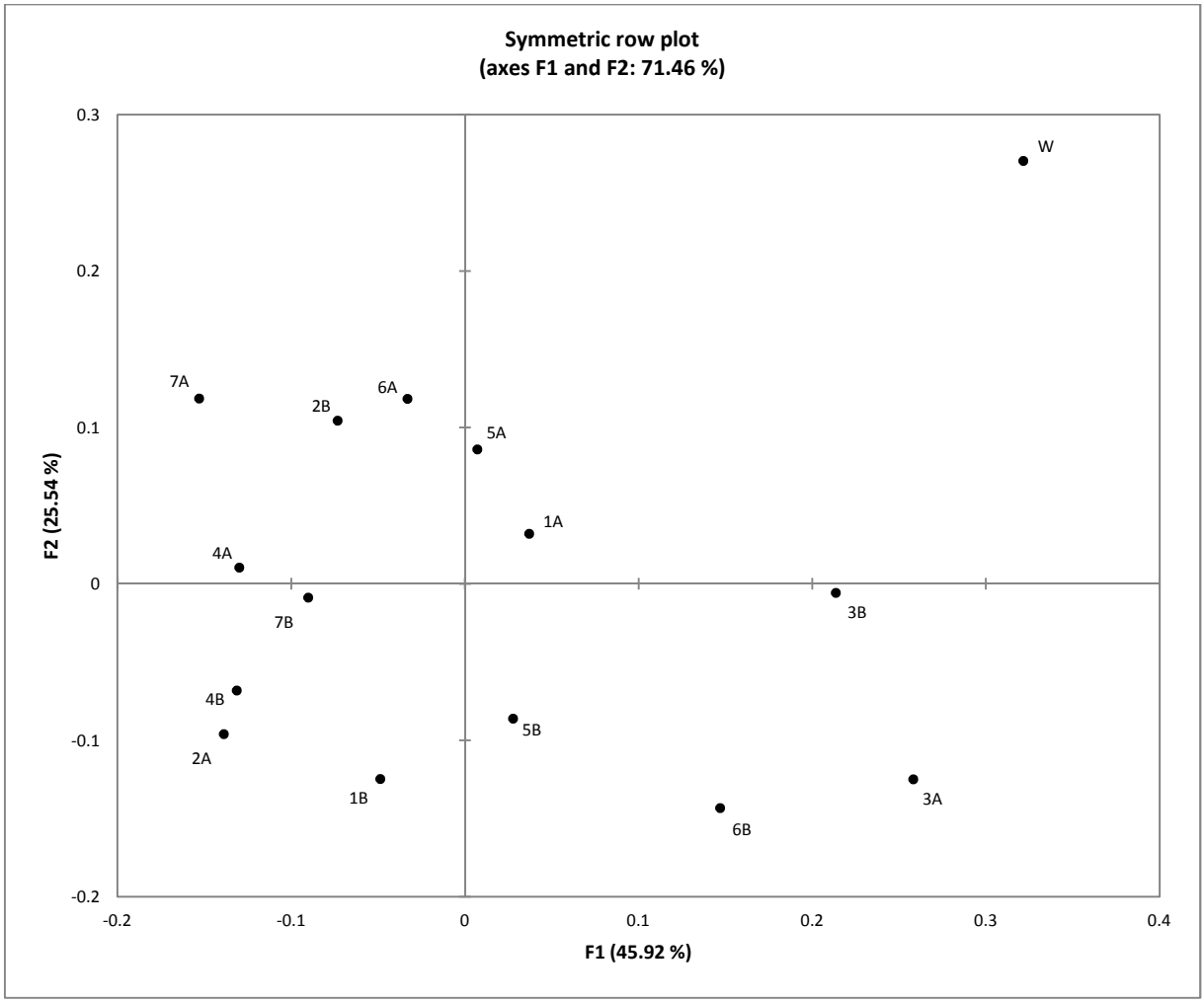


Figure 6.12. Non-symmetric correspondence analysis (NSCA) map representing the projection on F1 and F2 of 7 natural coffee treatments (1-7, with duplicates A and B) and washed coffee (W, assessed in duplicate), evaluated using the Descriptive Cupping method by a panel at the Agro-ecological Centre for Coffee, Mexico.

6.3.5 Comparison of both panels using MFA

The assessment of coffee samples by traditional descriptive panels from different countries has been compared using generalized Procrustes analysis by de Jong, Heidema & van der Knaap (1998), who found a high level of consensus between the panels. However, MFA has been preferred to compare data form different panels in studies where the data was obtained from freely-elicited descriptors, as the case of Lawrence et al. (2013) for wine.

In this study, the Descriptive Cupping results of both the New Zealand and the Mexico panel were compared using MFA. The 15 samples (7 natural treatments with 2 replicates each, plus

a washed witness) were used as observation rows. The contingency tables of both panels (Appendix 6) were used as active tables. The subgroups used for the New Zealand panel table were the 11 significant subgroups (χ^2>7, 6.3.4.1), while the subgroups used for the Mexico panel table were the 11 descriptor subgroups (6.3.4.2).

The correlation coefficient (RV) between both tables was 0.466, which is a similar level to the correlation for sensory data found in Chapter 4. Figure 6.13 shows the projection on F1 and F2 of the subgroups used by both panels. The combination of F1 and F2 explains a variability of 41.2%, which is lower than 50% and implies over half of the information obtained by both panels is still being left out of this graph, as only the most important sources of variation are being represented by F1 and F2. However, there is agreement in the use of some terms by both panels – for example, the vectors for *fermented* from both panels are in the same quadrant; *fruity* (from the Mexico panel, which lacks specific subgroups for fruitiness) is on the negative end of F2, like *tropical-fruity* from the New Zealand panel, and this is the same case for the term *floral* of both panels. However, a different use of descriptors by both panels is observed in several cases: the term *caramelly* used by the New Zealand group seems very different to the same term used by the Mexico panel (in the opposite quadrant) and probably more in line with the term *nutty* as used by the Mexico panel. These differences can be explained both by the cultural differences and the differences in training between both panels. The Mexico panel had more cupping experience in general, but less experience cupping natural coffees and no training in descriptors specific for natural coffees. In contrast, the New Zealand panel had a shorter training period, but was trained in specific descriptors for naturals. The specific descriptors for naturals include different subgroups of fruity flavour notes, as shown in the use of the terms *stone-fruity* versus *tropical-fruity*. The Mexico panel has only one category for fruitiness: *fruity*. Q Graders all over the world are mostly receiving training in descriptors for washed coffee (using Le Nez du Café® references), but are lacking the specific training in descriptors covering the fruity and winey characters of natural coffees. In the future, training of Q Graders should include descriptors and references for the characters specific to natural coffees, and ideally the set of Le Nez du Café® references should be upgraded to include these aroma notes. This would increase the level of agreement in the description of natural coffees and would prevent cuppers from assigning low quality scores to natural coffees unfairly due to an incomplete descriptive training.

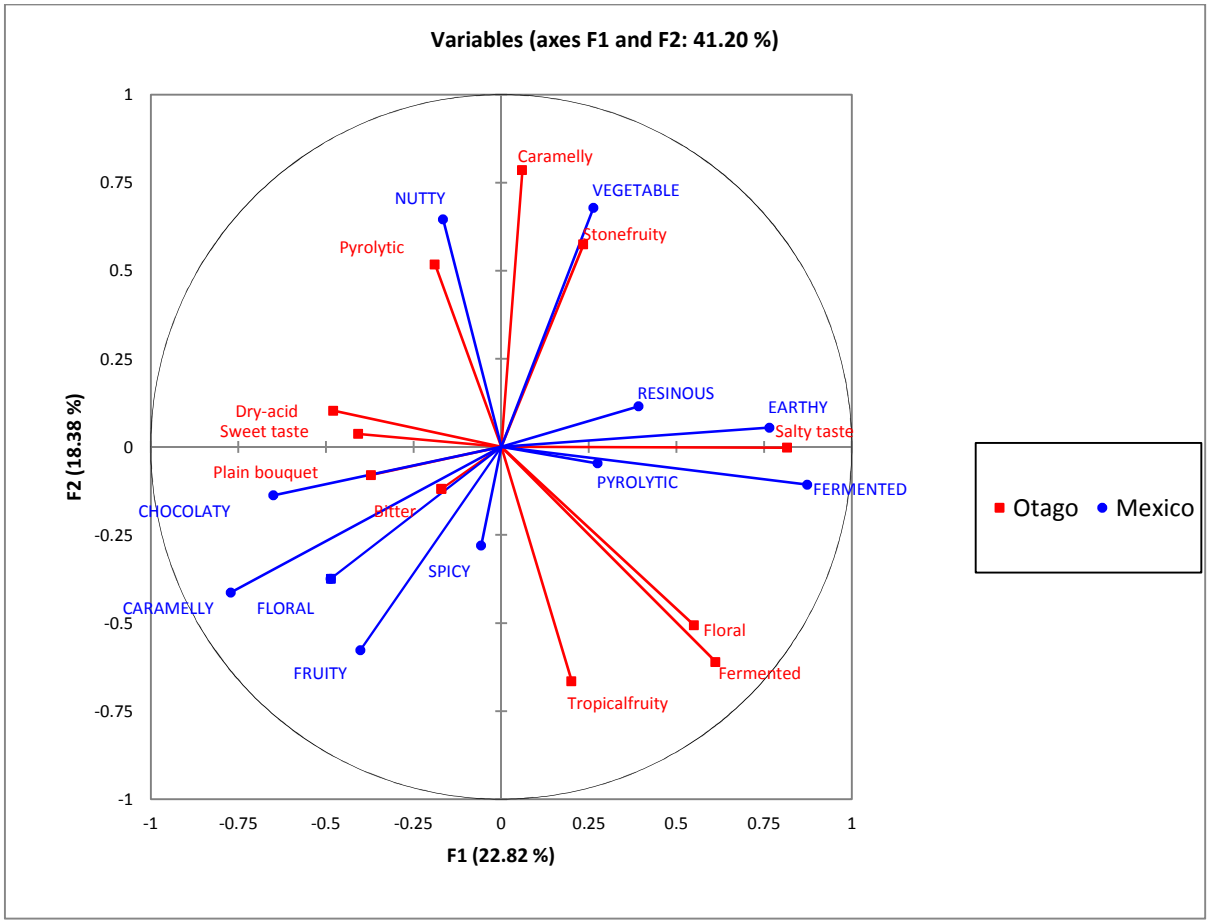


Figure 6.13. Multiple factor analysis (MFA) map representing the projection on F1 and F2 of significant descriptor subgroups elicited by the Otago, New Zealand panel (square) and bouquet subgroups used by the Mexico panel (circle, all-caps labels) from the Descriptive Cupping of 15 coffee samples of seven different natural coffee treatments and a washed witness.

Figure 6.14 shows the projection of the samples on F1 and F2 of the MFA. The washed witness (W) and the 'honeying' treatment are distinct for both panels. Both panels agree the 'honeying' treatment has a *fermented* character. Treatments on the negative side of F2 (treatments 4, 5 and 1, from left to right) are *fruity* for the Mexico panel or *tropical-fruity* for the New Zealand panel. Treatments 2 and perhaps 6 and 7 seem to be less fruity, although the placement of the duplicates for treatments 6 and 7 is not as clear. Overall, the MFA maps the treatments along F1 in a coherent order with the results from the field as the order of samples along F1 follows the same trends observed for microbial growth, pH and drying rates. It is also clear from Figure 6.14 that the washed and the 'honeying' treatments are the most distinct treatments.

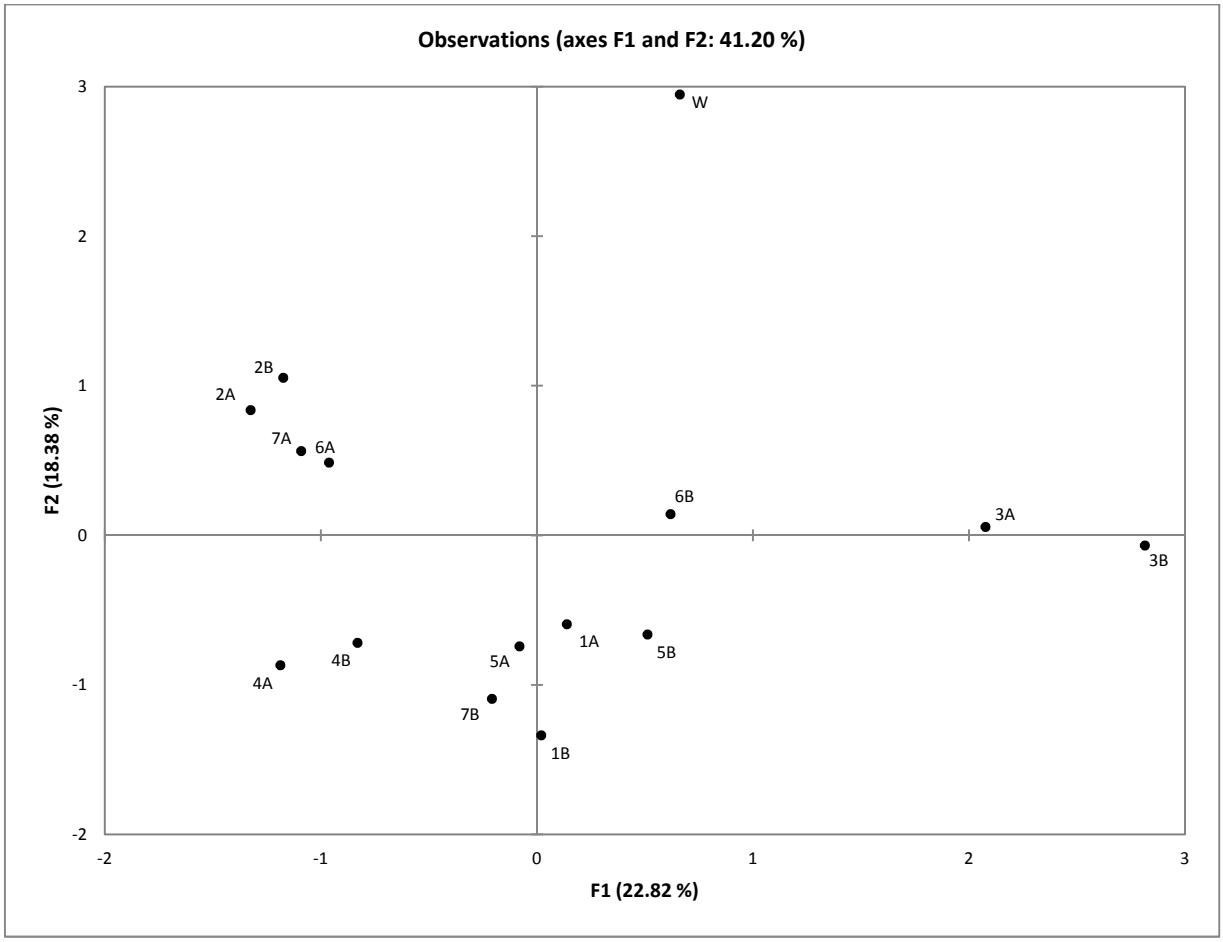


Figure 6.14. Multiple factor analysis (MFA) map representing the projection on F1 and F2 of 7 natural coffee treatments (1-7, with duplicates A and B) and washed coffee (W – averaged), evaluated using the Descriptive Cupping method in a panel from New Zealand and a panel from Mexico.

6.4 Conclusion

In this study, the drying rates during the drying period were controlled using low-technology methods available to any producer, such as the drying material and the depth of the coffee cherry layer, to produce 7 treatments with different drying protocols. The 7 drying protocols were selected with the objective of altering the type of microorganism growing and the rate of growth. A washed coffee witness was also prepared. The resulting samples were characterised using Descriptive Cupping by two independent panels – one in New Zealand and the other in Mexico. The results from both panels were compared to examine similarity of discrimination, similarity of descriptors use and cultural differences, to enable conclusions to be drawn on robustness and validity of Descriptive Cupping.

Asides from the washed witness, the most distinct treatment was the one termed 'honeying' treatment (treatment 3), which involved suspending the drying for the first two days. The 'honeying' treatment showed higher bacteria, yeasts and moulds populations during the process, which indicated a higher microbial activity. A distinct *fermented* character was found in the 'honeying treatment' by Descriptive Cupping, yet it was significantly saltier than other treatments. Further study of the 'honeying' process is needed in order to optimise the fruitiness without compromising other desirable characters.

Other natural treatments showed lower microbial populations and a varying degree of fruity and fermented sensory characters, depending on their drying rate. Fruitiness, for example, is not as pronounced in treatments with the highest drying rate (such as treatment 2). Chapter 7 will further explore these differences from the instrumental analysis point of view.

The panel comparison showed a satisfactory correlation between both panels and coherence in the use of descriptors such as *fermented*. However, a difference in the breadth and depth of the descriptors used was found. This difference may be due to cultural differences and also the differences in training and experience levels. Overall, Descriptive Cupping has been shown to be a rapid method for profiling coffee flavour with satisfactory levels of discriminating power, even in sample sets coming from the same raw material.

7 Volatile organic compounds as related to the sensory profile of natural coffee processed by varying drying rate treatments

7.1 Introduction

Chapter 6 presented the effect of different drying protocols on the fermentation of the coffee cherries and the flavour profile of natural coffees, assessed by Descriptive Cupping. Different drying treatments (7 in duplicate) were applied in the production of natural coffee in Mexico using the same raw material and a washed coffee witness was prepared. The samples were brought to the University of Otago and characterised. Chapter 6 focuses on the field experiment and the sensory characterisation of the samples through Descriptive Cupping. The present chapter focuses on the instrumental analysis of the samples' headspace and the multiple factor interpretation of both sensory and instrumental results.

Therefore the aim of this study was to investigate the effect of different drying protocols on the production of volatiles in the green bean and the roasted bean, and to link those volatiles to the sensory properties of the final coffee (*Objective 5*, 2.2.2.5), with focus on the Mocha character. Samples from the seven different treatments and the washed witness described in Chapter 6 were characterised using the same instrumental methods presented in Chapter 5, including analysis of the green bean headspace by PTR-MS and analysis of the roasted bean headspace using GC-MS/O. A multiple factor analysis comparing the instrumental results from the present chapter with the Descriptive Cupping results from Chapter 6 was also completed. These methods have been extensively described in the previous chapters and will only be briefly reviewed here.

7.2 Materials and methods

7.2.1 Samples

The samples of green and roasted coffee used in this study were the same samples used for Chapter 6 (Table 6.1). A subsample of the samples roasted at the University of Otago (6.2.4.1) was used for the instrumental analyses of roasted coffee headspace in this chapter.

7.2.2 Characterisation of green beans

Colour of the green beans was measured in triplicate in the 'CIE L* a* b*' colour space (daylight colour, D65 illuminant, 10° field of view), by filling a crucible with the beans and reading the colour using a Miniscan XE Plus colorimeter (HunterLab Inc., Reston, VA).

'Foxy' beans, which are defined as beans with a red silverskin (spermoderm) (SCAA, 2009b), were counted in a 350g subsample and reported as a percentage.

7.2.3 Headspace analysis of green coffee by PTR-MS

The composition of the volatile organic compounds (VOC) in the headspace of the green coffee beans was determined by high sensitivity proton-transfer-reaction mass spectrometry (PTR-MS), using the same methodology and equipment stated in 5.2.2.

7.2.4 Headspace analysis of roasted and ground coffee using headspace-solid-phase extraction (HS-SPE) and semi-quantification of odour-active compounds in HS-SPE concentrates through GC-MS

For the roasted coffees, the headspace of the roasted and ground beans was captured by a purge-and-trap methodology, using headspace-solid-phase extraction cartridges (HS-SPE), eluted with a solvent and concentrated, using the same methodology and equipment reported in 5.2.3.

The headspaces of the 15 samples (7 treatments in duplicate plus washed witness) were measured in duplicate and semi-quantified using GC-MS, following the same methodology and equipment reported in 5.2.5.

7.2.5 Semi-quantification of complementary compounds using static headspace gas chromatography-mass spectrometry (SH-GC–MS)

To enable the semi-quantification of those VOC with a retention index smaller than the solvent delay time, VOC composition of roasted and ground coffee headspace was analysed using static headspace gas chromatography-mass spectrometry (SH-GC–MS). The methodology and equipment used are reported in 5.2.6.

7.2.6 Data analysis

7.2.6.1 Univariate and multivariate analysis of green bean and roasted bean headspace data

The instrumental data analysis was first carried out as reported in 5.2.8. This implied separate analysis of the PTR-MS data from green coffee (see 5.2.8.1) and the GC-MS data from

roasted coffee (5.2.8.3). The odour active compounds semi-quantified in this study were the same found for the study in Chapter 5, and are shown in Table 5.4.

7.2.6.2 Analysis of specific fermentation-related compounds

A specific analysis of the fermentation-related compounds was then carried out, in order to investigate the role of fermentation on coffee flavour. The ratio of ethanol (m/z 47) to methanol (m/z 33) from the PTR-MS analysis of the green bean headspace was investigated (Gibson, 1974) and their profiles (after normalisation, (X-m)/s, where m is the mean and s is the standard deviation) were compared to the normalised peak area of a number of selected, known, fermentation-related compounds, using the Pearson (n-1) correlation coefficient. The compounds selected were products of valine degradation (2-methyl-propanal, 2-methylpropanoic acid), products of isoleucine degradation (2-methyl-butanal, 2-methylbutanoic acid), products of leucine degradation (3-methyl-butanal, 3-methylbutan-1-ol, 3-methylbutanoic acid), methyl esters (methyl formate, methyl acetate) and ethyl esters (ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl acetate) and compounds related to the oxidation of ethanol (acetaldehyde, acetic acid and the ethanol contents in roasted coffee headspace) (Roze et al., 2010; Thonning Olesen & Stahnke, 2004).

7.2.6.3 Multiple factor analyses, integrating field variables, instrumental data and cupping data

In order to understand the links between the different processing treatments, the profiles of odour active compounds and the coffee flavour, the different datasets were studied using multiple factor analysis (MFA) (Abdi et al., 2013; Lawrence et al., 2013). To be consistent with Chapter 5, the Otago panel Descriptive Cupping data were used for the MFA. Two sets of variables were kept as active tables in the MFA: the subgroup contingency table (in Appendix 6, Otago panel, 15 samples and subgroups with χ^2 above the critical value for p=0.05, as per 6.3.4.1) and the semi-quantification of the significant ($p<0.15$) odour-active compounds (7.2.6.1). These two data tables were included as active tables because they pertain to the same samples (the roasted and ground coffees). Several variable tables were considered supplementary data because they were not directly related to the roasted coffee flavour. Supplementary tables were the PTR-MS data of the significant m/z, the number of drying days, water activity (on the 2nd and on the 9th day of drying), pH (on the 6th and on the

10^{th} day of drying), the sample colour in green bean (L*, a*, b*) and the percentage of 'foxy' beans (see 7.2.2). The degree of correlation between the tables was investigated through the calculation of the regression vector (RV) coefficient.

7.3 Results and discussion

7.3.1 PTR-MS data from green coffee headspace

In this study, an ANOVA of the PTR-MS data identified 84 significant ($p<0.05$) m/z signals. The significant m/z were depurated using the sequential protocol in 5.2.8.1 (elimination of instrument-related m/z, elimination of mass ions with an average below 1 count per second and elimination of obvious isotopes, clusters with a water molecule and fragments of alcohols minus a water molecule). After the dataset depuration, 30 m/z were found relevant for use in the PCA.

Table 7.1 summarises the 30 m/z found after depuration and their tentative identification (20 m/z), based on the literature, where available. Table 7.1 also shows the number of sample groups found by the Tukey post-hoc test. Some (4) of the m/z are capable of separating samples in seven groups or more. Key examples for these highly discriminant, relevant m/z are 47 (ethanol), 57 (alcohol fragment), 71 (3-methylbutanol acetate) and 101 (Hexanal, 2,3-pentanedione, 3-methyl butenoic acid).

Table 7.1. Relevant m/z in green coffee headspace of coffee samples (7 natural process treatments in duplicate and 1 washed witness), analysed using PTR-MS – tentative identification and number of Tukey groups.

m/z ratio	Tentative identification	Number of groups of samples using Tukey post-hoc analysis
31	Formaldehyde fragment [d]	5
33	Methanol [a,b]	6
41	Alcohol/ester related fragment [d]	6
42	Acetonitrile [d]	5
45	Acetaldehyde [a]	4
47	Ethanol [a]	7
53	Unidentified	6

m/z ratio	Tentative identification	Number of groups of samples using Tukey post-hoc analysis
55	Butanal [c]	5
56	Unidentified	3
57	Alcohol fragment [b]	7
59	Acetone, propanal [a]	2
61	Propanol [a], acetic acid, methyl formate [b], acetate fragment [d]	6
63	Unidentified	2
67	Unidentified	3
69	Isoprene [a], octanal fragment, 1-octen-3-ol fragment [d]	5
71	Fragment of 3-methylbutanol acetate [e]	9
73	Isobutanal, Butanal, Butanone [a]	6
79	Unidentified	2
81	Unidentified	5
83	Unidentified	2
85	Methyl butenal [b], alcohol (1-hexenol, nonanol), ester fragment [d]	6
87	3-Methyl-2-buten-1-ol, 2*E*-butenoic acid, 3-methyl butanal, 2,3-butanedione [a]	6
91	Unidentified	5
93	Unidentified	4
97	2*E*,4*E*-Hexadienal [a]	4
101	Hexanal, 2,3-pentanedione, 3-methyl butenoic acid [a]	7
103	3-Methyl butanoic acid, pentanoic acid, 1-hexanol [a]	6
105	(Phenyl-ethyl alcohol+H^+) – H_2O [a]	3
107	Unidentified	2
117	2-heptanol, hexanoic acid [a]	4

(a) Identification reported by Yeretzian et al. (2002).

