EVALUATION AND MANAGEMENT OF COFFEE QUALITY DEFECTS IN RWANDA: CASE OF POTATO TASTE DEFECT

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ABSTRACT

EVALUATION AND MANAGEMENT OF COFFEE QUALITY DEFECTS IN RWANDA: CASE OF POTATO TASTE DEFECT

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Potato taste defect (PTD) is described as potato-like or peasy flavor that is perceived in coffee beverages. Consequently, it has been an obstacle in the coffee business affecting the value chain from producers to consumers. The quality of coffee is evaluated by professional cuppers who take decisions, that in turn determine the price of coffee. Hence, a good price is offered to a superior quality and defect-free coffee such as specialty coffee. Since PTD is detrimental to coffee quality, various research studies have been carried out to understand its causes. Pyrazines, particularly 2-isopropyl-3-methoxypyrazine (IPMP) and 2-isobutyl-3-methothoxypyrazine (IBMP), were identified as the main compounds associated with PTD in coffee. However, existing information on development of these compounds and their extent to produce detectable PTD are limited. Cupping is the only method that has been applied by the industry to detect PTD in coffee. However, its efficiency has not been documented. This project was conducted in Rwanda with the aim of investigating, predicting and managing the occurrence of PTD in coffee. Gas Chromatography - Mass Spectrometry (GCMS) was applied to identify and quantify IPMP and IBMP in green and roasted coffee beans collected from coffee washing stations in Rwanda. The occurrence of PTD in coffee was assessed by trained professional cuppers using the commercial standard cupping method. The same cuppers were assessed for their efficiency to detect PTD and determine sensory qualities of coffee using a method of generalizability theory. Furthermore, the best estimate thresholds (BET) of cuppers to detect IPMP, IBMP and a blend of IPMP-IBMP

dissolved in water and coffee beverage were determined. Based on GCMS analysis of 32 coffee samples, the mean concentrations of IPMP and IBMP were, respectively, 20.7 ± 1 ng/g and 85.8 ± 0.9 ng/g in green beans and 114.8 ± 0.7 ng/g and 158.1 ± 2.7 ng/g in roasted beans. Logistic regression analysis identified a relationship between PTD occurrence and two potential predictors, IPMP concentration in green beans and the ratio of IBMP to EDMP (2-ethyl-3,5-dimethylpyrazine) in roasted coffee beans. Coffee roasting impacted the contents of IPMP and IBMP in coffee a non-linear manner. Two main phases were observed, with loss of the two compounds at temperatures below 100°C, described by an exponential decay regression model; and formation of the compounds at roasting temperatures above 120°C, described by logistic dose response model. A panel of cuppers who were assessed demonstrated mean sensory detection thresholds of 0.7 ng/L, 1.3 ng/L, 1.4 ng/L for IPMP, IBMP and a blend of IPMP-IBMP in water, respectively. When the thresholds were measured in coffee, higher values were obtained with IPMP, IBMP and their blends detected at 110 ng/L, 384 ng/L, and 66.7 ng/L; respectively. The assessment of cuppers' efficiency has demonstrated a disagreement among cuppers to identify samples with detectable PTD, indicating a poor performance of cuppers. Generally, this project demonstrated that the prediction of PTD occurrence in coffee was influenced by the random distribution of PTD-associated pyrazines in coffee beans, the roasting profile and the variations in sensory sensitivity of cuppers to detect PTD. Consequently, regular refresher trainings of cuppers are recommended to improve PTD cupping efficiency. This research generated new knowledge with respect to the impact of roasting profiles on concentrations of PTD-associated compounds in coffee. The identified concerns that affect the efficient detection of PTD will open up opportunities for further research to enhanced understanding of the causes and origin of PTD in coffee.

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KEY TO ABBREVIATIONS

AFC: Ascending Forced-Choice

ASTM: American Society for Testing and Materials

BET: Best Estimate Threshold

BMP: 5-Bromo-2-methoxypyridine

CI: Confidence interval

EDMP: 2-ethyl-3,5-dimethylpyrazine

FFT: 2-furfurylthiol

GCMS: Gas Chromatography - Mass Spectrometry

IBMP: 2-Isobutyl-3-methothoxypyrazine

ID: Identification

IPMP: 2-Isopropyl-3-Methoxypyrazine

IRB: Institutional Review Board

MSD: Mass Selective Detector

n.a.: Not available

OA: Odor activity

PCA Principal Component Analysis

PTD: Potato Taste Defect

SEM: Standard error of the mean

SIM: Selected Ions Mode

TIC: Total Ion Chromatogram

CHAPTER 1. INTRODUCTION

After water, coffee is the second most popular beverage in the world (Donfrancesco et al., 2014) because of its stimulating effects and sensory qualities. In 2016, the European Union was identified as the main consumer of coffee worldwide, followed by the United States, Brazil and Japan (Samoggia and Riedel, 2018). Coffee, originating from Africa, has benefited many African farmers who are mainly smallholder coffee growers, earning income from their coffee plantations. In Rwanda, coffee is mainly grown for export and is one of the important cash crops that contribute to the Rwandan economy (NISR, 2015).

Since quality is a primary determinant of consumer preference, it is a crucial factor in the coffee business, requiring management and monitoring strategies along the processing and preparation operations from the plant in the field to the coffee beverage. The value chain of coffee is driven by the consumers who are the final buyers. Hence, their voices must be heard by the producers, processors and sellers. Currently, there is high demand for superior quality coffee, popularly known as "specialty" coffee (Barahona et al., 2020; McPherson, 2018; Poltronieri and Rossi, 2016) which is characterized by its unique flavor (Poltronieri and Rossi, 2016). Besides the unique geographical origin of coffee beans that contributes to flavor, specialty coffee is vigorously evaluated to identify any defect that might be perceived by consumers. Hence, to achieve the quality score of 80% or more according to the Specialty Coffee Association of America (SCAA) scale (SCAA, 2015), significant work needs to be done by producers, processors and quality assessors (Poltronieri and Rossi, 2016). The price of coffee is determined by its quality grade (Barahona et al., 2020; Pereira et al., 2018; Poltronieri and Rossi, 2016). Since quality of coffee matters, the Government of Rwanda has supported the specialty coffee sector by emphasizing

quality improvement in collaboration with various partners. The initiatives have included technical support to farmers and processors by establishing more coffee washing stations where coffee is grown, training of value chain actors and strengthening market linkages (Boudreaux, 2010). Despite these efforts, the coffee business in Rwanda and other parts of the African Great Lakes region still face quality defect challenges that affect market negotiations. The major quality challenge has been identified as Potato Taste Defect (PTD; Gueule et al., 2015; Jackels, et al., 2014; Gueule, et al., 2013; Czerny and Grosch, 2000).

Also known as "peasy flavor", PTD is characterized by an off-flavor similar to potato or pea flavor, which affects the taste of coffee (Scheidiget al., 2007). The suspected potential cause of PTD is from either the *Antestia* bug (*Antestiopsis orbitalis*) and/or bacteria (Gueule, et al., 2015; Kenneth, 2014; Gueule et al., 2013; Czerny and Grosch, 2000). However, previous research studies indicated that PTD coffee samples were associated with chemical compounds, mainly 2-isopropyl-3-methoxypyrazine and 2-isobutyl-3-methoxypyrazine (Jackels et al., 2014; Becker et al., 1987). These compounds are volatile and easily perceived by humans, having a very low odor threshold of 2 ng/L (Li et al., 2016). Consequently, PTD is a significant threat to the specialty coffee industry since any trace detected in coffee renders the coffee of poor quality, which is in turn rejected by the buyer. An example of the severity of PTD is experienced during the common competition of Cup of Excellence (ACE, 2021), where PTD samples are automatically excluded. The yearly basis competition conducted by ACE (Alliance for Coffee Excellence) aims at selecting the best quality coffee samples on basis of quality parameters mainly taste, aroma, and absence of defects among others.

Various studies have been conducted to better understand the occurrence of PTD in coffee and propose possible mitigation measures. For example, sorting of defective coffee beans has shown

promising results to reduce the occurrence of PTD (Mutarutwa et al., 2020). Color sorting machines can be used to separate physically damaged and defective coffee beans from intact beans (Gueule et al., 2013). The major challenge in the fight against PTD is associated with its detection since its occurrence is only confirmed in coffee beverages when assessed by a sensory analysis procedure known as cupping. This is the only quick method that is currently used in coffee quality analysis (Pereira et al., 2018; Donfrancesco et. al, 2014). Cupping is performed by trained people known as cuppers. They have a responsibility to classify coffee on quality grade basis, hence their decisions contribute to the price offered. Since the sensory sensitivity differs from one cupper to another, and most companies employ only one cupper (Pereira et al., 2018) to assess coffee quality, it is important to assess the reliability of results provided by the cuppers. Few previous studies have been conducted to assess the sensitivity of cuppers who evaluate coffee. Furthermore, there is no study that has been conducted on the effectiveness of different cuppers to detect PTD. The green beans of coffee have been reported to contain natural IPMP and IBMP (Mutarutwa et al., 2020) which contribute to the earthy or vegetative flavor (Pickering et al., 2007). Then, roasting introduces more flavor characteristic to roasted coffee. However, it is not known how the natural IPMP and IBMP of green beans and those developed in roasting might contribute to the occurrence of PTD. In addition, the extent to which IPMP and IBMP are formed during roasting is not known. Therefore, this study was conducted to evaluate and understand the occurrence of PTD in coffee.

AIM OF THE STUDY

This study was conducted to investigate, predict and control the occurrence of PTD in roasted coffee by using combined instrumental and sensory methods of analysis.

SPECIFIC AIMS

Aim 1: To predict the occurrence of PTD in roasted coffee by determining the relationship between the intensity of PTD and the IPMP and IBMP contents in coffee beans.

Hypothesis: The concentrations of IPMP and IBMP in coffee beans are correlated with the occurrence of PTD in roasted coffee beverages.

Aim 2: To assess the impact of roasting profile on PTD in coffee by determining the change in concentration of PTD responsible pyrazines upon roasting.

Hypothesis: The concentrations of IPMP and IBMP in coffee beans increase with roasting temperature.

Aim 3: To assess the reliability of cupping to detect PTD by determining the efficiency of cuppers to detect PTD responsible compounds.

Hypothesis: The detection of PTD in coffee beverages is influenced by the thresholds of IPMP and IBMP perceived by cuppers and consistency in reliability of cuppers.

SIGNIFICANCE OF THE STUDY

Potato taste defect is a serious quality defect affecting coffee businesses in Rwanda and neighboring countries. It makes coffee unappealing for consumers because of the unpleasant flavor. The defect not only affects consumers, but also the other value chain actors including farmers, processors and traders who experience losses due to rejection of coffee by international markets (Sualeh et al., 2014). In Rwanda and Burundi, PTD causes losses range between 30 and 40% of coffee beans (Miller, 2015). Coffee rejected due to PTD often is still sellable, but at very low prices due to poor quality. Various studies conducted on PTD have reported the role of IPMP and IBMP to generate PTD. These findings have been worthwhile in coffee research to orient scientists towards finding sustainable solutions to PTD. Despite these remarkable efforts, it is yet unknown how PTD and the responsible pyrazines behave during roasting of green coffee beans, and how cupping is efficient to detect the defect.

Roasting of coffee favors chemical reactions to form a variety of flavor compounds that impart the characteristic flavor profile of coffee. In addition, the temperature and time of the roasting profile (which can be defined as light, medium or dark roast) impacts the development of flavor compounds and the final quality of coffee. Furthermore, the fact that PTD is only perceived by human senses in roasted coffee suggests it is an indicator of potential changes in concentration or unmasking effects of IPMP and IBMP flavor compounds during roasting. In addition, the formation of pyrazines during coffee roasting was reported in previous studies (Ji and Bernhard, 1992; Misharina et al., 1992; Shibamoto and Bernhard, 1976; Murray and Whitfield, 1975; Maga and Sizer, 1973; Wang and Odell, 1973; Koehler and Odell, 1970).

Commercially, PTD is detected in coffee by professional cuppers (Mutarutwa et al., 2020; Jackels et al., 2014). It is costly to determine the quality of coffee at the end of the process because of

associated losses when a defect is detected in roasted coffee. In addition, it is important to use multiple professional cuppers to ensure reliability of results. However, it is costly to use multiple cuppers since they have special expertise (Pereira et al., 2018). Thus far, it is still unclear how PTD develops from the cherries to the coffee beverage. It is therefore indispensable to predetermine the occurrence and management of PTD at early stages of processing using the available information. Hence, the relationship between the green coffee or roast beans composition and the intensity of PTD are additional inputs to understand the development and occurrence of PTD.

Human senses are powerful tools to determine the extent of flavor compared to analytical equipment that only determines the presence and quantity of compounds responsible for a characteristic flavor. The complexity of the flavor profile of coffee and the many compounds that contribute to this profile is the reason for the intensive use of cupping to detect coffee defects. In the coffee industry, professional cuppers are trained to evaluate the quality for decision making prior to shipping. To determine the absence or presence of PTD is a priority among coffee defects in coffee. However, there have been concerns in the industry of non-homogeneity by cuppers in identification of PTD samples. Moreover, it is assumed that a small number of defective coffee beans can ruin a relatively large sample (Jackels et al., 2014; Gueule et al., 2013), but there has been no research conducted thus far to test this hypothesis. For this reason, it is important to evaluate the efficiency and sensitivity of different trained cuppers to identify PTD in coffee.

PTD in coffee is regarded as a mystery because of knowledge gaps, especially in understanding its development in coffee beans. The findings of this research will provide insights into the mechanisms of occurrence and detection of PTD in coffee. This research will improve our understanding of the impact of coffee roasting parameters on PTD occurrence. It is important to highlight that, to our knowledge, this is the first study to evaluate the sensory sensitivity and

consistency of coffee cuppers to detect PTD. Hence, these findings will be useful to the scientific community with additional information and knowledge to understand and manage the occurrence of PTD in coffee. In addition, the generated information will guide coffee value chain actors to manage the occurrence of PTD through optimum roasting and quality analysis. Finally, this study will identify interesting topics for further research.

CHAPTER 2. LITERATURE REVIEW

Coffee is a crop mainly grown in sub-tropical and equatorial regions where climate conditions favor its growth and determine the quality of coffee beverages. There are two main species of coffee, namely Arabica (*Coffea arabica*) which is more common, and Robusta (*Coffea canephora*). The coffee beverage is obtained after a series of operations starting with harvesting of coffee cherries, followed by primary and secondary processing (Figure 2.1).

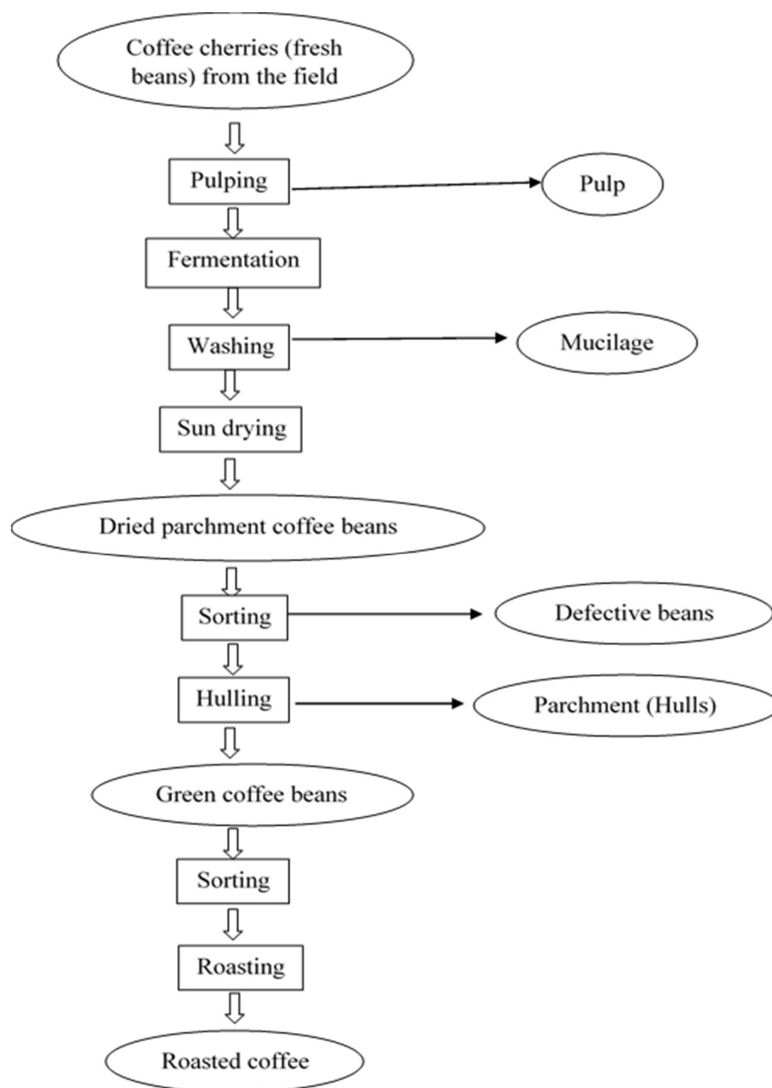


Figure 2.1: Flowchart of coffee processing unit operations (Wet method)

PROCESSING OF COFFEE CHERRIES (PRIMARY PROCESSING)

The quality of coffee beverages is dependent on various factors including the genetic variety, geographical origin (including climate, soil), and agricultural, processing and preparation practices among others (Sanz-Uribe et al., 2017). Coffee processing is a crucial step since it involves physico-chemical changes of cherries and green beans, which in turn determines the sensorial quality characteristics of the final coffee. Coffee processing involves two main steps including primary and secondary processing. Primary processing of coffee starts with ripe cherries or berries which are bright red in color (Figure 2.2), glossy and firm (Ghosh and Venkatachalapathy, 2014). It is carried out at coffee washing stations designed for coffee processing.

Figure 2.2: Ripe (red color) and unripe (green color) cherries on a coffee tree (photo from: https://perfectdailygrind.com/2017/06/coffee-cherries-go-unpicked-whats-the-solution/)

Harvesting

Red color which appears at stage three of ripening process (Figure 2.3) is the main indicator of ripe coffee cherries. Additional characteristics of cherry ripeness include but are not limited to the levels of soluble solids, sugars and moisture content (Sanz-Uribe et al., 2017).

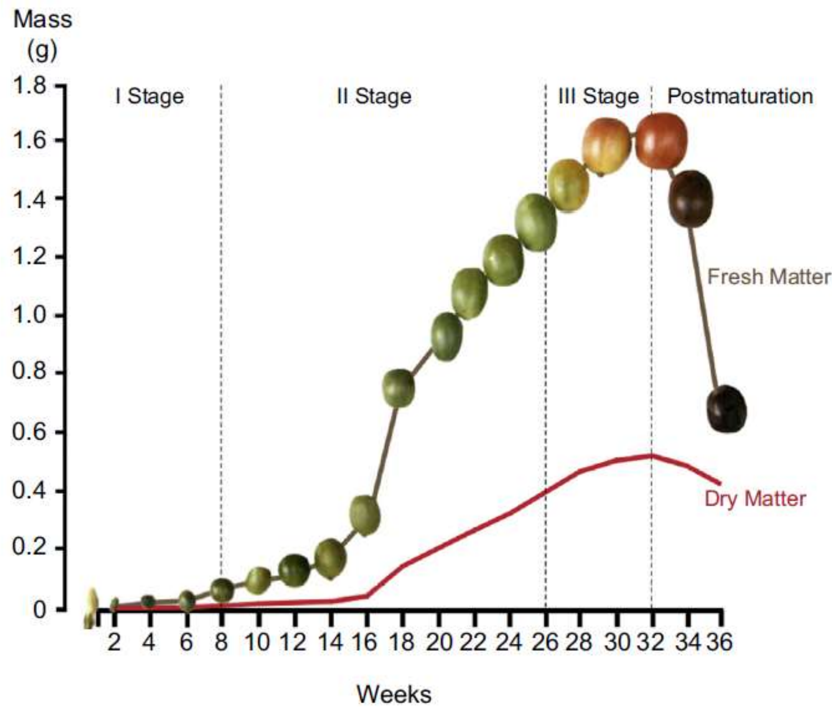


Figure 2.3: Ripening stages of coffee cherry as a function of mass (Sanz-Uribe et al., 2017)

Hand picking is the common method of coffee harvesting in Rwanda, since it preserves the quality of cherries (Ameyu, 2017), and allows selective picking of ripe berries. In addition, the landscape of many coffee plantations does not favor the use of mechanical harvesters. After harvesting, the cherries must undergo immediate processing to avoid chemical changes that negatively affect the quality (Sanz-Uribe et al., 2017). Coffee cherries are sorted (Figure 2.4) prior to processing to remove defective cherries and any other foreign materials. This step adds value to coffee quality such as in the production of specialty coffee (Ameyu, 2017).

A coffee cherry has four main layers around the seed (Figure 2.5), including pulp, mucilage, parchment and silver skin. These layers are removed during processing by two main methods, namely wet or dry method.