(b) Identification reported by Romano et al. (2014).

(c) Identification reported by Soukoulis et al. (2013).

(d) Personal communication – P. Silcock.

(e) Identification reported by Ezra et al. (2004).

In the PCA of the depurated m/z, a total of 88.5% of the variability was explained by the first two factors F1 (71.9%) and F2 (16.7%) (Figure 7.1). Of the 30 m/z, 26 were best represented by F1, as they had the largest square cosine for F1 compared to the other factors. F1 is best described by 3-methylbutanoic acid and alcohols, namely ethanol, as the main contributing

m/z to F1 were m/z 103 (3-methylbutanoic acid, pentanoic acid, 1-hexanol – 4.57%), m/z 41 (alcohol/ester related fragment – 4.42%), m/z 57 (alcohol fragment – 4.33%) and m/z 47 (ethanol – 4.30%).

F2 is best described by methanol, with the main contributing m/z being m/z 31 (formaldehyde fragment – 18.06%) and m/z 33 (methanol – 16.59%), followed by m/z 56 and m/z 83 (13.03% and 11.96%, respectively). The same two main contributing ions in F2 were found in the international appraisal sample set reported in Chapter 5. F1 and F2 suggest two different kinds of fermentation are taking place: ethanol fermentation along F1, probably combined with a degradation of amino acids, and methanol fermentation along F2.

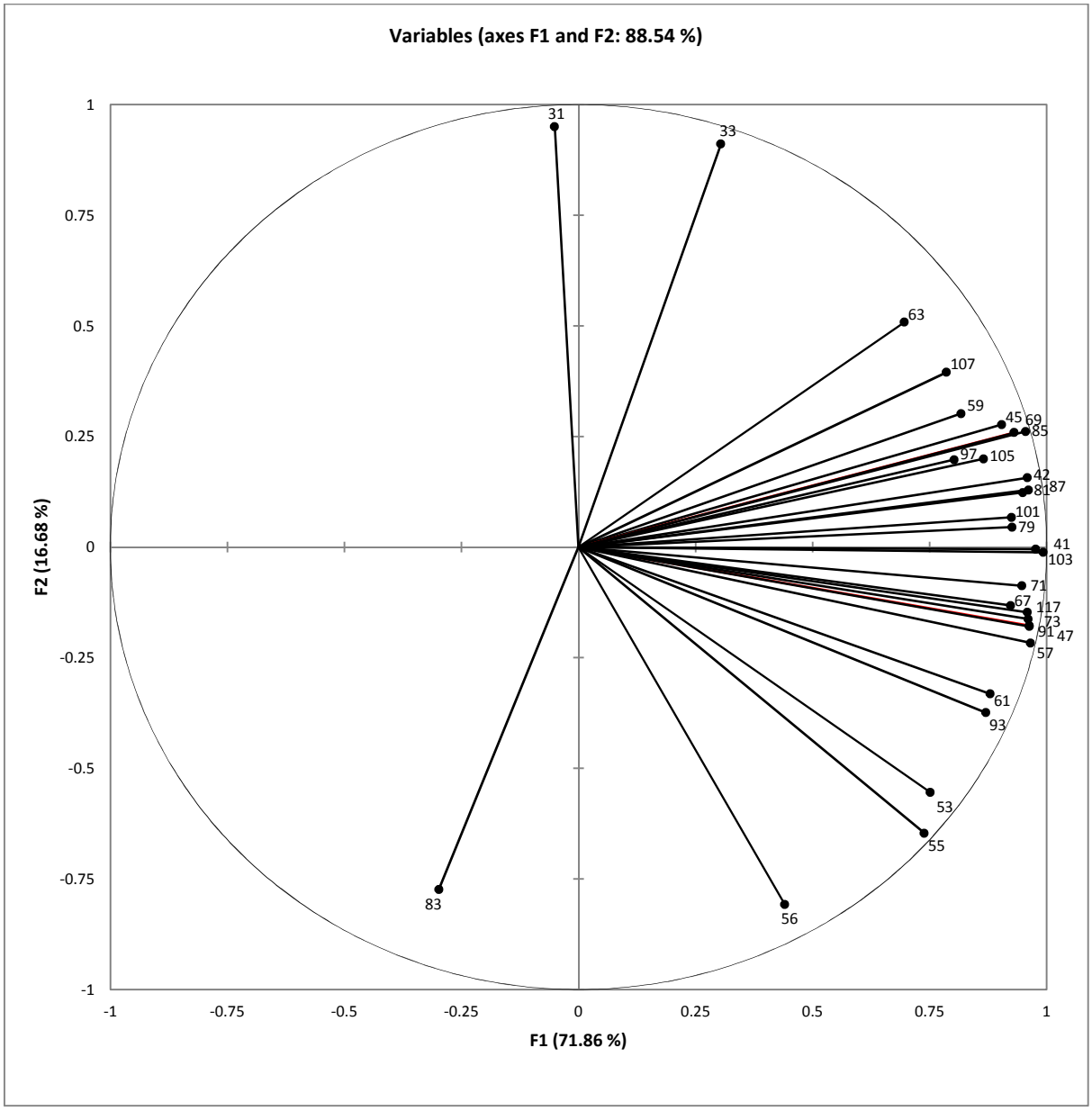


Figure 7.1. Principal component analysis (PCA) map representing the projection on F1 and F2 of the significant ($p < 0.05$), depurated m/z values for 7 natural coffee treatments in duplicate and 1 washed witness, evaluated using PTR-MS. See Table 7.1 for m/z identifications.

Figure 7.2 presents the projection of samples on F1 and F2 of the PCA of significant PTR-MS data. The placement of samples along F1 (71.86% of variability) shows four groups of samples: from left to right, sample W standing alone; next, an elongated group with most of the treatments (2A, 1A, 2B, 7B, 6A, 7A, 4B, 1B, 4A, 6B); next, treatment 5 (5B and 5A) and treatment 3 (3A and 3B – the 'honeying' treatment) near the right end. The placement of samples along F2 shows three groups of samples: from bottom to top, sample W stands alone;

next treatment 3 (3B and 3A), still on the negative side of F2, and then the rest of the samples. It is clear that, from the point of view of the green bean headspace, the washed witness (W) has a lower level of VOC emissions compared to the natural treatments, both along F1 and F2, except for m/z 83. This is associated with a common observation in the industry about green, natural coffee beans having an intense 'fruity' odour as opposed to the mild 'vegetable' odour of green, washed beans. The two treatments on the positive end of F1 (treatments 3 and 5) were the two treatments with a higher a_W, i.e., treatment 3, during the first days of drying and treatment 5, during the second week of drying (see Figures 6.4 and 6.5). These two treatments may have allowed an ethanol fermentation to proceed, which could have competed with the methanol catabolism. This is also supported by the high yeast count found with the equivalent treatment in 6.3.2 (treatment 23), which may be the reason for a lower level of methanol in treatment 3.

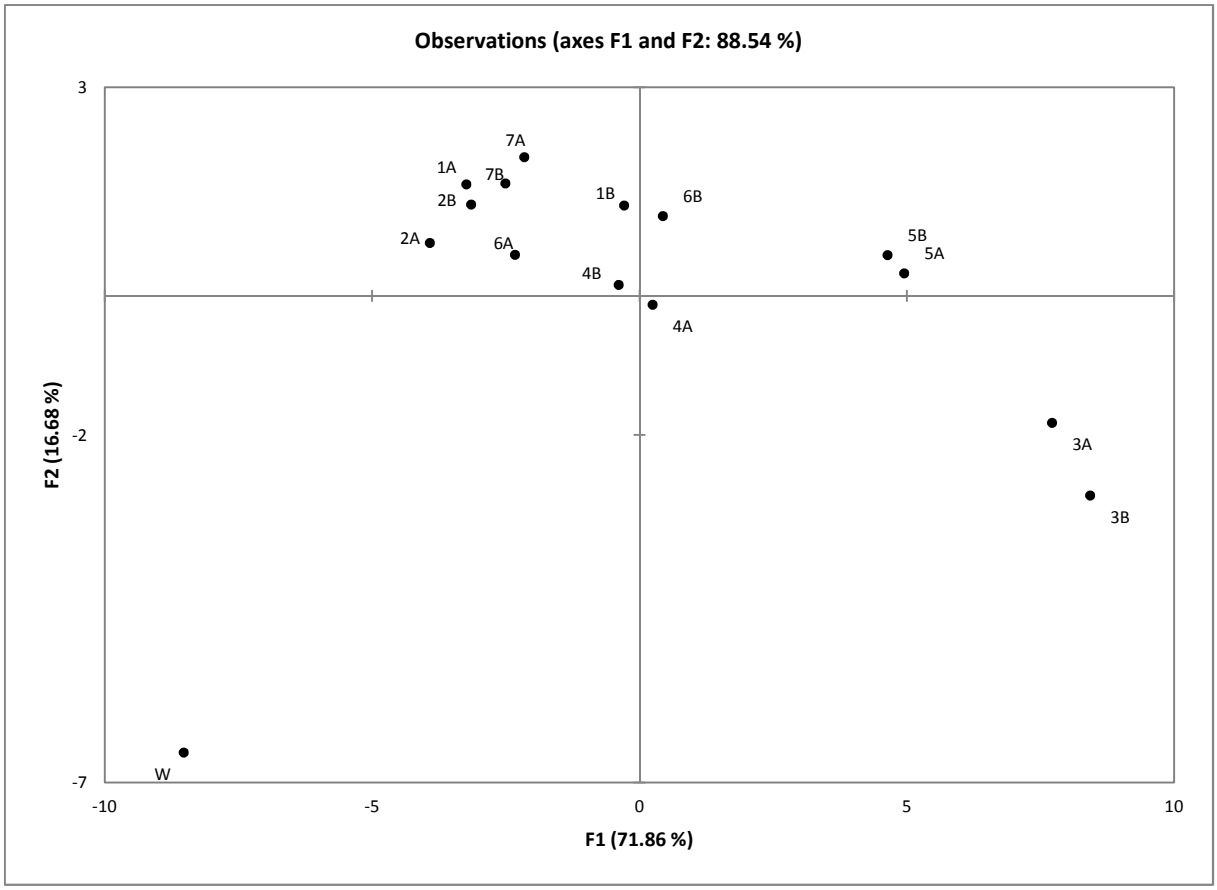


Figure 7.2. Principal component analysis (PCA) map representing the projection on F1 and F2 of 7 natural coffee treatments (1-7, with duplicates A and B) and washed coffee (W), evaluated using PTR-MS.

7.3.2 Semi-quantification of odour-active compounds in HE-SPE concentrates and static headspace of coffee grounds from GC-MS data

The GC-MS peak areas of the known, odour-active compounds of Table 5.6 were integrated (single-ion integration) and the resulting dataset was investigated using ANOVA. Only three compounds were found to be significant at a level of $p<0.05$: ethyl 2-methylbutanoate (peak 10b, strawberry), ethyl 3-methylbutanoate (peak 12, blueberry) and 3-Methylbutan-1-ol (peak 16b, stinky). Both fruity esters were highest in the 'honeying' treatment (treatment 3). 3-Methylbutan-ol was highest in the 'mould fermentation' treatment (treatment 5).

Three more compounds were found to be significant at a level of p between 0.05 and 0.15: methanethiol (peak 1, flatulence), 2-methylpropanal (peak 6, peanut/fruity/chocolate) and 2-methylbutanal (peak 7a, toasted bread/peanut/fruit). The number of significant compounds is much lower than the case of the international appraisal study (Chapter 5), which can be explained by the fact that the raw material was the same for all the samples in the present study, and thus the variability due to terroir and genetic effects was not present.

The semi-quantification data for all 6 compounds significant at values of $p<0.15$ (Appendix 8) were investigated using a PCA. High correlations ($r>0.95$, Pearson (n-1) coefficient) were found between 2-methylpropanal and 2-methylbutanal, and between ethyl 2-methylbutanoate and ethyl 3-methylbutanoate.

The PCA of the significant GC-MS/O data explains the variability in a total of 6 factors and 79.8% of the variability is explained by the first two factors (F1 and F2 – 53.6% and 26.3% respectively). Figure 7.3 presents the projection of the significant odour-active compounds on F1 and F2. The square cosines of four of the compounds (peaks 7a, 10b, 12 and 16b) were highest for F1, meaning they are best represented by F1. The main contributing compounds to F1 were peak 12 (ethyl 3-methylbutanoate, blueberry – 24.1%) and peak 10b (ethyl 2-methylbutanoate, strawberry – 23.4%). Therefore, F1 can be best described by the term 'fruity esters'.

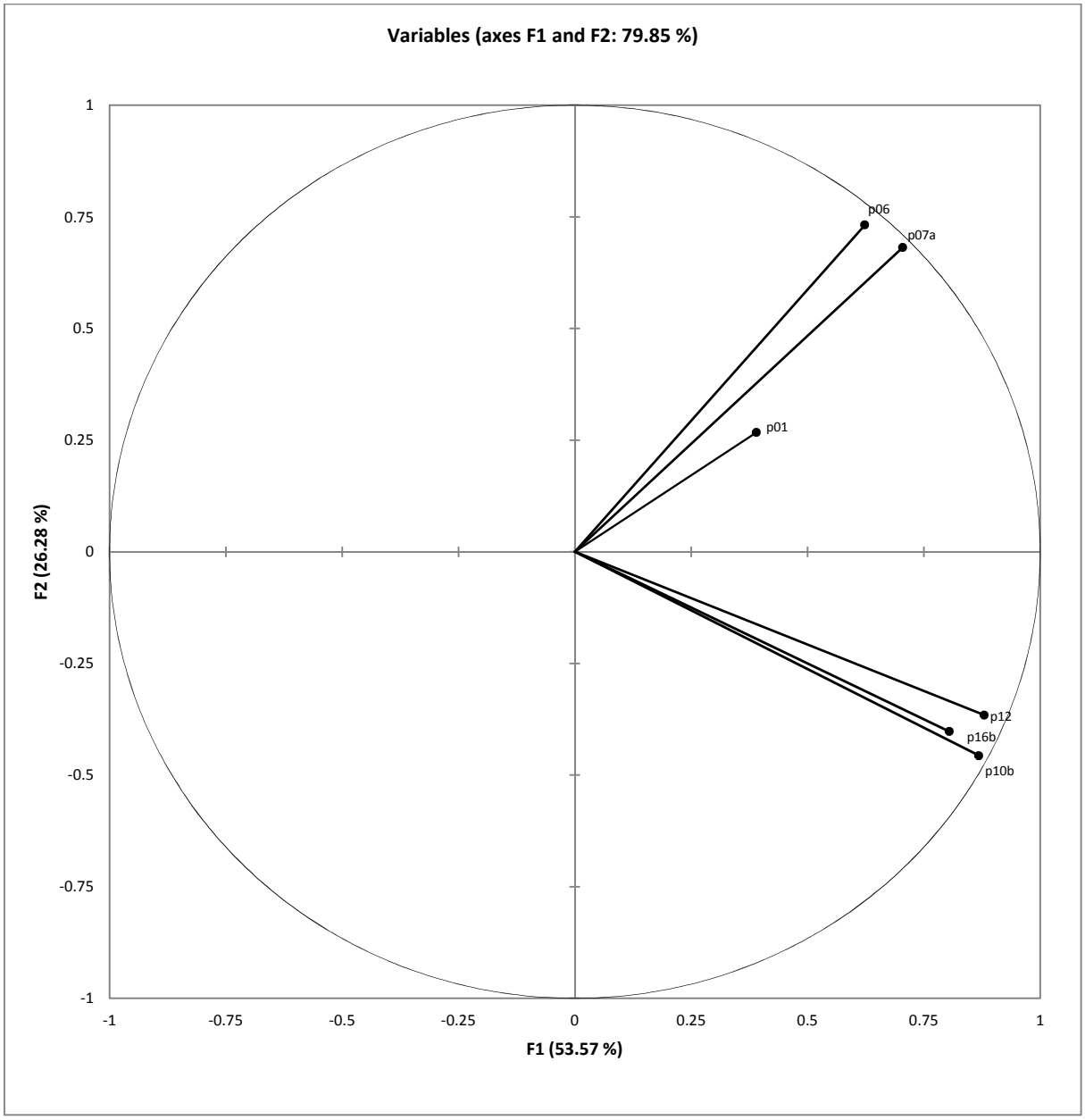


Figure 7.3. Principal component analysis (PCA) map representing the projection on F1 and F2 of significant odour-active compounds (p < 0.15) for 7 natural coffee treatments and 1 washed witness, evaluated using GC-MS. Peak legend: p06 – Propanal, 2-methyl; p07a – Butanal, 2-methyl; p01 – Methanethiol; p12 – Ethyl 3-methylbutanoate; p16b – 3-Methylbutan-1-ol; p10b – Ethyl 2-methylbutanoate (see Table 5.5 for odour character of peaks).

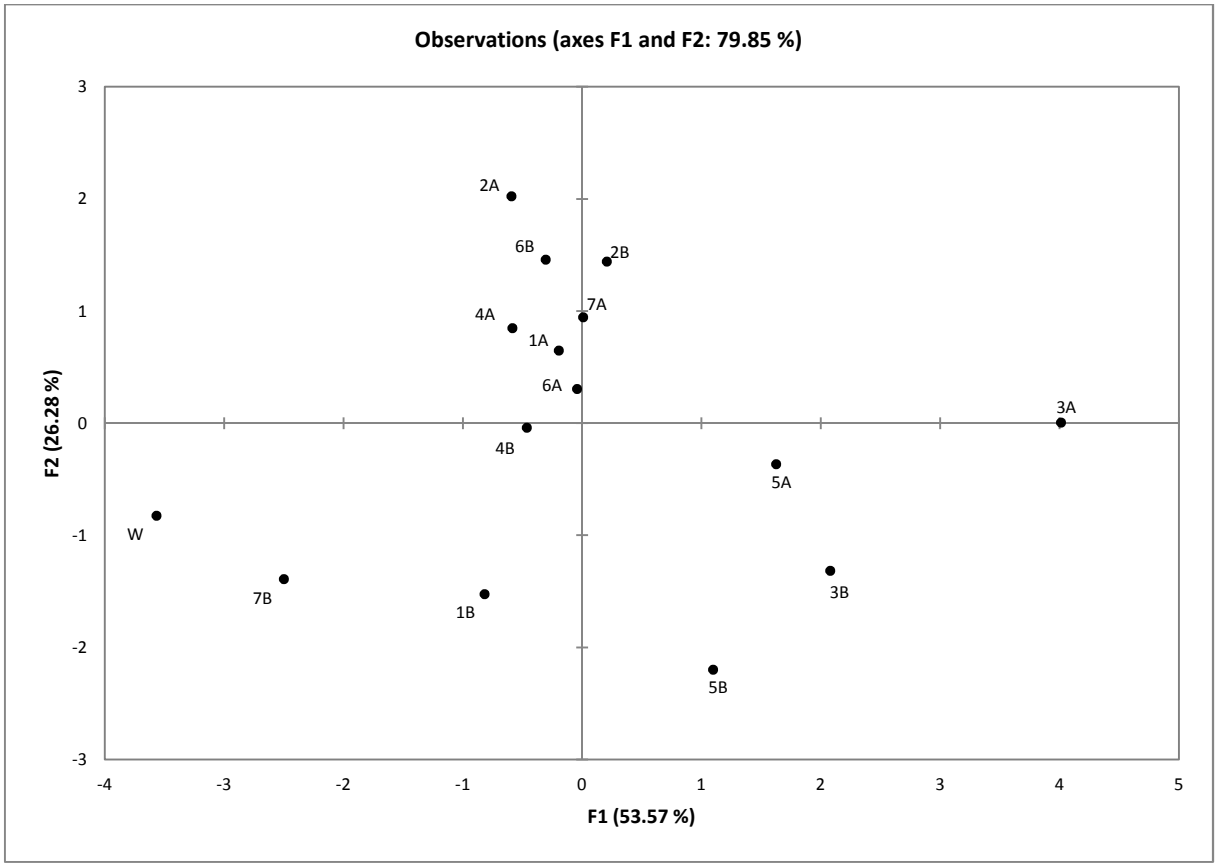


Figure 7.4. Principal component analysis (PCA) map representing the projection on F1 and F2 of 7 natural coffee treatments (1-7, with duplicates A and B) and washed coffee (W), evaluated using GC-MS (known, odour-active compounds significant at a level of p<0.15).

The square cosine of peak 6 (2-methylpropanal, peanut/fruity/chocolate) is highest for F2, and this compound is also the main contributor to F2 (34.0%). Since peak 7a (2-methylbutanal, toasted bread/peanut/fruit) also has a large contribution to F2, this factor can be described as 'fruity, branched-chain aldehydes'. Peak 1 (methanethiol, flatulence odour) is the main contributing compound to F3, with 79%.

Figure 7.4 maps the samples on F1 and F2 of the PCA. Treatment 7 presented different locations for both duplicates in this case and also in the case of the Descriptive Cupping analysis (6.3.4.1). In treatment 7, a thick layer of coffee cherries was used for the whole duration of the drying, which causes uneven drying (Berhanu et al., 2012). Uneven drying is likely to cause different placements for both duplicates of treatment 7.

The sample placement along F1 follows the same general pattern observed in the green coffee headspace: from left to right, sample W is found near the negative end, followed by most of

the samples clustered near the origin (in this case with sample 7B halfway in the negative side of F1), and then samples 5B and 5A on the positive side of F1, and 3B and 3A near the positive end. This placement suggests a link between the fermentation compounds observed in the first factor of the green coffee headspace analysis and the odour-active compounds contributing to F1 in the case of the roasted coffee, namely the fruity esters.

The reason for the placement of samples along F2 is not as clear. Treatments W, 5 and 3 are placed on the negative side of F2, while the rest of the treatments tend to be located on the positive side. However, a few of the duplicates (7B, 1B) are placed on the negative side.

7.3.3 Correlations among fermentation compounds

Gibson (1974) associated a particular flavour found in coffee from the Kenyan region of Solai with an ethanol to methanol ratio in the green beans larger than 1. 'Solai' flavour is described as being *"slightly fruity in character, or very slightly fermented in flavour. It is not to be confused with the characteristic fruitiness caused by bad processing conditions since these latter flavours are accompanied by a 'coarse' or 'common' flavour"* (Gibson, 1974). Flament and Bessière-Thomas (2002) suggest the ethyl derivatives formed during roasting could be responsible for that particular flavour. Thus, the ethanol/methanol ratio was calculated for the samples of this study, using the PTR-MS results from green coffee headspace for m/z 47 (ethanol) and m/z 33 (methanol). Figure 7.5 shows the effect of the treatment on the methanol/ethanol ratio in green coffee beans (PTR-MS).

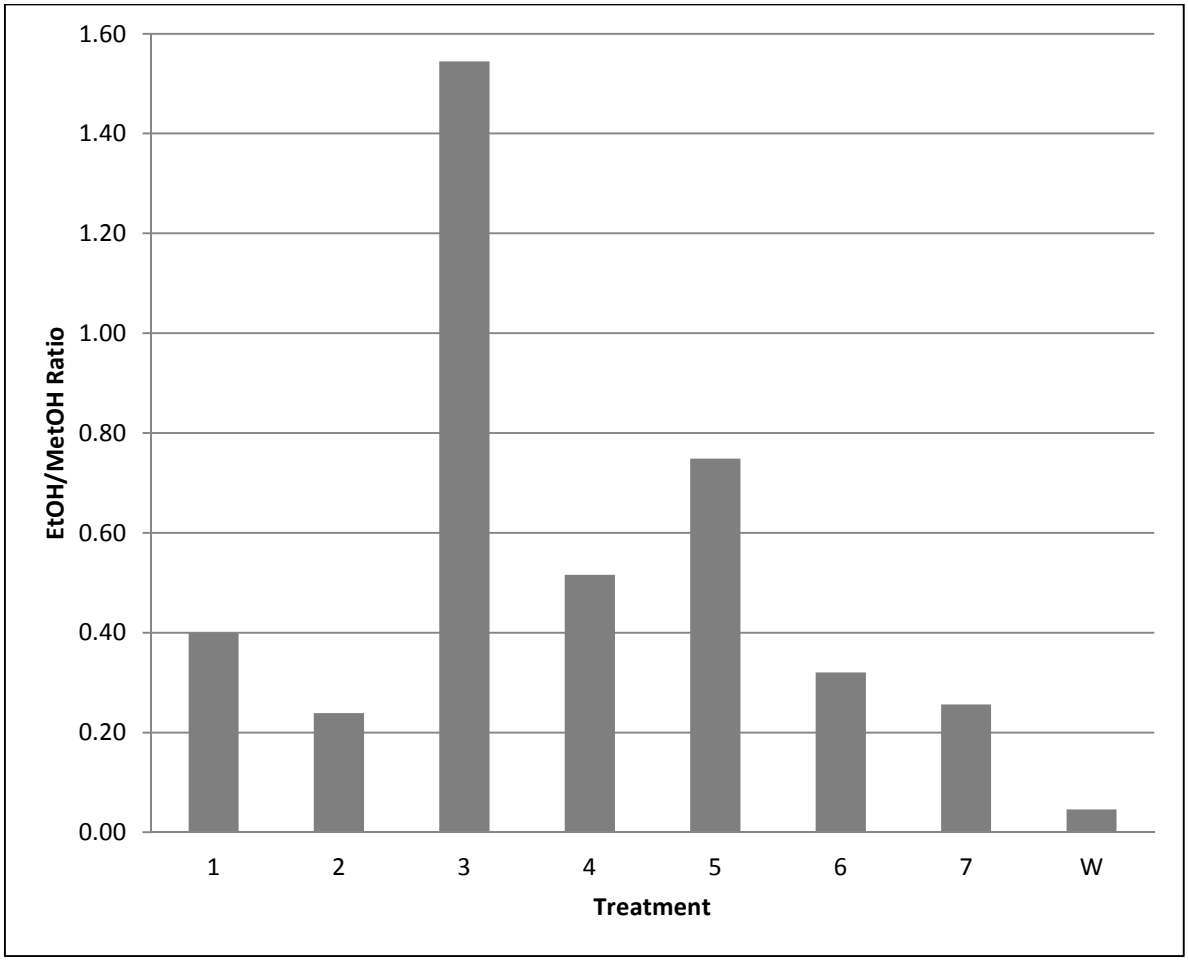


Figure 7.5. Effect of treatment on ethanol (m/z 47) / methanol (m/z 33) ratio (green beans, PTR-MS).

Holding samples for two days prior to drying ('honeying' - treatment 3) produced an ethanol/methanol ratio > 1 (1.54), whereas all other treatments presented ethanol/methanol ratio < 1. An ethanol/methanol ratio > 1 suggests ethanol fermentation dominates. This is probably due to a high yeast population as discussed in 6.3.2. Treatment 5 (slow drying rate during the second week) shows the next highest ethanol/methanol ratio (0.75). The rest of the natural treatments show an ethanol-methanol ratio between 0.25 and 0.55. The washed witness (W) presents the smallest ethanol/ratio (0.05). This order of the treatments reflects the general order followed by the first factor of the green bean headspace and the roasted bean headspace analyses.

The contents of ethanol after roasting is highly correlated (r=0.97, Pearson (n-1) coefficient) to the ethanol contents in the green beans, which suggests the ethanol is not completely volatilised by the high roasting temperatures and is retained encapsulated in the bean.

Table 7.2. Correlation indexes between selected fermentation compounds and derivatives (Pearson (n-1) coefficient)

Compounds	Methanol (green bean)	Ethanol (green bean)	Acetaldehyde	Methyl formate	Propanal, 2-methyl	Butanal, 2-methyl	Butanal, 3-methyl	Ethyl 2-methylbutanoate	Ethyl 3-methylbutanoate	3-Methylbutan-1-ol	Acetic acid	2-Methylpropanoic acid	3-Methylbutanoic acid	2-Methylbutanoic acid	Methyl acetate	Ethyl acetate
Methanol (green bean)	**1.00**[a]	0.14	0.17	0.32	0.14	0.12	-0.23	0.17	0.14	0.43	-0.25	0.09	0.11	0.09	0.37	0.25
Ethanol (green bean)	0.14	**1.00**	**0.60**	0.30	0.23	0.37	0.29	**0.97**	**0.95**	**0.72**	0.38	-0.07	-0.02	-0.11	**0.86**	**0.98**
Acetal-dehyde	0.17	**0.60**	**1.00**	**0.81**	**0.67**	**0.73**	**0.61**	**0.55**	**0.53**	0.47	0.29	0.20	0.28	0.21	**0.63**	**0.59**
Methyl formate	0.32	0.30	**0.81**	**1.00**	**0.85**	**0.80**	**0.63**	0.29	0.30	0.44	0.15	0.30	0.38	0.29	**0.58**	0.35
Propanal, 2-methyl	0.14	0.23	**0.67**	**0.85**	**1.00**	**0.95**	**0.87**	0.22	0.31	0.19	0.08	0.08	0.21	0.10	0.51	0.29
Butanal, 2-methyl	0.12	0.37	**0.73**	**0.80**	**0.95**	**1.00**	**0.89**	0.33	0.41	0.24	0.01	-0.05	0.07	-0.03	**0.59**	0.42
Butanal, 3-methyl	-0.23	0.29	**0.61**	**0.63**	**0.87**	**0.89**	**1.00**	0.24	0.36	0.07	0.10	-0.05	0.04	-0.03	0.38	0.28
Ethyl 2-methyl-butanoate	0.17	**0.97**	**0.55**	0.29	0.22	0.33	0.24	**1.00**	**0.97**	**0.81**	**0.54**	0.09	0.15	0.05	**0.82**	**0.94**
Ethyl 3-methyl-butanoate	0.14	**0.95**	**0.53**	0.30	0.31	0.41	0.36	**0.97**	**1.00**	**0.73**	0.51	0.01	0.08	-0.03	**0.85**	**0.93**
3-Methyl-butan-1-ol	0.43	**0.72**	0.47	0.44	0.19	0.24	0.07	**0.81**	**0.73**	**1.00**	**0.52**	0.22	0.27	0.15	**0.74**	**0.70**
Acetic acid	-0.25	0.38	0.29	0.15	0.08	0.01	0.10	**0.54**	0.51	**0.52**	**1.00**	**0.56**	**0.61**	0.49	0.20	0.28
2-Methyl-propanoic acid	0.09	-0.07	0.20	0.30	0.08	-0.05	-0.05	0.09	0.01	0.22	**0.56**	**1.00**	**0.98**	**0.99**	-0.05	-0.07
3-Methyl-butanoic acid	0.11	-0.02	0.28	0.38	0.21	0.07	0.04	0.15	0.08	0.27	**0.61**	**0.98**	**1.00**	**0.98**	0.03	-0.01

Compounds	Methanol (green bean)	Ethanol (green bean)	Acetaldehyde	Methyl formate	Propanal, 2-methyl	Butanal, 2-methyl	Butanal, 3-methyl	Ethyl 2-methylbutanoate	Ethyl 3-methylbutanoate	3-Methylbutan-1-ol	Acetic acid	2-Methylpropanoic acid	3-Methylbutanoic acid	2-Methylbutanoic acid	Methyl acetate	Ethyl acetate
2-Methyl-butanoic acid	0.09	-0.11	0.21	0.29	0.10	-0.03	-0.03	0.05	-0.03	0.15	0.49	**0.99**	**0.98**	**1.00**	-0.07	-0.09
Methyl acetate	0.37	**0.86**	**0.63**	**0.58**	0.51	**0.59**	0.38	**0.82**	**0.85**	**0.74**	0.20	-0.05	0.03	-0.07	**1.00**	**0.92**
Ethyl acetate	0.25	**0.98**	**0.59**	0.35	0.29	0.42	0.28	**0.94**	**0.93**	**0.70**	0.28	-0.07	-0.01	-0.09	**0.92**	**1.00**
Ethanol	0.30	**0.97**	**0.60**	0.31	0.21	0.35	0.19	**0.93**	**0.90**	**0.70**	0.27	-0.04	0.00	-0.06	**0.88**	**0.99**

(a) Values in bold are different from 0 with a significance level alpha=0.05.

Table 7.2 presents the correlation indexes between the selected fermentation-related compounds, calculated using the Pearson (n-1) coefficient. In the PCA analysis of significant, odour-active compounds shown in 7.3.2 it became clear two of the main discriminant ($p<0.05$) compounds contributing to the odour active compound variability are the two fruity esters, ethyl 2-methylbutanoate (strawberry note) and ethyl 3-methylbutanoate (blueberry note). Both fruity esters are correlated ($r>0.9$) to each other, to the ethanol contents (both in green and roasted form) and to the ethyl acetate contents. This implies the availability of ethanol in the green coffee largely impacts the formation of ethyl esters both before and during roasting. It also suggests the conditions that favour the formation of ethanol also favour the formation of the required acids for ester formation. These ethyl esters have a lower sensory threshold than their methyl counterparts (Flament & Bessière-Thomas, 2002), which would partly explain why the fruity character is more evident when the ethanol/methanol ratio is larger than 1 (Gibson, 1974). However, the methyl esters were not detected, which would also suggest the effect is not only due to a lower threshold, but actually to the esterification conditions.

Other high correlations ($r>0.9$) between the selected fermentation related compounds are 2-methyl-propanal with 2-methyl-butanal (aldehydes derived from the degradation of valine and isoleucine respectively, both with a fruity character); 2-methylpropanoic acid with 3-

methylbutanoic acid and 2-methylbutanoic acid (three branched-chain fatty acids derived from the degradation of valine, leucine and isoleucine respectively, all with a tropical fruit/fermented character), and methyl acetate along with ethyl acetate (possibly due to the availability of acetic acid for esterification). Methanol does not show a significant correlation with any of the other fermentation VOCs studied, not even with the methyl esters. Ethanol and acetic acid are not significantly ($p<0.05$) correlated either; the main reason for this may be the independent formation of acetic acid during roasting as a sugar-degradation product (Flament & Bessière-Thomas, 2002), as opposed to the catabolic oxidation of ethanol.

In the case of filamentous fungi metabolism, isoleucine, leucine and valine can be degraded to the aldehyde form (2-methylbutanal, 3-methylbutanal and 2-methylpropanal respectively) through the action of an aminotransferase and a 2-ketoacid decarboxylase, and from the aldehyde, the alcohol form can be generated through the action of an alcohol dehydrogenase. Alternatively, the acid forms (2-methylbutanoic acid, 3-methylbutanoic acid and 2-methylpropanoic acid respectively) can be generated from the amino acid form through the action of an aminotransferase and a 2-ketoacid dehydrogenase (Roze et al., 2010).

Therefore, three kinds of fermentation pathways seem to be functioning during processing in a relatively independent way or as a progression: (a) methanol fermentation, largely uncorrelated to the other selected compounds and highest in treatments 7 and 1; (b) ethanol fermentation, possibly linked to the yeast population, largely correlated to the ethyl esters and many times more intense in the 'honeying' treatment than in the other treatments, followed by treatment 5, which had a slow drying rate during the second week of drying, and (c) degradation of valine, isoleucine and leucine to the 2-methylpropanoic, 2-methylbutanoic and 3-methylbutanoic families of compounds; this is highest in treatment 6, followed by treatment 5 – the two treatments that involved suspension of drying for a few days. The presence of these amino acid fermentation compounds is also necessary for the formation of fruity esters of branched-chain fatty acids. However, the presence of ethanol will influence higher contents of ethyl derivatives as opposed to methyl derivatives. 'Honeying' (treatment 3) seems to favour the ethanol fermentation and inhibit the methanol one. 'Honeying' does not seem to make a difference on the valine, isoleucine and leucine degradation.

Further research is needed to link these fermentation pathways to specific microbial populations and growth conditions.

7.3.4 Multiple factor analyses, including the Descriptive Cupping dataset

The large number of different variable groups pertaining to this study were integrated in a multiple factor analysis (MFA) in order to understand the links between the treatment variables, the headspace volatiles and natural coffee flavour (sensory results – Chapter 6), with focus in the 'Mocha' character – meaning a fruity, winey character.

For the purpose of the MFA, and for the sake of consistency with Chapter 5, the sensory data produced by the University of Otago panel was used. Only two tables were kept active in the MFA – the subgroup table from the University of Otago panel (11 columns including the subgroups with $\chi^2>7$, as used in 6.3.4.1 – see Appendix 6) and the table with 6 odour-active compounds found significant with value of $p<0.15$ (7.4.2). Only these two tables were kept active as they are the only tables directly pertaining to the roasted coffee samples. The tables considered as supplementary include the table of depurated significant ($p<0.05$) PTR-MS m/z from the green bean headspace (7.3.1); the table with green bean aspect variables (colour as L*a*b* and percentage of 'foxy' beans – Appendix 7); the number of drying days for each sample; the table of water activity (a_W, on day 2 and day 9 of drying, section 6.3.1.2), and the table of pH (on day 6 and day 10 of drying, section 6.3.1.4). For the case of the three last tables, the washed witness was not considered in the MFA, since the washed process is not comparable to the natural process for these variables (they were measured on the coffee pulp and the washed witness was pulped).

Table 7.3 shows the RV coefficients between the active tables and the supplementary tables. The active tables have an RV coefficient with the MFA of 0.886 and 0.800 for the subgroup table and the odour-active compounds table, respectively, indicating both are well represented by the MFA. Both active tables have an RV coefficient between them of 0.429, lower than the one found in Chapter 5, but still indicating there is correlation between the descriptors and the GC-MS/O compounds. The RV coefficients between the subgroups table and the green bean headspace table (0.461) and between the odour-active compounds table and the green bean aspect table (0.488) are of the same order, between 0.400 and 0.500. However, there are also two much higher RV coefficients, in the order of 0.700-0.800, indicating correlations worth exploring. These are between the subgroup table and the green bean aspect table (bean colour and percentage of foxy beans), which have an RV coefficient of 0.744, and the odour-active compound table and the green bean headspace table, which have an RV coefficient of 0.719.

The bean colour has been traditionally assessed by the coffee industry as an indicator of quality (SCAA, 2009b). Bean colour in general has been found correlated to storage quality and to oxidative and enzymatic reactions affecting the flavour precursors (Borém et al., 2013). The reddish colour in particular and the presence of 'foxy' beans have been considered typical of fruity naturals by the industry (Diaz Pineda, 2012). On the other hand, the high RV coefficient found between the green bean headspace and the roasted bean headspace may be explained by the direct link between the green bean volatiles and the significant odour-active compounds for this study, as both are mostly related to differences in fermentation among the treatments.

Table 7.3. RV coefficients calculated between different variable tables, pertaining to 7 natural coffee treatments and one washed coffee witness.

Table	RV with the subgroup table (New Zealand panel, significant subgroups)	RV with the significant odour active compounds (GC-MS) table	RV with the MFA
Descriptors (New Zealand panel, significant subgroups)	1.000	0.429	0.886
Significant odour active compounds (GC-MS)	0.429	1.000	0.800
Green bean headspace (PTR-MS)	0.461	0.719	0.676
Green bean aspect (CIELAB colour and foxy beans)	0.744	0.488	0.792
Drying days [a]	0.229	0.231	0.266
a_W [a]	0.335	0.310	0.375
pH [a]	0.169	0.213	0.219

(a) The washed witness was not considered in the drying days, a_W and pH tables.

The RV coefficients between the field treatment variables (drying days, water activity, and pH) and the active tables are smaller – in the order of 0.150-0.350. The highest correlations in this group are found for the water activity table, which was in fact the main control parameter to achieve differentiation between treatments.

The MFA (from the active tables) resulted in a total of 14 factors (sum of eigenvalues =5.459). 83.1% of the total variability was represented in the first 5 factors. For the case of this section, the placement of samples on F1 and F2 of the MFA will be discussed before the variables, as this will help introduce the many variables involved. F1 represents 28.5% of the

variability and F2 represents 19.3% of the variability. The variability explained by the combination of F1 and F2 is 47.81%, which approaches 50% but still implies over half of the information from the active tables is not being explained by the plots of F1 and F2. Only the main sources of variability, as explained by F1 and F2 of the MFA, will be discussed below.

Figure 7.6 maps the samples on F1 and F2 of the MFA. All duplicates were placed next to each other along F1, with the possible exception of treatment 7 (7A and 7B). Treatment 7 has been shown to have duplicates that are most variable. Treatment 7 used a thick layer of piled coffee cherries, with a limited cherry turning frequency (once a day). This causes unevenness between the cherries on the top layer, exposed to the sunshine and the air, and the cherries in the bottom layers. The observations of treatment 7 during drying indicate an uneven appearance of cherries. As the cherries within the same batch were uneven, separate batches, like the case of 7A and 7B, are likely to be different from each other.

The general placement of samples along F1 is in line with what has been observed in the field and the instrumental results: from left to right, the washed witness on one end, followed by treatment 2, which was a fast-drying treatment, then the majority of the natural treatments (7, 6, 4, 1), then the treatment with the slowest drying rate during the second week (treatment 5) and last, the ‘honeying’ treatment (treatment 3), near the positive end of F1. The reasons for the placement of samples along F2 are not as clear; the treatments with a slower drying rate are spread wider apart along F2, while the faster rate treatments are closer together. This may suggest that slower drying introduces further complexity to the coffee matrix, through the role of fermentation and fruit respiration, possibly causing divergence between batches of the same treatment.

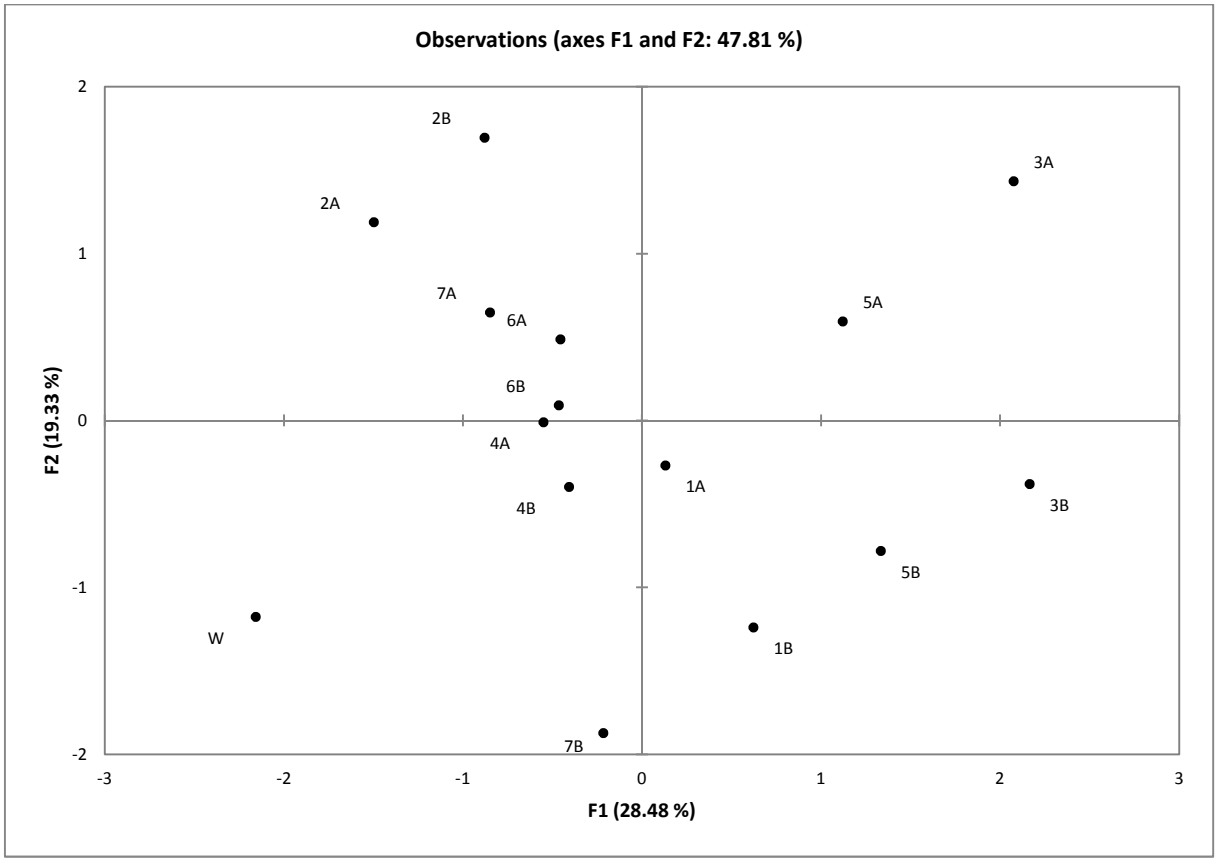


Figure 7.6. Multiple factor analysis (MFA) map representing the projection on F1 and F2 of 7 natural coffee treatments (1-7, with duplicates A and B) and washed coffee (W – averaged), evaluated using the Descriptive Cupping method and GC-MS.

Figure 7.7 maps the variables of the two active tables (subgroups and odour-active compounds) on F1 and F2 of the MFA. The subgroups most contributing to F1 are *fermented* (14.3%), *salty taste* (10.0%) and *tropical-fruity* (9.4%), all on the positive side of F1, while the odour-active compounds most contributing to F1 are peak 10b (ethyl 2-methylbutanoate, strawberry, 18.3%), peak 12 (ethyl 3-methylbutanoate, blueberry, 16.6%) and peak 16b (3-methylbutan-1-ol, stinky, 13.5%). The highest correlation indexes (Pearson (n-1) coefficient) between subgroups and odour active peaks are found between *salty taste* and peak 12 (r=0.865), between *salty taste* and peak 10b (r=0.813) and between *fermented* and peak 10b (r=0.713). It seems the industry's perception of a correlation between fruitiness/wineyness and salty taste in naturals (Davids, 2010) may be correct in some specific cases. Saltiness was only perceived in the 'honeying' sample. The 'honeying' treatment has proved to be different to the other natural treatments in all the analyses, from the intensity of the fermentation taking place (6.3.2), in the amount of VOCs emitted by the green beans (7.3.1) and in the contents of

significant odour-active compounds (7.3.2). This has been helpful to understand the potential of an initial fermentation stage prior to drying and the role of yeasts; however, even though this treatment has a clear fermented flavour, from the application point of view, the 'honeying' treatment given to 3A and 3B may have been too intense for the flavour change to be considered 'desirable'. The formation of ethanol at the expense of precursors such as sugars (Silva, 2014) may have been too intense. The relative absence of precursors may have allowed an 'undesirable' character, such as the salty taste, to become apparent. Future research should focus on a better understanding of the 'honeying' treatment, in order to optimise fruitiness without compromising sweetness.

In addition, the two fruity, branched-chain, ethyl esters (ethyl 2-methylbutanoate and ethyl 3-methylbutanoate), showing high correlations with *fermented* and *tropical-fruity* characters are also worth further investigation. The role and the formation conditions of these two esters have been subject to contradicting interpretations. They have been linked to over-fermented defects (Bade-Wegner, Bendig, Holscher, & Wollmann, 1997; Guyot & Vincent, 1982) but also to the fruity character in natural coffees (Hofmann, in a personal communication to Folmer (2014), without reporting methodology). It is clear from this study that these two esters play a key role in the formation of the 'Mocha' character, but more research is needed in order to optimise the 'desirable' fruity character from these esters without incurring over-fermentation.

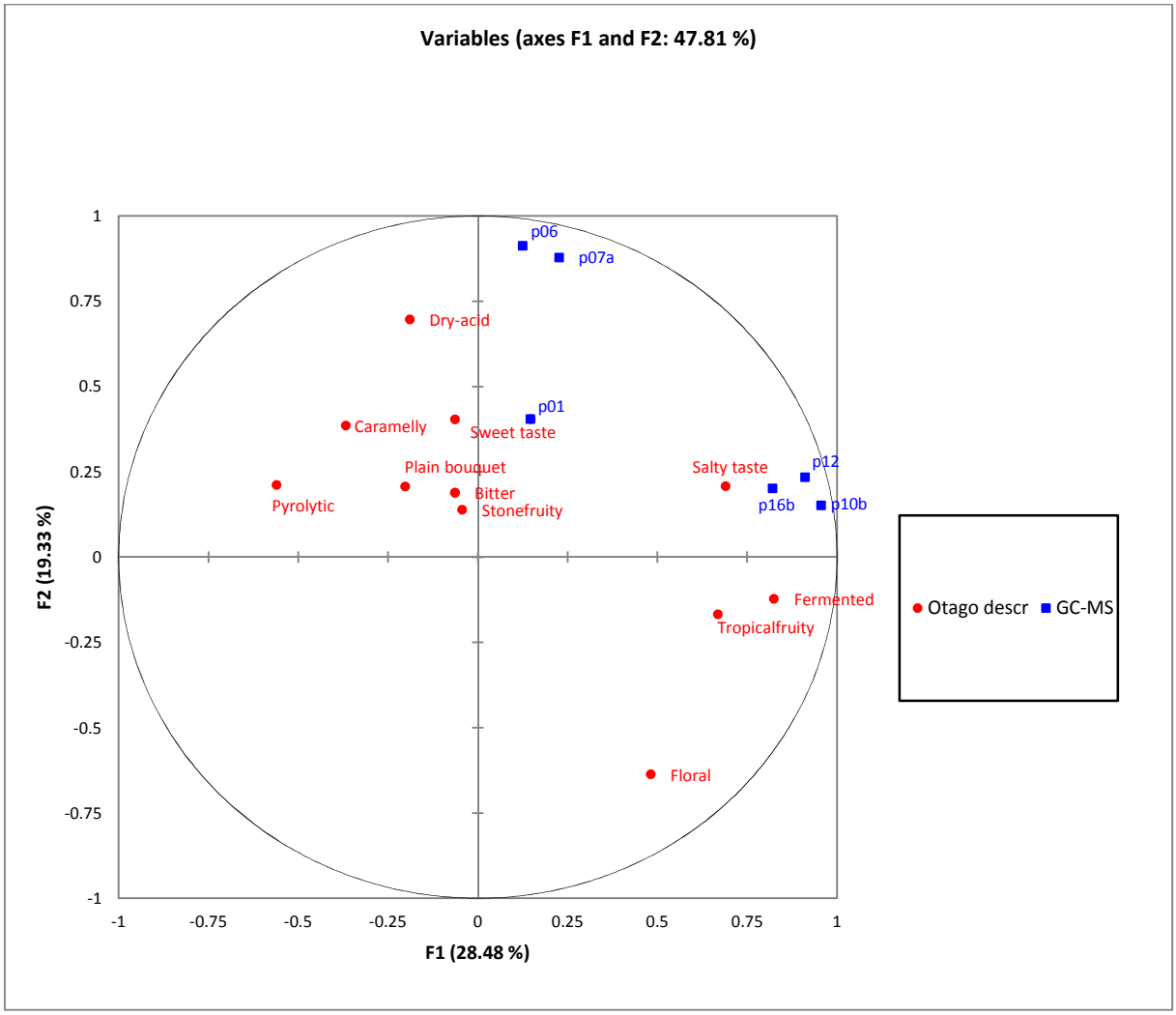


Figure 7.7. Multiple factor analysis (MFA) map representing the projection on F1 and F2 of descriptor subgroups (circle) and significant odour-active compounds (square). Peak legend: p01 – Methanethiol; p07a – Butanal, 2-methyl; p16b – 3-Methylbutan-1-ol; p06 – Propanal, 2-methyl; p10b – Ethyl 2-methylbutanoate; p12 – Ethyl 3-methylbutanoate; (see Table 5.5).