Figure 2.4: Sorting of coffee cherries prior to primary processing (Photo courtesy of Royal Coffee/The Crown)

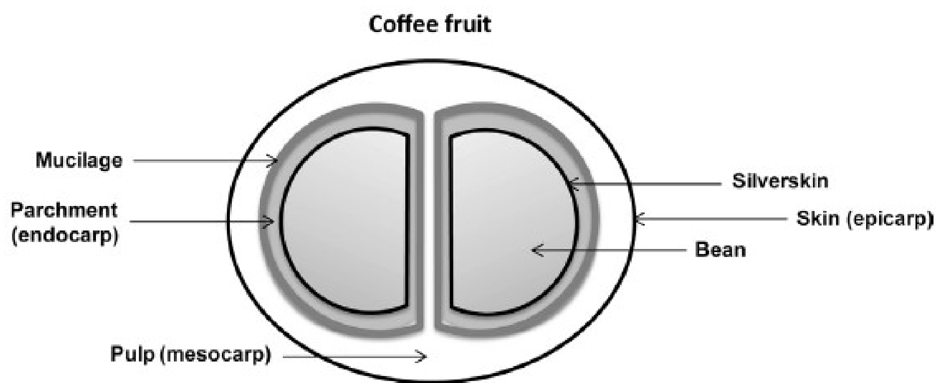


Figure 2.5: Structure of coffee cherry (Alves et., 2017)

Wet method of coffee processing

Pulping (Figure 2.6)**:** This is the first step of processing to mechanically remove the outer red skin and white fleshy pulp (Ghosh and Venkatachalapathy, 2014). The outcome is a seed with its remaining layers (Figure 2.7).

Figure 2.6: Pulping of coffee cherries (photo from: https://www.alamy.com/pulping-machine-coffee-harvest-in-la-zunga-ecuador-border-san-ignacio-department-of-cajamarca-peru-image217183253.html)

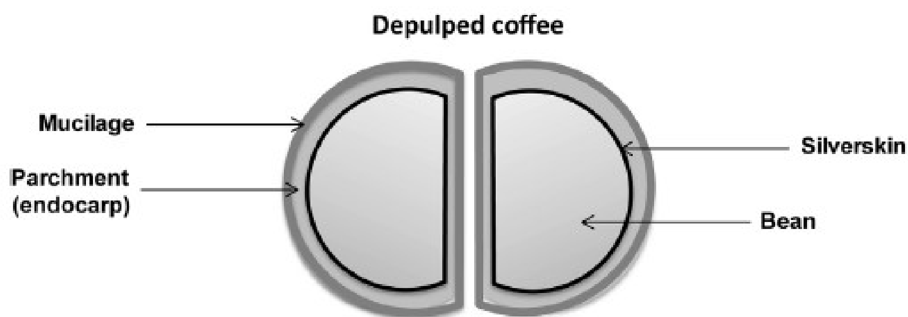


Figure 2.7: Structure of coffee bean after pulping (Alves et al., 2017).

Mucilage removal: This is mainly performed by wet fermentation for 24-48 hours. The mucilage is first degraded by enzymatic action of microbial pectinases (Sanz-Uribe et al., 2017) and then soaked in water. Fermentation improves the quality of coffee (Ghosh and Venkatachalapathy,

2014; Parliment, 2000) as a result of biochemical reactions (Sanz-Uribe et al., 2017). On the other hand, mucilage removal can be done mechanically resulting in a sharper acidity of resulting beans compared to a more juicy and fine acidity of beans obtained by fermentation (Sanz-Uribe et al., 2017). At the end of this process, washing is used to remove the remaining mucilage and a parchment coffee is obtained (Figure 2.8 and Figure 2.9).

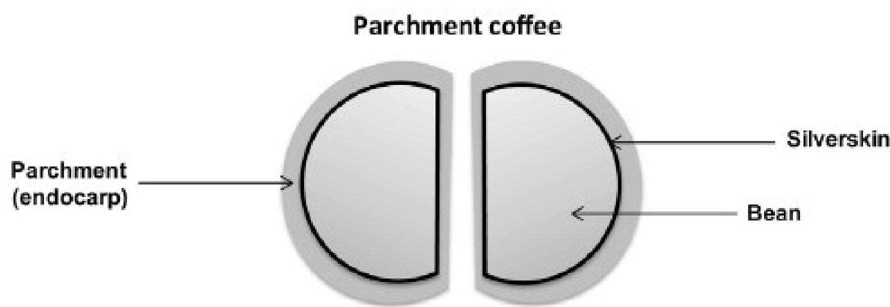


Figure 2.8: Structure of coffee bean after removing the mucilage (Alves et al., 2017).

Figure 2.9: Wet parchment coffee obtained after mucilage removal process (Photo courtesy of Royal Coffee/The Crown)

Drying: The parchment coffee is dried to a moisture content of 10-12 % (Sanz-Uribe et al., 2017; Ghosh and Venkatachalapathy, 2014). Sun drying (Figure 2.10) is the main approach used, but mechanical driers are also commonly used. Drying should be controlled to prevent the loss of flavors. At the end of drying process, well dried parchment coffee (Figure 2.11) is obtained.

Figure 2.10: Sun drying of parchment coffee in Rwanda (Left: indirect parabole drying at Muhondo coffee washing station; right: direct drying at Musasa coffee washing station)

Figure 2.11: Dried parchment coffee (Photo taken at Muhondo coffee washing station in Rwanda)

Dry method of coffee processing

The coffee cherries are directly dried with sun drying (Figure 2.12) to a moisture content of 12%, then the pulp and mucilage are separated from the seed by mechanical separation.

Figure 2.12: Sun drying of coffee cherries using dry method of coffee processing (photo from: https://www.baristainstitute.com/blog/jori-korhonen/january-2020/coffee-processing-methods-drying-washing-or-honey).

Sorting and hulling

Once the parchment is dry enough, defective beans (Figure 2.14) and any other extraneous materials are removed by sorting (Figure 2.15). Finally, the parchment coffee is hulled using a huller to obtain green beans (Figure 2.13 and Figure 2.16**Error! Reference source not found.**).

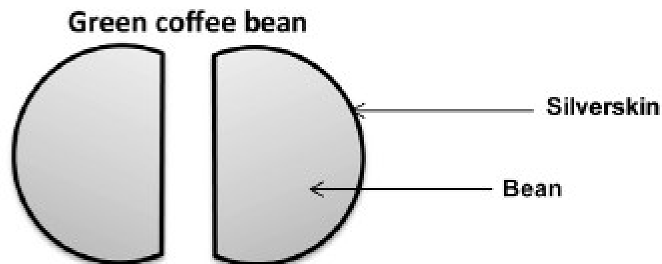


Figure 2.13: Structure of green coffee bean after hulling (Alves et al., 2017).

Figure 2.14: Defective beans obtained from coffee sorting (photo from: https://dailycoffeenews.com/2017/12/20/potato-taste-defect-what-roasters-need-to-know/).

Figure 2.15: Coffee sorting at Muhondo coffee washing station in Rwanda

Figure 2.16: Green coffee obtained after hulling

PRECURSORS OF COFFEE FLAVOR

Generally, for the perception of flavor, the responsible compounds should be unlocked from their precursors. The unlocking process occurs under defined conditions. The same mechanism applies to coffee flavor, which is developed during roasting. Roasting conditions highly contribute to physical and chemical changes of odorant compounds (Poisson et al., 2017; Schenker and Rothgeb, 2017). Besides flavor developed from newly formed or enhanced compounds, there is also a degradation of some other compounds to contribute to flavor of coffee. Coffee flavor development is a complex mechanism that produces more than 1000 flavor compounds contributing to the most important quality parameters of aroma and taste (Pereira et al., 2017; Poisson et al., 2017; Donfrancesco et al., 2014).

Flavor compounds fall into two categories, namely volatiles and non-volatiles. Non-volatile compounds contributing to coffee flavor are classified into carbohydrates, proteins, amino acids, lipids, phenolic acids and non-volatile acids (Poisson et al., 2017; Handayani, 2016). Non-volatile compounds provide multi-functional properties in coffee. For example, alkaloids such as caffeine and trigonelline are responsible for the bitter flavor and provide stimulating effects of coffee. Similarly, chlorogenic acids are responsible for astringency and bitterness of coffee and are at the same time antioxidants for human health (Sunarharum et al., 2014). The importance of non-volatile compounds in coffee quality was proven by findings of a study conducted on Brazilian coffee, indicating that a good quality coffee contains high amounts of chlorogenic acids and trigonelline. Carbohydrates and emulsions of lipids were also reported to carry and retain flavor volatiles in coffee (Sunarharum, et al., 2014). Volatile compounds are the most significant compounds that contribute to coffee flavor. The aroma of coffee is characterized by volatile compounds including furans, pyrazines, phenols and ketones, pyrroles, hydrocarbons, carboxylic acids, esters, alcohols

and aldehydes (Toci and Boldrin, 2018; Poisson et al., 2017; Handayani, 2016; Ku Madihah et al., 2013). Pyrazines are volatile compounds that have been reported to be the most significant flavor compounds in coffee (Toci and Boldrin, 2018; Handayani, 2016; Sunarharum et al., 2014; Ku Madihah et al., 2013).

COFFEE ROASTING (SECONDARY PROCESSING) AND QUALITY DEVELOPMENT

Roasting is a key processing operation for coffee since it determines the final overall quality of coffee beverage. It involves exposure of coffee beans to specified temperatures for a specific duration of time. The development of quality during roasting is a function of combination of temperature and time known as the roasting profile. Dry hot air in the roasting drum is used to increase the temperature and catalyze the change of physical and chemical characteristics of beans (Schenker and Rothgeb, 2017). The roasting profile is a specialty of each coffee roaster in particular, since coffee beans develop different flavors when exposed at different roasting conditions. Different roasting profiles have been reported in various studies, but generally the range of roasting temperature is between 160°C and 250°C, while the time ranges between 3 and 35 minutes (Cho et al., 2017; Schenker and Rothgeb, 2017; Sunarharum et al., 2014; Ku Madiha et al., 2013; Baggenstoss et al., 2008; Toci and Farah, 2008; Farah et al., 2006; Schenker et al., 2002; Mendes et al., 2001; Parliment, 2000). The concept consists of a combination of high temperature and short time or low temperature and longer time (Baggenstoss et al., 2008). In the coffee industry, there are three common types of roasts (Figure 2.17). They include light roast with sweet and nutty aromas, medium roast with balanced acidity/bitterness ratio, and dark roast with burnt, ashy, sour and pungent flavors (Sunarharum et al., 2014). In commercial roasting, the roasting profiles are different from one roaster to another depending on consumers' preferences.

For example, there are four main types of commercial roasting profiles such as light (170-205°C), medium (210-221°C), medium-dark (224-230°C) and dark roast (240-250°C) with roasting time ranging between 0.75 and 25 minutes (Lokker, 2017; Oden, Undated). The optimum time is between 1.5 and 6 minutes (Parliment, 2000).

Figure 2.17: Different roast levels of coffee. From left to right: green beans, light, medium, dark and extra dark (over-roasted) roast beans (photo from: https://www.jlhufford.com/blogs/newest-posts/what-do-different-coffee-roastings-mean).

Roasting is a process of three main steps as described by Handayani (2016). The process starts with feeding green beans to the roaster (Figure 2.18). The first stage consists of drying just after the beans are exposed to the roster temperature, and it is the beginning of color change as a result of Maillard reactions. In addition, flavors start to develop slowly during the first stage of roasting. The coffee bean is taken as a micro-reactor because it has a hard cell wall and limited intracellular spaces (Baggenstoss et al., 2008). These characteristics permit a second step corresponding to the first crack when the bean inside pressure increases as a result of temperature increase. At this point, rapid increase of bean temperature is recorded. It is then the appropriate time for flavor release from volatile compounds through chemical reactions. Finally, the temperature increases allowing

a third step marked by a second crack. At this stage, the woody cellulose of coffee bean is broken, and a smoky aroma is produced. Careful control should be taken at this stage because extended roasting leads to release of quinic acid, which is responsible for unfavorable sourness. This is the step where the degradation of pyrazines starts. Therefore, the extent of roasting should be controlled based on the desired quality of the resulting coffee beverage. For example, a pleasant and delightful aroma is obtained with medium roasting conditions. Finally, dark roast decreases body or mouthfeel and produces strong intensity of roasty note (Schenker and Rothgeb, 2017). In addition, fast roasting increases the rate of chemical reactions leading to increased amounts of gas generated and greater porosity of the roasted bean texture. The cup quality also becomes more acidic because of less degradation of chlorogenic acids with stronger flavor intensity (Bressanello et al., 2017; Poisson et al., 2017).

Figure 2.18: Probat coffee roaster

Roasting impacts the change of physical and chemical characteristics from unpleasant green beans to the pleasant state of roasted beans. Green coffee is composed of carbohydrates, proteins, lipids and organic acids which play a significant role in quality development during roasting (Moreira et al., 2017). Color is one of the important quality parameters of coffee and is used as a measure of

the extent of roasting. Non-roasted beans are referred to as green beans because of their characteristic greenish color, which changes to brown color as a result of Maillard and caramelization reactions (Schenker and Rothgeb, 2017; Ku Madihah et al., 2013). The shiny appearance of the outer surface of coffee is brought by migration of oil from inside to the outer surface of the beans (Poisson et al., 2017; Schenker and Rothgeb, 2017; Sunarharum et al., 2014). Acids undergo denaturation during roasting, and losses increase with extended time at high temperatures. The degradation of chlorogenic acid is an example. Sunarharum et al. (2014) reported that about 93% of chlorogenic acids are lost in dark roasting, hence explaining higher acidity in light compared to dark roast coffee (Poisson et al., 2017; Schenker and Rothgeb, 2017; Handayani, 2016; Sunarharum et al., 2014). Formation of aroma compounds is the most significant change that occurs in coffee beans during roasting. Hence, green and roasted beans differ significantly in flavor profile from pea-like flavor to developed roast pleasant flavor (Hashim and Chaveron, 1996).

COFFEE QUALITY AND DEFECTS

The pleasure and satisfaction that coffee provides to consumers is associated with its flavor, aroma, physiological and psychological effects (Agwanda et al., 2003). The quality of coffee beverages, which is described as cup quality, drinking quality or liquor quality, is a result of balanced combinations of flavor, body, color and aroma qualities particularly and importantly with a balanced acidity/bitterness ratio. Roasting is the most important operation to create this balance through controlled degradation of chlorogenic acids and quinic acids, and also development of bitterness from caffeine among other compounds (Schenker and Rothgeb, 2017; Handayani, 2016; Sunarharum et al., 2014). Arabica coffee is generally preferred over Robusta because of its

balanced acidity/bitterness as a result of low contents of caffeine and chlorogenic acid (Handayani, 2016). Moreover, the texture and mouthfeel of coffee beverages are essential parameters for coffee preference. They are termed as body, which is regarded as viscosity that brings joy sensation in the mouth (Handayani, 2016). Triglycerides and carbohydrates found in coffee are responsible for coffee body (Sunarharum et al., 2014).

Quality is a leading parameter to determine the price of coffee, either as green or roasted beans. The model of price driven by quality encourages farmers and processors to monitor and improve the quality at critical points such as agronomic practices, processing and roasting practices that are pre-quality determinants (Moreno et al., 1995). Although more efforts are oriented to quality improvements, some coffee beans still exhibit quality defects. Defective beans include black, sour or brown, immature, bored or insect damaged, and broken beans and any other extraneous matter mixed with beans (Farah et al., 2006). These defective beans are usually the sources of unpleasant flavors and odors in coffee beverages.

PTD - POTENTIAL SOURCES AND CAUSES

Potato taste defect is one of the common quality defects identified in East African coffee (Gueule et al., 2015; Jackels et al., 2014; Gueule et al., 2013) and is devastating coffee business in the region. Recently, a number of studies revealed the insect commonly called "*Antestia* bug" (*Antestiopsis orbitalis*) as the potential source of PTD in coffee. The insect introduces either chemical compounds classified as pyrazines, which are believed to be responsible for PTD, or bacteria that produce similar pyrazines in coffee cherries (Gueule et al., 2015; Kenneth, 2014; Czerny and Grosch, 2000). Pyrazines are chemical compounds that are responsible for characteristic odors in food. The main pyrazines identified as the causes of PTD in coffee were 2-

isopropyl-3-methoxypyrazine (IPMP) and 2-isobutyl-3-methoxypyrazine (IBMP). They were isolated from defective green and roasted coffee beans (Mutarutwa et al., 2020; Gueule et al., 2015; Jackels et al., 2014; Gueule et al., 2013; Czerny and Grosch, 2000). Grapes and chilled fish can also contain IPMP, which was reported to contribute to musty potato flavor and odor (Alberts et al., 2013; Miller et al., 1973). The pyrazines IPMP and IBMP were also identified in river water causing earthy or musty off-flavors (Li et al., 2016). The suspected source of IPMP reported by An et al. (2012) was among the metabolites of soil *actinomycetes,* while IBMP was suspected to come from anaerobic degradation of grass. The potato-like odor was also reported to originate from bacteria producing alkly-methoxy pyrazines (Gueule et al., 2015). Some examples of pyrazine producing bacteria include *Pseudomonas perolens*, which produces IPMP, and *Serratia rubidaea*, which produces 3-isopropyl-2-methoxy-5-methylpyrazine (Cheng et al., 1991). The latter compound was also identified in defective coffee samples originating from Mexico (Czerny and Grosch, 2000). In a study conducted on Arabica coffee, Gueule et al. (2015) isolated the organism *Pantoea coffeiphila,* which also produces IPMP and may be another possible contributor to potato-like odor. Additionally, *Pantoea agglomerans* (also named *Enterobacter agglomerans*), was reported by Gueule et al. (2013) to be involved in the development of potato taste. Since the *Antestia* bug is considered as the main source of PTD in coffee, scientists are focusing on managing the insect in plantations. In addition, emphasis has been placed on identifying and understanding chemical compounds that are suspected to induce PTD.

CHARACTERISTICS OF PYRAZINES AS RESPONSIBLE COMPOUNDS OF COFFEE FLAVOR AND PTD

Pyrazines are important flavor compounds in coffee that highly contribute to coffee flavor with their characteristic nutty, earthy, green and roasty aroma (Seninde and Chambers, 2020; Poisson et al., 2017; Sunarharum et al., 2014). Pyrazines in coffee are commonly the products of roasting through the Maillard reaction and Strecker's degradation (Franca et al., 2009). They are also found in green beans (Mutarutwa et al., 2020). However, they are affected by high temperatures as their concentrations decrease with extended roasting (Franca et al., 2009). The main PTD pyrazine compounds are IPMP and IBMP, which have very low flavor thresholds characteristic to most pyrazines. Their properties are described in Table 2.1 and chemical structures in Figure 2.19 and Figure 2.20. Van Gemert (2011) has conducted a literature review on previous studies that reported thresholds measured in water of 0.0002-10 ng/L and 0.001-10 ng/L for IPMP and IBMP, respectively. When measured in air, the thresholds were 0.0005-0.005 ng/L and 0.002-0.005 ng/L. IBMP was reported as a potent pyrazine compound in green coffee beans (Sunarharum et al., 2014; Franca et al., 2009) among others presented in Table 2.2.

Table 2.1: Properties of IPMP and IBMP (Li et al., 2016)

Physical/chemical property	IPMP	IBMP
Chemical formula	$C_8H_{12}N_2O$	$C_9H_{14}N_2O$
Molecular weight	152.19	166.22
Aqueous solubility (mg/L), 25°C	2438.0	1034.0
Odor threshold concentration (ppb)	0.002	0.002
Density (g/mL) 5°C	0.996	0.990
Boiling point (°C)	120-125	83-86

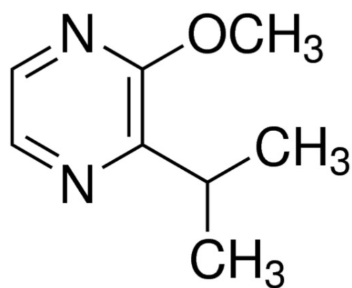


Figure 2.19: Chemical structure of IPMP

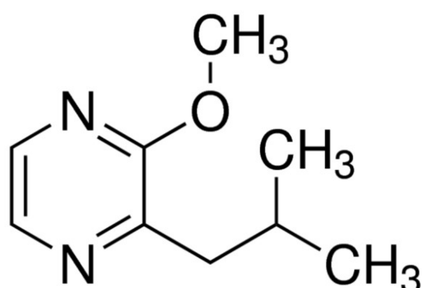


Figure 2.20: Chemical structure of IBMP

Table 2.2: Key odorant pyrazines identified in Arabica coffee samples (Sunarharum et al., 2014).

Compound	Concentration (ppb)	Threshold (ppb)	Medium for threshold measurement	Aroma description
2,3-diethyl-5-methylpyrazine	73-95	0.09	Water	Nutty-roast
2-ethenyl-3,5-dimethylpyrazine	52	0.000012	Air	Earthy
2-ethenyl-3-ethyl-5-methylpyrazine	18	0.000014	Air	Earthy
2-ethyl-3,5-dimethylpyrazine	55-330	0.04	Water	Nutty-roast
2-methoxy-3,5-dimethylpyrazine	1.1	0.006	Water	Earthy
2-methoxy-3-isopropylpyrazine (IPMP)	2.4	0.002	Water	Earthy, roasty
3-isobutyl-2-methoxypyrazine (IBMP)	59-97	0.002	Water	Peasy

QUALITY EVALUATION OF COFFEE

Quality control in the food industry is commonly performed by analytical methods using instruments and/or sensory evaluation using human senses. In the coffee industry, sensory evaluation is the common method used to assess the quality of coffee. The method of sensory evaluation in the coffee industry is termed "cupping" and is considered as the standard measurement of coffee quality when it is conducted by well-trained assessors who are known as "cuppers" (Seninde and Chambers, 2020; Pereira et al., 2018; Donfrancesco et al., 2014; Sunarharum et al., 2014). The assessment involves scoring or rating quality attributes of coffee beans or grinds as well as coffee beverages. Most importantly, the attributes consist of color, cleanliness, smell or aroma, flavor, aftertaste, acidity, body, and overall quality or balanced quality (Bressanello et al., 2017; Riberio et al., 2009).