Figure 7.8 projects on F1 and F2 the significant m/z measured by PTR-MS in the green bean headspace, considered here as supplementary data. Most m/z are directly correlated with F1 and thus with the fermentation intensity, the concentration of fruity esters and the *fermented* character. As discussed in 7.3.3, the ethanol fermentation is linked to the formation of the ethyl esters. The fact that most m/z are directly correlated with F1 implies that ethanol and other fermentation-related volatile organic compounds detected in the green bean headspace can potentially be used as predictors of the fruity and fermented characters in the roasted product.

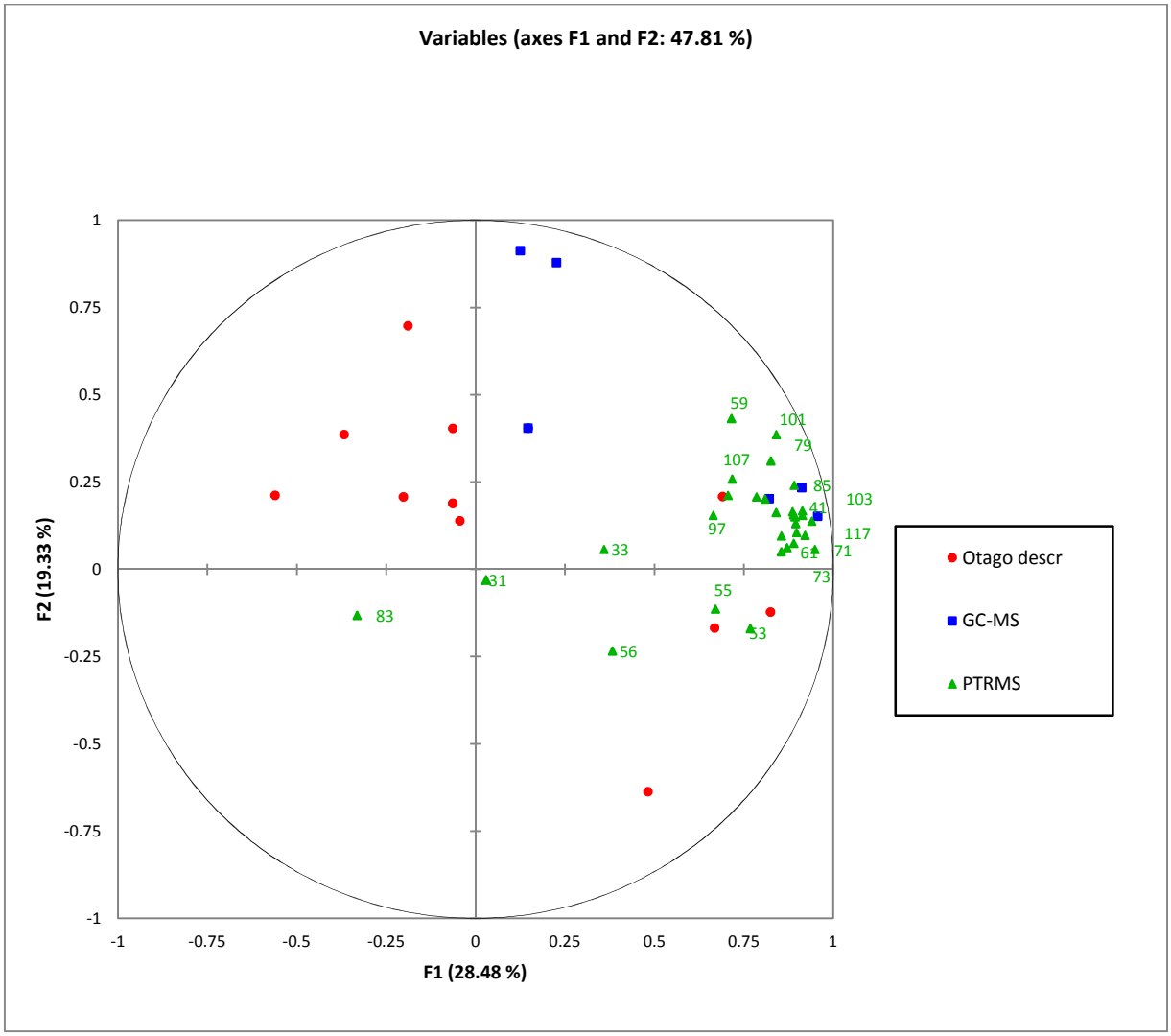


Figure 7.8. Multiple factor analysis (MFA) map representing the projection on F1 and F2 of descriptor subgroups (circle) and significant odour-active compounds (square) as active tables, and significant PTR-MS m/z values (triangle) as supplementary table. Only key labels are shown, to improve readability.

Figure 7.9 projects the variables pertaining to the green bean aspect (CIELAB colour and percentage of foxy beans) on F1 and F2 of the MFA. Both the a* colour parameter and the percentage of foxy beans have a high square cosine on F1 (0.623 and 0.595, respectively) and a high loading on F1 (0.789 and 0.771 respectively). An increase in a* means the bean colour is less green and more red (Negueruela, 2012). The percentage of foxy beans is a similar indicator, as it is the fraction of visually reddish beans (the 'foxy' beans). This implies the reddish colour of the beans is linked to a more intense fermentation and also to a more pronounced *fermented* character (see the figures for Treatment 3 in Appendix 7), which validates the industry's perception about the foxy beans and the reddish colour as an indicator

of fruitiness (Diaz Pineda, 2012). Further research is needed to understand the formation of reddish colour on the beans and the silverskin during fermentation.

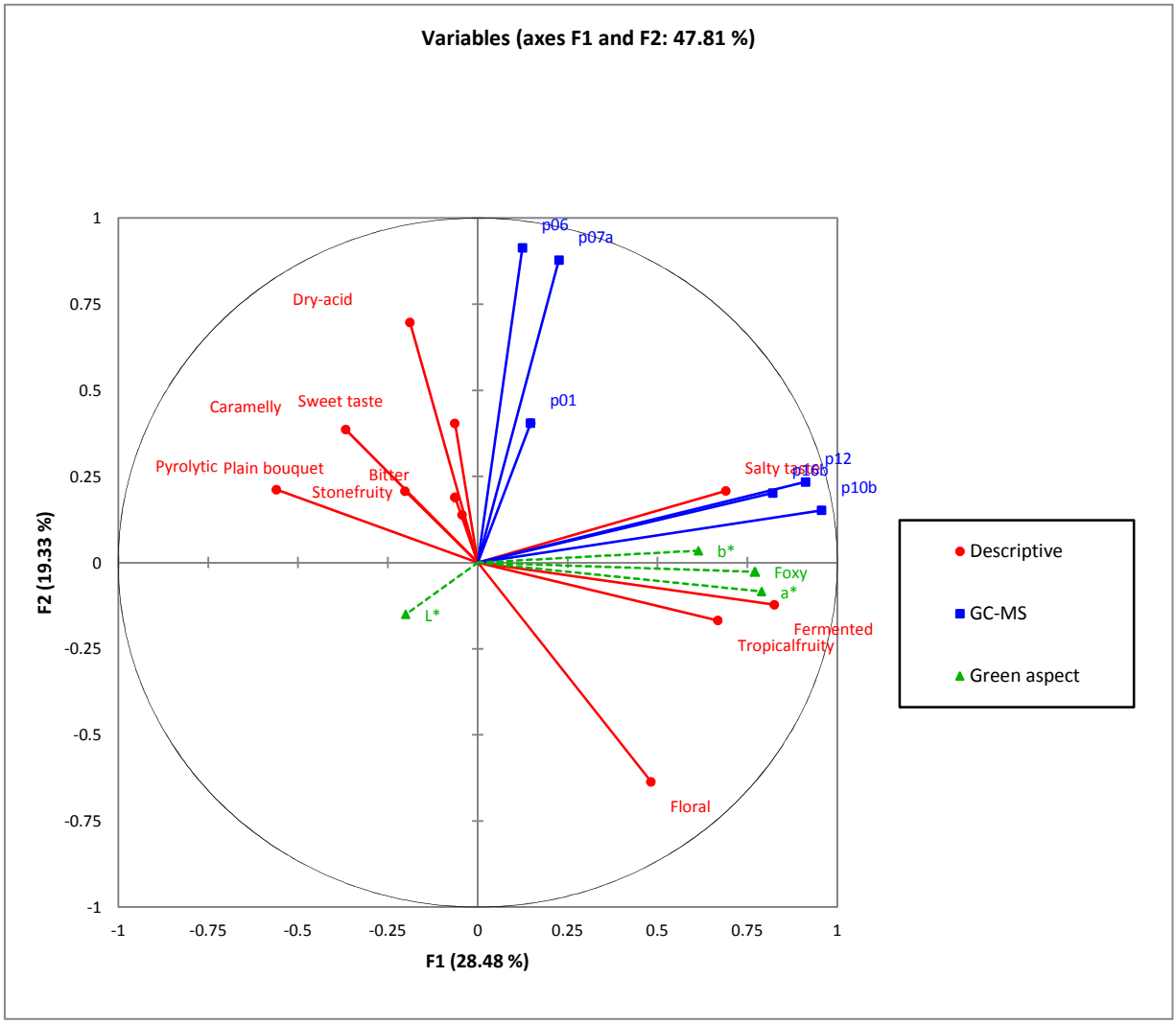


Figure 7.9. Multiple factor analysis (MFA) map representing the projection on F1 and F2 of descriptor subgroups (circle) and significant odour-active compounds (square) as active tables, and green bean aspect variables (triangle – bean CIELAB colour and percentage of foxy beans) as supplementary table.

Figure 7.10 projects the field treatment variables (drying days, water activity and pH) as supplementary data on F1 and F1 of the MFA. The washed witness was not considered in this analysis because the processing differences meant these variables were not comparable. The largest correlations between these variables and the active table were found between the number of drying days and peak 1 (methanethiol, r=0.730, Pearson (n-1) coefficient) and between the water activity on the 9th day of drying and peak 16 b (3-methylbutan-1-ol, r=0.737, Pearson (n-1) coefficient). Since methanethiol is a derivative of methionine

pyrolysis, the first of these two correlations may imply a link between drying time, coffee fruit metabolism and the contents of free amino acids, as proposed by Selmar et al. (2014). The latter of these correlations suggests a catabolism of leucine when enough water is available during the second week of drying.

The projection of these supplementary variables on F1 and F2 also shows a link between a higher water activity and a *fermented* character, as well as an inverse relationship between pH and fruity flavour. There may be a cause-effect chain starting at a higher water activity during drying, which allows microbial growth (namely yeast growth) and promotes ethanol fermentation. The fermentation results in a pH drop because of the oxidation of ethanol to acetic acid, together with the production of other acids. The availability of ethanol increases the contents of ethyl esters, which contribute to the *fermented* character. Overall, microbial fermentation plays an important role in the development of the Mocha character, characterised by *fruity* and *fermented* flavour notes.

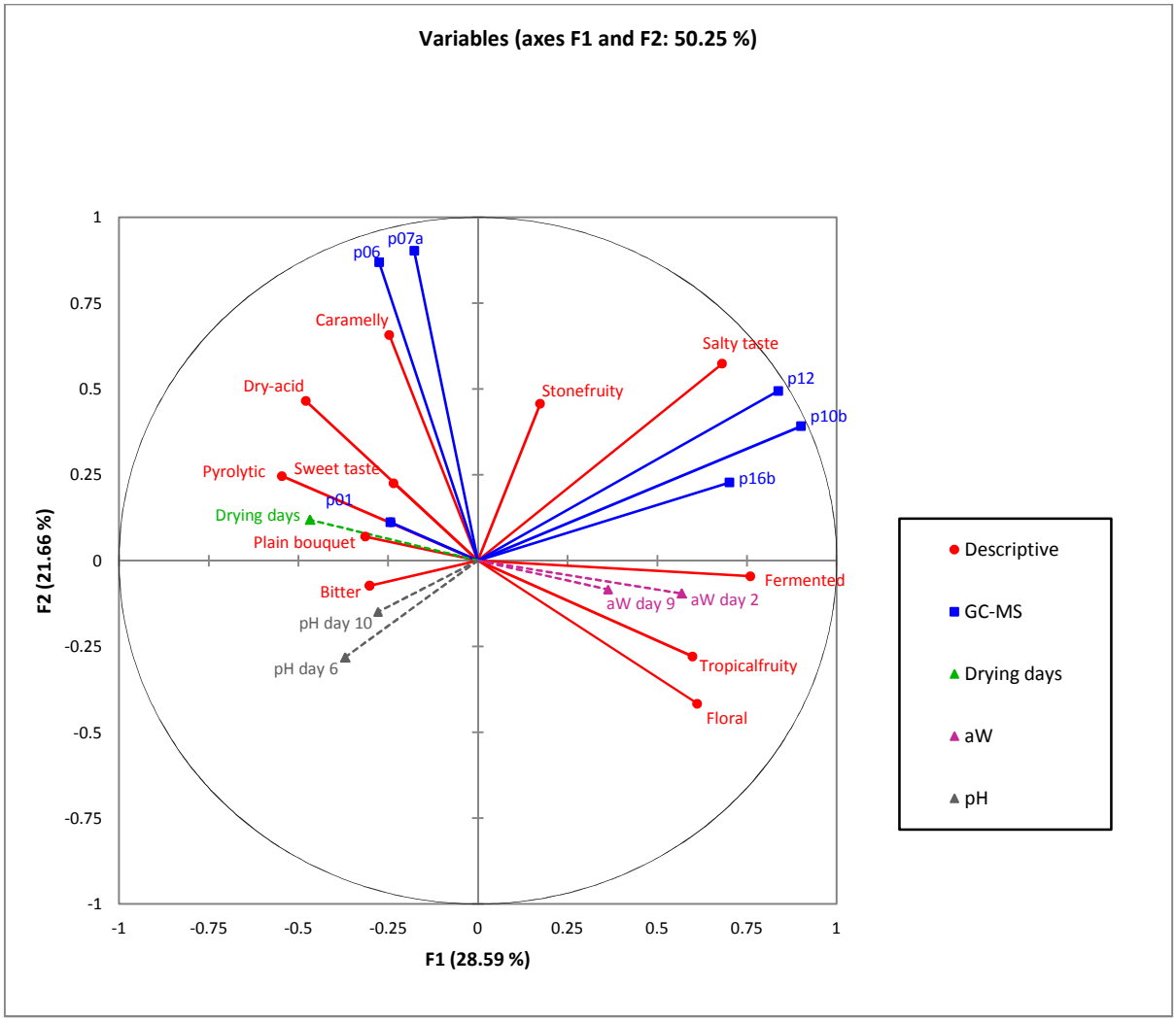


Figure 7.10. Multiple factor analysis (MFA) map representing the projection on F1 and F2 of descriptor subgroups (circle) and significant odour-active compounds (square) as active tables, and number of drying days, water activity (on day 2 and day 9) and pH (on day 6 and day 10) as supplementary tables (triangle). The washed witness has been excluded from this MFA.

7.4 Conclusion

In this study, the samples (15) produced using different natural coffee treatments (7) and a washed witness (as reported in Chapter 6) were characterised through the analysis of the headspace of the green and roasted coffee beans by PTR-MS and GC-MS respectively, and these analytical results were interpreted together with their aroma and flavour profiles (from Chapter 6) using multiple factor analysis (MFA).

Aside from the washed witness, the most distinct treatment was the one termed ‘honeying’ treatment, which involved suspending the drying for the first two days. This resulted in an intense fermentation with a high production of ethanol. Ethanol becomes available for esterification, namely with branched-chain fatty acids coming from valine, isoleucine and leucine metabolism, producing ethyl, fruity esters. Fruity esters contribute to the fruity and winey character of ‘Mocha’ style naturals, but a too intense fermentation may deplete the levels of important precursors for sugar pyrolysis and Maillard reactions. Further study of the ‘honeying’ treatment is needed in order to optimise the fruitiness without compromising other desirable characters.

A suspension of drying during the second week of sun-drying (after the water activity of the cherry had dropped below 0.800 and the batch weight had dropped to nearly half the initial weight) also resulted in a distinctive product, considering all the variables involved. This may be a safer, more controllable way to develop the ‘Mocha’ character than ‘honeying’. At any rate, it is clear that for the ‘Mocha’ character to be developed there must be some degree of catabolic degradation of valine, isoleucine and leucine, as their derivatives play a key role in the development of fruity and winey characters in natural coffees. Fast drying (and thus machine-drying) does not allow the time for these catabolic reactions to occur.

Treatments involving thick layers of cherries and limited turning frequency during drying resulted in a larger degree of unevenness within each batch and between duplicates of the same treatment. For this reason, these methods do not seem adequate for controlling the drying rate. Complete suspension of drying during a few days (for example, by bagging the batch) seems a more homogeneous and controllable method for decreasing the drying rate.

More research is needed to understand the role and the growth conditions of the different microbial populations, as well as their relationship with methanol fermentation, ethanol fermentation and amino acid degradation.

8 General discussion

8.1 Integration of analytical methods and Descriptive Cupping

Chapter 2 introduced the concept of using a sensory-directed approach to interpreting the flavour of complex products such as coffee and to understanding the abundant analytical data. Interpreting analytical data without the guidance of sensory tools can potentially be misleading. An example of this last situation is the study by Gonzalez-Rios et al. (2007a), who concluded *"coffee produced by the traditional wet method seemed to have a better aroma quality than coffee produced by the mechanical method"* based solely on the number of 'pleasant' and 'unpleasant' compounds as detected by GC-O. Cases like this are the reason why Sunarharum et al. (2014) insist that *"matching or creating a comprehensive link on all components of coffee flavor and sensory quality will lead to a deeper understanding of coffee flavor"*.

The work by Charles et al. (2015) is a choice example of a study integrating both sensory and instrumental analyses, which serves to illustrate the importance of the matrix effect on flavour. These authors investigated the effect of roast degree and sugar addition on aroma release and perception in espresso coffee. The sensory aspect was measured through Temporal Dominance of Sensations (TDS), a method that records the dominant attribute perceived (from a list of 9 attributes) along time, for 1 minute after the espresso consumption starts. The analytical aspect was measured through analysis of the coffee headspace (by PTR-ToF-MS) and through sampling of the 'nosespace' of assessors at the same time that they rated the espresso samples. This ensured both the sensory and instrumental analyses were done on the same sample, the same subject and the same conditions. The addition of sugar did not affect the aroma release of coffee analytically, but it changed the flavour perception of coffee, with increased intensity of the caramel, roasted and nutty flavour notes perceived. This is explained as a cross-modal interaction between taste and flavour and could not be predicted from the headspace analysis alone.

In the present research, an effort to integrate analytical and sensory data was made. Analytical data were collected from the green and roasted samples using PTR-MS and GC-MS/O respectively. In this case, the analytical and sensory data were not collected at the same time, but the same roasted samples were used for both analyses. Methodologically speaking, the

main innovation in the present research was the introduction of Descriptive Cupping as a sensory method (*Objective 1*, 2.2.2.1 – see also Chapters 3, 4 and 6).

Descriptive Cupping has some advantages as a sensory method, but also some limitations. Its main advantages are the availability of a large global corpus of cupping data which may be analysed retroactively and the availability of thousands of licensed 'Q-Graders'. Q-Graders are available in many coffee-producing countries, most of which do not have access to trained sensory panels. In addition, Q-Graders have been screened, tested with a licensing exam and have been trained in a common descriptor vocabulary. This training includes the use of descriptive terms in conjunction with reference standards (Le Nez du Café®) in a manner similar to descriptive analysis. In most cases, Q-Graders are continually assessing coffee and thus little or no further training is required. As it has been shown in Chapter 3, the use of a small number of Q-Graders can still produce coherent results. This is probably due to their high level of expertise in coffee assessment. The comparison of the Descriptive Cupping results from two panels, in Chapter 6, demonstrated the method is largely reproducible, regardless of the cultural and training differences among cuppers.

Nevertheless, the perception of attributes by cuppers is influenced by their own cupping training, which emphasises the scoring of coffees based on quality criteria. This situation may be an advantage of Descriptive Cupping when the link between the flavour profile and the perception of coffee 'quality' is being investigated. However, it may also become a limitation for gathering 'objective' and 'unbiased' data. There may be a tendency of cuppers to focus on taints or undesirable flavour notes, such as the earthy notes in Chapter 3 or the phenolic notes in Chapter 4. Anyhow, any kind of sensory assessor training unavoidably implies a degree of bias, as there is an interplay between perception and the descriptive vocabulary learned during training (Lawless & Heymann, 2010). Though the Descriptive Cupping results are coherent, the validity needs to be further investigated through comparison with sensory methods. The investigation examining the relationship between Descriptive Cupping and descriptive sensory methods is outside the scope of the present research but is currently being carried out at the University of Otago by another student, showing a good agreement between both methods.

This research was conducted under the principle that the best guidance in the understanding of an extremely complex food matrix, such as coffee, is the sensory interpretation. Analytical

methods such as the details of the SPE extraction methodology were developed with the guidance of sensory assessments. The Descriptive Cupping results, be it by themselves or within an MFA, guided the interpretation of all analytical results.

8.2 The flavour of natural coffee in broad context

Along this research, analytical and sensory methods have been combined with a focus on the flavour of natural coffees. Is there such a thing as a 'natural coffee character' (*Objective 2*, 2.2.2.2)? The answer is not simple. The results from both Chapter 3 and Chapter 4 suggest there is a large range of common flavours in the coffees produced by different methods, while there are some flavour descriptors that tend to be specific to a processing method.

The area of common flavours between the different processing methods is what could be considered the most 'characteristic' coffee flavour notes. In Chapter 3, the common flavours are most clearly found around the *vegetable*, *caramelly*, *chocolate*, *spicy*, *resinous* and *earthy* subgroups (Figures 3.2 and 3.3), as samples from any processing method can show these characters. All samples in Chapter 3 come from the same Mexican state and thus variability due to the effect of terroir should not be as important as variability from the processing methods. In the case of Chapter 4 (Figures 4.1 and 4.2), the terroir effect is larger than the effect of processing and samples tend to be distributed geographically. Nevertheless, there is also flavour overlap of samples from different processing methods around the following subgroups: *vegetable*, *dried-fruity*, *caramelly*, *nutty*, *toasty*, *chocolaty spicy*, *pyrolytic*, *resinous*, *pungent*, *fungal*, *phenolic*, *woody*, *bitter-taste*, *dry-acid*, *astringent*, *earthy*, *past-croppish* and *rough body*. The variability of the sample set is wider for the study in Chapter 4; nevertheless, all the subgroups which were found to be common to the different processing methods in Chapter 3 were also found in Chapter 4. *Caramelly*, *nutty*, *toasty* and *chocolaty* flavour notes are all closely related to Maillard reactions and are thus at the core of the 'characteristic' coffee flavour. In Chapter 5, these flavour notes were linked mostly to pyrazines. Even though the processing method can influence the composition of flavour precursors – namely sugars and amino acids as precursors of the Maillard reaction (Selmar et al., 2014) – the resulting flavour of some coffees still falls in this region irrespective of their processing method.

Nevertheless, there are some areas in the coffee flavour spectrum that seem populated only by samples from a specific processing method. In the case of Chapter 3 (Figures 3.2 and 3.3), these areas seem to be the *floral* subgroup for washed coffees, and the *fermented* and *fruity* subgroups for the natural coffees. In Chapter 4 (Figures 4.1 and 4.2), the *floral* and *citrus-like* subgroups seem to be populated exclusively by washed coffees, while *red-fruity*, *tropical-fruity* and *sweet-acid* are populated exclusively by naturals. These results are consistent for the two sample sets, which were evaluated in two different countries by two different panels.

The study of the *floral* flavour note for the washed coffees is outside the scope of this research. The study in Chapter 5 showed some flowery GC-O compounds (Table 5.6), but none of them were linked to particular *floral* samples. On the other hand, the case of the *fermented* and *fruity* subgroups as specific to natural coffees has been discussed extensively in the preceding chapters and will be further explored in the following sections.

Summarising – no, there is not such a thing as the 'natural coffee' character. There are natural coffees that could be easily confused for washed coffees as they may lack all the attributes considered 'characteristic' of natural coffees, such as fruitiness or wineyness. These are natural coffees predominantly chocolaty or caramelly, or perhaps vegetable or phenolic. Some taints and defects, in particular, can appear due to any processing method. Then again, there are natural coffees that unmistakeably display the 'characteristic' natural coffee profile, yet that does not mean all these 'characteristic' coffees have the same flavour. Some of them are dried-fruity, some are red-fruity, some seem tropical-fruity yet others could be described as winey, fermented, fungal or a combination of these characters.

8.3 The 'Mocha' character as a natural coffee 'style'

In complex products, such as beer, categorisation according to 'style' is essential in order to understand a market's preference for a particular profile as well as to analyse and compare the wide range of flavour expressions in a coherent manner. In the case of beer, 'style' is a concept *"used to differentiate and categorise beer by various factors such as colour, flavour, strength, ingredients, production method, recipe, history, or origin"*, although a single classification of beer styles is impossible to make, as different associations classify beers differently (Arfelli & Sartini, 2014). Since coffee is a tropical product originated outside the main consuming countries, the concept of 'coffee style' has mostly been used to describe a

roasting method or a manner of brewing and serving coffee. Green coffee, on the other hand, has mostly been defined by its region of origin instead of by a deliberate 'style' (Ukers, 1922). The only categorisation of green coffees that approaches the concept of style is the classification, according to processing method, in natural, washed and pulped natural coffees (Brando & Brando, 2014). However, a broad category, such as 'natural coffee', has been described in conflicting terms, often within the same document. For example, Ukers (1922) said *"generally speaking, washed coffees will always command a premium over coffees dried in the pulp"*, while he also stated that *"for many generations, Mocha coffee* [a natural coffee] *has been recognized throughout the world as the best coffee obtainable"*. The author was clearly distinguishing 'Mocha' coffee from generic naturals. In other words, 'Mocha' has been acknowledged as a specialty 'style' within the natural coffees, even though the definition of the term 'Mocha' is very vague and has changed over time.

The use of the term 'Mocha' is particularly relevant for the scope of this research. 'Mocha' is the name of a Yemeni port on the Red Sea. Between the 15th and the 17th Centuries, Mocha was the main exporting port for all coffee trade. Coffee exported from this port was named 'Mocha'. A Dutch coffee and tea price list from 1774 lists the *"Levants of Mocha"* coffee at a price between 50% and 260% higher than other origins (Haggam, 2012). The term 'Mocha' has been associated with coffee for so long that it effectively means completely different things in different countries and contexts. Nowadays, the term 'Mocha' can mean a mixture of coffee and chocolate, a use first recorded in 1849 (Harper, 2015). 'Moka' is also the brand name of the stovetop coffee maker, patented by Alfonso Bialetti in 1933. In Brazil, 'Moca' is the name given to the 'peaberry', which is a coffee bean with a characteristic rod shape (Mendonça et al., 2005). In Colombia, 'Moka' is the name of a coffee varietal with very small beans (XUECafé, 2012).

For the purpose of this research, the term 'Mocha' refers to a specific natural coffee flavour or style, originally related to the flavour of Yemeni coffees that were exported from the port of Mocha, but now referring to any coffee with the same character. Ukers (1922) described the flavour of 'Mocha' beans as having a "*distinctive winy flavour and is heavy with acidity* […], *smooth and delicious*". The flavour of coffee from Harrar, Ethiopia (another natural to this date) was described as having *"cup characteristics resembling Mocha, and by some are preferred to Mocha because of their winier cup flavour*". The Mexican Tepic, which is still

mostly natural, was described as the "*so called 'Mexican Mocha'*" (Ukers, 1922). For Akiyama et al. (2008), the 'Mocha' character implies an elegant, fermented, fruity, winey and spicy odour.

This research has viewed the flavour of natural coffee from different points of view. Chapter 3 focused on the differences between the main processing methods (*Objective 2*, 2.2.2.2) within a single processing region; Chapter 4 focused on characterising the flavour of natural coffee from different countries (*Objective 3*, 2.2.2.3), using other processing methods as a point of reference only, and Chapter 6 looked at different natural coffee treatments using a uniform, controlled raw material (*Objective 5*, 2.2.2.5). In all cases, fruitiness and wineyness – the 'Mocha' character – have stood out both as characters strongly influenced by processing and as highly discriminant characters. 'Mocha' coffees are distinct from other washed and pulped-natural coffees and even from some natural coffees that do not present fruitiness. In Chapter 4, it is particularly evident that naturals not presenting fruitiness (such as the studied Brazilian samples) actually approach more the flavour of their washed counterparts than the flavour of 'Mocha' style naturals (such as the Nicaraguan samples studied). Among all the samples studied, only natural coffee samples have shown fresh red-fruity and tropical-fruity characters. While the different processing methods seem to have a common flavour for most characters, only naturals seem able to present a clear 'Mocha' style profile (see, for example, Chapter 3). This represents an opportunity for natural coffee producers to differentiate through the different expressions of the 'Mocha' character.

The χ^2 per-cell analyses from the characterisation studies show that, in fact, the fruitiness or wineyness of a natural coffee can range greatly in its expression. This range can go from a dried-fruit character, still shared by some pulped naturals (samples 26N and 29PN from Chapter 3), to a tropical-fruity character (samples 3N, 15N, 51N in Chapter 3, and sample NNB4 in Chapter 4), a red-fruity character (samples NNB5, NNC, NNR from Chapter 4) or even a fermented character, usually implying a degree of wineyness (sample 21PN from Chapter 3 – a case of a pulped natural sharing this character, and sample NNB2 from Chapter 4). Any of these samples could have been classified as 'Mocha'. A better understanding of natural coffee flavour will lead to a better categorisation of the natural coffee styles. An abstract generalisation of these categories is proposed in Figure 8.1. The coffees currently meeting the 'Mocha' style could be further categorised into those with a fresh fruity character

(including red-fruity and tropical-fruity) and fermented character (including winey/fermented and fungal). This categorisation would help to better understand the links between processing and flavour, and would also facilitate the marketing of those natural coffees.

Figure 8.1 also shows an arrow from left to right, from the non-fruity character to the fermented character, showing the general sense of fermentation intensity during processing. Without going into the specifics, there is a general relationship between the drying rate, the degree of fermentation and the resulting flavour. The Brazilian samples used (Chapter 4), for example, which were dried in under a few days using mechanical dryers, tend to be the least fruity samples, if not completely 'non-fruity'. The so-called 'honeying' treatment (Chapter 6), which was fermented for two days prior to drying, is a good example of the 'winey' or 'fermented' category. Of course, other characteristic coffee flavour notes, such as chocolaty, caramelly and pyrolytic, may still be present in fruity naturals. Combinations of, for example, red-fruity and chocolate are not uncommon in naturals and many samples of this study have been described as such. Samples between two styles are not uncommon either; for example, sample 3A in Chapter 6, combines both tropical-fruity and fermented characters. This figure is a broad generalisation, yet it is useful to convey the concept of different natural coffee styles to producers, other members of the coffee industry and consumers.

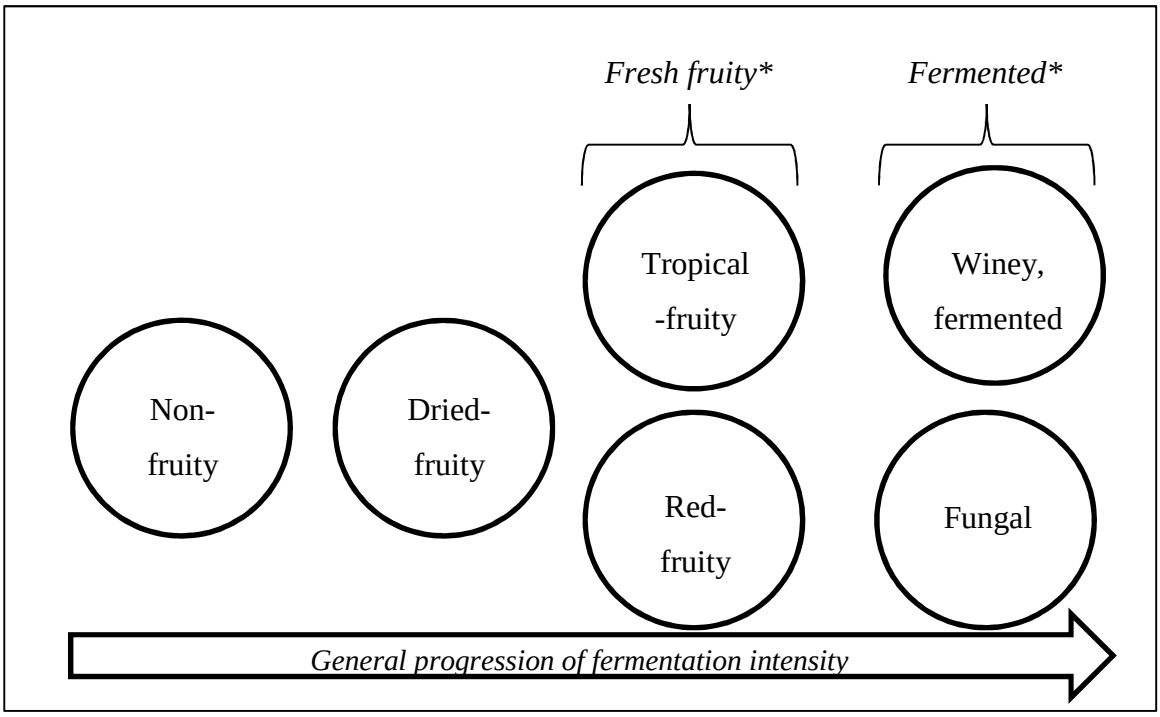


Figure 8.1. Generalisation of natural coffee styles. (*) - Currently considered 'Mocha' naturals.

8.4 Formation of the 'Mocha' character in naturals

The research conducted at the Institute of Plant Biology (IPB) of Technical University Braunschweig (Germany) for over a decade has made it very clear that the processing method (natural, washed, pulped natural) strongly influences the metabolic reactions in the living coffee bean during processing (including the initiation of seed germination), while these reactions in turn affect the composition of flavour precursors in the bean, namely amino acids, sugars and chlorogenic acids (Bytof et al., 2007; Bytof et al., 2005; Bytof, Selmar, & Schieberle, 2000; Knopp et al., 2006; Selmar et al., 2001; Selmar, Bytof, Knopp, & Breitenstein, 2006; Selmar et al., 2014). These differences in the flavour precursors (2.1.2.2) in turn can explain the presence of different flavour compounds after roasting. Both amino acids and sugars are the main precursors of Maillard reactions. Thus, the different profiles of amino acids and sugars due to different processing methods cause differences in the composition of Maillard products after roasting (Selmar et al., 2014), which are key to coffee flavour. Therefore, flavour differences in coffee processed by different methods have been explained as caused mostly by metabolic differences of the bean during processing.

On the other hand, the importance of microbial fermentation during natural coffee processing has been indicated by a research group from the Universidade Federal de Lavras (Brazil) (Evangelista et al., 2014; Silva, 2014; Silva et al., 2008; Silva, Schwan, Sousa Dias, & Wheals, 2000). The microbiota present during the drying of natural coffee processing in Brazil has been characterised (Silva, 2014; Silva et al., 2008) and the effect of specific yeast strains inoculated prior to drying on the flavour and volatiles profiles has been assessed. Out of four coffees inoculated with separate yeast strains (*Saccharomyces cerevisiae* UFLA YCN727, *S. cerevisiae* UFLA YCN724, *Candida parapsilosis* UFLA YCN448 and *Pichia guilliermondii* UFLA YCN731), only the coffee inoculated with UFLA YCN727 produced a distinct fruity beverage, while the coffee inoculated with UFLA YCN724 produced a fermented flavour note (Evangelista et al., 2014).

The flavour of complex products like coffee is affected by many factors and these two research approaches are complementary. The study of the coffee bean physiology from the IPB explains many of the differences between the processes from the flavour precursor point of view, but does not explain why some naturals are fruitier or are more intense in winey character than others. The multidisciplinary studies of natural coffee fermentation in Brazil, involving microbiology characterisation, headspace analysis and sensory analysis have made it clear that something as subtle as the specific strain of *Saccharomyces cerevisiae* yeast inoculated will affect the coffee flavour. However, to the best of our knowledge, the possible metabolic or chemical links between the coffee fermentation and the resulting flavour characters have not been stated by any researcher.

Thus, the main contribution of the present research to the understanding of flavour formation in natural coffees (*Objective 4*, 2.2.2.4 and *Objective 5*, 2.2.2.5) is the proposed link between the ‘Mocha’ character (fruitiness and/or wineyness) and two separate types of catabolism. On one side, the catabolism of valine, isoleucine and leucine (probably microbial, although it could also be due to the coffee bean metabolism) gives way to the three families of volatile organic compounds summarised in Table 8.1 (Roze et al., 2010; Thonning Olesen & Stahnke, 2004). Even though some of these compounds, namely the aldehydes, can also be created during roasting through Strecker degradation (Flament & Bessière-Thomas, 2002) the main formation pathway of these compounds is catabolic, as the headspace study of the green beans suggests (5.3.1, 7.3.1).The odour character of most of those compounds is fruity or fermented,

as has been confirmed in the GC-MS/O study (5.3.2), and more importantly, their presence is correlated to the *fruity* character.

Table 8.1. Volatile compounds derived from the catabolism of branched-chain amino acids and their odour character. Adapted (Roze et al., 2010; Thonning Olesen & Stahnke, 2004).

Chemical class	From the catabolism of **valine**	From the catabolism of **isoleucine**	From the catabolism of **leucine**
Alcohol	2-methylpropan-1ol	2-methyl-1-butanol *Ethereal-fruity* [a] Stinky [b]	3-methyl-1-butanol *Fruity, winey* [a]
Aldehyde	2-methylpropanal *Overripe fruit* [a] Peanuty/Fruity/Chocolate [b]	2-methylbutanal *Fruity, fermented* [a] Toasted bread/Peanut/Fruit [b]	3-methylbutanal *Fruity* [a] Toasted bread/Peanut/Fruit [b]
Carboxylic acid	2-methylpropanoic acid *Acid odour, fruity* [a] Tropical fruit [b]	2-methylbutanoic acid *Fruity* [a] Fermented/Tropical fruit [b]	3-methylbutanoic acid *Fruity, cheesy* [a] Fermented/Tropical fruit [b]
Ethyl ester	Ethyl 2-methylpropanoate *Ethereal, fruity, sweet, pine-cone* [a]	Ethyl 2-methylbutanoate *Apricot, apple, strawberry* [a] Strawberry [b]	Ethyl 3-methylbutanoate *Blueberry, fruity* [a] Blueberry [b]

(a) Aroma descriptors in italic taken from Flament and Bessière-Thomas (2002).
(b) Underlined descriptors taken from GC-MS/O study (5.3.2)

On the other side, the ethanol fermentation (most likely by yeasts – Chapter 7) enables the production of ethyl esters, for example ethyl acetate. Ethyl esters have a lower odour threshold (they are more odour-active) than methyl esters (Flament & Bessière-Thomas, 2002) and also present a fruity character. Ethyl 2-methylbutanoate and ethyl 3-methylbutanoate – the ethyl esters of the branched-chain acids from Table 8.1 – are the two esters most closely related to the red-fruity character of some naturals, as shown in Chapters 5 and 7. These two esters have been associated in the past with the over-fermented defect (Bade-Wegner et al., 1997; Guyot & Vincent, 1982). Nevertheless, these two esters are so highly odour-active that – provided that the fermentation has not depleted the sugars and free amino acids as suggested by Silva (2014) – they can still impart a fresh fruity character without affecting the cup quality.

As the understanding of these flavour formation pathways evolves in the future, technologies will become available for producers to deliberately create and maintain their own natural coffee 'style'. The coffee drying curves can be controlled using low-level technology tools that are available to any coffee farmer, as it was shown in Chapters 6 and 7. It should be

possible, for example, to consistently produce enough fruity esters for the coffee to be noticeably fruity without incurring over-fermentation. More sophisticated drying systems and specific fermentation inocula may further help obtain a particular flavour outcome. Nevertheless, these processes could only be able to modify the substrate given by the combination of environmental and genetic factors that produce a unique coffee in every terroir.

8.5 Future directions of research

From the methodological point of view, a validation of the Descriptive Cupping methodology is required. This research has demonstrated the coherence and meaningfulness of the Descriptive Cupping methodology when compared to analytical analyses. Nevertheless, a comparison with accepted descriptive sensory methodologies needs to be made. This study is currently being carried out at the Department of Food Science of the University of Otago – so far showing a good agreement between both methods – and is expected to be completed during 2015.

From the point of view of natural coffee flavour, there are many unresolved questions that should be addressed by future researchers. They are listed below in bullet format.

- In this research, the instrumental analyses focused exclusively on the coffee headspace. However, non-volatile compounds also play a key role in coffee flavour. Future research should include the analysis of non-volatile compounds – namely organic acids – and how they are affected by the processing.
- It has been proposed in this research that the catabolism of valine, isoleucine and leucine produces derivatives that play a key role in the development of fruitiness. Is this catabolism mostly microbial or is it due to the coffee bean metabolism during drying? If it is microbial processes, what are the main populations responsible for it and under which conditions? If it is due to the coffee bean metabolism, what are the conditions for it?
- It has been shown that ‘honeying’ or fermenting the coffee cherries during the first few hours after harvest involves the production of ethanol (most likely by yeasts), which plays a role in the formation of fruity esters. How can this fermentation be better controlled so that the formation of fresh fruity flavour notes does not affect the

other cup qualities? This may require the inoculation of a specific strain, as shown by Evangelista et al. (2014) or simply a better control of the 'honeying' conditions using low-level technology.

- This research also suggests that the suspension of drying for a few days once a mid-level water activity has been achieved (the case of Treatment 5 in Chapter 6) can also produce a fruity character. It remains to link this either to microbial or to coffee bean metabolism and to further characterise the mechanism. Suspension of drying at this level of water activity is not as risky as 'honeying' in the sense that a sudden over-fermentation is not as likely. Thus, this technique could prove to be of interest for controlling the final flavour outcome.
- This research also generates the opportunity for new coffee processing types to be explored, for example, a controlled 'honeying' prior to pulping, which could result in a fruity washed coffee.

In general, one question must be addressed – how can the final flavour outcome of coffee be controlled? In such a complex product as coffee, it is obviously unrealistic to believe the complete flavour profile can be fully controlled. However, it would be sufficient for the producer to be able to aim at a 'style', such as red-fruity, tropical-fruity, dried-fruity or winey. That would be a huge advancement compared to the current situation, in which producers basically do not know why their coffee has a particular flavour outcome.

9 Conclusions

The overall aim of this research was to understand the flavour of Arabica natural coffee as a product class and to relate that flavour to its main mechanism of formation during the post-harvest drying process. In order to do that, three different sets of coffee samples were characterised using sensory and analytical methods. To achieve the sensory approach, a methodology called Descriptive Cupping was developed. Descriptive Cupping involves the statistical analysis of the descriptors elicited by coffee cuppers and can be regarded as a rapid profiling of green coffee samples. The analytical methodology involved the headspace analysis of coffee beans, using multiple techniques. The main focus was on the odour-active volatiles of roasted coffee (using SH-GC-MS/O and SPE-GC-MS/O), while the headspace of the green beans was studied as complementary data using PTR-MS to help understand flavour formation.

The first set of coffee samples originated from a single Mexican state (the State of Guerrero), where four processing treatments (natural process, pulped natural process, and two variations of the washed process: fermentation under water and dry fermentation) were applied at different farms. Since the region of origin was relatively compact, the effect of the processing method became apparent. Natural coffees tended to show red fruity, dried fruity, tropical fruity and fermented flavours (Mocha profile); washed coffee (from both washed treatments) tended to show more floral, spicy and nutty flavours; pulped-naturals, as an intermediate method between washed and natural, could be found approaching either profile. Some overlapping cases whereby natural coffees were more similar to the washed profile were also found. This study largely clarified the main effects of the processing method on coffee flavour yet it did not allow the relative importance of these effects to be placed in a broader geographical context.

Therefore, in order to characterise the flavour of natural coffees in a broader geographical context, the second sample set included 22 natural coffee samples from 7 producing countries. Additional samples (10 samples – 9 washed and 1 pulped natural) for use of points of reference from the same farms as some of the natural coffees were included. This study showed a larger geographical origin effect than the processing effect on coffee flavour. Together with the geographical factors, it must be noted that the origin co-varies with different farming systems, as well as different harvesting and processing technologies. These cultural factors may be playing a role at least as important as the physical environment where

coffee is grown in the development of the flavour. Only when natural coffees show a 'Mocha' character (fruitiness or wineyness) do they become clearly different from their matched washed coffee counterparts. The absence or presence of the 'Mocha' character appears more important for flavour variability as a whole than origin itself. Moreover, its absence or presence is also one of the main sources of variation within the natural coffees as a group.

The headspace of the second sample set was characterised analytically. In roasted coffee, important effects on the headspace profile were found from thermal degradation products of sugars and Maillard reaction products (involving amino acids and sugars). However, the main factors explaining the Mocha character seem to be related to both amino acid catabolism (of valine, isoleucine and leucine) and ethanol fermentation. The esters produced by the ethyl esterification of 2- and 3-methylbutanoic acid are more potent odorants than their methyl counterparts and present a fresh, red-fruity character (strawberry, blueberry), which is the most common description of specialty natural coffees.

The third sample set consisted of coffee samples coming from the same raw material and processed through seven different natural treatments. The drying rates of the treatments along the drying period were controlled using low-technology methods available to any grower. These treatments produced different drying curves, which implied a different availability of water for microbial growth and thus different degrees of fermentation of the outer layers of the coffee cherry. Asides from a washed coffee witness, the most distinct treatment was the one termed 'honeying' treatment, which involved slowing down the drying for the first two days. This resulted in an intense fermentation with a high production of ethanol, which confirms ethanol plays a key role in the formation of fruity esters. However, a safer, more controllable way to develop the 'Mocha' character than 'honeying' may be a suspension of drying during the second week of sun-drying, which also resulted in a distinct fruity product. At any rate, a likely explanation for the development of fruitiness or wineyness is the catabolic degradation of valine, isoleucine and leucine (which were not measured), as their metabolic derivatives play a key role in the development of fruity and winey characters in natural coffees. Fast drying (and thus mechanical drying) does not allow the time for these catabolic reactions to occur.

The panel comparison showed a satisfactory correlation between both panels and coherence in the use of descriptor subgroups such as *fermented*. Overall, Descriptive Cupping has been

proved to be a rapid method for profiling coffee flavour with satisfactory levels of discriminating power.

The current specialty coffee market is demanding high-quality naturals possessing a distinct fruity or winey flavour, yet also showing sweetness, acidity, body and a clean cup, free of taints from over-fermentation. This study has shown that the final flavour profile of a natural coffee can be deliberately affected by the processing method, which effectively means it makes sense to begin speaking of natural coffee 'styles', as there are beer or wine styles. Each producer should find the adequate style suitable for his own terroir conditions, his market and his product differentiation strategy. At any rate, this research suggests that deliberately allowing for specific types of microbial growth to occur during drying will have a positive impact in the development of the characteristic fruitiness and wineyness of naturals, as long as the extent of the fermentation is controlled. This also generates the opportunity for new processing types to be explored, for example, a controlled 'honeying' prior to pulping, which could result in a fruity washed coffee.

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11 Appendices

11.1 Appendix 1 – Chapter 3 – Contingency Table

Table 11.1. Contingency table of coffee samples (rows) and descriptor subgroups (columns) for the study in Chapter 3.