Currently, cupping is the method used to detect coffee defects such as PTD. It is perceived through both oral and nasal cavities of the cupper. However, cuppers are not able to detect PTD unless coffee is roasted and extracted in water. Some experienced cuppers may perceive PTD by smelling roasted coffee beans or powder. However, human senses alone are not sufficient to identify defects in coffee. A number of studies have been conducted on identification and quantification of flavor compounds that are believed to produce PTD. Hence, researchers have successfully identified and quantified IBMP and IPMP using various methods such as gas chromatography-olfactometry or gas chromatography - mass spectrometry (Poisson et al., 2017; Jackels et al., 2014). Gas chromatography-mass spectrometry has gained popularity in coffee flavor analysis due to its efficiency. It was identified as a powerful analytical method due to its sensitivity to trace levels of volatile chemicals with very low thresholds. The method has been used to identify and quantify volatile chemicals such as pyrazines in various foods. For example, Hjelmeland et al. (2016) has

applied Gas Chromatography - Mass Spectrometry (GCMS) to analyze methoxypyrazines in wine. Mutarutwa et al. (2018) have applied GCMS coupled with solvent extraction and SPME (Solid Phase Micro-extraction) to quantify 3-alkyl-2-methoxypyrazines such as IBMP and IPMP in green bell pepper, green pea, carrot and cucumber. Since coffee has a number of volatile chemicals, GCMS was identified as a suitable method of analysis to determine the chemical profile of different types of coffee. In addition, previous researchers have applied GCMS to study defects of coffee such as PTD (Mutarutwa et al., 2020; Jackels et al., 2014). The principle of GCMS involves two main steps of components separation and identification. The sample is introduced and heated in the GC (gas chromatograph) to vaporize a mixture of volatile chemical components. The later are carried by an inert gas through a column for separation based on chemical affinity with the inner surface of the column. Once the compounds are separated, they are transferred to MS (mass spectrometry) and ionized for identification based on molecular mass.

CHAPTER 3. GCMS QUANTIFICATION OF IPMP AND IBMP FOR PTD PREDICTION IN COFFEE

ABSTRACT

Cupping is the common method to evaluate the sensory quality of coffee. Although instrumental analysis has been applied to determine the chemical composition of coffee, it is not common in quality evaluation of coffee. The pyrazine compounds, 2-isopropyl-3-methoxypyrazine (IPMP) and 2-isobutyl-3-methoxypyrazine (IBMP) are believed to be primary contributors to “potato taste defect” (PTD) in coffee. The purpose of this study was to investigate the relationship between the occurrence of PTD in coffee beverages and the concentrations of IPMP and IBMP in green and roasted coffee beans. The study entailed quantification of pyrazines in 32 coffee samples collected in Rwanda. The green coffee beans and corresponding roasted beans were analyzed with Gas Chromatography - Mass Spectrometry (GCMS) after aqueous-solvent extraction. The same roasted samples were cupped by 11 professional cuppers to determine the probability of PTD occurrence. The findings of this study showed a low frequency of 0.13 of PTD occurrence in the samples, with one sample that showed the highest probability of 0.39. The results generated by GCMS indicated variations in concentrations of IPMP and IBMP in both green and roasted beans. On average, the green beans contained 20.7 ± 1 ng/g of IPMP and 85.7 ± 0.8 ng/g of IBMP, while the roasted beans contained 114.8 ± 0.7 ng/g of IPMP and 158.1 ± 2.6 ng/g of IBMP. Logistic regression analysis identified a relationship between PTD occurrence and two potential predictors, IPMP concentration in green beans and the ratio of IBMP to EDMP (2-ethyl-3,5-dimethylpyrazine) in roasted coffee beans. However, a mismatch between cupping and GCMS results was observed. In addition, a higher odor activity (OA) of FFT (2-furfurylthiol) was believed

to contribute to masking effects of PTD-associated compounds. More research is needed to improve prediction of PTD based on coffee beans composition.

INTRODUCTION

Potato Taste Defect is a flavor defect that is commonly perceived in coffee and is believed to develop from the presence of high amount of IPMP and IBMP in coffee (Mutarutwa et al., 2020; Gueule et al., 2015; Jackels et al., 2014; Gueule et al., 2013; Czerny and Grosch, 2000). The defect was first reported in the East African region coffee (Gueule et al., 2015; Jackels et al., 2014; Gueule et al., 2013) and has severe consequences on coffee quality and subsequent marketability (Mutarutwa et al., 2020; Miller, 2015; Sualeh et al., 2014).

In line with changing demands of consumers, the current specialty coffee sector demands high quality and defect-free coffee (Samoggia and Riedel, 2018). Consequently, one cup of coffee identified with detectable PTD during cupping is enough to exclude the whole lot from specialty grade. Hence, cupping of coffee which is performed by cuppers is key in quality evaluation, especially to efficiently detect PTD that is only detected in roasted coffee. This shows the power of sensory tests in food quality evaluation since cupping is the only common method of quality evaluation for coffee. However, the cuppers must be trained and possess high sensory sensitivity to achieve reliable results (Barahona et al., 2020; Pereira et al., 2017; Donfrancesco et al., 2014). Due to differences in sensory sensitivity (Lawless, 2010), it is likely that the cuppers score differently as a result of the complexity of coffee flavors that make cupping problematic (Mutarutwa et al., 2020; Sunarharum et al., 2014). More than 800 flavor compounds in coffee have been reported, but only 20 to 30 aroma compounds were found to be important for a single type of coffee (Sunarharum et al., 2014). The same authors have also pointed out that pyrazines are among

the key flavor compounds in coffee. The importance of pyrazines in coffee flavor is supported by studies conducted on Arabica coffee (Toci and Boldrin, 2018; Handayani, 2016; Ku Madihah et al., 2013), which reported that IPMP and IBMP are among pyrazines that produce off-flavors in coffee, hence their contribution to PTD.

Due to limited research, it is not yet clear how PTD is associated with IPMP and IBMP in coffee. For example, a study conducted by Jackels et al. (2014) demonstrated that IBMP was identified in green beans of both PTD-suspected and non-PTD coffee samples. However, they found that higher concentrations of IPMP were obtained in PTD suspected samples than non-PTD samples. Similarly, a recent study of Mutarutwa et al. (2020) reported higher concentrations of IPMP in PTD suspected samples. Both studies quantified IPMP and IBMP with GCMS technique. In addition, GCMS technique was applied by Franca et al. (2009), Riberio et al. (2009), Toci and Farah (2008), Akiyama et al. (2007), Sanz et al. (2002), Maeztu et al. (2001), and Spadone et al. (1990) to determine and assess coffee quality. Their findings indicated that GCMS is efficient to determine the volatile compounds including pyrazines in coffee. In addition to cupping, the use of instrumental analysis to detect PTD could be an alternative to reduce the variability of cupping results (Mutarutwa et al., 2020) and cost of hiring professional cuppers (Pereira et al., 2018). Hence, this study was conducted to investigate the potential to predict the occurrence of PTD by determining the relationship between the intensity of PTD in coffee beverages and the amount of IPMP and IBMP in coffee beans. This study applied GCMS to determine the concentrations of IPMP and IBMP in the interior of both green and roasted coffee beans (Jackels et al., 2014).

MATERIALS AND METHODS

Chemicals

Standards of IPMP, IBMP, FFT and EDMP (>97 % purity; Sigma-Aldrich, Saint Louis, USA) were purchased and used to identify and quantify similar compounds in coffee samples. The internal standard 5-bromo-2-methoxypyridine (BMP; 95% purity; Fisher Scientific, USA) was added to take into account the variability of GCMS in data analysis. Ethyl acetate (99.9% purity; Fisher Scientific, USA) was used to extract organic compounds from coffee samples. The Milli-Q water was purified with E-pure model D4641 (Barnstead International, Iowa, USA). Acetone (99.6%; Fisher Scientific, USA), ethyl acetate (99.9%; Fisher Scientific, USA) and methanol (99.8%; Sigma-Aldrich, Saint Louis, USA) were used to clean the sample injector syringe.

Screening of coffee samples

The screening of samples consisted of collecting parchment coffee samples from various coffee washing stations in different geographical regions of Rwanda. The purpose was to collect as many samples as possible and evaluate them by cupping to identify PTD suspected coffee beans. Following intensive cupping sessions of 458 coffee samples by three professional cuppers, suspected PTD samples were selected for the purpose of this study. In order to select the right samples (i.e., coffee samples having detectable PTD), a non-probability purposive sample selection method was applied. Finally, 24 samples suspected of PTD and 8 samples suspected as non-PTD were collected. The collection of samples involved well dried parchment beans of Arabica coffee.

Roasting of green beans and preparation of coffee beverage

Green beans were produced by hulling the parchment coffee with a coffee huller model DRC-2X (Pinhalense S/A, Brazil). From each sample, 400 g of green beans were roasted at medium (or

moderate) roasting profile (175-190°C for 8-10 min) with a Probat BRZ2 roaster (Gimborn Maschinenfabrik GmbH, Germany). The roasting temperatures were measured in the roasting chamber (drum) in which the beans were rotating to allow uniform roasting. After 12-24 h of rest as recommended by the Specialty Coffee Association of America (SCAA, 2015), 20 g of roasted beans were placed in a cupping cup and the beans were ground and sieved in a Ditting KR1203 model grinder (Ditting Maschinen AG, Switzerland). The sieve was set at 4.5, corresponding to Nº 40 US standard size. Cupping was performed in three replications, and five cups of the sample were prepared for each replication as recommended by the Specialty Coffee Association of America (SCAA, 2015). The coffee beverages were prepared by the infusion method by pouring 250 mL of boiled water (90-95°C) in a cup of ground coffee. The beverages were held for 4 min to allow extraction before cupping.

Cupping

Procedures for the sensory evaluation of coffee samples by cupping were reviewed and approved by the Michigan State University Institutional Review Board. In this study, the preparation and cupping method developed by the Specialty Coffee Association of America (SCAA, 2015) was adopted. Prior to cupping, the crust formed at the top of the coffee beverage was broken to assess the aroma, and then removed to facilitate cupping. Cupping was performed when the coffee beverage reached a temperature between 50 and 60°C by a panel of 11 cuppers who were randomly selected from a group of professional cuppers. The professional cuppers were identified as cuppers who were well trained in cupping with at least one national or international cupping certificate such as the “Q-grader”, and who have previously participated in a national jury of at least one cup of excellence competition.

The cuppers were supplied with coded samples in random order, and each sample was presented with five cups. One by one, the cuppers evaluated the cups by introducing a sample of coffee beverage in the mouth using a spoon. After rotating the sample in the mouth, it was spitted in a prepared vessel. The cuppers have recorded the results based on their judgement using a cupping form developed by the Specialty Coffee Association of America (SCAA, 2003). The cuppers were requested to identify any perceived coffee defects, especially the occurrence of PTD, a category for which was added on the form. The cuppers also evaluated quality attributes described on the cupping form (Appendix 1). The quality attributes evaluated consisted of aroma, flavor, aftertaste, acidity, intensity, body, uniformity, balance, clean cup, sweetness, and the overall quality (overall score).

GCMS analysis

After cupping, the same 32 samples of green and roast coffee beans were analyzed with GCMS in 3 replications. An aqueous-solvent extraction was performed to extract organic compounds including pyrazines from coffee samples. The parameters of GCMS method were developed by amending the parameters from previous studies conducted by Bressanello et al. (2017), Jackels et al. (2014) and Riberio et al. (2009).

Extraction of pyrazines

A sample of 10 g of green or roasted coffee beans was ground with a Krups burr grinder GVX212 (Krups, USA) to coarse particles that passed through a Nº 20 US standard sieve size. To extract coffee compounds, 1 g of ground coffee was weighed with a precision balance model PG503-S (Mettler Toledo LLC, Ohio, USA) and dissolved in 2 mL of Milli-Q water. After mixing, the solution was heated at 50°C for 2 min in a dry bath incubator (Fisher Scientific, USA). The solution was immediately cooled down on ice for 1 min to reduce the risk of pyrazines vaporization, then

1 mL of ethyl acetate was added and mixed for 10 min at 300 rpm in an Innova 4900 multi-tier environmental shaker (New Brunswick Scientific, New Jersey, USA). The supernatant from each sample was pipetted into a 1.7 mL microcentrifuge tube, and then centrifuged in an AccuSpin micro centrifuge (Fisher Scientific, Pennsylvania, USA) at 16,060 × *g* for 5 min to separate the supernatant from the precipitate. From the upper layer supernatant, 90 µL were pipetted into a GC vial, then 10 µL of 100 µM BMP standard solution was added to obtain a final concentration of 10 µM BMP. The obtained sample was ready for GCMS analysis.

Sample injection

The instrumental analysis was performed with a gas chromatograph (Agilent 6890N; Agilent Technologies, Inc., USA) coupled with an inert mass selective detector (Agilent 5973; Agilent Technologies, Inc.). Prior to and post aliquot collection of the extract, the syringe installed in the Agilent 7683 auto sampler injector G2613A (Agilent Technologies, Inc., USA) was rinsed using acetone, ethyl acetate and methanol. For each injection, an aliquot of 1 µL was injected in the GC with a fast plunger speed. A splitless mode was selected with inlet parameters of temperature (250°C), pressure (7.58 psi), purge flow (10 mL/min), purge time (0.75 min), total flow (13.8 mL/min), gas saver flow (25 mL/min) and gas saver time (5 min). During the analysis, a blank sample of ethyl acetate was run after each 3 runs of replication of sample to clean the column.

Separation of volatile compounds

Compounds were separated by gas chromatography using a capillary column (Rtx 5MS; Model Restek 12623; Crossbond 5% diPhe 95% DiMe Polysilox; 30 m Length, 250 µm Diameter, 0.25 µm Film thickness; Restek Corporation, USA). The column was set at constant flow mode with initial flow of 1 mL/min and the oven temperature was set at initial temperature of 40°C. The

temperature was increased to 100°C at 25°C/min, then to 150°C at 5°C/min, and finally increased at 40°C/min to 270°C and held for 5 min. The total run time was 20.4 min.

Identification and quantification of compounds of interest and data collection

The identification of compounds of study, handling and collection of raw data were performed with Agilent MSD ChemStation G1701DA D.01.00 (Agilent Technologies, Inc., USA) in combination with the spectral NIST 02 MS Library and AMDIS 2.1 program. Following a separation process in the column, the compounds ions were detected by MSD, quadrupole mass spectrometer with SCAN mode. The main masses used for qualitative analysis were in order of m/z 137, 152, 124 for IPMP; 124, 151, 94 for IBMP; 135, 136, 42 for EDMP; 81, 53, 114 for FFT and 150, 135, 149 for BMP. However, the quantification masses were m/z 137, 124, 135, 81 and 150 for IPMP, IBMP, EDMP, FFT and BMP, respectively. The spectra were recorded with masses ranging between m/z 45 and 200. The data were produced in form of chromatograms with retention time and peak areas. The TIC showed chromatograms of all eluted peaks of volatile compounds based on intensities of all mass spectral peaks. The retention time obtained from each of the standard compounds was used to identify the corresponding compounds from a large number of peaks eluted from the sample. Data were collected as peak areas that were later adjusted with the internal standard BMP by calculating the ratio between the peak area of the sample and that of BMP. The concentrations of IPMP and IBMP in coffee were calculated from equations obtained from the standard curves of the same pure compounds. Single standard curves were developed from the GCMS analysis of standards IPMP and IBMP. The pure standards were diluted with ethyl acetate at various concentrations. The concentrations included 0.05 µM, 0.1 µM, 0.2 µM, 0.5 µM, 1 µM, 10 µM and 50 µM. The GCMS analysis followed a method used for coffee samples. A standard curve for each standard compound was developed and the line equation was produced,

which was later used to quantify the compounds in sample. The final concentrations were estimated in ng of compound per 1 g of coffee powder.

Statistical analysis

The statistical analysis of data was performed with IBM SPSS statistics version 25. Cupping data were subjected to descriptive statistics to determine the frequencies of detecting PTD in samples. In addition, descriptive statistics were performed to determine the mean concentrations of IPMP and IBMP in coffee samples. A multiple comparison was performed to determine the extent of differences between replications and differences between cuppers. Logistic regression analysis was performed to determine the relationship between the occurrence of PTD in coffee beverages and the concentrations of PTD-suspected compounds in both green and roasted coffee. The model was fitted with data obtained from cupping of coffee beverages as the dependent variable (PTD occurrence) and GCMS data obtained by measuring the concentrations of studied predictors in green coffee (IPMP and IBMP) and roasted coffee (IPMP, IBMP, EDMP and FFT). Since FFT and EDMP are potent coffee compounds that might mask the PTD-associated compounds, the concentration ratios of IPMP/EDMP, IPMP/FFT, IBMP/EDMP and IBMP/FFT in roasted coffee were also included in the predictors. The best predictors that fit with the logistic regression model equation 3.1 were obtained with a forward stepwise (likelihood ratio) method. In addition, Principal Component Analysis (PCA) procedure with varimax rotation was performed to provide more information on the relationship between the studied variables.

$$Ln\frac{p}{(1-p)} = \alpha + \beta_1 * \chi_1 + \beta_2 * \chi_2 + \ldots + \beta_n * \chi_n \quad (3.1)$$

p: Probability of detectable PTD

α: Intercept of the model equation

β_n: Slope of the model equation for predictor "1, 2, …, n"

χ_n: Concentration (ng/L) of predictor "1, 2, …, n"

RESULTS AND DISCUSSION

Detection of PTD by cupping

The frequencies to detect (or not detect) PTD in coffee by trained cuppers, which were calculated as a ratio of number of cases PTD was (not) detected to the total number of responses, are presented in Table 3.1. Generally, the results showed a low frequency of occurrence of PTD in the cupped coffee samples. Among the cuppers, the frequency of detecting PTD in coffee was between 0.08 and 0.19. The 32 coffee samples assessed for PTD by the 11 cuppers had previously been screened by three cuppers for potential PTD and included 24 PTD-suspect and 8 non-PTD samples based on the initial screening. However, subsequent assessment by the larger group of cuppers found a relatively low prevalence of PTD among the samples. The low frequency of detecting PTD may indicate that these samples of coffee beans did not contain sufficient concentrations of PTD-associated compounds to be consistently perceived by the cuppers. In addition, processing methods such as roasting promote the development of complex flavors in coffee, hence making the evaluation by cuppers problematic (Pereira et al., 2017). During cupping, the observed practice of pushing aside a cup identified with detectable PTD might have facilitated bias among cuppers, hence every cupper was aware of the occurrence of PTD in the cup since the commercial cupping

method is typically conducted in a common room at the same time. This could also explain the lack of significant differences among the cuppers to detect PTD. The efficiency of cuppers to detect the PTD-associated compounds is an additional factor responsible for low PTD frequency due to differences in sensory sensitivity, level of training and cupping experience (Pereira et al., 2017). As shown in Figure 3.1, the frequency of identifying PTD in the 32 samples differed among the replicates (different cupping sessions). A significant difference ($p < 0.05$) in PTD detection was found between replication 1 and 2, and between 1 and 3; while the results in replications 2 and 3 were not significantly different ($p > 0.05$). Inconsistencies in identification of PTD coffee has been reported by other researchers. Mutarutwa et al. (2020) reported a similar concern when a PTD suspected sample from Ethiopia was PTD-free after re-cupping.

Cupping as a method of sensory evaluation is influenced by the efficiency of cuppers and nature of samples (Pereira et al., 2018). Similarly, the findings of this study demonstrated a poor efficiency among the cuppers to perceive PTD in coffee beverage. The cupping results for all coffee samples are presented in Table 3.2. Overall, PTD was perceived with a frequency of 0.13 after cupping 32 samples (Table 3.2), and the sample ID 21 had the highest frequency (0.39) of being identified as PTD. According to the cupping method of SCAA (2015), once one cup has detectable PTD, the lot of coffee from which the sample was taken is automatically excluded from the specialty grade. Hence, in this study 20 samples were identified with detectable PTD as indicated by at least 1 cupper in at least 1 replicate of cupping. Among these samples, only 12 samples were identified with detectable PTD at a frequency of more than 0.2. Based on the findings of this study, it is not yet clear how PTD was not detected in the samples that were previously screened with detectable PTD (data not shown). In addition, certain samples that were previously clean, were later identified with detectable PTD. Alternatively, these results were in line with the

assumption of Mutarutwa et al. (2020) and Jackels et al. (2014) that a single bean that was infected by *Antestia* bug or contains sufficient PTD-associated compounds, is enough to contaminate a whole sample. However, this assumption is not confirmed by any research study to indicate the size of sample that is affected by one PTD bean. The inefficiency of cupping to provide reliable results also might be attributed to the composition of coffee beans used that might not have had detectable PTD, and the low sensitivity of cuppers to detect PTD at low thresholds (Pereira et al., 2018). In addition, the methods used to roast and cup the samples might have contributed to variations observed between replications. For example, grinding and infusion of coffee in separate cups might have contributed to quality differences of similar samples among the cups. The commercial roaster that was used during roasting did not have an automatic regulator for roasting temperature and time, hence it was the task of the technician to control the roasting conditions and decide on the end of roasting. This might also be a source of heterogeneity in coffee characteristics between replications, since the roasting temperature varied between 175 and 190°C and a change in one unit of temperature can result to changes in physico-chemical characteristics of coffee.

Table 3.1: Frequency of detecting PTD by 11 cuppers in 32 coffee samples cupped in three replications.