Sample	Floral-Ar	Vegetable-Ar	Fruity-Ar	Tropical-Ar	RedFruits-Ar	Caramels-Ar	Chocolates-Ar	Nutty-Ar	DriedFruit-Ar	Spicy-Ar
2N	0	0	1	0	1	3	5	1	0	2
2Wh	0	0	2	0	1	2	3	0	0	1
2Wd	3	0	2	0	1	3	2	1	0	3
3Wh	1	0	1	0	0	1	5	2	0	1
3Wd	2	1	3	0	0	2	3	2	1	1
3N	1	0	1	1	2	2	2	2	0	2
5Wd	2	0	0	0	1	1	5	1	0	2
5N	1	0	3	0	0	2	3	0	0	0
7Wh	0	0	1	0	0	0	4	2	0	2
7Wd	0	0	0	0	0	2	4	2	0	0
7PN	0	0	2	0	0	2	5	1	0	1
7N	0	1	0	1	0	0	3	1	0	0
9Wh	1	0	2	0	0	4	2	2	0	2
9N	1	0	1	2	1	0	7	2	1	1
12PN	1	4	0	0	0	2	2	0	0	3
12N	2	0	1	0	2	1	6	1	2	1
15Wh	0	0	1	0	0	2	4	1	0	3
15PN	0	0	1	0	0	1	5	2	0	1
15N	1	0	3	3	1	0	3	0	0	2
16Wh	0	0	1	0	0	1	7	0	0	3
16N	1	0	3	2	0	2	4	0	1	1
18Wh	1	0	1	0	1	2	5	1	0	1
18N	1	0	3	1	0	2	4	1	0	2
21Wh	2	2	1	0	0	3	3	2	0	3
21Wd	0	0	0	0	0	1	4	2	0	1
21PN	0	1	1	0	0	3	3	1	1	1
21N	3	0	2	0	0	2	5	1	0	2
24PN	0	1	0	0	0	2	5	2	0	0
24N	1	0	1	0	0	3	6	1	1	2
26Wh	0	0	0	0	0	2	3	1	0	0
26Wd	1	1	2	0	0	3	3	0	0	3
26N	0	0	2	0	0	1	4	1	2	0

Sample	Floral-Ar	Vegetable-Ar	Fruity-Ar	Tropical-Ar	RedFruits-Ar	Caramels-Ar	Chocolates-Ar	Nutty-Ar	DriedFruit-Ar	Spicy-Ar
29PN	1	0	2	0	1	2	5	0	1	1
29N	1	0	1	2	1	2	6	0	1	3
36Wh	0	0	1	0	0	3	3	2	0	2
36PN	1	0	2	0	0	3	5	1	0	3
36N	0	0	1	1	0	0	5	3	0	1
49Wh	1	0	0	0	1	4	3	2	0	1
49PN	0	2	0	1	1	1	5	2	0	2
49N	3	0	3	0	0	2	5	1	1	2
51PN	2	0	1	0	0	2	4	2	0	3
51N	0	0	3	1	0	2	4	2	0	0
52PN	3	1	1	0	0	1	5	2	0	1
52N	0	0	1	0	0	2	5	3	0	2
53Wd	0	0	1	0	0	4	4	1	0	1
53PN	0	0	1	0	0	3	6	2	0	0
53N	0	0	3	0	0	3	3	1	0	2
54PN	1	1	1	0	0	5	3	1	0	1
54N	0	0	0	0	0	2	6	2	0	1
55PN	2	2	1	0	0	3	6	2	0	1
55N	1	0	1	0	0	2	4	4	0	1
57Wd	2	1	0	0	0	0	3	3	0	2
57PN	0	1	0	0	0	1	5	2	0	0
57N	0	0	0	0	0	0	3	1	0	0
60PN	1	0	1	1	1	0	5	3	0	3
60N	0	0	1	0	0	3	6	3	0	4

Sample	Pyrolytic-Ar	Resinous-Ar	Fermented-Ar	Phenolic-Ar	Earthy-Ar	Floral-Fl	Vegetable-Fl	Fruity-Fl	Tropical-Fl	RedFruit-Fl
2N	0	0	0	0	2	0	1	1	0	0
2Wh	1	1	0	0	0	0	0	0	0	0
2Wd	0	0	0	0	0	1	0	1	0	0
3Wh	4	0	0	0	2	0	0	0	0	0
3Wd	0	0	0	0	0	0	1	4	0	0
3N	0	2	3	1	0	0	0	1	2	1
5Wd	0	1	0	0	0	0	1	0	0	0
5N	1	0	3	1	0	0	0	1	0	0

Sample	Pyrolytic-Ar	Resinous-Ar	Fermented-Ar	Phenolic-Ar	Earthy-Ar	Floral-Fl	Vegetable-Fl	Fruity-Fl	Tropical-Fl	RedFruit-Fl
7Wh	0	0	0	0	0	0	0	0	0	0
7Wd	1	2	0	0	2	0	0	0	0	0
7PN	0	0	1	1	1	0	1	2	0	0
7N	0	2	0	0	5	0	2	0	0	0
9Wh	1	0	0	0	0	0	0	2	0	0
9N	0	1	1	0	0	0	0	3	0	1
12PN	0	0	1	0	0	0	4	0	0	0
12N	0	2	1	0	0	0	0	3	0	0
15Wh	3	0	0	0	1	0	1	0	0	0
15PN	0	2	0	0	2	1	0	2	0	0
15N	0	1	2	1	0	0	0	3	0	1
16Wh	0	0	0	0	0	1	1	1	0	0
16N	0	1	3	0	0	1	0	1	0	0
18Wh	3	0	0	0	1	0	0	0	0	0
18N	0	2	1	0	2	0	1	2	1	0
21Wh	0	1	0	0	0	0	1	1	0	0
21Wd	1	0	0	0	1	1	1	0	0	0
21PN	0	1	3	0	1	0	0	0	0	0
21N	0	0	0	0	0	1	0	3	0	0
24PN	4	1	0	0	0	0	0	0	0	0
24N	0	1	1	0	0	0	0	2	0	0
26Wh	0	0	0	1	0	0	1	1	0	0
26Wd	1	1	0	0	0	0	1	3	0	0
26N	3	0	2	1	0	0	0	1	0	0
29PN	2	1	2	0	1	0	0	0	0	1
29N	2	0	2	0	0	0	0	1	1	1
36Wh	0	1	0	1	0	0	0	1	0	0
36PN	0	1	0	0	0	0	1	0	0	0
36N	3	2	3	1	0	0	0	1	0	0
49Wh	0	2	0	0	0	0	0	0	0	0
49PN	0	2	0	0	2	0	1	1	1	1
49N	1	1	2	1	0	1	0	1	0	1
51PN	0	2	2	0	0	3	0	1	0	0
51N	0	3	1	0	0	0	0	1	2	0
52PN	0	1	0	0	0	1	1	1	0	0
52N	1	0	2	0	0	0	0	2	0	0
53Wd	5	1	0	0	1	0	0	0	0	0
53PN	2	2	1	2	0	0	0	0	0	0

Sample	Pyrolytic-Ar	Resinous-Ar	Fermented-Ar	Phenolic-Ar	Earthy-Ar	Floral-Fl	Vegetable-Fl	Fruity-Fl	Tropical-Fl	RedFruit-Fl
53N	1	2	0	0	1	0	0	0	0	0
54PN	0	3	0	0	2	0	1	2	0	0
54N	0	0	0	0	2	0	0	1	0	0
55PN	0	1	0	0	2	0	2	3	0	0
55N	0	1	0	0	0	2	0	2	0	0
57Wd	0	2	0	0	0	0	0	2	0	0
57PN	0	0	0	0	4	0	1	1	0	0
57N	6	1	0	1	0	0	0	0	0	0
60PN	0	1	0	0	1	0	0	1	0	0
60N	1	1	0	0	3	0	0	1	0	0

Sample	Caramels-Fl	Chocolates-Fl	Nutty-Fl	DriedFruit-Fl	Spicy-Fl	Pyrolytic-Fl	Resinous-Fl	Fermented-Fl	Phenolic-Fl	Earthy-Fl	Hard cup-Fl
2N	2	3	0	0	0	0	1	0	0	0	0
2Wh	0	2	1	0	1	0	2	0	1	0	1
2Wd	0	1	0	0	1	1	0	0	0	0	0
3Wh	1	2	0	0	1	2	2	0	0	0	0
3Wd	1	2	0	0	0	0	0	0	0	0	0
3N	1	2	0	0	1	1	1	1	1	0	0
5Wd	1	3	1	0	0	0	1	0	0	0	1
5N	0	1	0	0	0	3	3	1	2	0	0
7Wh	0	2	0	0	1	2	0	0	0	0	2
7Wd	1	3	0	0	0	1	1	0	0	0	1
7PN	1	5	1	0	0	0	0	0	0	0	0
7N	0	2	1	0	0	0	2	0	0	4	0
9Wh	2	2	1	0	0	0	0	0	0	0	0
9N	1	1	0	0	0	1	1	2	0	1	0
12PN	1	2	0	0	0	0	0	2	0	0	0
12N	1	4	0	0	0	0	0	1	0	0	0
15Wh	1	2	1	0	1	0	0	0	0	0	1
15PN	0	4	1	0	1	0	1	0	0	2	0
15N	0	4	0	0	0	0	0	3	1	0	0
16Wh	0	2	1	0	1	0	0	0	0	0	0
16N	1	2	1	0	0	1	1	3	1	0	0
18Wh	1	2	0	0	1	1	1	0	0	0	0

Sample	Caramels-Fl	Chocolates-Fl	Nutty-Fl	DriedFruit-Fl	Spicy-Fl	Pyrolytic-Fl	Resinous-Fl	Fermented-Fl	Phenolic-Fl	Earthy-Fl	Hard cup-Fl
18N	1	3	0	0	0	0	1	1	0	1	0
21Wh	2	1	0	0	1	1	0	0	0	0	0
21Wd	1	1	0	0	0	0	0	0	0	1	0
21PN	2	1	0	1	0	0	0	2	0	2	0
21N	1	1	2	0	0	0	1	0	0	0	0
24PN	0	2	2	0	0	1	2	0	0	0	0
24N	0	4	1	0	2	0	0	0	1	0	0
26Wh	3	2	1	0	0	0	0	0	0	0	0
26Wd	3	2	0	0	1	1	0	0	0	0	0
26N	0	1	1	0	0	4	0	2	1	0	0
29PN	0	0	0	3	0	2	1	1	0	0	0
29N	2	4	2	0	0	0	1	1	1	0	0
36Wh	2	2	1	0	0	0	1	0	0	0	1
36PN	0	2	0	0	0	0	1	0	0	0	0
36N	2	5	0	0	0	2	0	1	0	0	0
49Wh	2	3	0	0	0	0	0	0	0	0	0
49PN	1	2	1	0	0	0	0	2	0	0	0
49N	1	2	1	1	0	0	0	1	0	0	0
51PN	2	3	1	0	1	0	1	1	0	0	0
51N	0	1	0	0	0	2	0	1	1	0	0
52PN	4	1	2	0	0	0	0	0	0	0	0
52N	2	1	2	0	1	2	0	0	0	0	0
53Wd	1	1	0	0	0	4	1	0	3	0	0
53PN	1	4	0	0	0	3	0	2	1	0	0
53N	1	2	0	0	0	3	0	0	2	0	0
54PN	2	3	0	0	1	0	1	0	0	2	0
54N	1	3	1	1	0	0	0	0	0	1	0
55PN	0	2	2	0	0	0	0	0	0	2	0
55N	1	2	2	0	0	0	0	0	0	0	0
57Wd	1	2	0	0	2	0	1	0	0	0	0
57PN	1	2	0	1	1	0	1	0	0	4	0
57N	0	3	1	0	0	3	1	1	0	0	0
60PN	1	3	1	0	0	0	0	1	1	2	0
60N	2	4	0	0	0	1	0	1	0	0	0

11.2 Appendix 2 – Chapter 4 – Training program of cupping panel

11.2.1 Training Session 2 – Body

11.2.1.1 Objectives

1. Practice focusing on the sense of smell while building up olfactory vocabulary.
2. Comprehend the concept of *body* in its two dimensions: *heaviness* and *texture*.
3. Practice a full specialty cupping protocol.

11.2.1.2 Session schedule

Time	Activity
2:00 – 2:30	Odour recognition exercise & discussion
2:30 – 2:55	Body heaviness exercise
2:55 – 3:20	Body texture exercise
3:20 – 4:10	Cupping (Focus: Body)
4:10 – 4:30	Discussion

11.2.1.3 Activities

1. Give each participant a concealed aroma mug, coded with a 3-digit random number. Have participants write down descriptors. Rotate mugs until everybody has done all 10 of them.
2. Write down descriptors for each mug on whiteboard. Agree on ‘standard’ descriptor.
3. Present concept of Body heaviness. Participants sample references and practice assessment procedure. Brief discussion.
4. Present concept of Body texture. Participants sample references and practice assessment procedure. Brief discussion.
5. Cup two samples. Focus on body (heaviness and texture).
6. Discuss body results. Discuss other attributes.

11.2.1.4 Key concepts

Body heaviness: The degree to which the coffee mouthfeel is viscous/heavy.

Body smoothness: The degree to which the coffee's texture feels homogeneous and smooth.

Body roughness: The degree to which the coffee's texture feels grainy, like coarse powder.

11.2.1.5 Assessment procedures

Heaviness: Slurp coffee to assess needed strength. Swivel tongue.

Texture: Rub tongue around hard palate.

11.2.1.6 Coffee samples

1. High body reference: *Java Grade 1*
2. Low body reference: *PNG Sigri A*

11.2.1.7 Olfactory references

The olfactory references were presented in a mug, covered by pierced aluminum foil to conceal the references, and capped with a Petri dish to avoid odour release when not in use.

1. Dark chocolate (Whitaker's 70 % cocoa Ghana; 1 square) #477
2. Toasted bread (freshly toasted white bread; ¼ slice) #709
3. Maple syrup (Cottees Maple Syrup Flavoured; 10mL) #774
4. Black earth (from local garden; 20g)) #047
5. Cinnamon (PAMS Ground Cinnamon; 5g) #962
6. Coriander seed (freshly ground coriander seed; 10g) #214
7. Molasses (Blue Label Molasses; 15mL) #475
8. Almond essence (1mL) #186

9. Cucumber peel (15g) #764

10. Beef stock (Sensient Protex 3496; 1g) #926

11.2.1.8 Body (mouthfeel) references

The following references were served in 60 mL clear plastic cups and presented to the cuppers in a classroom setting. The cuppers assessed heaviness by slurping the reference to assess needed strength and swiveling the tongue. Texture was assessed by rubbing the tongue around hard palate. After assessing the references, the panel leader led the discussion.

Smoothness: Diluted drinking yoghurt (1:5).

Roughness: Turkish coffee – prepared with coffee ground to a fine powder and brewed using 14 g of coffee for each 60 mL water.

Wateriness: Drinking water.

Heaviness: Diluted coffee creamer (30 g /100 mL).

11.2.2 Training Session 3 – Acidity

11.2.2.1 Objectives

1. Build up olfactory vocabulary by focusing on identification of odours.

2. Comprehend the concept of *acidity* in its two dimensions: *intensity* and *quality*.

3. Practice a full specialty cupping protocol.

11.2.2.2 Session schedule

Time	Activity
2:00 – 2:30	Odour recognition exercise & discussion
2:30 – 2:45	Acidity intensity exercise
2:45 – 3:20	Acidity quality exercise
3:20 – 4:10	Cupping (Focus: Body)
4:10 – 4:30	Discussion

11.2.2.3 Activities

1. Give each participant a concealed aroma mug, coded with a 3-digit random number. Have participants write down descriptors. Rotate mugs until everybody has done all 10 of them.

2. Write down descriptors for each mug on whiteboard. Agree on 'standard' descriptor.

3. Present concept of Acidity intensity. Participants sample references and rank their sourness. Brief discussion.

4. Present concept of Acidity quality. Participants taste control coffee brew and describe it. Then taste spiked brews and describe them. Brief discussion.

5. Cup two samples. Focus on Acidity (intensity and quality).

6. Discuss acidity results. Discuss body results. Discuss other attributes.

11.2.2.4 Key concepts

Acidity intensity: The perceived intensity of sour taste in a brew (SCAA, 2009a).

Juicy or sweet acidity: the one modulated by sweetness and often related to malic acid. It is considered desirable.

Sharp or bright acidity: a very clean, almost mineral acidity, often related to phosphoric acid. It is considered desirable.

Sour or winey acidity: a pungent acidity related to volatile acids such as acetic acid. It is considered undesirable when it is overpowering.

Grassy or herbal acidity: a very dry, astringent acidity related to quinic acid. It is considered undesirable.

11.2.2.5 Assessment procedures

Acidity intensity: focus on the intensity of sour taste; for some people, it becomes clearer at the sides of the tongue, but is perceived all over tongue.

Acidity quality: focus on the way the sour taste is modulated by other tastes, on any perceived pungency, tingling or astringency associated to the perceived acidity.

11.2.2.6 Coffee samples

1. Juicy acidity reference: *Guatemala Huehuetenango (86.8% roast/Green ratio).*
2. Sharp acidity reference: *Kenya AB (86.8% roast/Green ratio).*

11.2.2.7 Olfactory references

The olfactory references were presented in a mug, covered by pierced aluminum foil to conceal the references, and capped with a Petri dish to avoid odour release when not in use.

1. Buttery (Ezipop Popcorn Microwave Extra Butter; 20g) #370
2. Lemon (lemon flavour; 50µL on a cotton ball) #454
3. Caramel (Monin Caramel Sauce; 10mL) #819
4. Biscuit (Arnotts Plain Biscuits Marie; 20g) #394
5. Liquorice (Black Knight Licorice Twist; 1 twist) #047
6. Beeshoney (Pams Clover Honey; 10g) #502
7. Redcurrant jelly (Barkers Red Currant Jelly; 20g) #642
8. Hazelnut (Hazelnut flavor; 50µL on cotton) #743
9. Smokey flavour (50µL on a cotton ball) #018
10. Açaí flavour (50µL on a cotton ball) #381

11.2.2.8 Acidity intensity references

The references for acidity intensity were presented to cuppers in 60 mL clear plastic cups, in a classroom setting. Cuppers slurped the references and assessed the intensity of the sour taste. Cuppers ranked the references from the lowest to the highest intensity. The panel leader led the discussion of the results.

Low acidity – Citric acid solution 0.25g/L

Medium acidity – Citric acid solution 0.50g/L

High acidity – Citric acid solution 1.00g/L

11.2.2.9 Acidity quality references

A control brew (2L) was prepared using Brazilian coffee (Moreninha Formosa, 40g/L). The control brew was presented to the cuppers (120 mL, in cupping mugs). After the cuppers assessed the control brew by slurping it and tasting it, each one of five cups containing 200 mL of the control brew was spiked with 2 mL of a different organic acid (0.1 Molar solutions of Acetic, Citric, Lactic, Malic and Phosphoric acids). The cuppers slurped the spiked brews and focused on the way the sour taste is modulated by other tastes, on any perceived pungency, tingling or astringency associated to the perceived acidity. The perceptions were then discussed in a discussion led by the panel leader.

11.2.3 Training Session 4 – Aftertaste

11.2.3.1 Objectives

1. Assess recall of previous odour descriptors.

2. Comprehend the concept of *aftertaste* in its two dimensions: *duration* and *quality*.

3. Practice a full specialty cupping protocol.

11.2.3.2 Session schedule

Time	Activity
2:00 – 2:30	Odour recognition exercise & discussion
2:30 – 3:00	Explanation of Aftertaste
3:00 – 4:00	Cupping (Focus: Aftertaste)
4:00 – 4:30	Discussion

11.2.3.3 Activities

1. Give each participant a concealed aroma mug (already known from previous sessions), coded with a 3-digit random number. Have participants write down descriptors. Rotate mugs until everybody has done all 10 of them.

2. Write down descriptors for each mug on whiteboard. Agree on ‘standard’ descriptor.

3. Present concept of Aftertaste. Explain duration and quality of aftertaste.

4. Cup two samples. Focus on Aftertaste (duration and quality).

5. Discuss aftertaste results. Discuss acidity and body results. Discuss other attributes.

11.2.3.4 Key concepts

> *“Aftertaste is defined as the length of positive flavor (taste and aroma) qualities emanating from the back of the palate and remaining after the coffee is expectorated or swallowed. If the aftertaste were short or unpleasant, a lower score would be given” (SCAA, 2009a).*

11.2.3.5 Assessment procedures

When the sample has cooled to about 70º C, 8-10 minutes from infusion, evaluation of the liquor should begin. The liquor is aspirated into the mouth in such a way as to cover as much area as possible, especially the tongue and upper palate. Because the retro nasal vapors are at their maximum intensity at these elevated temperatures, Aftertaste is rated at this point.

11.2.3.6 Coffee samples

1. Long aftertaste reference: *Ethiopian Harrar.*

2. Short aftertaste reference: *Mexico Altura.*

11.2.3.7 Olfactory references

The olfactory references were presented in a mug, covered by pierced aluminum foil to conceal the references, and capped with a Petri dish to avoid odour release when not in use.

1. Buttery (Ezipop Popcorn Microwave Extra Butter; 20g) #092

2. Lemon (lemon flavour; 50µL on a cotton ball) #746

3. Redcurrant jelly (Barkers Red Currant Jelly; 20g) #250

4. Biscuit (Arnotts Plain Biscuits Marie; 20g) #209

5. Liquorice (Black Knight Licorice Twist; 1 twist) #339
6. Coriander seed (freshly ground coriander seed; 10g) #487
7. Molasses (Blue Label Molasses; 15mL) #298
8. Almond essence (1mL) #545
9. Cucumber peel (15g) #794
10. Maple syrup (Cottees Maple Syrup Flavoured; 10mL) #811

11.2.4 Training Session 5 – Flavour

11.2.4.1 Objectives

1. Build up olfactory vocabulary by focusing on identification of odours.
2. Comprehend the concept of *flavour* in its two dimensions (*intensity* and *quality)* and its two main components (*taste* and *nose*).
3. Practice a full specialty cupping protocol.

11.2.4.2 Session schedule

Time	Activity
2:00 – 2:30	Odour recognition exercise & discussion
2:30 – 2:50	Taste recognition and ranking exercise
2:50 – 3:20	Taste blends exercise
3:20 – 4:10	Cupping (Focus: Body)
4:10 – 4:30	Discussion

11.2.4.3 Activities

1. Give each participant a concealed aroma mug, coded with a 3-digit random number. Have participants write down descriptors. Rotate mugs until everybody has done all 10 of them.
2. Write down descriptors for each mug on whiteboard. Agree on ‘standard’ descriptor.

3. Present concept of *Flavour*. Present concepts of *taste*, *basic taste*s and *taste intensity*. Participants sample references, classify them under sour, sweet or salty, and rank their intensity. Brief discussion.

4. Present concept of *taste modulation*. Participants taste blends of basic tastes and describe them. Brief discussion.

5. Cup two samples. Focus on Flavour (intensity and quality; taste and nose).

6. Discuss flavour results. Discuss other attributes.

11.2.4.4 Key concepts

> *"Flavor represents the coffee's principal character, the 'mid-range' notes, in between the first impressions given by the coffee's first aroma and acidity to its final aftertaste. It is a combined impression of all the gustatory (taste bud) sensations and retro nasal aromas that go from the mouth to nose. The score given for Flavor should account for the intensity, quality and complexity of its combined taste and aroma, experienced when the coffee is slurped into the mouth vigorously so as to involve the entire palate in the evaluation" (SCAA, 2009a).*

Taste: Taste is the sensation produced when a substance in the mouth reacts chemically with receptors of taste buds.

Basic tastes: Salty, bitter, sweet, sour and umami. In the case of coffee, umami is not relevant.

Taste modulation: the effect that the combination of different tastes has on the overall perceived taste. The perception of a taste can be either enhanced or reduced by the presence of compounds that are not directly related to that taste.

Nose: The sensation of the vapours released from brewed coffee as they are exhaled by the movement of the larynx while swallowing (Lingle: *Coffee Cuppers Handbook*).

11.2.4.5 Assessment procedures

When the sample has cooled to about 70º C, 8-10 minutes from infusion, evaluation of the liquor should begin. The liquor is aspirated into the mouth in such a way as to cover as much area as possible, especially the tongue and upper palate. Because the retro nasal vapors are at

their maximum intensity at these elevated temperatures, Flavor and Aftertaste are rated at this point.

11.2.4.6 Coffee samples

1. Complex flavour: *Ethiopia Yirgacheffe*..
2. Simple flavour: *Brazil Moreninha Formosa.*

11.2.4.7 Olfactory references

The olfactory references were presented in a mug, covered by pierced aluminum foil to conceal the references, and capped with a Petri dish to avoid odour release when not in use.

1. Ground cloves (5g) #996
2. Apricot (Apricot jam; 20g) #393
3. Jasmine tea (4g) #160
4. Prunes (20g) #377
5. Raisins (20g) #446
6. Beeshoney (Pams Clover Honey; 10g) #651
7. Dark chocolate (Whitaker's 70 % cocoa Ghana; 1 square) #171
8. Cinnamon (PAMS Ground Cinnamon; 5g) #941
9. Smokey flavour (50µL, on cotton) #402
10. Açaí flavour (50µL on cotton ball) #015

11.2.4.8 Basic tastes references

Basic taste references, prepared as shown below, were presented to the cuppers in a classroom setting, in 60 mL clear plastic cups coded with a three-digit number and in a random arrangement. The cuppers were asked to (a) identify the basic taste of each reference and (b)

rank the references of the same taste in order of intensity. After the exercise, the answers were provided by the panel leader.

Sour I – Citric acid solution 0.25g/L	#338
Sour II – Citric acid solution 0.50g/L	#428
Sour III – Citric acid solution 1.00g/L	#183
Sweet I – Refined sugar solution 7.50g/L	#142
Sweet II – Refined sugar solution 15.00g/L	#282
Sweet II – Refined sugar solution 22.50g/L	#275
Salty I – NaCl 1.00g/L	#236
Salty II – NaCl 2.00g/L	#440
Salty III – NaCl 3.00g/L	#673

11.2.4.9 Taste blends references

Three references containing blends of the basic tastes, prepared as shown below, were presented to the cuppers in 60 mL clear plastic cups. The cups were coded with three-digit numbers and presented in a random arrangement, in a classroom setting. The cuppers were asked to describe the blend, including the basic tastes and their intensities. The correct answers were discussed in the group.

Blend #507 – Sour III + Sweet I

Blend #227 – Sour II + Sweet II

Blend #433 – Sour I + Salty II

11.2.5 Training Session 6 – Fragrance/Aroma

11.2.5.1 Objectives

1. Assess recall of previous odour descriptors.

2. Comp rehend the concepts of *fragrance* and *aroma* in their two dimensions (*intensity* and *quality)* and their three moments of assessment (dry, wet and crust).

3. Practice a full specialty cupping protocol.

11.2.5.2 Session schedule

Time	Activity
2:00 – 2:30	Odour recognition exercise & discussion
2:30 – 3:20	Le Nez du Café exercise (if available)
3:20 – 4:10	Cupping (Focus: Fragrance/Aroma)
4:10 – 4:30	Discussion and homework

11.2.5.3 Activities

1. Give each participant a concealed aroma mug, coded with a 3-digit random number. Have participants write down descriptors. Rotate mugs until everybody has done all 10 of them.

2. Write down descriptors for each mug on whiteboard. Agree on 'standard' descriptor.

3. Explain the assessment procedure for Fragrance and Aroma while cupping.

4. Cup two samples. Focus on Fragrance/Aroma (intensity and quality; dry, wet and crust).

5. Discuss flavour results. Discuss other attributes.

6. Give cupping homework with cupping results in closed envelope.

11.2.5.4 Key concepts

Fragrance/Aroma: The aromatic aspects include Fragrance (defined as the smell of the ground coffee when still dry) and Aroma (the smell of the coffee when infused with hot water). One can evaluate this at three distinct steps in the cupping process: (1) sniffing the grounds placed into the cup before pouring water onto the coffee; (2) sniffing the aromas released while breaking the crust; and (3) sniffing the aromas released as the coffee steeps. Specific aromas can be noted under 'qualities' and the intensity of the dry, break, and wet aroma aspects noted on the 5-point vertical scales. The score finally given should reflect the preference of all three aspects of a sample's Fragrance/Aroma (SCAA, 2009a).

11.2.5.5 Assessment procedures

1. Within 15 minutes after samples have been ground, the dry fragrance of the samples should be evaluated by lifting the lid and sniffing the dry grounds.

2. After infusing with water, the crust is left unbroken for at least 3 minutes but not more than 5 minutes. Breaking of the crust is done by stirring 3 times, then allowing the foam to run down the back of the spoon while gently sniffing. The Fragrance/Aroma score is then marked on the basis of dry and wet evaluation.

11.2.5.6 Coffee samples

1. Complex, intense aroma: *Washed Ethiopia Yirgacheffe, Grade 2.*

2. Subtle aroma: *slightly aged Guatemala.*

3. Homework coffee: *slightly aged Guatemala.*

11.2.5.7 Olfactory references

The olfactory references were presented in a mug, covered by pierced aluminum foil to conceal the references, and capped with a Petri dish to avoid odour release when not in use.

No	Reference	Code
1	Ground cloves (5g)	522
2	Apricot (Apricot jam; 20g)	759
3	Jasmine tea (4g)	575
4	Prunes (20g)	230
5	Raisins (20g)	260
6	Cinnamon (PAMS Ground Cinnamon; 5g)	461
7	Molasses (Blue Label Molasses; 15mL)	926
8	Maple syrup (Cottees Maple Syrup Flavoured; 10mL)	037
9	Biscuit (Arnotts Plain Biscuits Marie; 20g)	449
10	Hazelnut (Hazelnut flavor; 50µL on cotton ball)	973

11.2.6 Training Session 7 – Balance

11.2.6.1 Objectives

1. Build up olfactory vocabulary by focusing on identification of odours.

2. Comprehend the concept of *balance* in its two dimensions (*attribute intensity* and *attribute balance).*

3. Practice a full specialty cupping protocol.

11.2.6.2 Session schedule

Time	Activity
2:00 – 2:30	Odour recognition exercise & discussion
2:30 – 2:50	Presentation about balance
2:50 – 3:40	Cupping (Focus: Balance)
4:10 – 4:30	Discussion of cupping; discussion of last homework and homework for next session.

11.2.6.3 Activities

1. Give each participant a concealed aroma mug, coded with a 3-digit random number. Have participants write down descriptors. Rotate mugs until everybody has done all 10 of them.

2. Write down descriptors for each mug on whiteboard. Agree on 'standard' descriptor.

3. Explain the assessment procedure for Balance while cupping.

4. Cup two samples. Focus on Balance.

5. Discuss balance results. Discuss other attributes.

6. Discuss previous homework results.

7. Give cupping homework with cupping results in closed envelope.

11.2.6.4 Key concepts

Balance: How all the various aspects of Flavor, Aftertaste, Acidity and Body of the sample work together and complement or contrast to each other is Balance. If the sample is lacking in certain aroma or taste attributes or if some attributes are overpowering, the Balance score would be reduced (SCAA, 2009a).

11.2.6.5 Assessment procedures

1. As the coffee continues to cool (160º F - 140º F; 70º C - 60º C), the Acidity, Body and Balance are rated next. Balance is the cupper's assessment of how well the Flavor, Aftertaste, Acidity, and Body fit together in a synergistic combination.

2. The cupper's preference for the different attributes is evaluated at several different temperatures (2 or 3 times) as the sample cools. To rate the sample on the 16-point scale, circle the appropriate tick-mark on the cupping form. If a change is made (if a sample gains or loses some of its perceived quality due to temperature changes), remark the horizontal scale and draw an arrow to indicate the direction of the score.

11.2.6.6 Coffee samples

1. Balanced coffee: *Colombia (RACAFE & Cia.).*

2. Subtle aroma: *Sumatra organic.*

3. Homework coffee: *Tanzania organic.*

11.2.6.7 Olfactory references

The olfactory references were presented in a mug, covered by pierced aluminum foil to conceal the references, and capped with a Petri dish to avoid odour release when not in use.

No	Reference	Code
1	Ground black pepper (1g)	645
2	Ground allspice (1g)	590
3	Ground cardamom (1g)	704
4	Dates (30g)	759
5	Passionfruit flavour (250µL on cotton ball)	580
6	Orange peel (from 1 fresh orange)	761
7	Malt powder (30g)	113
8	Peanut butter (30g)	767
9	Vanilla extract (1g)	263
10	Dried figs (30g)	629

11.2.7 Training Session 8 – Overall

11.2.7.1 Objectives

1. Comprehend the concept of *overall assessment*.
2. Practice a full specialty cupping protocol,
3. Re-cup samples, in order to verify what was discussed.

11.2.7.2 Session schedule

Time	Activity
2:00 – 2:15	Practice with aroma mugs
2:15 – 2:30	Presentation about overall.
2:30 – 3:20	Cupping (Focus: Overall)
3:20 – 3:40	Cupping discussion
3:40 – 4:10	Re-cupping of same samples.
4:10 – 4:30	Discussion of previous homework

11.2.7.3 Activities

1. Practice aroma recognition with only three aroma mugs.
2. Explain the concept of *Overall* assessment.

3. Cup two samples. Focus on Overall.

4. Discuss Overall results. Discuss other attributes.

5. Cup same samples again, as a validation of what was discussed.

6. Discuss previous homework results.

7. Give cupping homework with cupping results in closed envelope.

11.2.7.4 Key concepts

> *"The Overall score is determined by the cupper and given to the sample as 'Cupper's Points' based on ALL of the combined attributes" (SCAA, 2009a).*

11.2.7.5 Coffee samples

1. Complex coffee: *Ethiopia Harrar (Natural).*

2. Simple coffee: *Brazil Moreninha Formosa (natural).*

3. Homework coffee: *Peru 'Lunya Grande' – Fair Trade-Organic (washed).*

11.2.7.6 Olfactory references

The olfactory references were presented in a mug, covered by pierced aluminum foil to conceal the references, and capped with a Petri dish to avoid odour release when not in use.

No	Reference	Code
1	Passionfruit flavour (250µL on cotton ball)	794
2	Dried figs (30g)	703
3	Malt powder (30g)	102

11.2.8 Training Session 9 – 'Catador®' App use

11.2.8.1 Objectives

1. Learn use of the 'Catador' iPad application.

2. Practice a full specialty cupping protocol,
3. Re-cup samples, in order to verify what was discussed.

11.2.8.2 Session schedule

Time	Activity
2:00 – 2:15	Practice with aroma mugs
2:15 – 2:30	Presentation about application.
2:30 – 3:20	Cupping (Focus: iPad usage)
3:20 – 3:40	Cupping discussion
3:40 – 4:10	Re-cupping of same samples.
4:10 – 4:30	Discussion of previous homework

11.2.8.3 Activities

1. Practice aroma recognition with only three aroma mugs.
2. Explain the use of the *Catador* App.
3. Cup two samples. Focus on iPad use.
4. Discuss results.
5. Cup same samples again, as a validation of what was discussed.
6. Discuss previous homework results.
7. Give cupping homework with cupping results in closed envelope.

11.2.8.4 Key concepts

The *Catador* App is an iPad application that replicates the SCAA cupping ballot in iPad form, so that the assessment results can then be emailed as a comma-delimited file.

11.2.8.5 Coffee samples

1. *Brazil Perla (washed).*
2. *Brazil Cerrado (semi-washed, meaning pulped natural)*
3. Homework coffee: *Peru COCLA, Quillabamba, Cusco (washed organic).*

11.2.8.6 Olfactory references

The olfactory references were presented in a mug, covered by pierced aluminum foil to conceal the references, and capped with a Petri dish to avoid odour release when not in use.

No	Reference	Code
1	Ground allspice (1g)	408
2	Peanut butter (30g)	134
3	Vanilla extract (1g)	374

11.3 Appendix 3 – Chapter 4 – Contingency Table

Table 11.2. Contingency table of international coffee samples (rows) and descriptor subgroups (columns) for the study in Chapter 4.

Sample	Astringent	Bitter_taste	Caramelly	Chocolaty	Citrus-like	Complex	Driedfruity	Dry-acid	Earthy	Fermented	Floral
BNI	2	0	3	2	0	0	1	1	0	0	0
BNI	1	1	2	1	1	0	0	2	5	0	2
BNO	0	0	3	2	0	0	0	2	2	0	0
BNO	1	1	3	3	1	0	0	0	1	1	0
BNX	1	2	2	3	0	0	1	1	1	0	0
BPW	3	0	4	1	0	0	3	0	0	0	0
BWI	1	0	6	6	1	0	1	0	0	1	1
BWI	1	2	3	1	0	0	2	0	2	0	2
BWW	2	1	2	3	2	0	1	1	1	0	1
BWW	1	2	3	2	2	0	1	1	1	0	0
CNX1	1	1	2	6	2	1	3	1	0	1	1
CWX1	0	0	1	2	1	0	3	1	0	1	0
CWX2	3	1	2	1	3	0	1	1	1	0	6
DNT	3	1	2	0	3	0	0	1	1	0	1
DWT	1	1	3	3	1	0	0	1	3	0	1
ENX	2	2	1	2	1	0	1	0	1	0	1
HNX	3	1	3	4	1	0	1	1	1	0	0
HNX	1	1	4	5	1	1	0	0	0	0	0
HWX	1	0	1	4	1	0	0	0	2	1	0
HWX	1	1	3	3	4	0	1	1	1	0	3
MNM	0	0	3	2	0	0	1	1	2	2	1
MNT	2	1	2	3	0	0	1	0	2	1	1
MWM	1	0	3	1	1	1	3	1	0	1	1
MWT	1	0	2	2	2	0	3	1	1	0	2
MWT	0	0	2	3	1	1	0	0	0	0	1
NNB1	0	0	1	1	1	0	1	0	1	2	1
NNB1	1	0	1	3	2	1	0	0	0	1	0
NNB2	1	1	4	3	2	1	1	0	0	3	2
NNB2	2	1	1	4	2	0	1	0	0	3	1
NNB3	1	1	3	3	2	1	1	0	1	3	0
NNB3	0	0	1	3	0	0	1	0	0	1	0
NNB4	3	1	2	3	2	0	0	1	1	1	2
NNB4	1	0	0	3	1	0	0	0	1	1	1

Sample	Astringent	Bitter_taste	Caramelly	Chocolaty	Citrus-like	Complex	Driedfruity	Dry-acid	Earthy	Fermented	Floral
NNB5	1	1	3	4	1	1	2	0	1	0	2
NNB5	2	0	0	2	3	1	0	1	0	2	3
NNC	0	0	1	3	1	0	0	1	1	1	1
NNC	1	1	0	3	2	0	1	0	0	1	2
NNH	1	0	2	1	2	1	1	1	0	0	1
NNH	1	0	2	2	1	0	1	0	1	1	1
NNP1	1	1	2	2	0	0	2	1	2	0	0
NNP2	2	0	2	2	1	0	0	0	1	1	2
NNR	1	0	2	4	0	2	1	0	2	0	1
NNR	0	1	0	3	1	0	0	0	1	1	1
NNY	0	0	1	3	0	0	0	1	0	0	2
NNY	0	0	2	2	3	1	1	0	2	0	3
PNG	0	0	0	1	0	0	0	0	0	1	2
VNG	3	2	1	2	2	0	1	0	0	0	2
VWG	2	1	2	2	3	0	1	2	2	0	2

Sample	Fruity	Fungal	Acid	Long	Medium-acid	Nutty	Past-croppish	Phenolic	Pungent	Pyrolytic	Redfruity
BNI	0	0	0	0	0	1	2	4	0	2	0
BNI	0	2	0	0	0	1	2	2	0	1	1
BNO	0	1	0	0	2	0	2	3	0	1	1
BNO	0	0	0	1	1	1	0	3	0	1	0
BNX	2	0	0	0	1	1	0	1	0	4	0
BPW	2	0	0	0	0	2	0	1	1	1	1
BWI	0	2	0	0	2	3	0	1	0	0	0
BWI	0	1	0	0	1	1	0	2	0	1	0
BWW	0	0	1	0	1	1	1	3	0	1	0
BWW	0	0	0	1	1	2	1	4	0	2	2
CNX1	3	1	0	0	3	2	0	1	0	0	5
CWX1	0	2	0	1	5	2	0	2	1	1	3
CWX2	0	0	2	0	5	3	0	2	2	0	1
DNT	1	0	1	0	2	2	1	1	0	1	0
DWT	0	1	1	0	1	4	0	2	1	2	0
ENX	1	2	0	0	1	1	3	1	0	0	3
HNX	0	0	0	0	1	1	0	4	0	3	0

Sample	Fruity	Fungal	Acid	Long	Medium-acid	Nutty	Past-croppish	Phenolic	Pungent	Pyrolytic	Redfruity
HNX	2	1	0	0	1	2	1	2	0	1	0
HWX	1	0	0	0	2	1	1	0	0	0	2
HWX	1	2	0	1	2	2	0	2	1	1	2
MNM	0	0	0	1	2	2	0	1	0	0	1
MNT	1	0	0	1	1	2	0	0	0	1	2
MWM	1	0	0	1	4	1	0	1	0	0	1
MWT	1	0	0	0	3	4	0	1	1	2	1
MWT	2	0	0	0	1	2	0	1	0	1	1
NNB1	2	2	1	0	0	2	0	0	0	0	4
NNB1	2	0	0	1	4	0	0	1	0	2	5
NNB2	1	0	1	1	4	0	0	0	0	2	4
NNB2	3	0	0	0	2	1	0	1	0	0	5
NNB3	3	0	0	0	2	0	0	0	0	0	5
NNB3	1	0	0	1	3	1	0	0	0	3	3
NNB4	2	1	0	2	4	0	0	0	0	1	3
NNB4	1	0	0	0	2	1	0	0	0	1	3
NNB5	4	1	0	1	1	2	0	0	0	3	5
NNB5	2	2	0	1	2	0	1	1	0	1	6
NNC	1	0	0	0	2	1	0	0	0	2	3
NNC	1	0	0	0	3	0	0	1	0	1	6
NNH	4	0	0	0	4	2	0	0	0	0	3
NNH	2	0	0	1	1	2	0	0	0	0	2
NNP1	2	4	0	0	2	1	0	0	1	0	4
NNP2	3	0	0	2	3	1	0	0	0	2	2
NNR	1	0	0	1	4	1	0	0	0	1	6
NNR	2	0	0	1	3	0	0	0	0	1	5
NNY	2	0	1	1	2	2	0	1	0	1	3
NNY	2	0	0	0	3	0	0	0	0	2	3
PNG	1	0	0	0	1	1	0	0	0	0	1
VNG	0	0	1	1	4	2	0	1	0	3	4
VWG	1	3	0	1	2	0	3	1	1	2	1

Sample	Resinous	Rough_body	Smooth_body	Spicy	Stonefruity	Sweet-acid	Sweet_taste	Toasty	Tropicalfruity	Wood	Vegetable
BNI	0	0	0	0	0	0	0	3	1	0	2
BNI	0	1	0	3	0	0	1	3	0	2	1
BNO	1	2	2	2	0	0	1	3	0	2	3
BNO	0	2	0	3	0	0	0	2	0	0	1
BNX	0	3	0	3	1	0	0	2	0	0	0
BPW	0	0	2	0	1	1	0	3	0	0	0
BWI	0	1	1	1	0	0	0	4	0	2	1
BWI	1	0	2	3	0	0	0	5	0	2	0
BWW	2	2	0	2	1	0	0	1	0	1	0
BWW	0	2	0	0	1	0	0	2	0	0	1
CNX1	0	0	1	2	0	3	0	2	2	0	1
CWX1	2	2	2	2	1	0	0	3	1	0	0
CWX2	0	1	0	3	0	1	2	2	0	1	2
DNT	1	0	1	1	0	0	1	4	0	0	2
DWT	0	1	2	2	1	0	0	3	0	2	0
ENX	0	1	0	1	2	0	0	1	1	2	0
HNX	0	2	0	3	0	0	0	4	0	1	2
HNX	0	2	2	2	0	0	0	4	0	0	0
HWX	1	1	0	2	0	0	1	1	0	1	1
HWX	1	0	1	0	0	1	0	2	0	1	1
MNM	0	0	0	3	2	0	0	3	1	0	0
MNT	0	2	0	2	1	0	0	0	2	0	0
MWM	0	0	3	2	0	1	1	3	0	0	0
MWT	0	0	1	2	2	2	1	4	0	0	0
MWT	0	0	0	3	1	0	0	4	0	0	1
NNB1	0	1	2	2	1	2	0	0	1	0	1
NNB1	2	0	3	1	1	4	1	0	2	0	0
NNB2	1	1	2	2	0	2	2	4	3	0	0
NNB2	0	0	2	0	0	2	0	1	3	0	0
NNB3	0	0	3	0	1	3	2	1	3	0	0
NNB3	0	0	4	2	1	1	0	2	2	0	0
NNB4	2	0	1	0	1	0	3	3	2	0	1
NNB4	0	0	2	1	2	2	0	1	4	0	0
NNB5	0	0	2	0	0	3	1	3	2	0	0
NNB5	0	1	0	0	0	1	1	2	3	0	0
NNC	0	0	0	2	1	1	0	2	2	0	0
NNC	0	0	1	0	0	2	0	2	2	0	0
NNH	0	0	3	2	0	1	3	3	3	0	0

Sample	Resinous	Rough_body	Smooth_body	Spicy	Stonefruity	Sweet-acid	Sweet_taste	Toasty	Tropicalfruity	Wood	Vegetable
NNH	1	0	0	0	1	1	0	2	1	0	0
NNP1	1	0	0	1	0	0	0	2	1	0	1
NNP2	0	0	1	0	0	3	0	2	2	0	0
NNR	1	1	4	3	4	1	1	2	3	0	1
NNR	0	0	1	1	3	3	0	1	2	0	1
NNY	1	3	0	1	0	1	0	3	1	1	0
NNY	1	1	2	1	0	2	1	2	1	1	0
PNG	1	0	0	0	0	0	0	1	2	0	0
VNG	0	3	1	2	0	0	0	4	0	0	0
VWG	1	4	0	1	1	1	0	4	0	0	3

11.4 Appendix 4 – Chapter 5 – Peak areas of significant compounds

Table 11.3. Peak areas of significant compounds (p<0.15), semi-quantified using GC-MS in Chapter 5.

Sample	p01	p02	p03	p04	p06	p07a	p10b	p12	p16b	p17a
	Methane-thiol	Acetal-dehyde	Di-methyl sul-phide	Methyl formate	2-Methyl-pro-panal	2-Methyl-butanal	Ethyl 2-methyl-buta-noate	Ethyl 3-methyl-buta-noate	3-Methylbutan-1-ol	Pyr-azine
BNI	2365337	7359600	444057	2892382	5569567	6558257	202	15369	121732	500729
BNO	1720507	8454736	443699	3364447	5323461	6221126	0	8148	52438	449502
BNX	1880097	10249298	449470	3459507	5759647	6261719	0	35559	90582	346295
BPW	1821866	7507551	860220	3821444	5559258	6599412	490	44910	92961	408574
BWI	1867275	9194958	553851	3301295	5775332	6604514	191	35730	87050	505552
BWW	1805054	8537264	603139	3441646	5324897	6093744	0	16300	51163	426563
CNX1	1716466	13227686	2928598	5577980	7424625	8017949	730	52069	88985	239612
CNX2	2701405	11768840	3086416	5159700	8234987	9411770	11453	148964	125714	306693
CWX1	1993025	10287281	2641303	4559892	7303343	8221740	0	27055	70953	315050
CWX2	2075153	12514605	3108411	5482923	6824240	7385442	301	17304	98542	335277
DNT	2280634	10169680	1021506	3918610	6408836	7038304	181	25475	94449	423564
DWT	1769881	9033390	767164	3301873	5330271	6187956	0	38640	85009	505862
ENX	2170999	11251611	722451	5355862	6561803	7381375	1435	64392	122020	332395
HNX	2092940	8877499	1382205	3800198	6894542	7590535	0	20549	77777	567952
HWX	2005564	10088111	3099992	4830885	6175313	7159473	0	27047	93658	371246
MNM	2327608	12237734	1132116	6368020	8648795	8785584	1604	65100	115526	294266
MNT	2169962	10931781	600932	4648965	7396328	8028190	6230	86523	142302	379110
MWM	1804227	15487489	884068	6926428	6154521	5885580	0	50910	77218	365352
MWT	1631212	12092752	1040543	5101113	6236840	6293462	0	19569	67845	444691
NNB1	1884495	12045129	939173	5209961	7768057	8373176	16330	114307	128948	299227
NNB2	1636908	11588877	865278	5107419	8042529	8719764	19615	90396	111571	483881
NNB3	1321207	12391955	669263	5397901	8440209	9344870	17067	100924	128781	383553
NNB4	1581592	12792220	823808	5587443	8147734	8821283	14703	97113	163702	399939
NNB5	1891168	12564194	1289796	5416826	7872900	8523070	6641	56455	127223	369478
NNC	1974699	10964163	627661	4357801	7003167	7583366	6167	66018	114453	411238
NNH	1689747	10811949	669351	4348515	6928878	7385857	1355	16680	84823	246866
NNP1	2220201	13752152	961539	6568741	8391131	8501701	4616	60986	129928	380457
NNP2	2051152	16152099	1288004	6112700	7645706	7566577	5839	85362	128305	424906
NNR	1406933	14634549	795315	6967898	8217057	8905537	10224	127944	160586	335049
NNY	1747045	10590733	624304	4268195	7087423	7619880	1621	52216	94036	288920

Sample	p01	p02	p03	p04	p06	p07a	p10b	p12	p16b	p17a
	Methane-thiol	Acetaldehyde	Dimethyl sulphide	Methyl formate	2-Methylpropanal	2-Methylbutanal	Ethyl 2-methylbutanoate	Ethyl 3-methylbutanoate	3-Methylbutan-1-ol	Pyrazine
PNG	2268071	12278874	1447225	5501264	7594150	8592662	15077	118383	162240	225563
VNE	2605140	11153895	736573	4590888	6296614	6655458	1631	77342	140319	691888
VNG	2281173	12604649	1296459	5773281	7579944	8048293	1942	47807	191086	579442
VWG	2016684	12092295	1041589	4591961	7109262	7481436	0	34433	83072	210517