Cupper ID	**Frequency of detecting PTD**	**Frequency of not detecting PTD**
1	0.08	0.92
2	0.17	0.83
3	0.14	0.86
4	0.11	0.89
5	0.11	0.89
6	0.15	0.85
7	0.16	0.84
8	0.19	0.81
9	0.11	0.89
10	0.13	0.88
11	0.12	0.88

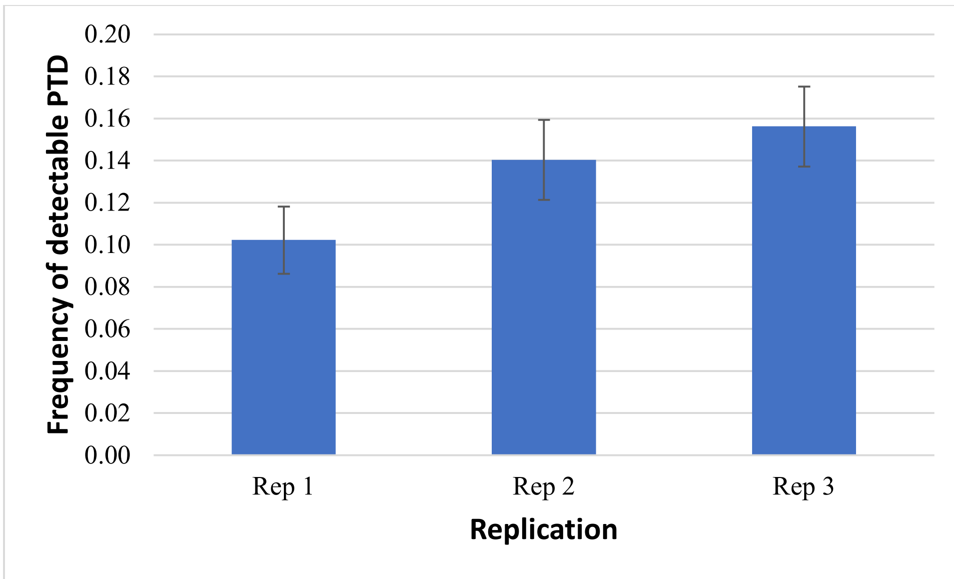


Figure 3.1: Comparison of occurrence of PTD in three replications when 32 coffee samples were cupped by 11 cuppers.

Table 3.2: The occurrence of PTD evaluated by 11 cuppers and concentrations of IPMP, IBMP, EDMP and FFT (ng/g of coffee powder) in green and roasted beans of 32 coffee samples that were evaluated in three replications.

Sample ID	Coffee beverage		Green beans		Roasted beans			
	Frequency of detectable PTD	**Frequency of undetectable PTD**	**IPMP (Mean ± SEM in ng/g)**	**IBMP (Mean ± SEM in ng/g)**	**IPMP (Mean ± SEM in ng/g)**	**IBMP (Mean ± SEM in ng/g)**	**EDMP (Mean ± SEM in ng/g)**	**FFT (Mean ± SEM in ng/g)**
1	0.03	0.97	9.2 ± 0.4	52 ± 1.1	101.1 ± 0.4	252.6 ± 22	1577 ± 108	7765 ± 316
2	0.00	1.00	10.3 ± 1.0	108 ± 0.6	108.9 ± 1.3	196.1 ± 2.6	2113 ± 133	8008 ± 269
3	0.06	0.94	58.4 ± 2	128 ± 1.9	103.1 ± 1.8	118 ± 10.1	3739 ± 221	9806 ± 340
4	0.33	0.67	12.8 ± 0.5	97.7 ± 1.9	95.8 ± 1.5	189 ± 10.8	899 ± 25	6098 ± 251
5	0.33	0.67	12 ± 0.7	102.1 ± 1.8	105.2 ± 1.7	159.9 ± 10.6	1254 ± 79	6581 ± 96
6	0.25	0.75	12 ± 0.8	113.5 ± 3.3	117 ± 3.8	112 ± 3.7	2949 ± 133	9059 ± 316
7	0.06	0.94	11.1 ± 0.3	40.4 ± 2	107.7 ± 2.4	54 ± 6.5	1839 ± 122	9652 ± 328
8	0.03	0.97	12.7 ± 0.6	100.4 ± 0.1	112.5 ± 0.7	188.1 ± 9.6	1502 ± 58	7452 ± 226
9	0.00	1.00	8.2 ± 0.4	93.2 ± 1.6	134 ± 6.3	153.1 ± 13.4	1426 ± 123	10661 ± 642
10	0.03	0.97	14.3 ± 0.5	115.4 ± 1.8	126.6 ± 1.9	119.4 ± 15.6	1687 ± 136	10616 ± 347
11	0.00	1.00	19.0 ± 1.5	111.2 ± 1.6	105.4 ± 4.1	193.9 ± 18.8	1815 ± 119	9255 ± 162
12	0.00	1.00	14.3 ± 1	86.4 ± 2.8	118.7 ± 3.6	138.2 ± 12.7	1494 ± 175	8177 ± 325
13	0.33	0.67	28.5 ± 5.5	78.5 ± 2.2	115.7 ± 7.4	123.8 ± 2.9	2046 ± 105	8810 ± 353
14	0.03	0.97	11.2 ± 0.8	108.2 ± 2.6	132.8 ± 4.4	131.9 ± 14	1934 ± 114	7978 ± 382
15	0.33	0.67	5.7 ± 0.6	49.4 ± 1.1	132.3 ± 2.8	172 ± 10.3	1459 ± 78	8580 ± 168
16	0.33	0.67	17 ± 0.4	111.4 ± 1.8	103.7 ± 3.1	145.3 ± 5.7	1392 ± 74	7557 ± 34
17	0.09	0.91	66.5 ± 6.1	98.6 ± 1.4	121.3 ± 4.7	123 ± 12.3	1685 ± 140	8101 ± 577
18	0.06	0.94	54.4 ± 2.8	105.2 ± 4.3	133.2 ± 8.9	220.5 ± 14.1	1177 ± 70	7183 ± 366
19	0.00	1.00	9.2 ± 0.2	74.3 ± 2.5	108.7 ± 3.8	120.6 ± 12.9	1607 ± 66	7783 ± 135
20	0.00	1.00	6.7 ± 0.9	61.0 ± 0.4	113.7 ± 0.3	101.3 ± 6.1	1175 ± 69	6177 ± 221
21	0.39	0.61	22.6 ± 3	65.5 ± 2.6	108.8 ± 2.7	198.3 ± 4	1181 ± 76	6619 ± 145

Table 3.2 (cont'd)

22	0.21	0.79	11.5 ± 0.6	82 ± 1.9	144 ± 1.5	178.7 ± 21.7	2101 ± 113	9006 ± 158
23	0.30	0.70	113 ± 21.5	69.6 ± 1.7	121.4 ± 3.4	212.6 ± 11.6	1641 ± 98	7907 ± 218
24	0.00	1.00	12.1 ± 0.3	102.9 ± 1.6	128.3 ± 3.2	130.1 ± 3.6	2172 ± 64	10411 ± 441
25	0.00	1.00	26.0 ± 0.9	71.9 ± 0.7	105.9 ± 2	214.6 ± 28	1159 ± 21	7358 ± 485
26	0.34	0.66	16.3 ± 0.3	103.6 ± 1.3	94 ± 1.2	182.8 ± 8.7	1506 ± 90	7694 ± 254
27	0.00	1.00	11.5 ± 0.2	58.6 ± 0.5	120.2 ± 2.2	145.8 ± 8.5	1133 ± 74	7439 ± 371
28	0.36	0.64	4.2 ± 0.1	73.1 ± 2.7	109.6 ± 1.6	180.6 ± 7	1206 ± 82	6221 ± 126
29	0.00	1.00	8.8 ± 0.2	59.6 ± 1.5	115 ± 3.1	187 ± 7.5	1268 ± 83	6559 ± 143
30	0.00	1.00	8.8 ± 0.5	75.1 ± 1.3	114.8 ± 1.8	138.8 ± 4	1467 ± 89	7647 ± 396
31	0.00	1.00	22.9 ± 1.7	82.3 ± 3.1	122.5 ± 1.1	138.6 ± 26.5	1722 ± 19	7892 ± 138
32	0.33	0.67	11.4 ± 1.2	62.9 ± 1.7	91 ± 4	135.7 ± 10.7	1300 ± 64	7299 ± 184
Overall	0.13	0.87	20.7 ± 1	85.7 ± 0.8	114.8 ± 0.7	158.1 ± 2.6	1644 ± 25	8043 ± 66

Quantification of IPMP and IBMP in coffee beans

According to Mutarutwa et al. (2020), Gueule et al. (2015), Jackels et al. (2014), Gueule et al. (2013), Czerny and Grosch (2000), IPMP and IBMP have been identified as the main pyrazines in coffee beans that are associated with PTD since they were identified as having green or bell pepper-like flavor. In the present study, the concentrations of IPMP and IBMP were quantified in coffee beans before and after roasting. The results presented in Table 3.2 show that the concentrations of IPMP and IBMP in green beans varied from 4.2 to 113.1 ng/g and 40.4 to 128 ng/g respectively. In roasted beans, the concentrations varied between 91 and 144 ng/g for IPMP, while the concentrations of IBMP varied between 54 and 252.6 ng/g. The results in the current study were in line with quantification results for IBMP in green coffee samples obtained from different countries in the study conducted by Mutarutwa et al. (2020), who used GCMS coupled with headspace solid phase microextraction. Mutarutwa et al. (2020) reported the amount of IBMP in green coffee samples ranging from 11.6 to 138.5 ng/g. Contrary to our results, Mutarutwa et al. (2020) detected low amounts of IPMP ranging between 1.1 and 3.2 ng/g in green coffee samples. In the same study, when they analyzed seven samples of insect-damaged beans from Rwanda, they found that IBMP concentration was between 23.8 and 29.1 ng/g, while IPMP was between 2.8 and 5.2 ng/g with two samples having extremely large concentrations of IPMP (543.2 ng/g and 4237 ng/g).

There are large variations in IPMP and IBMP concentrations reported in the literature for coffee samples, which may be due to differences in geographical origin, production and processing practices of coffee beans (Mutarutwa et al., 2020; Poltronieri and Rossi, 2016; Donfrancesco et al., 2014). Differences in roasting conditions, specifically variations in temperature and time profiles, can impact the concentrations of IPMP and IBMP in roasted coffee. The results in the

present study found considerably higher mean concentrations of IPMP and IBMP upon roasting, with IPMP increasing from 20.7 to 114.8 ng/g and IBMP increasing from 85.7 to 158.1 ng/g. According to previous studies, pyrazines in general are formed at high temperatures as a result of Maillard and Strecker's degradation reactions (Franca et al., 2009).

Relationship between the occurrence of PTD and concentrations of IPMP and IBMP

A logistic regression analysis was performed with a forward stepwise (likelihood ratio) method to evaluate the possibility of predicting the occurrence of PTD in coffee beverage based on the concentrations of IPMP or IBMP in coffee beans. However, the results of the logistic regression found there was no evidence of good fit of the statistical model to data as indicated by the Chi-square statistically significant difference ($p < 0.05$) of Hosmer and Lemeshow test, which is an indicator of poor fit model with small p-value.

Although the Hosmer and Lemeshow test indicated a poor fit model, a stepwise logistic regression procedure identified three statistically significant ($p < 0.05$) potential predictors of PTD as shown in Table 3.3. The identified potential predictors of PTD are IPMP of green beans, FFT of roasted beans and IBMP/EDMP ratio in roasted beans. Note that predictors that were considered in the model included the concentrations of IPMP and IBMP of green beans; IPMP, IBMP, EDMP and FFT of roasted beans; and the concentration ratios of IPMP/EDMP, IPMP/FFT, IBMP/EDMP and IBMP/FFT in roasted beans. The output of the statistical analysis (Table 3.3) showed that FFT was statistically significant ($p < 0.05$). However, the value 1 (standing for detectable PTD) which falls within the 95% confidence interval (CI) for the odds ratio of FFT, might be an indicator of lack of evidence of significant impact of the predictor FFT of roasted beans on the detectable PTD. In addition, the statistical analysis provided a null B-coefficient (slope of the model equation) for FFT. Hence, there was no evidence of relationship between FFT of roasted beans and the

occurrence of PTD. On the other hand, IPMP of green beans and the ratio of IBMP/EDMP in roasted beans were significantly different with a 95% CI for the odds ratio excluding the value 1. Hence, they are potential predictors that show possible evidence of relationship to PTD in coffee beverages. However, IPMP of green beans had a value 1 outside a 95 % CI for the odds ratio but very close to the interval. This indicates that IPMP of green beans might have a slight impact on the occurrence of detectable PTD. The model equation of potential predictors of PTD is described in equation 3.2.

$$Ln\frac{p}{(1-p)} = -0.064 - 4.294 * \chi_1 - 0.017 * \chi_2 \qquad (3.2)$$

p: Probability of detectable PTD

χ_1: Ratio concentration (ng/L) of IBMP/EDMP in roasted coffee

χ_2: Concentration (ng/L) of IPMP in green coffee

The data were also analyzed using PCA to identify potential correlations between the probability of detecting PTD and the concentrations of IPMP and IBMP. Although the Kaiser-Meyer-Olkin value (0.545) was slightly above 0.5, the Bartlett's sphericity test was significant ($p < 0.05$), indicating the suitability to conduct PCA. Two components were extracted after varimax rotation and were plotted as shown in Figure 3.2. The total variance explained by the two components was 49.4 % which is low compared to the desired minimum of 75 %. However, the PCA results indicated that PTD had weak influence on the extracted components as shown in Figure 3.2. This is an indicator of weak relationship between PTD and the proposed predictors.

With observational comparison, a mismatch of cupping and GCMS results was obtained. For example, the sample ID 21 which was identified with the highest frequency of detectable PTD (0.39), had low or moderate concentrations of IPMP (22.6 ng/g in green beans and 108.8 ng/g in roasted beans) and IBMP (65.5 ng/g in green beans and 198.3 ng/g in roasted beans) as shown in Table 3.2. In another example, the sample ID 28 was identified with detectable PTD at a frequency of 0.36, while its corresponding green beans had the lowest IPMP concentration (4.2 ng/g). In a similar way, the sample ID 3 had the highest IBMP concentration (128 ng/g) in green beans while the sample ID 7 had the lowest IBMP concentration (40.4 ng/g) in green beans. However, both samples were similarly identified with detectable PTD at a frequency of 0.06. Another case was identified in sample ID 1 which was identified with detectable PTD of frequency 0.03 while its corresponding roasted beans had the highest IBMP concentration of 252.6 ng/g. Finally, our results were in accordance with the findings of Mutarutwa et al. (2020) who also discovered a mismatch between the concentrations of IPMP and IBMP with cupping results. Pearson correlation coefficients were calculated to determine the extent to which IPMP and IBMP concentrations in green and roasted beans were themselves related. The results of this analysis did not show evidence of any significant correlation as shown by small correlation coefficients (Table 3.4).

Table 3.3: Logistic regression parameters (at each step of stepwise forward analysis) determining the relationship between the occurrence of PTD and the concentrations of IPMP and IBMP in coffee beans. The B coefficient describes the slope of logistic regression

Steps of predictor selection	Predictor	Coffee sample	B coefficient	Standard error	Wald	df	Significance	Exp (B)	95% C I for Odds ratio (EXP (B))	
									Lower	Upper
1	IBMP/EDMP ratio	Roasted beans	-2.798	1.149	5.934	1	0.015	0.061	0.006	0.579
	Constant		-1.540	0.155	99.348	1	0.000	0.214	-	-
2	IPMP	Green beans	-0.016	0.006	6.008	1	0.014	0.984	0.972	0.997
	IBMP/EDMP ratio	Roasted beans	-2.779	1.155	5.788	1	0.016	0.062	0.006	0.597
	Constant		-1.273	0.180	49.876	1	0.000	0.280	-	-
3	IPMP	Green beans	-0.017	0.007	6.987	1	0.008	0.983	0.970	0.996
	FFT	Roasted beans	0.000	0.000	6.600	1	0.010	1.000	1.000	1.000
	IBMP/EDMP ratio	Roasted beans	-4.294	1.350	10.117	1	0.001	0.014	0.001	0.192
	Constant		-0.064	0.500	0.016	1	0.898	0.938	-	-

Table 3.4: Correlation coefficients between IPMP and IBMP in green and roasted beans

	IPMP in roasted beans	IBMP in roasted beans
IPMP in green beans	0.136	0.016
IBMP in green beans	-0.015	-0.033

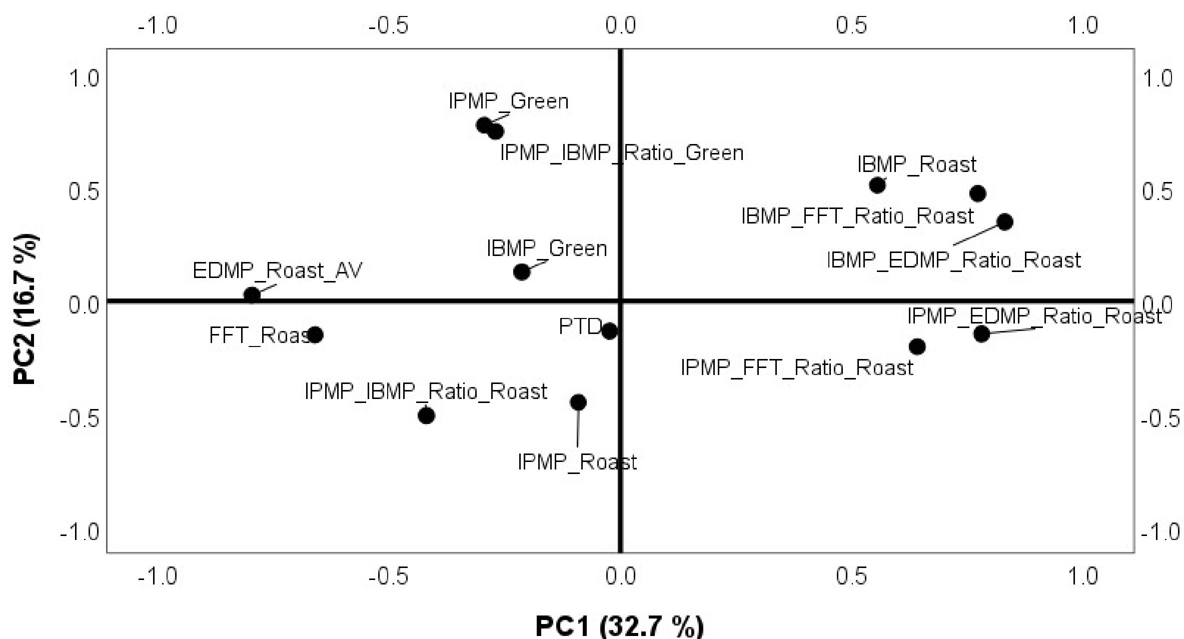


Figure 3.2: PCA loading biplot (after varimax rotation) of PTD and concentrations of proposed predictors in 32 coffee samples. PC1 is explained by 32.7 of variance while PC2 is explained by 16.7 % of variance.

Masking effect of coffee flavor compounds on IPMP and IBMP

Numerous potent flavor compounds are reported to contribute to coffee flavor, with several of these compounds having low sensory thresholds and relatively high concentrations in coffee beverages. Due to the complexity of the coffee flavor profile, there is potential for certain flavor compounds to mask the flavor of other compounds. In this study we have investigated the flavor masking impact on IPMP and IBMP in coffee by calculating their corresponding odor activities (OA) as the ratio of concentration of a compound to its corresponding threshold (Franca et al.,

2009). In addition, the OAs of FFT and EDMP as the potent flavor compounds were calculated. FFT is a thiol compound which was described with roasty aroma and has a threshold of 10 ng/g (Handayani, 2016; Sunarharum et al., 2014) while EDMP has nutty-roast flavor and a threshold of 40 ng/g (Sunarharum et al., 2014). The results in Table 3.5 showed that FFT had the highest OA (804,316) followed by IBMP (79,026) in roasted beans. Therefore, it is likely that FFT flavor has masked PTD contributing to difficulties of cuppers to detect PTD as indicated by low detection frequency and disagreement as detailed in the above sections. However, when we determined the correlation between PTD occurrence and OAs of volatile compounds (Table 3.6), there was no evidence of correlation between the occurrence of PTD and OAs of measured compounds.

Table 3.5: Average odor activity of PTD responsible pyrazines (IPMP and IBMP) and other potent odorants (EDMP and FFT) of coffee

Flavor compound	Sample	OA (Mean ± SEM)
IPMP	Green beans	10,363 ± 501
IBMP	Green beans	0.02 ± 0
IPMP	Roasted beans	57,400 ± 358
IBMP	Roasted beans	79,026 ± 1,289
EDMP	Roasted beans	41,092 ± 622
FFT	Roasted beans	804,316 ± 6,626

Table 3.6: Odor activity (OA)-based correlation coefficients of PTD occurrence, PTD pyrazines and potent flavor compounds of green and roast coffee. The significance was determined at 0.05 level.

Odor activity	Sample	PTD occurrence	
		Pearson Correlation	**Significance**
IPMP	Green beans	-0.07	0.02
IBMP	Green beans	-0.002	0.94
IPMP	Roasted beans	0.01	0.67
IBMP	Roasted beans	-.07	0.02
EDMP	Roasted beans	0.02	0.48
FFT	Roasted beans	-0.04	0.26

CONCLUSION

This was the first study to quantify the PTD responsible pyrazines in corresponding green and roasted coffee beans to improve understanding of the occurrence of PTD. The samples of coffee green beans were roasted, cupped and analyzed with GCMS. The findings revealed a mismatch between the occurrence of PTD and the concentrations of IPMP and IBMP in both green and roast beans. The low probability (0.13) of occurrence of detectable PTD in samples and variability in replicated samples were attributed to the composition of coffee beans, the possible low sensitivity of cuppers and the inconsistent procedures of roasting and cupping. Additionally, the low maximum frequency of occurrence of detectable PTD of 0.39 indicated a disagreement to detect PTD among cuppers. Furthermore, the findings from this study showed that FFT had higher OA than IPMP and IBMP, which might have masked the sensitivity of cuppers to identify PTD in these samples. Roasting of coffee beans increased IPMP concentrations from 20.7 to 114.8 ng/g and IBMP concentrations from 85.65 to 158.05 ng/g. Logistic regression analysis has shown evidence of analytical prediction of PTD from the composition of IPMP in green beans and concentration ratio of IBMP/EDMP in roasted beans. More research is recommended to further elucidate the potential relationships between concentrations of flavor compounds in coffee and incidence of PTD.