Sample	p21	p22b	p24c	p26a	p26b	p32	p33b	p38	p43	p45	p50
	2-Butanone, 3-hydroxy	2-Propanone, 1-hydroxy	2-Ethylpyrazine	2-Ethyl-6-methylpyrazine	2-Ethyl-5-methylpyrazine	Pyrazine, 3-ethyl-2,5-dimethyl-	Furfural	2-Furanmethanol, acetate	5-Methyl furfural	Dimethyl sulfoxide	γ-Butyrolactone
BNI	1259453	165912	192046	576422	419433	141133	384282	1467876	409527	12117	598391
BNO	1120705	189819	178658	562716	389201	128103	457206	783596	429035	23160	473095
BNX	1063888	226578	187891	675562	490472	184378	565891	1190730	584995	3151	488575
BPW	1638247	407160	163593	448112	358971	75520	822997	437304	479399	61088	492223
BWI	1369791	237542	187266	495897	376444	99557	539746	818949	442744	26888	388577
BWW	1267662	201935	163289	462302	346631	90651	479308	1061043	465201	60720	441435
CNX1	1281881	410575	128467	431374	327319	96223	1231380	880519	804953	174768	473198
CNX2	1452442	295400	128477	330745	258959	67852	635777	1533652	568707	197850	683973
CWX1	1339469	255201	144353	402959	312976	77647	614988	1225798	527662	144065	544545
CWX2	1594230	356569	151873	466839	332873	101606	890244	1448268	779299	173215	502899
DNT	1549045	291055	173175	481824	363781	94060	636488	879512	519673	37433	681697
DWT	2141531	348013	194450	542777	387833	96280	762764	1634918	612194	124731	757104
ENX	1742305	383846	135334	329497	310947	82352	1030932	842349	688694	3447	693321
HNX	1887003	228914	221471	563058	489703	152380	570389	993870	508419	26090	637216
HWX	1991943	404261	142695	369870	274391	69193	924084	1306984	620003	220032	620062
MNM	1300891	301018	134761	394930	298534	100433	723299	1024823	539713	90546	595918
MNT	1627467	271016	177422	452652	460339	158776	838360	708431	650353	19867	574771
MWM	1944766	525157	153764	441747	327331	79076	1219987	1404669	768417	116333	579975
MWT	2124508	523318	202437	622686	417515	120615	1456698	1103902	879702	89078	455039
NNB1	1335846	286982	157656	500485	354265	107072	789818	1273410	576738	42637	560726
NNB2	1848459	460637	227578	741457	484605	160180	1059280	475968	499214	26651	555866
NNB3	1710976	492627	209646	702974	483696	167639	1255989	1094599	807648	43652	575285
NNB4	1693852	430289	198573	642270	457497	156279	1122920	1069719	737909	62201	571108

Sample	**p21**	**p22b**	**p24c**	**p26a**	**p26b**	**p32**	**p33b**	**p38**	**p43**	**p45**	**p50**
	2-Butanone, 3-hydroxy	2-Propanone, 1-hydroxy	2-Ethylpyrazine	2-Ethyl-6-methylpyrazine	2-Ethyl-5-methylpyrazine	Pyrazine, 3-ethyl-2,5-dimethyl-	Furfural	2-Furanmethanol, acetate	5-Methyl furfural	Dimethyl sulfoxide	γ-Butyrolactone
NNB5	1546223	366021	180750	597715	409029	125153	933403	740112	657852	55322	592660
NNC	1683005	428285	209105	653468	479558	148264	1100402	964887	794111	60126	539863
NNH	1224466	271596	140504	449389	330368	95196	847178	665000	582954	18150	325881
NNP1	1512018	278730	157838	349238	362561	87328	765152	894491	570365	7457	718099
NNP2	1688061	437353	180357	511322	388106	109976	1040160	1337438	718977	129378	600448
NNR	1732364	398212	157251	467632	354017	102875	1061001	1419486	771566	49973	730037
NNY	1259600	273125	161715	546963	402787	124581	788588	925015	643524	11848	470238
PNG	1020293	210598	131072	426600	331129	111152	497645	965043	452021	23885	484014
VNE	2005103	392131	215769	607892	423079	136516	740271	1697769	624677	99604	1158430
VNG	2576430	619482	233751	665285	500302	162299	1208538	1557808	872382	192273	908974
VWG	1188741	271273	126496	377240	305762	71474	708179	1094378	619140	35738	561745

11.5 Appendix 5 – Chapter 6 – Weather conditions during experiment; drying curves and a_W of microbiological experiment

11.5.1 Weather conditions during the drying experiment

Table 11.4. Summary of weather during study

Day from harvest (main experiment)	Day from harvest (microbiology experiment)	Date	Weather
1		27/01/2013	Sunny
2		28/01/2013	Sunny
3		29/01/2013	Sunny
4		30/01/2013	Sunny
5		31/01/2013	Rainy
6		1/02/2013	Cloudy afternoon
7		2/02/2013	Rainy afternoon
8		3/02/2013	Cloudy. Rainy evening.
9		4/02/2013	Sunny
10		5/02/2013	Sunny
11		6/02/2013	Sunny
12		7/02/2013	Sunny
13		8/02/2013	Cloudy
14		9/02/2013	Sunny
15		10/02/2013	Sunny
16	0	11/02/2013	Sunny
17	1	12/02/2013	Sunny
18	2	13/02/2013	Sunny. Cloudy evening.
19	3	14/02/2013	Cloudy. Rainy evening.
20	4	15/02/2013	Rainy
21	5	16/02/2013	Rainy
22	6	17/02/2013	Rainy
23	7	18/02/2013	Sunny
24	8	19/02/2013	Sunny

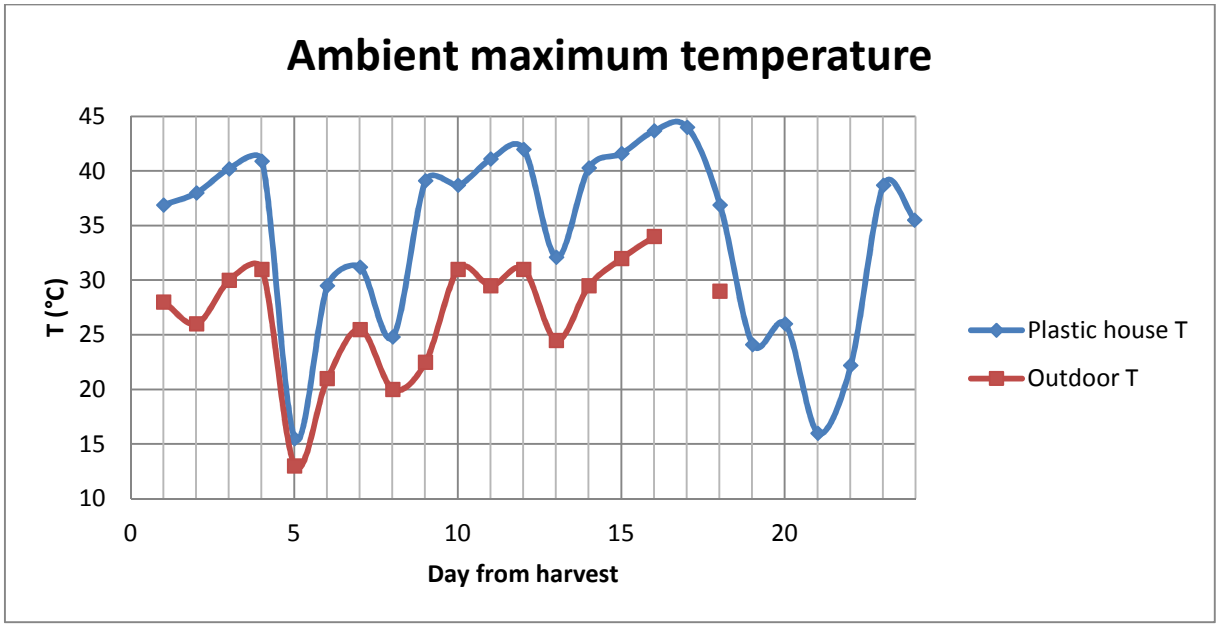

Figure 11.1. Ambient maximum temperature during drying experiments.

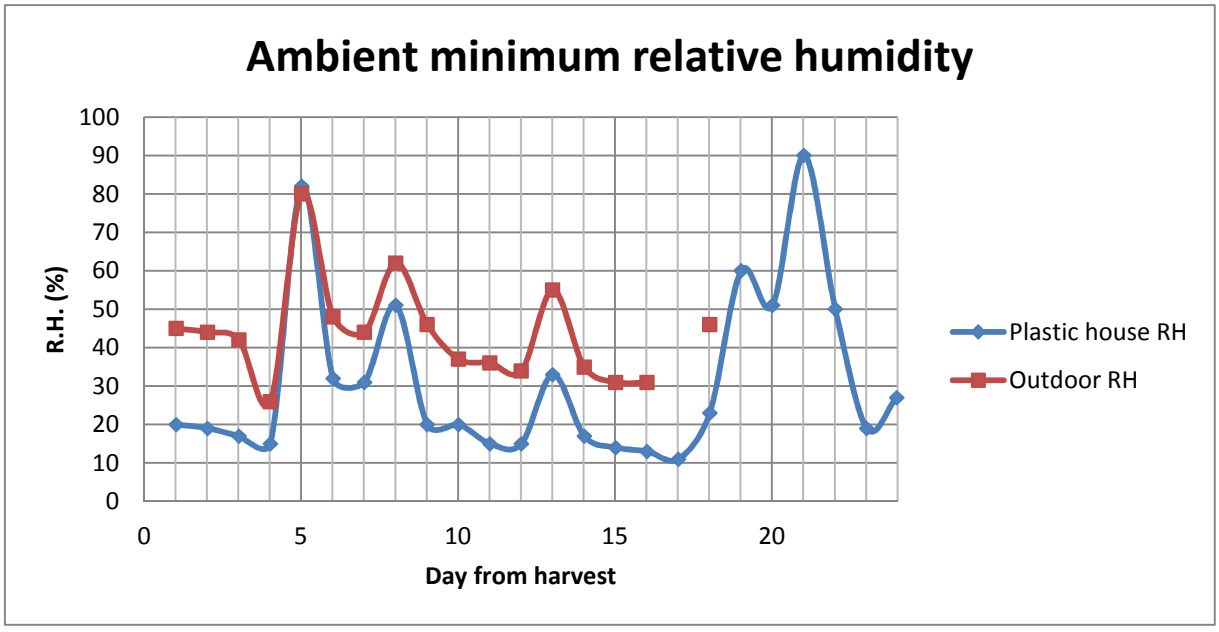

Figure 11.2. Ambient relative humidity during drying experiments.

11.5.2 Drying curves and evolution of a_W during microbiological experiment

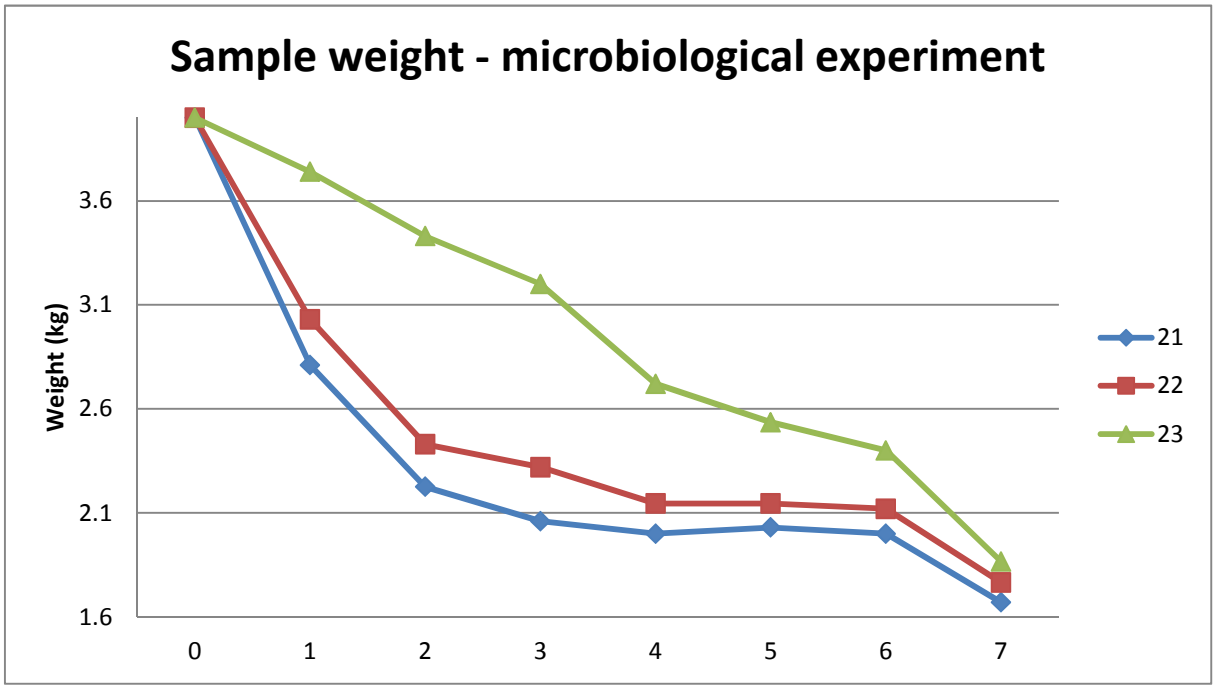

Figure 11.3. Sample weight during microbiological experiment.

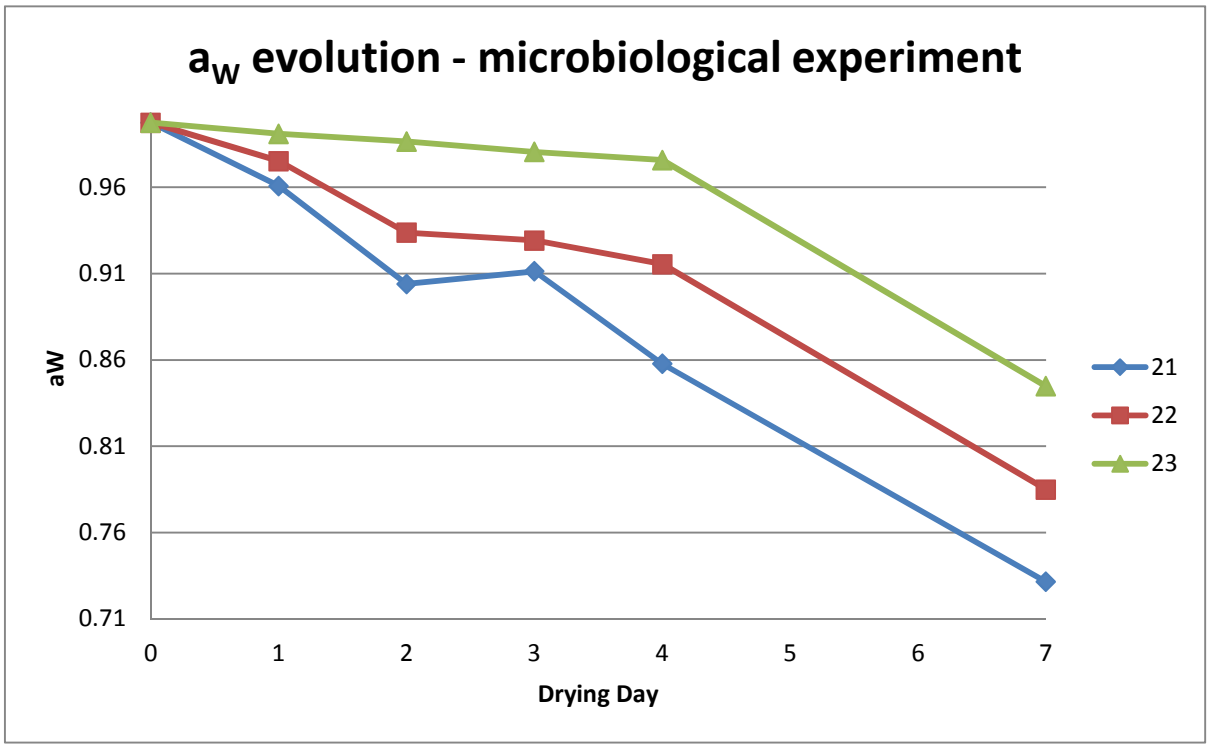

Figure 11.4. Sample a_W during microbiological experiment.

11.6 Appendix 6 – Chapter 6 – Contingency tables

11.6.1 University of Otago (New Zealand) panel

Table 11.5. Contingency table of 7 drying treatments in duplicate and 1 washed witness (rows) and descriptor subgroups (columns) from the University of Otago cupping panel. Significant subgroups (χ^2 > 7.0) in *italics*.

Sample	Astringent	*Bitter*	*Caramelly*	Chocolatey	Citrus-like	Driedfruity	*Dry-acid*	Earthy	*Fermented*	*Floral*
1A	1	0	2	2	2	1	1	1	1	1
1A	1	1	1	3	0	1	0	1	1	1
1B	0	1	2	1	3	2	0	0	1	2
1B	1	1	1	4	1	1	1	0	1	1
2A	1	0	4	3	2	1	3	1	0	0
2A	0	0	3	2	2	1	0	1	0	1
2B	1	1	5	4	2	1	1	1	1	0
2B	1	0	4	3	3	1	1	2	0	0
3A	0	1	3	3	1	1	1	0	1	0
3A	1	0	3	4	2	1	0	1	1	2
3B	0	0	4	3	3	1	0	1	1	3
3B	0	0	2	3	3	3	0	1	2	2
4A	1	0	2	3	3	1	1	2	1	2
4A	0	0	2	2	1	0	0	1	1	0
4B	0	1	2	4	3	3	0	2	0	1
4B	0	1	2	2	2	2	0	1	1	1
5A	0	1	2	2	2	1	1	1	1	0
5A	3	0	2	2	2	0	1	0	1	1
5B	1	0	3	2	3	1	0	0	1	0
5B	1	0	2	3	2	2	0	1	1	2
6A	1	1	1	3	3	3	0	0	0	0
6A	1	1	3	2	2	1	1	0	1	0
6B	0	1	4	1	3	1	0	1	1	1
6B	0	2	2	1	3	2	0	2	1	1
7A	0	1	2	4	1	1	0	1	0	0
7A	1	1	4	3	2	3	1	1	0	1
7B	0	0	2	3	3	0	0	2	1	2
7B	0	0	3	2	1	3	0	1	0	3
W	0	0	4	4	0	2	0	0	0	1
W	1	0	4	2	1	0	0	2	0	0

Sample	**Fruity**	**Fungal**	**Medium-acid**	**Nutty**	**Other**	**Phenolic**	***Plain bouquet***	***Pyrolytic***	**Redfruity**	**Resinous**
1A	2	0	3	2	1	0	0	1	2	1
1A	3	0	2	2	0	1	0	1	3	1
1B	2	0	2	2	0	0	0	1	2	2
1B	1	0	0	3	0	0	0	1	1	1
2A	2	0	1	2	2	0	0	2	0	0
2A	1	0	2	0	1	0	0	2	2	3
2B	1	0	2	1	0	1	1	0	2	1
2B	1	0	1	2	1	0	0	1	1	1
3A	0	0	1	2	2	1	0	1	2	0
3A	3	0	3	0	0	2	0	1	3	0
3B	2	0	2	0	0	0	0	1	4	1
3B	1	0	1	2	0	0	0	1	3	0
4A	1	1	2	1	1	0	0	1	1	1
4A	3	1	2	3	0	1	2	2	2	1
4B	2	0	2	1	0	0	0	2	3	1
4B	1	0	2	3	0	0	0	1	0	0
5A	4	0	1	2	0	0	0	0	3	2
5A	2	0	1	1	0	1	0	1	3	0
5B	2	0	3	3	1	0	0	0	1	1
5B	2	1	3	1	0	2	0	0	0	2
6A	2	1	1	1	1	1	0	1	2	1
6A	1	0	2	2	0	0	0	1	3	1
6B	3	0	1	0	0	1	0	2	2	1
6B	2	0	1	2	1	0	0	1	2	1
7A	3	0	3	2	0	0	0	2	3	2
7A	1	0	1	2	1	0	0	2	1	1
7B	1	0	3	2	0	0	0	0	1	0
7B	2	1	1	2	0	0	0	0	2	0
W	2	0	2	2	0	0	0	3	1	1
W	2	0	2	2	0	0	0	1	1	0

Sample	**Rough body**	***Salty taste***	**Smooth body**	**Spicy**	***Stone-fruity***	***Sweet taste***	**Sweet-acid**	**Toasty**	***Tropical-fruity***	**Vegetable**	**Wood**
1A	1	0	2	2	0	0	1	2	1	0	0
1A	2	0	1	1	0	0	0	2	1	0	2
1B	1	0	1	1	0	0	1	3	2	0	2
1B	0	0	0	1	0	0	0	1	2	2	0
2A	1	0	0	2	0	1	0	1	1	1	0
2A	0	0	3	2	0	0	1	1	0	1	0
2B	1	0	2	1	2	2	0	4	1	0	0
2B	2	0	1	3	1	1	0	3	0	0	1
3A	1	1	0	2	0	0	1	1	1	0	0
3A	0	0	1	1	1	1	0	0	1	0	1
3B	0	1	3	2	0	0	1	2	2	0	0
3B	0	0	1	2	2	0	1	1	0	0	1
4A	1	0	0	2	0	0	0	1	1	0	1
4A	0	0	0	2	0	0	0	1	0	0	0
4B	1	0	2	3	0	1	0	2	1	0	0
4B	1	0	2	2	0	0	0	3	0	0	0
5A	1	0	2	1	0	2	1	2	2	0	1
5A	0	0	2	1	0	0	1	1	2	1	1
5B	2	0	0	3	0	1	0	2	1	0	0
5B	1	0	2	2	1	0	0	1	2	1	2
6A	1	0	2	2	1	1	1	2	1	0	1
6A	1	0	1	3	0	1	0	2	0	1	0
6B	1	0	1	0	0	0	1	4	1	1	1
6B	2	0	1	2	0	0	1	3	0	1	0
7A	2	0	1	2	1	1	0	4	2	1	0
7A	0	0	1	1	0	0	0	1	0	1	0
7B	0	0	2	2	0	1	1	2	1	1	1
7B	1	0	1	1	1	1	1	1	1	0	0
W	0	0	1	1	2	0	1	1	0	1	0
W	0	0	1	1	0	0	1	1	0	0	1

11.6.2 CAFECOL (Mexico) panel

Table 11.6. Contingency table of 7 drying treatments in duplicate and 1 washed witness (rows) and descriptor subgroups (columns) from the CAFECOL (Mexico) cupping panel.

Sample	FLORAL	FRUITY	VEGETABLE	NUTTY	CARAMELLY	CHOCOLATY	SPICY	RESINOUS	PYROLYTIC	EARTHY	FERMENTED
1A	3	10	2	3	6	9	0	0	5	2	5
1B	2	14	0	2	7	6	0	1	0	0	5
2A	0	18	0	4	8	9	0	1	1	0	1
2B	3	9	3	1	8	13	1	0	2	1	2
3A	0	10	2	0	1	4	0	0	6	4	13
3B	0	7	4	0	1	6	2	2	2	4	9
4A	5	14	3	1	13	7	3	0	0	1	0
4B	2	18	1	0	9	11	2	1	1	0	2
5A	3	7	3	0	5	9	0	2	3	1	3
5B	1	12	0	2	6	8	0	2	1	0	8
6A	3	9	3	4	5	12	0	0	5	0	1
6B	1	11	1	3	4	3	0	2	1	0	10
7A	0	8	1	1	11	16	0	1	1	0	2
7B	3	11	0	0	12	10	1	1	1	0	5
W	1	0	8	6	1	2	0	2	0	1	4

11.7 Appendix 7 – Chapter 7 – Characterisation of green beans

Table 11.7. Foxy beans (%) and CIELAB colour parameters of seven natural coffee treatments in duplicate and one washed witness (W).

Sample	Foxy beans (%)	L	a	b
1A	13%	41.55	2.65	19.95
1B	31%	43.24	3.63	21.09
2A	0%	42.31	1.75	18.71
2B	2%	46.01	1.89	19.13
3A	47%	43.32	4.01	20.30
3B	49%	44.30	4.06	20.36
4A	32%	43.81	3.08	19.87
4B	10%	45.16	2.96	21.14
5A	7%	43.42	2.52	19.88
5B	21%	45.04	2.28	20.32
6A	6%	44.41	2.11	19.74
6B	8%	44.26	2.25	19.95
7A	15%	43.24	2.28	20.55
7B	7%	44.22	2.50	19.25
W	0%	45.94	1.49	16.87

11.8 Appendix 8 – Chapter 7 – Peak areas of significant compounds (p<0.15) semi-quantified using GC-MS

Table 11.8. Peak areas of significant compounds (p<0.15), semi-quantified using GC-MS in Chapter 7.

Sample	p01	p06	p07a	p10b	p12	p16b
	Methanethiol	2-Methyl-propanal	2-Methyl-butanal	Ethyl 2-methyl-butanoate	Ethyl 3-methyl-butanoate	3-Methylbutan-1-ol
1A	1347106	5636612	5903021	21644	112774	155140
1B	1500621	4805838	5188361	27797	157458	161110
2A	1449200	5864818	6112897	8016	80273	115878
2B	1654751	5691313	6011655	18057	108638	146972
3A	1523071	6014309	6439556	67282	307748	227719
3B	1451185	5306361	5864575	63001	273895	187512
4A	1723037	5198708	5822302	18019	75450	136266
4B	1683240	5004121	5642958	22259	115375	150479
5A	1578970	5545367	5835729	40671	172507	234492
5B	1668891	4771986	5353192	45348	173892	265256
6A	1639785	5429040	5638112	23965	103254	171954
6B	1716254	5509369	5940705	12331	83212	138892
7A	1520372	5595746	5947062	14873	107195	170862
7B	1316844	4572889	5124718	16374	72200	127159
W	1245213	4628438	5070141	1546	44241	85342