CHAPTER 4. PERFORMANCE ASSESSMENT OF PROFESSIONAL COFFEE CUPPERS TO DETECT POTATO TASTE DEFECT IN COFFEE

ABSTRACT

Professional cuppers are key actors in the coffee value chain since they take decisions that determine the price of coffee on basis of quality grade. A quality evaluation by cuppers is a function of personal judgment and acquired training, hence they are expected to provide reliable results and correct decisions. Currently, superior quality and defect-free specialty coffee is highly demanded in the international market. However, the occurrence of potato taste defect (PTD) in coffee results in an automatic exclusion from the specialty category. Since the occurrence of PTD in the specialty coffee industry is determined by sensory evaluation by trained cuppers, this study was conducted to assess their efficiency to detect PTD in coffee. Samples of Arabica coffee were collected and roasted with a medium roasting profile. A panel of 12 professional cuppers evaluated ten coffee samples in three replications by identifying samples with detectable PTD and evaluating the quality attributes of flavor, sweetness, acidity and general acceptability. The data were analyzed based on Generalizability theory to assess the discrimination of samples, consensus of cuppers and reproducibility of results. The findings showed a small Generalizability coefficient (G) of 0.37 and small index of dependability (D) of 0.31 for PTD detection. Similar findings were obtained for coffee quality attributes with G-coefficients ranging from 0.17 to 0.34 and D-coefficients from 0.11 to 0.26. The small G and D coefficients were attributed to small variance (30.9%) between coffee samples. Among the cuppers, a moderate variance of 15.7% was obtained to detect PTD in coffee. Although the findings demonstrated a consistent repeatability of results across cupping sessions; the variances among cuppers and small generalizability coefficients have demonstrated a moderate disagreement among cuppers, hence a poor performance to detect PTD

in coffee. Consequently, regular refresher trainings of cuppers on PTD detection are recommended.

INTRODUCTION

Coffee is a popular beverage all over the world with increasing global consumption. This is particularly associated with the pleasure acquired from a cup of coffee as a leading key to preference by most consumers (Samoggia and Riedel, 2018). The pleasure from coffee originates from a combination of quality parameters, mainly taste and aroma that are influenced by the geographic origin, processing and preparation procedures of coffee beans (Mutarutwa et al., 2020; Poltronieri and Rossi, 2016). A cup of coffee is ultimately derived from coffee that is planted, harvested and processed into green beans which are later roasted and brewed into a beverage. Coffee quality evaluation is performed organoleptically by trained individuals to determine the quality grade, which in turn determines the price of coffee. This evaluation is done by cupping, a popular method to evaluate the quality of coffee and performed by professional cuppers or experts who are well trained on coffee quality and defects. As elucidated by Tomic et al. (2013), the judgment of cuppers is mainly based on the acquired training, personal judgment and experience. Hence, they are expected to generate accurate cupping results conducive to take the right decision with confidence. It is important to highlight that a wrong decision on coffee quality can lead to economic losses to either the producer or the buyer. Since the price of coffee is correlated to its sensory quality (Barahona et al., 2020), cuppers are actors whose decisions significantly impact (Mutarutwa et al., 2020) the coffee value chain. Their task is to detect any change in coffee quality (Donfrancesco et al., 2014) and identify defects that may not even be perceived by consumers

(Thomas et al., 2017). In addition, the cuppers play a role of sharing information that guide the value chain actors to improve the quality of coffee as per consumers' needs.

Currently, specialty coffee is gaining popularity due to its uniqueness in flavor quality (Barahona et al., 2020; Samoggia and Riedel, 2018; Poltronieri and Rossi, 2016; Donfrancesco et al., 2014). It is a high-quality, defect-free coffee scoring higher than 80% on the Specialty Coffee Association of America cupping form (SCAA, 2015). Hence, the role of cuppers is to detect any sensory defects such as unpleasant flavors and aroma (Thomas et al., 2017), and to grade the coffee. For instance, the occurrence of PTD, a prevalent defect found in coffee from the African Great Lakes region, specifically Rwanda and Burundi (Mutarutwa et al., 2020; Gueule et al., 2015; Jackels et al., 2014; Gueule et al., 2013), is vigorously evaluated since it makes the resulting coffee beverage unpleasant. Although various research studies have been conducted to investigate PTD, its origin is not yet clear. However, researchers (Gueule et al., 2015; Jackels et al., 2014; Gueule et al., 2013; Czerny and Grosch, 2000) have reported that pyrazines such as IPMP (2-isopropyl-3-methoxypyrazine) and IBMP (2-isobutyl-3-methothoxypyrazine) are responsible for generating PTD in coffee beverages. Thus far, cupping is the only method to identify coffee quality defects (Donfrancesco et al., 2014) including PTD. Although humans are regarded as powerful instruments in sensory evaluation, it happens that they generate inconsistent results due to various factors that Meilgaard (2016) categorized into physiological and psychological factors. Similar cases were identified in coffee cupping with inconsistency of PTD evaluation results. The findings from previous studies have assumed that one defective bean may be sufficient to ruin a large sample of non-defective beans (Jackels et al., 2014). However, this hypothesis has not yet been thoroughly investigated. Similarly, cuppers have reported inconsistency of PTD distribution in coffee cups from a single sample. Unfortunately, no study has investigated this research topic.

In order to understand the relationship between PTD and chemical composition of coffee beans, research findings reported in chapter three of this dissertation demonstrated that there was no correlation between the concentrations of PTD pyrazines and cupping results. Consequently, the efficiency of cuppers to detect PTD was questioned. Pereira et al. (2018) and Pereira et al. (2017) have reported that the sensory sensitivity of cuppers is of significant relevance in quality evaluation, especially when dealing with taste and smell. Since different cuppers have different sensory sensitivity, it is of interest to evaluate their efficiency to perform cupping and identify specific quality defects such as PTD. The performance evaluation of sensory assessors is commonly conducted in the food industry to evaluate their ability to produce reliable results (Raithatha, 2018). Among methods used to assess the reliability of sensory tests, generalizability theory has been recommended and applied by various studies. This method is built on product discrimination or accuracy, agreement between cuppers on scoring and reproducibility of the test (Raithatha, 2018; Verhoef et al., 2015; Tomic et al., 2013; Derndorfer et al., 2005; Brennan, 2001). Since there is no previous study that has been conducted to assess the inconsistency of coffee cupping results with respect to PTD, this study was conducted to investigate the reliability of cuppers to detect PTD in coffee.

MATERIALS AND METHODS

Screening of coffee samples

Hundreds of arabica coffee samples were randomly collected from various coffee washing stations and coffee companies in Rwanda and were screened to obtain PTD-suspected samples and corresponding non-PTD samples. The fully washed parchment samples were hulled with a coffee huller (model DRC-2x; Pinhalense S/A, Brazil) to obtain green beans which were not sorted for

defective beans to increase the chances of PTD occurrence. The green beans were roasted using a medium roasting profile (175-190°C for 8 to 10 min) using a Probat BRZ2 roaster (Gimborn Maschinenfabrik GmbH, Germany). The roasted coffee beans were milled and sieved through a sieve set at 4.5 corresponding to Nº 40 US standard size with a coffee grinder (Ditting KR1203; Ditting Maschinen AG, Switzerland). Coffee beverages were prepared at a ratio of 20 g of coffee powder dissolved in 250 mL of boiled water (90-95°C). The coffee beverages were cupped by two professional cuppers to identify the samples with detectable PTD. From the screening process, five samples suspected with PTD were selected for the subsequent study using a larger group of professional cuppers. In addition, five more non-PTD samples were collected for comparison purposes. Hence, each PTD sample had a corresponding non-PTD sample with similar geographical origin, resulting in a total of 10 samples of green coffee.

Roasting of green beans and preparation of coffee beverages

The green coffee beans obtained from the screening process were roasted using an "IKAWA" pro digital micro roaster (Ikawa Ltd; London, United Kingdom) using a profile described in Table 4.1 and Table 4.2. The samples were roasted 24 hours prior to cupping as recommended by Pereira et al. (2018). Grinding of coffee beans, sieving of ground coffee and preparation of coffee beverages were carried out as described above in the screening of samples. The coffee beverages were prepared one hour prior to cupping.

Table 4.1: Time and temperature points of roasting profile programmed with "IKAWA" roaster

Time (min)	Temperature (°C) of roasting chamber
00.00	180
03.30	140
05.00	155
07:00	180
08:00	190
10:00	200

Table 4.2: Time and fan speeds of roasting profile programmed with "IKAWA" roaster

Time (min)	Fan speed (%)
00.00	76
01.19	76
02:09	76
03:00	72
03:18	60
07:56	65
10:00	65

Cupping of coffee beverages

Cupping of 10 coffee samples was performed by a panel of 12 cuppers who were randomly selected from professional coffee cuppers located in Rwanda and who agreed to participate. Professional cuppers were defined as trained and qualified people having at least one certificate of coffee cupping and working in the coffee industry as cuppers. A cupper having less than one year of experience was excluded from the selection pool. Cupping was carried out in three sessions corresponding to three replications, with a break of two hours between sessions. During cupping, the cuppers were presented with 3-digit coded cups of coffee samples of temperature ranging between 30 and 40°C. The cuppers were requested to taste the samples to identify any having PTD and record their judgement by indicating "Yes" (presence of PTD) or "No" (Absence of PTD). A vessel was provided to spit out the tasted sample since the cuppers do not swallow. The cuppers also evaluated the quality attributes of flavor, acidity, sweetness and the general acceptability and scored these parameters using a seven-point hedonic scale (Appendix 3). This study was reviewed and approved by the Michigan State University Institutional Review Board.

Data analysis

Descriptive statistics

Descriptive statistics were performed to determine the probability of PTD occurrence. The scores of quality parameters were reported as means with standard errors. The statistics were performed using SAS software version 9.4.

Performance of cuppers

The performance of individual cuppers and the whole panel was assessed on three main factors including discrimination, consensus and repeatability. Hence, the generalizability theory recommended by Talsma (2016), Verhoef et al. (2015) and Brennan (1992) was applied to determine the performance. According to the generalizability theory, the discrimination associated with coffee samples was investigated to determine the ability of cuppers to differentiate between the samples. Hence, the better performance was built on maximized variances between the samples. Consensus that was associated with cuppers was used to determine the agreement of cuppers on identified PTD-suspected coffee and scoring the quality parameters. The reliability of cuppers to provide consistent results across the cupping sessions was also determined by assessing reproducibility of results.

To assess the three factors, a G-study analysis was performed with a crossed design ($p*s*c$) involving the coffee samples as the objects of measurement (p), cupping sessions (s), the cuppers (c) and their interactions as described by the Venn diagram in Figure 4.1.

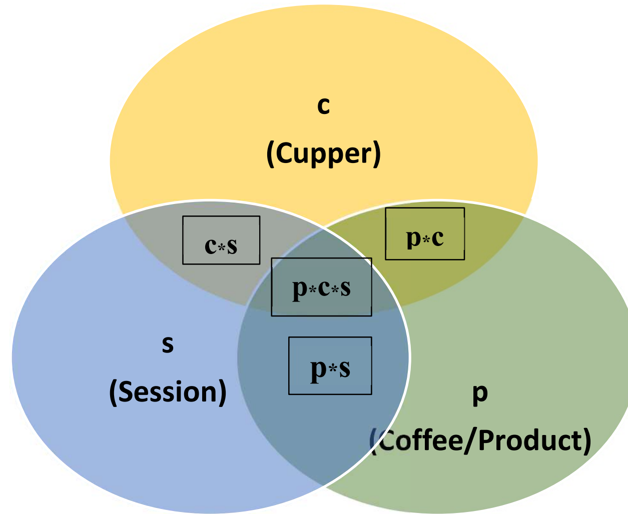


Figure 4.1: Venn diagram describing the crossed G-study design. The main effects are presented in the circles, and interaction effects in the intersections of circles.

Calculation of variance components

The G-study concept consists of identifying and estimating the variations of levels and their interactions of the three facets of sample, session and cupper. Hence, the components of interest were described in the linear model equation 4.1.

$$x_{prc} = \mu + \nu_p + \nu_s + \nu_c + \nu_{ps} + \nu_{pc} + \nu_{sc} + \nu_{psc} \quad (4.1)$$

x_{prc}: Observable score

μ: Grand mean

ν_p: Effect of coffee

ν_s: Effect of cupping session

ν_c: Effect of cupper

ν_{ps}: Effect of interaction coffee-session

ν_{pc}: Effect of interaction coffee-cupper

ν_{sc}: Effect of interaction session-cupper

ν_{psc}: Effect of interaction coffee-cupper-session

Since the G-theory utilizes variances, they were estimated with the method of minimum variance quadratic unbiased estimation (MIVQUEO) using SAS software version 9.4. For efficient calculation, Verhoef et al. (2015) recommended to set a negative variance value of the component to zero. The linear model of variances of components is described in equation 4.2. The variance components were reported as variance component percent of the total variance. The total variance was obtained by summing up all variance components.

$$\sigma^2(\chi_{prc}) = \sigma^2{}_p + \sigma^2{}_s + \sigma^2{}_c + \sigma^2{}_{ps} + \sigma^2{}_{pc} + \sigma^2{}_{sc} + \sigma^2{}_{psc} \quad (4.2)$$

$\sigma^2(\chi_{prc})$: Total observed variance

$\sigma^2{}_p$: Variance due to effect of coffee

$\sigma^2{}_s$: Variance due to effect of cupping session

$\sigma^2{}_c$: Variance due to effect of cupper

$\sigma^2{}_{ps}$: Variance due to effect of interaction coffee-session

$\sigma^2{}_{pc}$: Variance due to effect of interaction coffee-cupper

$\sigma^2{}_{sc}$: Variance due to effect of interaction session-cupper

$\sigma^2{}_{psc}$: Variance due to effect of interaction coffee-cupper-session

Calculation of performance coefficients

The performance of cuppers was assessed with generalizability coefficient (G) and Index of dependability (D). The coefficients were calculated using equations 4.3 and 4.4 (Talsma, 2016; Verhoef et al., 2015) for the whole panel of cuppers and then for reduced panel by excluding one cupper. The performance of each individual cupper was determined by comparing the coefficients of the reduced panel (when the cupper of interest was excluded) to the whole panel.

$$G = \frac{\sigma^2{}_p}{(\sigma^2{}_p + \sigma^2{}_{ps} + \sigma^2{}_{pc} + \sigma^2{}_{psc})} \tag{4.3}$$

$$D = \frac{\sigma^2{}_p}{(\sigma^2{}_p + \sigma^2{}_s + \sigma^2{}_c + \sigma^2{}_{ps} + \sigma^2{}_{pc} + \sigma^2{}_{sc} + \sigma^2{}_{psc})} \tag{4.4}$$

RESULTS AND DISCUSSION

Cuppers

A study conducted by Pereira et al. (2018) suggested a minimum of six professional cuppers were required to produce reliable results in coffee cupping. Hence, our study used a sufficient number of 12 cuppers who were composed of nine females and three males. The large number of females was an added value since they have been reported to generally perform better in sensory analysis than males (Michon et al., 2009). At the time of the experiment, all cuppers were working as cupping professionals in coffee companies with experience ranging from 2 to 17 years. Two cuppers had less than five years of experience, but the rest of the panel had more than 10 years of cupping experience.

Reliability of cupping test and sample discrimination

G and D coefficients as determinants of reliability of cupping tests were calculated for the whole and reduced panels. The coefficients were used to assess how the panel agreed on detecting PTD and scoring quality attributes of coffee. According to Talsma (2016), excellent performance by cuppers would correspond to a coefficient close to 1, with an acceptable minimum being 0.8 (Verhoef et al., 2015), but this might vary depending on differences in quality characteristics among the samples. The D-coefficient, which is applied in food industries for screening excellent

food assessors (Talsma, 2016), was used to assess the performance of individual cuppers in addition to G-coefficient. The results presented in Table 4.3 showed that the whole panel used in this study performed with relatively small coefficients for PTD detection and quality attributes ranging from 0.17 to 0.37 and 0.11 to 0.31 for G and D coefficients, respectively. Hence, these findings were indicative of poor performance of the whole panel and individual cuppers to agree on scoring quality attributes of coffee samples (Talsma, 2016; Verhoef et al., 2015).

Table 4.3: Generalizability (G) coefficients and Dependability (D) coefficients calculated from cupping scores of coffee samples. In the row of cupper ID, "None" describes the coefficients of the whole panel of 12 cuppers, while the numbers correspond to the panel ID who was excluded for a reduced panel.

ID of excluded cupper	**G-coefficient**					**D-coefficient**				
	PTD	**Flavor**	**Acidity**	**Sweetness**	**Acceptability**	**PTD**	**Flavor**	**Acidity**	**Sweetness**	**Acceptability**
None	0.37	0.34	0.17	0.31	0.31	0.31	0.26	0.11	0.22	0.25
1	0.33	0.33	0.18	0.30	0.31	0.28	0.26	0.12	0.22	0.25
2	0.34	0.36	0.19	0.32	0.33	0.29	0.28	0.12	0.23	0.27
3	0.41	0.36	0.21	0.34	0.33	0.34	0.31	0.17	0.28	0.28
4	0.38	0.33	0.17	0.31	0.29	0.32	0.25	0.11	0.22	0.24
5	0.37	0.32	0.17	0.31	0.32	0.31	0.24	0.11	0.21	0.26
6	0.45	0.39	0.20	0.33	0.36	0.39	0.30	0.13	0.23	0.28
7	0.37	0.33	0.16	0.31	0.28	0.30	0.24	0.10	0.22	0.22
8	0.33	0.33	0.17	0.29	0.28	0.28	0.25	0.11	0.20	0.22
9	0.33	0.32	0.14	0.28	0.29	0.28	0.25	0.09	0.20	0.23
10	0.33	0.33	0.16	0.31	0.30	0.28	0.26	0.10	0.22	0.24
11	0.35	0.34	0.17	0.32	0.30	0.30	0.27	0.11	0.23	0.24
12	0.42	0.33	0.17	0.32	0.32	0.38	0.26	0.12	0.24	0.27

The G- and D-coefficients for PTD were 0.37 and 0.31, respectively, which indicated a relatively low agreement of the panel members to detect PTD in coffee samples. According to Talsma (2016), the performance by panels can be affected by the nature of samples under study, such as small quality differences between samples. Therefore, it is important to highlight that the small G coefficient observed in this study could be attributed to small variance (30.9%) of PTD (Table 4.4) between coffee samples, which also indicated a poor discrimination between samples. This is in line with the explanation of Verhoef et al. (2015), who stated that the samples with close similarities show small differences. It is worth noting that a larger G coefficient goes together with larger sample variance, which is also an indicator of good sample discrimination (Verhoef et al., 2015).

Under certain assumptions, we cannot forget the realistic possibility of inherent low occurrence or absolute absence of PTD in collected samples as well as the influence of cuppers that contributed to small variance of samples. For example, the results from descriptive statistics (Figure 4.2) demonstrated a low frequency (0.2) of PTD occurrence in the 10 coffee samples. Among the 10 coffee samples assessed in this study, five were previously selected because they had detectable PTD during a preliminary cupping session using two cuppers (data not shown). During the screening of samples, the sample ID 10 was among non-PTD samples; however, in this study it was reported with detectable PTD by most of the cuppers (Table 4.5). A similar situation of PTD inconsistency was reported by other researchers (Mutarutwa et al., 2020; Jackels et al., 2014; Gueule et al., 2013) who hypothesized that a single PTD grain can affect a sample. Furthermore, the variations in cuppers' potential to detect PTD has contributed to small coefficients. For example, only 3 cuppers detected PTD with a probability of PTD occurrence of 0.4 or more (Table 4.6) in coffee samples, which indicated a disagreement between cuppers.

Table 4.4: Variance of main components and their interactions obtained from panel assessment of coffee samples.

Quality parameter	Variance (%)						
	Coffee	Cupper	Session	Coffee-Cupper	Coffee-session	Cupper-session	Coffee-cupper-session
PTD	30.9	15.7	0.2	21.6	0	0	31.6
Flavor	26.5	21.9	0	18.1	0	0.4	33.2
Acidity	11.5	32.3	0	13.5	0.5	1.8	40.4
Sweetness	22.3	27.7	0	11.6	0.1	0.6	37.8
Overall acceptability	25.1	18.3	0	18.4	0	0.8	37.5

Table 4.5: Frequency (%) of PTD occurrence in 10 coffee samples that were evaluated by 12 cuppers in 3 replications.

Sample ID	Frequency of detectable PTD (%)	Frequency of undetectable PTD (%)
1	8.3	91.7
2	5.6	94.4
3	13.9	86.1
4	13.9	86.1
5	11.1	88.9
6	16.7	83.3
7	8.3	91.7
8	22.2	77.8
9	11.1	88.9
10	86.1	13.9

Table 4.6: Frequency (%) among 12 cuppers to detect PTD in 10 coffee samples evaluated in three replications.

Cupper ID	Frequency of PTD detection (%)	Frequency of undetectable PTD (%)
1	10	90
2	13.3	86.7
3	40	60
4	3.3	96.7
5	10	90
6	46.7	53.3
7	20	80
8	10	90
9	10	90
10	10	90
11	6.7	93.3
12	56.7	43.3

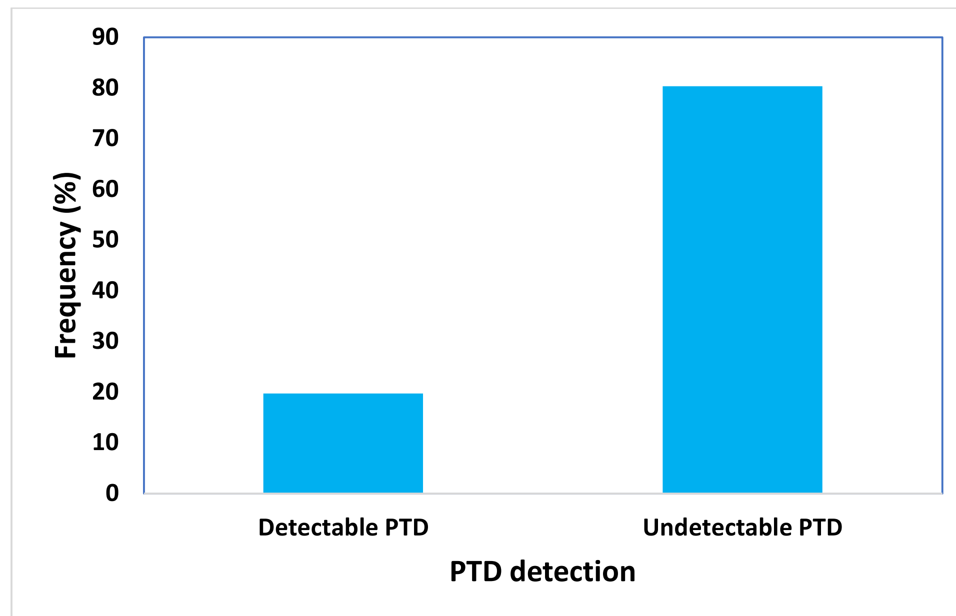


Figure 4.2: Frequency of PTD detection in 10 coffee samples evaluated by 12 cuppers in three replications.

Besides PTD, a poor agreement on scoring flavor, acidity, sweetness and general acceptability of coffee samples was also identified with small G and D coefficients (Table 4.3). Again, this can be explained by close similarities of coffee samples as demonstrated by small variances associated with these parameters in the coffee samples (Table 4.4). Consequently, the small variances of evaluated quality attributes have resulted in a poor discrimination of coffee samples. This is supported by close scores attributed to quality attributes as shown in Table 4.7. These results demonstrated that nine samples were slightly liked by cuppers with average score of 5, while one sample was slightly disliked with average score of 3. Hence, the small differences in flavor, acidity, sweetness and general acceptability between samples have contributed to poor test reliability.

Further analyses on the poor performance of the whole panel were performed by determining the contribution of each panelist. This was determined by exploring the G and D coefficients of reduced panels presented in Table 4.3. The assessment procedure described by Talsma (2016) involved a comparison of G and D coefficients of the whole panel with reduced panel when one cupper was excluded. The same author recommended that an increase of 0.1 (or more) of D-coefficient for a reduced panel is an indicator of poor performance of the excluded cupper, who is immediately excluded from the study. In this study, changes in D-coefficients were less than 0.1 for each quality parameter when each of the cuppers was excluded. This is an important finding which demonstrated that the cuppers used in this study were at a comparable poor performance level. Although the variability of individual cuppers' performance was not significant according to the concept of Talsma (2016), the findings showed that six cuppers have positively contributed to the performance of panel to detect PTD. This was demonstrated by the results in Table 4.3 that showed a decrease of coefficients of reduced panels when the cuppers with ID 1, 2, 8, 9, 10 and 11 were excluded. On the other hand, the cuppers with ID 3, 4, 6 and 12 demonstrated a negative

contribution to the performance of the panel since the G and D coefficients of reduced panels were increased when these cuppers were excluded. Given that discrimination performance and test reliability are associated with sample variance (Talsma, 2016; Verhoef et al., 2015; Tomic et al., 2013; Derndorfer et al., 2005; Shavelson and Webb, 2005; Brennan, 1992), it is important to evaluate factors contributing to sample variance. Accordingly, Talsma (2016) proposed a way of calculating the combined contribution of variances of cuppers, cupping sessions and their interactions which was expressed in percentage as "α" in equation 4.5. Hence, the findings of this study showed a considerable contribution of the three components to the small D coefficients as indicated by large α in **Error! Reference source not found.**. The panel results showed that the 3 components contributed 51.6%, 84.1%, 297%, 127% and 76.2% of coffee samples variances for PTD, flavor, acidity, sweetness and acceptability, respectively. These results demonstrate that the variance between samples was extremely low, hence resemblance of samples in quality characteristics.

$$\alpha = 100 \times \left(\frac{1}{D} - \frac{1}{G}\right) \tag{4.5}$$

Table 4.7: Mean scores (± SEM) of quality attributes of 10 coffee samples evaluated by 12 cuppers in three cupping sessions. The evaluation used a 7-point hedonic scale from 1 (dislike very much) to 7 (dislike very much).

Sample ID	Flavor	Acidity	Sweetness	General acceptability
1	5.6 ± 0.2	5.7 ± 0.2	5.5 ± 0.2	5.6 ± 0.2
2	5.7± 0.2	5.9 ± 0.2	6.0 ± 0.1	5.8 ± 0.2
3	5.7 ± 0.2	5.7 ± 0.1	5.6 ± 0.2	5.8 ± 0.2
4	5.2 ± 0.2	5.4 ± 0.2	5.7 ± 0.2	5.5 ± 0.3
5	5.6 ± 0.2	5.6 ± 0.2	5.8 ± 0.2	5.6 ± 0.2
6	5.0 ± 0.2	4.9 ± 0.2	5.2 ± 0.2	5.1 ± 0.2
7	5.5 ± 0.2	5.7 ± 0.2	5.6 ± 0.2	5.7 ± 0.2
8	5.3 ± 0.2	5.5 ± 0.3	5.4 ± 0.3	5.3 ± 0.2
9	5.8 ± 0.3	5.8 ± 0.3	5.8 ± 0.2	5.8 ± 0.3
10	3.1 ± 0.3	4.3 ± 0.3	3.7 ± 0.2	3.2 ± 0.3

Table 4.8: The calculated α (%) indicating the contribution of variances of cuppers, cupping sessions and their interactions to the variance of coffee samples. In the column of cupper ID, "0" indicates a whole panel of 12 cuppers, while the other numbers correspond to the ID of the cupper who was excluded for a reduced panel.

ID of excluded cupper	PTD	Flavor	Acidity	Sweetness	Acceptability
0	51.6	84.1	297	127	76.2
1	59.2	83.3	282	127	71.5
2	59.2	78.9	269	123	66.5
3	49.4	41.5	95	66.4	46.2
4	46.5	94.1	309	130	86.2
5	53.7	99.7	362	142	78.5
6	39.1	81.3	277	130	73.8
7	58.8	111.6	348	130	100.7
8	59.2	94.2	338	160	99.4
9	59.2	93.5	389	157	85.3
10	59.2	88.5	375	138	80.7
11	53.5	69.0	307	120	80.2
12	27.8	82.8	286	107	57.2

Consensus of cuppers on scoring

Excellent agreement between the panelists in scoring the samples is demonstrated by small variance components associated with the panelists (Talsma, 2016; Verhoef et al., 2015). Hence, the components of interest of this study were cuppers, interaction of cupper-session, interaction of cupper-coffee, and interaction of cupper-session-coffee. Their corresponding variances for PTD are presented in Table 4.4. A moderate variance (15.7%) of the main effect of cuppers was obtained, which indicated a moderate agreement between cuppers to identify samples with detectable PTD. However, a disagreement between cuppers was affected by the effect of coffee samples, which resulted to slight increase of the variance (21.6%) of cuppers when interacted with coffee samples. In addition, the results in Table 4.6 demonstrate there was moderate disagreement among cuppers to identify samples as having PTD. For example, the cupper ID 12 who detected more PTD demonstrated the highest frequency of detecting PTD (0.57), while the cupper ID 4

showed a frequency of 0.03. However, the results in Table 4.5 show that the sample ID 10 was reported with detectable PTD by most of the cuppers with an overall frequency of 0.86. It is important to note that some cuppers may have missed PTD in samples because it was not there while others missed it when it was there. As a result, this scenario revealed an existing challenge to only rely on cupping to detect PTD in coffee. It is important to highlight the challenge of PTD in the coffee business since the economic damage it creates in the coffee industry is huge (Mutarutwa et al., 2020; McPherson, 2018). Some examples include the rejection of coffee at the market, leading to economic losses among the value chain actors (Mutarutwa et al., 2020). Consequently, variances in cupping reliability among cuppers may be indicative of the likelihood of incorrect decisions taken based on wrong results on PTD in coffee, which in turn affect the price of coffee beans (Pereira et al., 2018; Pereira et al., 2017). The lack of variance attributable to the interaction of cupper-session demonstrated the consistency of results among three cupping sessions contributed to the excellent consensus of cuppers among the sessions. However, the slight increase of variance (31.6 %) attributed to the cupper-coffee-session interaction as shown in Table 4.4, was impacted by the variances of both coffee sample and cupper main effects. The lack of agreement among the cuppers on detection of PTD was also indicated by the variations among cuppers to detect PTD in coffee samples (Table 4.6). In case of flavor, acidity, sweetness and overall acceptability of coffee samples; similar findings of moderate disagreement among cuppers were also obtained (Table 4.4). This was demonstrated by the moderate variances of main effects of cupper, interaction of cupper-coffee and interaction of cupper-coffee-session. However, the variance of interaction cupper session was negligible as a result of small session variance.

Repeatability

For excellent repeatability, the variance components associated with cupping session should be as small as possible (Talsma, 2016; Verhoef et al., 2015; Tomic et al., 2013; Derndorfer et al., 2005; Shavelson and Webb, 2005; Brennan, 1992). The findings presented in Table 4.4 revealed a negligible variance (0.2%) was attributable to PTD between the cupping sessions. A lack of variance for flavor, acidity, sweetness and overall acceptability between the cupping sessions was observed between cupping sessions. This is an indicator of excellent repeatability i.e.; the panel consistently scored the samples across the cupping sessions. The good repeatability was also demonstrated by the negligible variances of the cupper-session interaction. However, the variances of coffee samples and cupper main effects have impacted a slight increase of variance of interaction of cupper-coffee-session (31.6%). The comparison of cupping results showed that there was no significant difference ($p > 0.05$) between cupping sessions, indicating good repeatability of the test by cuppers. Hence, the findings of this study have demonstrated that the cuppers have provided consistent results when a similar sample was evaluated more than once.

CONCLUSION

The ability of cuppers to efficiently and reliability identify coffee quality defects is essential for the global coffee business. Hence, it is indispensable to monitor the performance of cuppers to ensure reliable results. This study assessed the ability of cuppers to consistently detect PTD and evaluate the quality attributes of flavor, acidity, sweetness and general acceptability of coffee. The findings demonstrated excellent competencies of cuppers to provide consistent results when the samples were evaluated in three replications. This was indicated by 0.2% variance of the session or replication main effect to detect PTD and 0.0% variance for scoring other quality attributes.

However, the assessment on consensus among cuppers to detect PTD revealed a moderate variance of 15.7% of cupper main effect, indicating a moderate agreement among cuppers due to their differences in sensorial sensitivity. On the other hand, the variance in PTD occurrence among the samples was small (30.9%). However, the occurrence of PTD was expected to be more than this since 50% of samples were previously identified with potential detectable PTD, hence indicating a poor discriminability of samples. In addition, the small generalizability coefficients of 0.37 for G and 0.31 for D coefficients indicated a poor reliability of cupping to detect PTD. The quality attributes were also scored with moderate disagreement due to small generalizability coefficients. The small differences in quality characteristics described by small variances of main effect of sample, have contributed to the poor discriminability of coffee samples. As a general conclusion, the study on evaluation of performance of cupping has revealed a poor reliability of cuppers to detect PTD. Consequently, the variances in cupping reliability among cuppers may indicate improper decisions taken based on wrong results on PTD in coffee, which in turn affects the coffee business. During coffee cupping, a larger panel of cuppers is recommended to reduce the variations resulting from differences in sensitivity among the cuppers. In addition, calibration of cuppers using PTD standard solutions prior to cupping sessions and regular refresher trainings are key to enhance the efficiency of cuppers to detect PTD. When assessing the performance of cuppers, it is recommended for future studies to consider using coffee samples that have been processed with different methods to increase the variations among samples.

CHAPTER 5. ESTIMATED THRESHOLDS FOR UNPLEASANT FLAVORS OF IPMP AND IBMP DETECTED BY PROFESSIONAL CUPPERS IN WATER AND COFFEE

ABSTRACT

Sensory analysis is a popular method to assess and grade the quality of food using human senses. In coffee, the method is commonly known as cupping, performed by trained professional cuppers. An additional major task of cuppers is to detect any defects that affect the sensory quality of coffee beverages. Potato taste defect (PTD) has been identified among the defects contributing to off flavor minimizing coffee quality. Previous studies have demonstrated that the pyrazines 2-isopropyl-3-methoxypyrazine (IPMP) and 2-isobutyl-3-methothoxypyrazine (IBMP) are potentially responsible for PTD in coffee. To evaluate the sensory sensitivity of cuppers to detect PTD, this study was conducted to determine the sensory thresholds of coffee cuppers to detect IPMP and IBMP in both water and coffee beverage. Samples were prepared from 10^6 ng/L concentration of stock solutions of IPMP, IBMP and a blend of IPMP-IBMP. The samples were serially diluted in water yielding concentrations from 25.6 to 0.3 ng/L and in coffee yielding concentrations from 2500 to 10.2 ng/L. The solutions were evaluated by 12 professional cuppers using three-ascending forced-choice (3-AFC) method of limits. The thresholds were estimated as best estimate threshold (BET) for individual cuppers and the whole panel. This study revealed panel BET values of 0.7 ng/L, 1.3 ng/L and 1.4 ng/L, for IPMP, IBMP and a blend of IPMP-IBMP in water; respectively. When the thresholds were measured in coffee, the BET values were 110 ng/L, 384 ng/L and 66.7 ng/L, for IPMP, IBMP and a blend of IPMP-IBMP, respectively. Overall, large differences in sensory sensitivity were observed among cuppers who also demonstrated larger thresholds to detect IPMP and IBMP, hence low reliability to detect PTD in coffee.

INTRODUCTION

Coffee has attractive pleasant flavors that develop in a series of operations from the field to the cup. Hundreds of volatile compounds are responsible for coffee flavors (Pereira, et al., 2017; Donfrancesco et al., 2014). However, these flavors are perceived in accordance with their respective thresholds and intensity in coffee. Lawless and Heymann (2010) have defined a detection threshold in sensory analysis as the minimum concentration of a substance that is detected by 50% of the assessors.

The complexity of coffee flavor compounds, which are primarily developed during roasting, influences the task of quality assessors (Pereira et al., 2017; Poltronieri and Rossi, 2016). Consequently, a unique method of sensory analysis known as “cupping” was established to evaluate coffee quality (Pereira et al., 2017). Cupping is performed by professional tasters, known as “cuppers”, who translate consumer needs into quality requirements (Thomas et al., 2017). Cuppers have a crucial responsibility in the coffee business since the price paid by consumers is relative to sensory quality of coffee they buy (Barahona et al., 2020; Pereira et al., 2018; Poltronieri and Rossi, 2016). Among other tasks, cuppers have a duty to detect defects and categorize coffee batches into quality grades such as specialty. Specialty coffee is a category of defect-free coffee which is currently in high demand since consumers are looking for superior quality and are willing to pay high prices for such products (Barahona et al., 2020; McPherson, 2018; Pereira et al., 2018; Samoggia and Riedel, 2018; Poltronieri and Rossi, 2016; Donfrancesco et al., 2014). Hence, coffee producers, processors and cuppers are concerned to monitor, enhance and maintain the sensorial qualities of coffee.

The process of screening and monitoring for coffee defects starts from raw fresh coffee beans. However, there are defects that are only perceived in the cup, yet efforts to eliminate these defects

must occur during production and processing of coffee. This is the case for PTD, an unacceptable defect that is only detected in prepared coffee beverages. This defect is common in coffee from Rwanda and Burundi, and contributes to economic losses due to rejection of tons of coffee each year (Miller, 2015). This undesirable flavor has been characterized as potato-like flavor, peasy off-flavor or green peas-like flavor (Mutarutwa et al., 2020; McPherson, 2018; Gueule et al., 2015; Jackels et al., 2014; Gueule et al., 2013), and is perceived by tasting coffee beverages. With experienced people such as cuppers, the potato-like aroma can be perceived during roasting or grinding when higher intensities of its precursors are present in coffee beans. There is a very low threshold of IPMP and IBMP required to cause PTD as reported by Gueule et al. (2015), Jackels et al. (2014), Gueule et al. (2013), and Czerny and Grosch (2000). Previous studies have reported that green, earthy, herbaceous and bell pepper-like flavors are generated by IPMP and IBMP (Franca et al., 2009; Pickering et al. 2007; Murray and Whitfield, 1975). The same authors also reported that these methoxypyrazines are common in green bell pepper and fresh beans. Although they are desirable in some foods as such as green bell pepper, they are undesirable in others such as grape juice (Pickering et al., 2008) and wine (Pickering et al., 2007).

A major challenge to detect PTD arises from the chemical properties of coffee, which includes numerous flavor compounds that are difficult to separate by sensory analysis. Currently, there is no efficient method other than cupping that is used to detect off-flavors in coffee beverages (Donfrancesco et al., 2014). Thus, cuppers are key to taste and evaluate coffee quality on behalf of consumers prior to shipping (Pereira et al., 2017; Thomas et al., 2017; Donfrancesco et al., 2014) since they understand consumer preferences. In view of their responsibilities and expectations from their task, cuppers are expected to have high sensory sensitivity and knowledge to recognize, describe, and measure the intensities of quality parameters of coffee (Thomas et al.,

2017; Donfrancesco et al., 2014). Furthermore, the experience in cupping is an added value for reliable results (Van Gemert, 2011). Due to interactions between flavors in coffee and subsequent loss of sensory perception of individual volatile compounds (Grosch, 2001), IPMP and IBMP are not efficiently detected even though they have a low sensory threshold (Pickering et al., 2007). The threshold is used to mathematically determine the aroma contribution of a compound in a mixture of volatile compounds (Grosch, 2001). It can also be used to select sensory assessors based on their sensitivity for a particular component (Lawless and Heymann, 2010).

Meilgaard et al. (2016) and Lawless (2010) have well described the three types of thresholds of detection, recognition and difference threshold. A detection threshold was defined as the smallest concentration of a compound described as stimulus that produces a sensory sensation, while a recognition threshold was defined as a stimulus level of a compound that can be detected and identified. A difference threshold was defined as the change in concentration of a compound to produce a detectable difference. Variations of these thresholds exist among sensory assessors due to differences in sensory sensitivity (Lawless, 2010). A similar challenge has been observed in coffee cupping. The findings in chapters 3 and 4 of this dissertation showed a disagreement among cuppers to identify coffee samples that had detectable PTD. In addition, a review of previous studies conducted by Van Gemert (2011) identified various thresholds of IPMP and IBMP less than 10 ng/L. It is also important to highlight that the threshold values may vary depending on measurement method and the training level of assessors. Among numerous methods to determine thresholds in food, the ascending forced-choice method of limits has been reported as the most common method (Giguère et al., 2016; Lawless, 2010; Lawless and Heymann, 2010; Pickering et al., 2008; Pickering et al., 2007; Eisele and Semon, 2005; ASTM, 2004). Besides water and air, thresholds are also measured in actual foods and beverages (Meilgaard et al., 2016). For instance,

the thresholds of IPMP have been measured in both grape juice (Pickering et al., 2008) and wine (Pickering et al., 2007). Thus far, there is no study that has determined the IPMP or IBMP threshold using coffee as a medium of test. Since the price of coffee is quality dependent and consumers demand defect-free coffee, cuppers have an essential role to accurately and consistently assess coffee quality with respect to PTD. However, the low reliability reported in chapter 4 indicates the importance of investigating sensitivity of cuppers to detect PTD responsible compounds. Hence, this study was conducted to determine the efficiency of coffee cuppers by determining their sensory detection thresholds for IPMP and IBMP when added in water and coffee. The thresholds of cuppers will be used to predict the detection of PTD by comparing thresholds with the concentrations of these compounds measured in coffee samples. In addition, the findings of this study will create awareness to enhance the PTD sensory sensitivity of cuppers.

MATERIALS AND METHODS

Chemicals

Food grade standards of IPMP and IBMP (purity ≥ 99%; Sigma-Aldrich, Saint Louis, USA) were purchased. Dilution water was prepared by steam distillation (GFL 2008; Gesellschaft fur labortechnik mbH, Burgwedel, Germany). Food grade alcohol (purity > 98%) was purchased by Parmeshwar Impex Pvt Ltd (Gujarat, India).

Preparation of stock solutions

The initial stock solutions of IPMP, IBMP and a blend of IPMP-IBMP (50:50) were prepared referring to previous similar experiments carried out by Pickering et al. (2008) and Pickering et al. (2007). Pure standards of each compound were dissolved in ethanol to prepare initial stock solutions of 1 mg/mL concentration. The initial stock solutions were diluted with distilled water

to make working stock solutions of 10^6 ng/L concentration. All stock solutions were stored in a refrigerator (4°C).

Preparation of coffee beverages

A parchment sample of arabica coffee that was previously processed using the wet (fully washed) method was collected from a coffee washing station in Rwanda and hulled with a coffee huller model DRC-2X (Pinhalense S/A, Brazil) to obtain green beans. Roasting of coffee was carried out with a Probat BRZ2 roaster (Gimborn Maschinenfabrik GmbH, Germany) using a medium profile (175-190°C, for 8-10 min). Roasting was carried out within 24 hours prior to cupping as recommended by Pereira et al. (2018). Roasted beans were ground and sieved in a Ditting KR1203 model (Ditting Maschinen AG, Switzerland). The sieve was set at 4.5, corresponding to N° 40 US standard size. The coffee beverage was prepared by infusion method consisting of pouring boiled water (90-95°C) on ground coffee at a ratio of 1 g coffee/12.5 mL water. The solution was kept for 4 min to allow extraction of water-soluble components. The coffee beverage was filtered to separate the extraction liquid from coarse coffee particles.

Preparation of samples

Two types of samples were prepared. The first sample was obtained by diluting the working stock solution with distilled water, while a second sample was obtained by spiking coffee beverage with working stock solution. A geometric mean of 2.5 recommended by ASTM (2004) was applied to determine concentration series of each sample. The water-based samples were prepared by diluting the working stock solution to six concentration series from to 25.6 to 0.3 ng/L. The starting concentrations were pre-determined by two bench-tests conducted by two professional cuppers. Simultaneously, the working stock solutions were spiked in coffee beverage to obtain seven concentration series of coffee-based samples. Following a bench test that was carried out by three

professional cuppers, the concentrations of study ranged from 2500 to 10.2 ng/L. Note that the same cuppers had previously cupped the coffee beverage to make sure it was free of defects.

Cupping of samples

The participants in this experiment called “cuppers”, were randomly selected from a group of professional coffee cuppers who were working in the coffee industry as cuppers at the time of experiment. Professional cuppers were defined as cuppers who were trained, had at least one coffee cupping certificate and had at least one year of cupping experience. Applying these selection criteria, twelve professional cuppers (three males and nine females) were identified and agreed to participate in the study. The cupping experiment was performed in two sessions that were carried out on two different days in a laboratory designed for coffee cupping. The first session involved water-based samples while the other involved coffee-based samples. All the three compounds of study were assessed in each cupping session.

At the beginning of cupping session, the cuppers were introduced to the experiment to understand the purpose and procedure of the test since it was different from the usual cupping procedure. The recommended 3-AFC method of sensory analysis was applied to determine the thresholds of the studied compounds (Giguère et al., 2016; Lawless, 2010; Lawless and Heymann, 2010; Pickering et al., 2008; Pickering et al., 2007; Eisele and Semon, 2005; ASTM, 2004). Each cupper received six or seven sets of samples depending on the session. A set of samples consisted of three cups, one containing a sample with compound of study, and the other two cups containing blank samples. A set of three cups containing 50 mL of sample in each 3-digit coded cup was presented to the cuppers at random. The samples were prepared 30 minutes prior to cupping and were served to cuppers at ambient temperature of around 25°C for water-based samples and between 40-50°C for coffee-based samples. The cuppers were forced to choose the odd samples from each of the three

samples based on their judgment after tasting using a coffee cupping spoon. The cuppers were required to start with the lowest concentration and progressively taste to the highest concentration. The cuppers introduced each sample in the mouth using a spoon and were not allowed to swallow. Hence, a vessel was provided to spit out the samples after tasting. Water was provided to rinse the mouth and clean the cupping spoon before tasting the next sample. The cuppers were allowed a break of 30 seconds between two samples, and two hours between compounds of study. The design of experiment involved evaluation of all the provided samples. A cupping form (appendix 2) was provided, and the cuppers were requested to encircle the 3-digit code corresponding to the odd sample. Since this study involved human beings, it was reviewed and approved by the Michigan State University Institutional Review Board.

Estimation of panel and individual detection thresholds using ASTM-E679 method

The calculation of threshold for both individual cuppers and the whole panel was performed using the ASTM-E679 method (ASTM, 2004). For the purpose of verification, logistic regression analysis was performed to determine the sensory threshold of the panel (Giguère et al., 2016; Lawless, 2010; Lawless and Heymann, 2010; ASTM, 2004). Descriptive statistics and logistic regression analyses were performed using IBM SPSS statistics version 25. In accordance with the ASTM-E679 method, the threshold was determined as the best estimate threshold (BET). The method consisted of recording data by indicating the choices of the cupper for each concentration, with the "+" sign to indicate the right choice, and "0" value to indicate the wrong choice. The cupper's individual BET was calculated as a geometric mean of consecutive lowest and highest concentrations. The lowest concentration was the last missed concentration, and the highest was the first detected concentration with a condition of continuous selection of correct choices. Hence,

the equation 5.1 was used to calculate the individual BETs while the panel BET was calculated as the geometric mean of individual BET values as shown in equation 5.2.

$$BET_{ind}=\sqrt{a^2 \times b^2} \quad (5.1)$$

BET_{ind}: Individual BET

a: Last missed concentration

b: First detected concentration

$$BET_{panel}= \sqrt[n]{(\chi_1 \times \chi_2 \times \ldots \chi_n)} \quad (5.2)$$

BET_{panel}: Group or panel BET

n: Number of cuppers

χ_n: Individual BET value of cupper

Estimation of panel detection threshold using psychometric function

An additional method was used to estimate the panel BET using the psychometric function. This is an empirical method that uses a probability function to determine a detection threshold (Lawless and Heymann, 2010). It consists of plotting the logarithmic concentrations against the percentages of chance-correct choices calculated using equation 5.3. Hence, the threshold is obtained by interpolation with reference to the arbitrary percentage of correct responses derived from 50% detection level (Giguère et al., 2016; Lawless, 2010; Lawless and Heymann, 2010; Harvey, 1986). Since it was assumed that some cuppers might have guessed to select the odd sample, the Abbott's formula described in equation 5.4 was applied to adjust the data (Lawless, 2010; Lawless and Heymann, 2010). To calculate the panel BET with psychometric function, the data were first fitted with logistic regression model to investigate the relationship between the proportion of correct choices and the logarithmic concentrations of study compounds. As a result, the model parameters

were obtained from regression equations. Finally, the logistic regression model equation 5.5 was used to calculate the panel BET value with reference to the required proportion obtained by equation 5.6 with P_{corr} of 0.5 (Giguère et al., 2016; Lawless, 2010; Lawless and Heymann, 2010; Harvey, 1986).

$$P = \frac{C}{R} \quad (5.3)$$

P: Proportion correct

C: Number of correct responses

R: Total number of responses (Number of cuppers within concentration)

$$P_{corr} = \frac{(P - Pc)}{(1 - Pc)} \quad (5.4)$$

P_{corr}: Chance-corrected proportion

P_c: Chance probability (For the case of 3-AFC, the chance probability is 1/3= 0.333). (Giguère et al., 2016; Lawless, 2010; Lawless and Heymann, 2010; Harvey, 1986).

$$Ln \frac{p}{(1-p)} = \alpha + \beta\chi \quad (5.5)$$

p: Proportion correct

$\propto$: Intercept of the model equation

β: Slope of the model equation

χ: Concentration of the compound of study (ng/L)

$$P_{req} = P_c + [P_{corr} \times (1 - P_c)] \quad (5.6)$$

P_{req}: Observed proportion that is required to achieve 50 % level of performance

RESULTS AND DISCUSSION

Individual detection BETs for IPMP, IBMP and blend of IPMP-IBMP dissolved in water

The data were collected on six concentration series that were pre-determined (data not shown) with bench tests when the compounds of study were dissolved in water. Individual detection BET values of water-based samples were calculated and presented in Figure 5.1, Figure 5.2 and Figure 5.3. There was considerable variation in detection thresholds of the pyrazines by cuppers, with BET values ranging from 0.2 - 6.5 ng/L, 0.4 - 6.5 ng/L, and 0.4 - 6.5 ng/L for IPMP, IBMP and a blend of IPMP-IBMP when diluted in water, respectively. These results were in line with threshold values reported in previous studies such as values compiled by Van Gemert (2011) varying from 0.0002 - 10 ng/L and 0.001 - 10 ng/L for IPMP and IBMP, respectively. To assess the performance of cuppers, the whole panel BET was arbitrarily set as a maximum reference. Hence, individual BET values below the whole panel BET is indicative of good performance compared to the panel. In addition, when more than 50% (Tempere et al., 2011; Lawless & Heymann, 2010) of cuppers scored below the whole panel BET, it was an indicator of good panel performance. Further, a low BET value was indicative of good performance in sensitivity of the cupper (Meilgaard et al., 2016). Among the studied compounds, the results of the present study demonstrated a better performance of cuppers to detect IPMP in water, since 67% of cuppers have scored below the whole panel BET with or less than 0.4 ng/L (Figure 5.1). The current study has also highlighted an agreement between cuppers to detect IPMP in water since more than 50% of cuppers showed a low BET value. On the other hand, the results showed a disagreement to detect IBMP in water with variability in BET values among the cuppers. Figure 5.2 clearly shows that 50% of cuppers have performed above the panel BET with three cuppers who showed a high BET value of 6.5 ng/L. As a result, these findings demonstrated that the cuppers performed well to detect IPMP with low

threshold in water than IBMP. The comparison of thresholds between IPMP and IBMP conducted by Pickering et al. (2007) has also revealed a lower orthonasal aroma threshold of IPMP than IBMP. When IPMP and IBMP were combined and dissolved in water, the detection performance of cuppers improved compared to that of IBMP alone. As a result, a satisfactory performance with 75% of cuppers who scored below the panel BET was achieved as shown in Figure 5.3. Alternatively, this was considered as the contribution of IPMP due to synergy effect (Meilgaard et al., 2016) with its low BET in water when tested alone.

Some cuppers have individually demonstrated good performance, while others showed a poor performance. For example, the cupper ID 12 demonstrated high competence to detect IPMP in water (Figure 5.1) and correctly detected all the odd samples among the prepared concentrations. However, this has raised a concern to calculate the individual BET value. A similar situation was addressed in the standard document of ASTM (2004) by first determining the hypothetical concentration which can either be downward or upward. When the cupper has detected or missed all the odd samples, the hypothetical concentration is assumed as the highest or lowest concentration that a cupper would have detected or missed (Tempere et al., 2011; Lawless and Heymann, 2010; ASTM, 2004; Bi and Ennis, 1998). The hypothetical concentration is then calculated by dividing or multiplying the lowest or highest concentration by the geometric factor. Hence, the BET value of cupper ID 12 was calculated as the geometric mean of the downward hypothetical concentration and the lowest concentration, resulting in a BET of 0.2 ng/L.

It is important to highlight that some cuppers in this study demonstrated difficulties to detect the compounds of study diluted in water at low concentrations. This is illustrated in Figure 5.2, where 23% of cuppers had IBMP BET values of more than 6 ng/L. Similarly, the cuppers ID 1 and 9 showed a relatively large BET (6.5 ng/L) when the IPMP and IBMP were blended (Figure 5.3). It

was also found that the cupper ID 9 demonstrated BET values above the panel BET values for all studied compounds, showing a poor performance to detect the compounds in water. Moreover, the cupper ID 6 has shown BET values above the panel BET for both IPMP and IBMP.

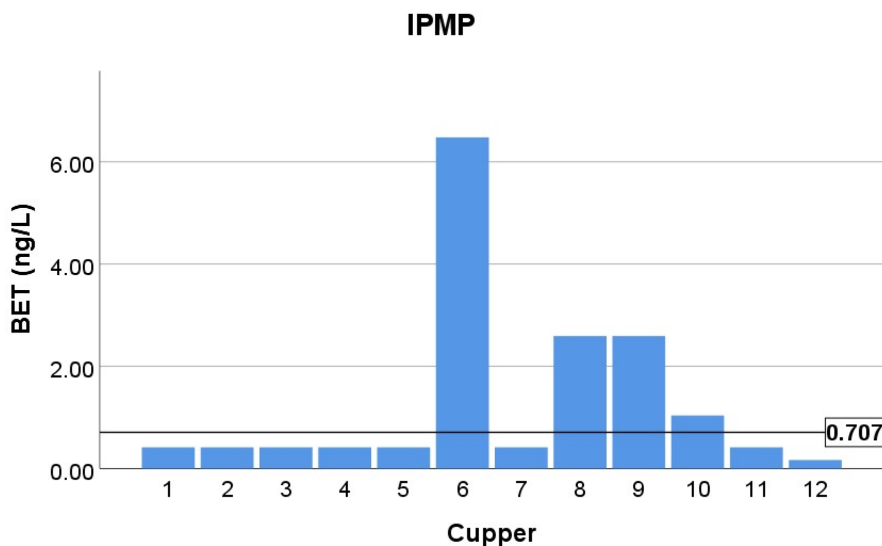


Figure 5.1: Individual detection BET values of 12 cuppers after cupping six concentration series of IPMP diluted in water. The x-axis shows the cupper's ID, and the y-axis shows the BET value calculated using the ASTM E679 method. The horizontal line indicates the whole panel BET value to determine the performance of cuppers against the panel, with poor performance when the individual BET value is above the line.

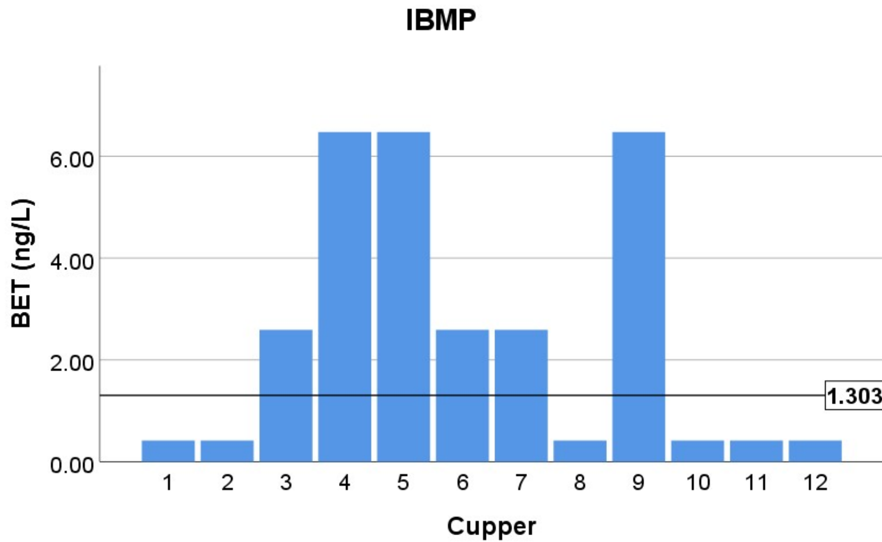


Figure 5.2: Individual detection BET values of 12 cuppers after cupping six concentration series of IBMP diluted in water. The x-axis shows the cupper's ID, and the y-axis shows the BET value calculated using the ASTM E679 method. The horizontal line indicates the whole panel BET value to determine the performance of cuppers against the panel, with poor performance when the individual BET value is above the line.

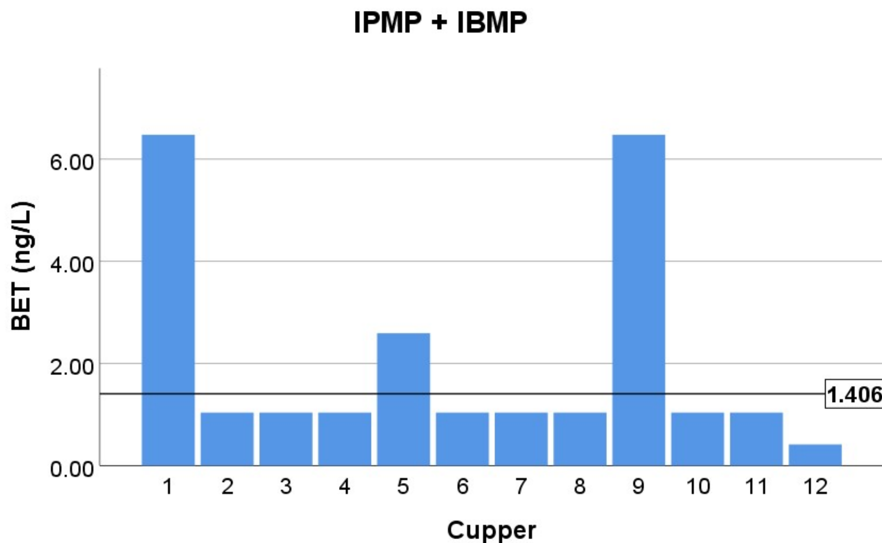


Figure 5.3: Individual detection BET values of 12 cuppers after cupping six concentration series of a blend of IPMP-IBMP (50:50) diluted in water. The x-axis shows the cupper's ID, and the y-axis shows the BET value calculated using the ASTM E679 method. The horizontal line indicates the whole panel BET value to determine the performance of cuppers against the panel, with poor performance when the individual BET value is above the line.

Individual detection BETs for IPMP, IBMP and blend of IPMP-IBMP spiked in coffee

When the compounds of study were spiked in coffee beverage, seven concentration series were pre-determined (data not shown) and cupped by 12 cuppers. The present study revealed individual detection BETs by the cuppers ranging from 16.2 - 1581 ng/L, 40.5 - 3953 ng/L and 6.5 - 633 ng/L for IPMP, IBMP and a blend of IPMP-IBMP, respectively. The current study has demonstrated considerably larger BET values for cuppers to perceive these compounds in coffee beverages than in water. It is important to note that the sensitivity of a single flavor compound is lost in a mixture (Donfrancesco et al., 2014; Grosch, 2001) containing a variety of flavors as described by spatial and temporal filtering mechanisms detailed by Laing (1994). This was the case of roasted coffee, which has been reported to contain more than 800 chemical flavor compounds (Pereira et al., 2017; Poisson et al., 2017) that are generated during roasting, hence competing with added IPMP and IBMP with masking effects (Grosch, 2001; Laing, 1994).

As shown in Figure 5.4, 50% of cuppers have demonstrated IPMP BETs below the whole panel BET. However, in case of IBMP, 42% of cuppers scored below the whole panel BET (Figure 5.5). When IPMP and IBMP were blended and spiked in coffee, 33% of cuppers scored below the panel BET (Figure 5.6). These findings showed that the performance of cuppers was moderate with variances in individual performances. For example, the cupper ID 12 showed an excellent performance by detecting all odd samples containing a blend of IPMP-IBMP, leading to determination of a downward hypothetical concentration that was used to calculate the individual BET value. The same cupper demonstrated a great performance for identifying the three compounds when dissolved in water. On the other hand, the cupper ID 4 failed to identify the odd samples of coffee spiked with IBMP, which led to determination of the upward hypothetical concentration as the BET value for this cupper. The performance of cupper ID 4 to identify the

individual compounds or mixture in coffee was poor compared to other cuppers (Figure 5.4, Figure 5.5 and Figure 5.6). For this reason, the poor performance of this cupper has contributed to the large panel BET value. In contrast, the same cupper was relatively proficient in identifying IPMP (Figure 5.1) and the blend of IPMP with IBMP (Figure 5.3) in water solutions. Further, cupper 4 has adequate experience in cupping of more than 10 years.

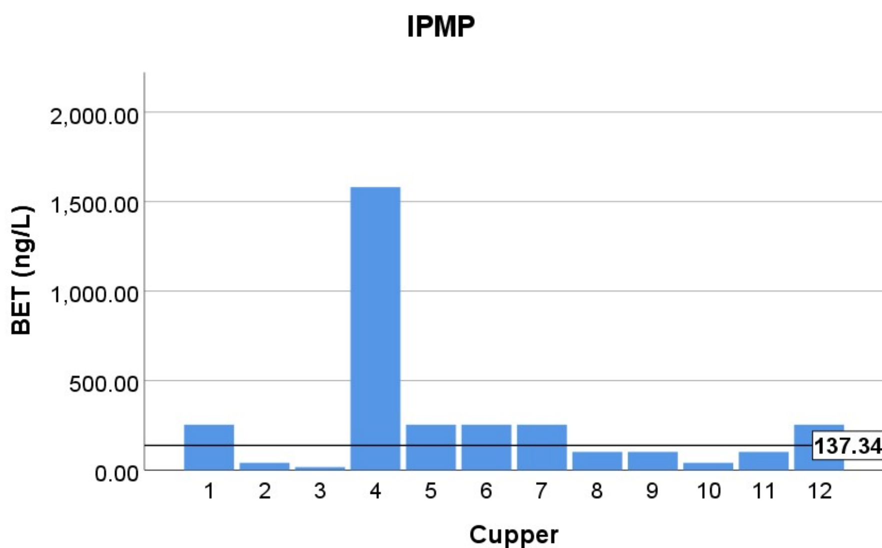


Figure 5.4: Individual detection BET values of 12 cuppers after cupping seven concentration series of IPMP spiked coffee. The x-axis shows the cupper's ID, and the y-axis shows the BET value calculated using the ASTM E679 method. The horizontal line indicates the whole panel BET value to determine the performance of cuppers against the panel, with poor performance when the individual BET value is above the line.

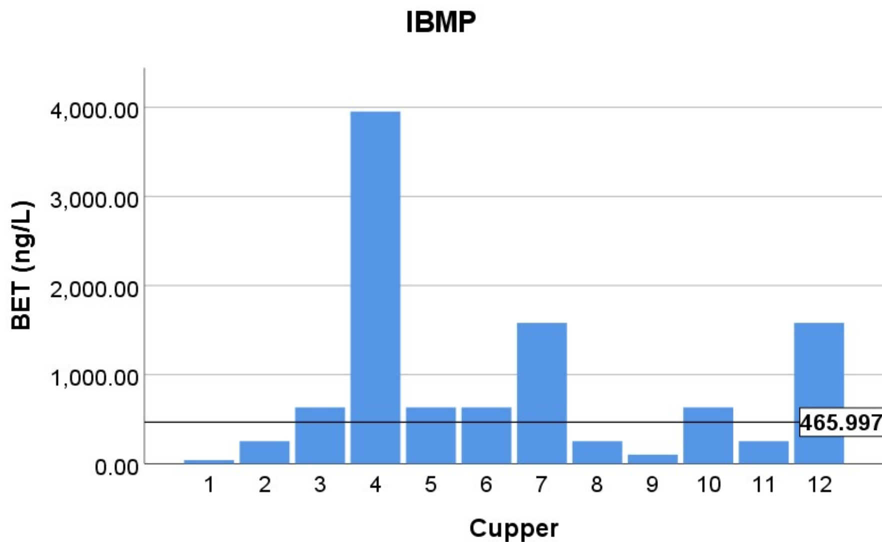


Figure 5.5: Individual detection BET values of 12 cuppers after cupping seven concentration series of IBMP spiked coffee. The x-axis shows the cupper's ID, and the y-axis shows the BET value calculated using the ASTM E679 method. The horizontal line indicates the whole panel BET value to determine the performance of cuppers against the panel, with poor performance when the individual BET value is above the line.

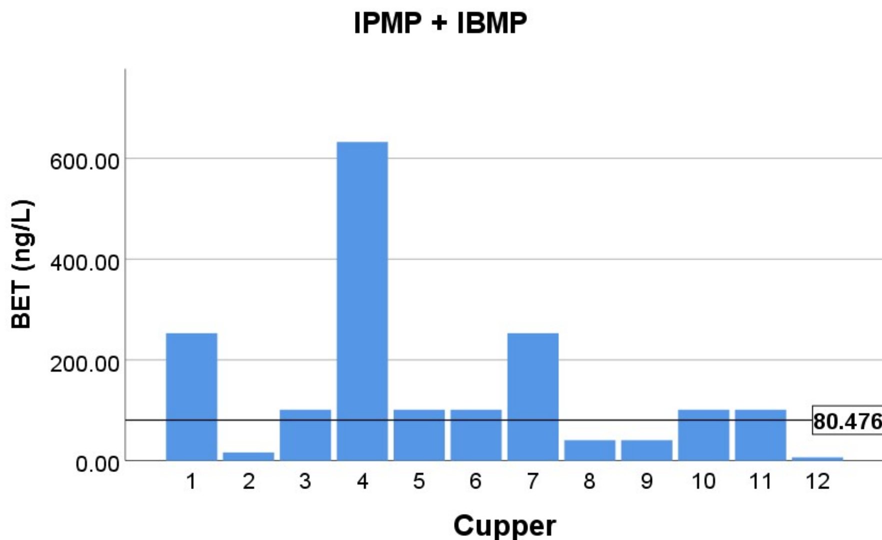


Figure 5.6: Individual detection BET values of 12 cuppers after cupping seven concentration series of blended IPMP-IBMP spiked coffee. The x-axis shows the cupper's ID, and the y-axis shows the BET value calculated using the ASTM E679 method. The horizontal line indicates the whole panel BET value to determine the performance of cuppers against the panel, with poor performance when the individual BET value is above the line.

Panel detection BETs for IPMP, IBMP and blend of IPMP-IBMP in water and coffee

With ASTM-E679 method, the findings revealed panel BET values of study compounds in coffee were considerably higher compared to that when the compounds were diluted in water (Table 5.1). As described above, the chemical composition of coffee and its ability to mask perception of the pyrazines contributed to these larger values. This study demonstrated a poor performance of certain cuppers, such as cupper ID 4. When cupper ID 4 was excluded from the analysis (reduced panel in Table 5.1), the panel BET values obtained using the ASTM-E679 method for IPMP, IBMP, and blend of IPMP-IBMP dropped from 137 to 110 ng/L, 466 to 384 ng/L, 80.5 to 66.7 ng/L respectively. As a result, these values can be considered as the BETs of the panel when the compounds were dissolved in coffee.

Table 5.1: Panel detection BET values of IPMP, IBMP and a blend of IPMP-IBMP dissolved in water and coffee. The table shows both BET values calculated by ASTM E679 method and psychometric function. The reduced panel represents a panel when cupper ID 4 was excluded (n.a.: Non-available).

Study compound	Medium of experiment	Detection BET (ng/L)		
		ASTM E679 method		Psychometric function of whole panel
		Whole panel	Reduced panel	
IPMP	Water	0.7	n.a.	0.7
IBMP	Water	1.3	n.a.	1.7
IPMP + IBMP	Water	1.4	n.a.	1.7
IPMP	Coffee	137	110	211
IBMP	Coffee	466	384	670
IPMP + IBMP	Coffee	80.5	66.7	86

Since various methods are applied to determine the thresholds, an additional psychometric function method was applied to support the ASTM-E679 method. Following a logistic regression model fitting as shown in Figure 5.7, Figure 5.8, Figure 5.9; the results showed a moderate association between the proportion of correct choices and the concentration series of IPMP ($R^2 = 0.54$) and

IBMP (R^2 = 0.52) in water. In other words, 54.2% of the variance in proportion correct was explained by the concentration of IPMP, while the concentration of IBMP explained 52.4% of the variance in proportion correct. However, for the blend of IPMP-IBMP in water, 70.9% of the variance in proportion correct was explained by the concentration of the blend, indicating a strong association (R^2 = 0.71). The associations were also supported by the significance ($p < 0.05$) of the predictor, i.e., the concentration of study compounds. The panel BET values of water-based samples were calculated using the logistic regression parameters determined in equations 5.7, 5.8 and 5.9. Hence, the results presented in Table 5.1 indicate close water-based BET values generated by the two methods.

$$Ln \frac{p}{(1-p)} = -0.973 + 1.332 * (\chi_1) \quad (5.7)$$

$$Ln \frac{p}{(1-p)} = -1.086 + 0.626 * (\chi_2) \quad (5.8)$$

$$Ln \frac{p}{(1-p)} = -2.229 + 1.279 * (\chi_3) \quad (5.9)$$

$$Ln \frac{p}{(1-p)} = -1.055 + 0.005 * (\chi_4) \quad (5.10)$$

$$Ln \frac{p}{(1-p)} = -1.340 + 0.002 * (\chi_5) \quad (5.11)$$

$$Ln \frac{p}{(1-p)} = -0.776 + 0.009 * (\chi_6) \quad (5.12)$$

p = Proportional correct

χ_1 = Concentration IPMP in water (ng/L)

χ_2 = Concentration IBMP in water (ng/L)

χ_3 = Concentration IPMP − IBMP in water (ng/L)

χ_4 = Concentration IPMP in coffee (ng/L)

χ_5 = Concentration IBMP in coffee (ng/L)

χ_6 = Concentration IPMP − IBMP in coffee (ng/L)

When the compounds of study were spiked in coffee, the logistic regression analysis produced psychometric curves illustrated in Figure 5.10, Figure 5.11 and Figure 5.12. This analysis revealed a moderate association ($R^2 = 0.5$) between the proportion of correct choices and the concentration series of IPMP. On the other hand, a weaker association was found for IBMP ($R^2 = 0.4$) and a slight moderate association for a blend of IPMP-IBMP ($R^2 = 0.55$). It is important to point out that both Figure 5.11 and Figure 5.12 showed irregularities of s-shaped psychometric curves. This was assumed to result from difficulties of cuppers to detect the study compounds in coffee, hence variations in sensory sensitivity. The logistic regression parameters were used to calculate BET values (Peng et al., 2012; Tempere et al., 2011; Shavelson & Webb, 2005) which were relatively higher compared to those obtained using the ASTM-E679 method (Table 5.1) as a result of poor association between the proportion of correct choices and the concentration series.

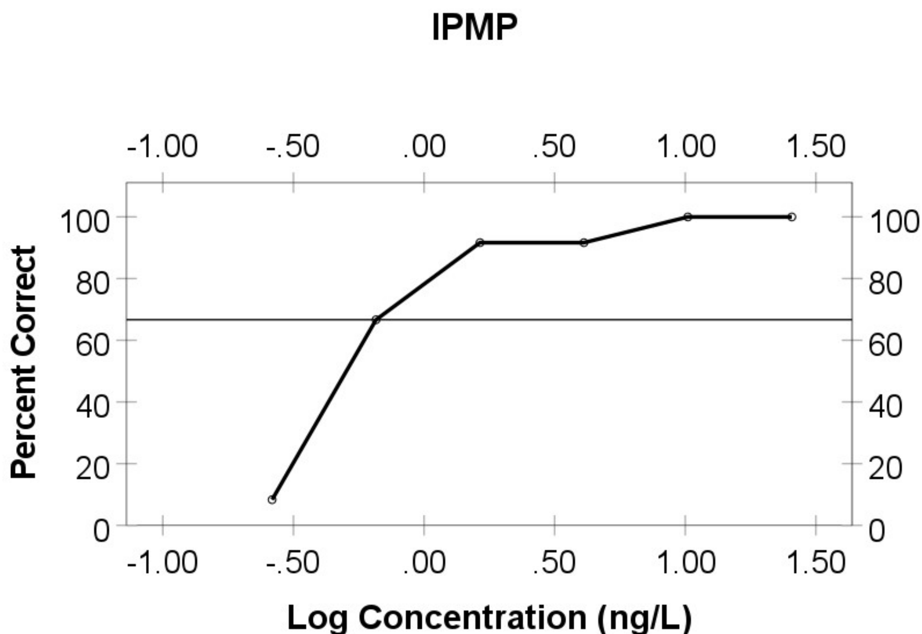


Figure 5.7: Psychometric curve of percentage of correct choices and logarithmic IPMP concentration (ng/L) in water. The data were obtained from 12 cuppers. The BET value is determined by plotting a vertical line that crosses the intersection between the curve and the horizontal line (indicating the proportion that is required to achieve 50% level of performance).

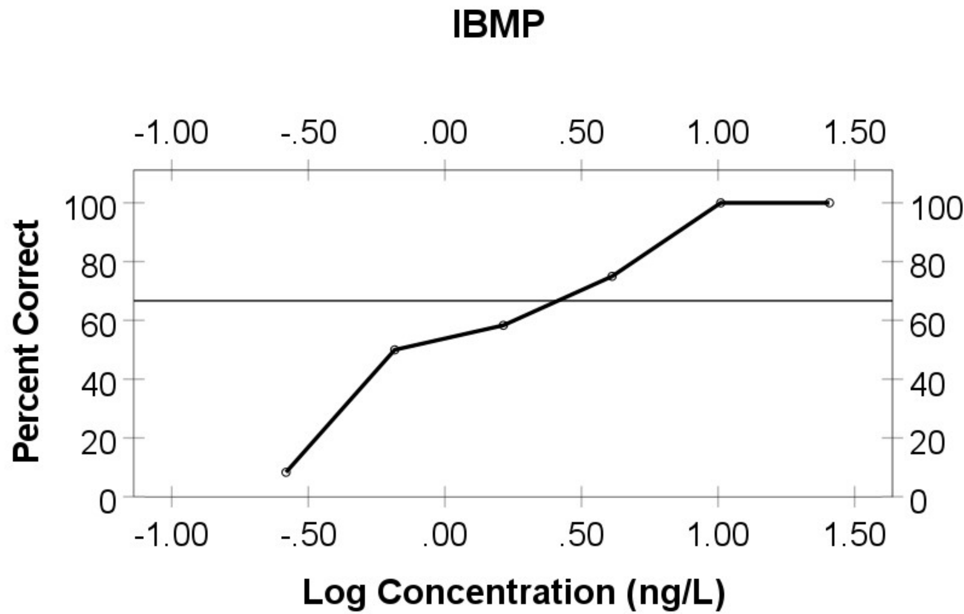


Figure 5.8: Psychometric curve of percentage of correct choices and logarithmic IBMP concentration (ng/L) in water. The data were obtained from 12 cuppers. The BET value is determined by plotting a vertical line that crosses the intersection between the curve and the horizontal line (indicating the proportion that is required to achieve 50% level of performance).

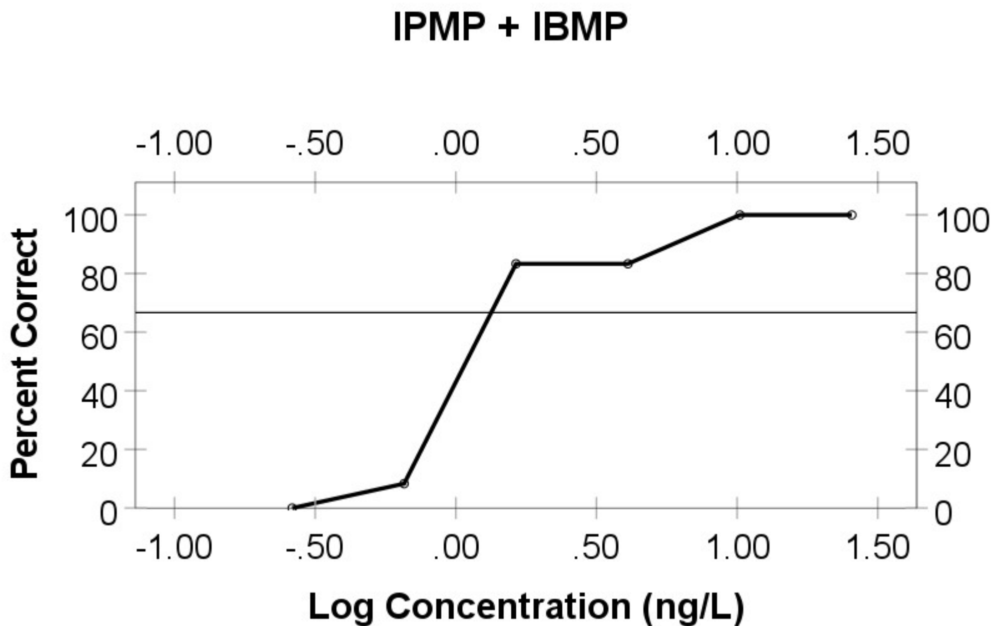


Figure 5.9: Psychometric curve of percentage of correct choices and logarithmic blend of IPMP-IBMP concentration (ng/L) in water. The data were obtained from 12 cuppers. The BET value is determined by plotting a vertical line that crosses the intersection between the curve and the horizontal line (indicating the proportion that is required to achieve 50% level of performance).

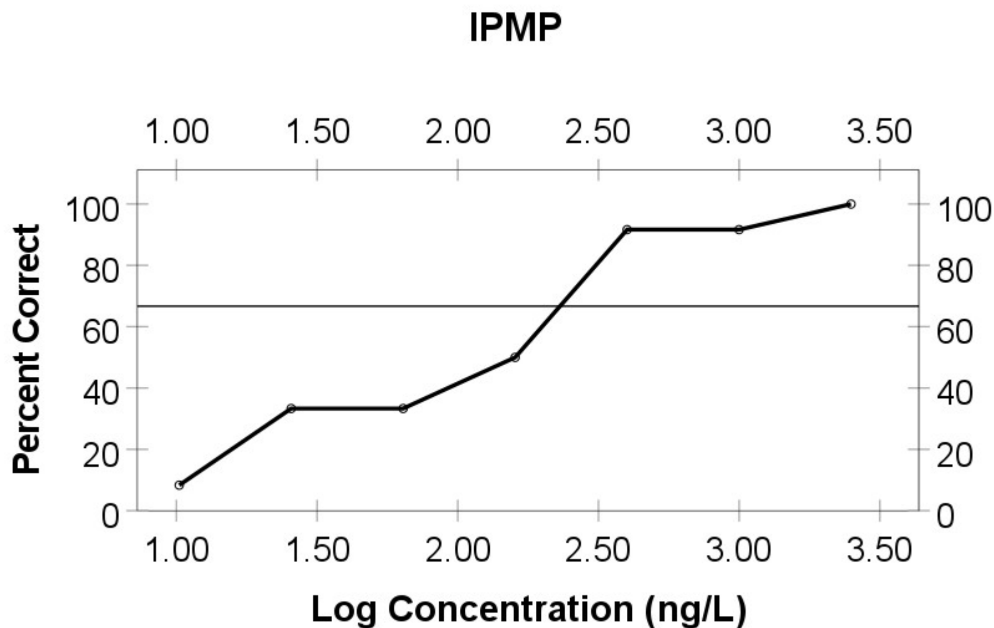


Figure 5.10: Psychometric curve of percentage of correct choices and logarithmic IPMP concentration (ng/L) in coffee. The data were obtained from 12 cuppers. The BET value is determined by plotting a vertical line that crosses the intersection between the curve and the horizontal line (indicating the proportion that is required to achieve 50% level of performance).

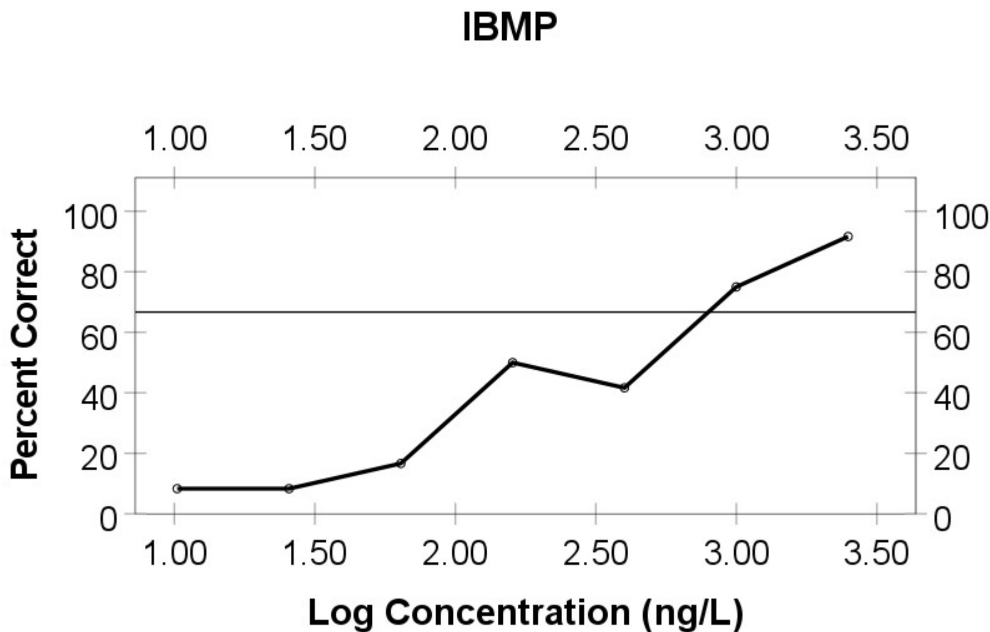


Figure 5.11: Psychometric curve of percentage of correct choices and logarithmic IBMP concentration (ng/L) in coffee. The data were obtained from 12 cuppers. The BET value is determined by plotting a vertical line that crosses the intersection between the curve and the horizontal line (indicating the proportion that is required to achieve 50% level of performance).

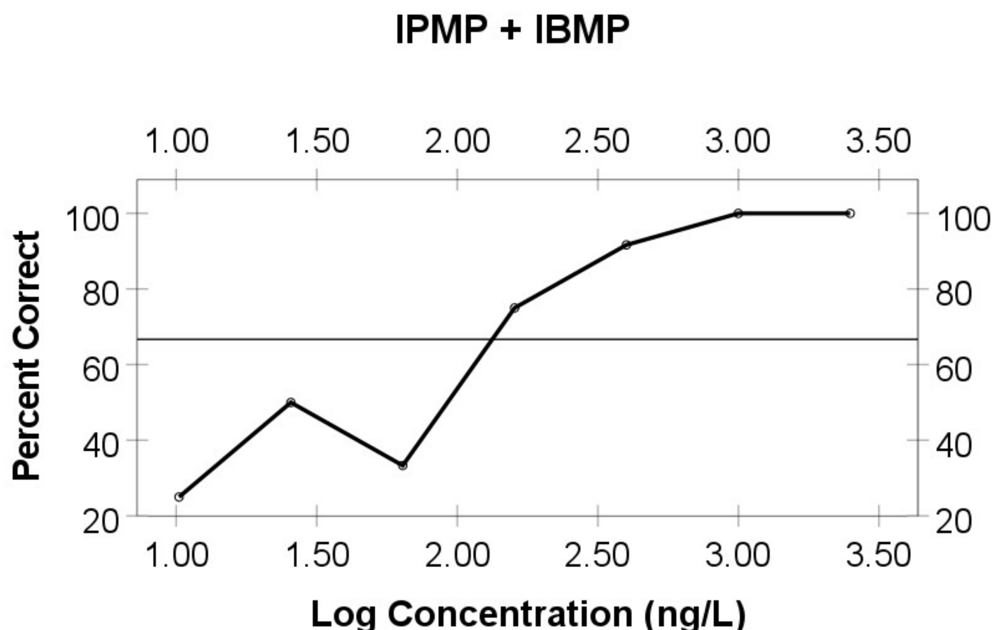


Figure 5.12: Psychometric curve of percentage of correct choices and logarithmic blend of IPMP-IBMP concentration (ng/L) in coffee. The data were obtained from 12 cuppers. The BET value is determined by plotting a vertical line that crosses the intersection between the curve and the horizontal line (indicating the proportion that is required to achieve 50% level of performance).

CONCLUSION

For the first time, the present study has estimated thresholds of coffee cuppers to detect IPMP and IBMP. The detection thresholds of 12 cuppers were measured in water and reported as BET values ranging between 0.2 - 6.5 ng/L for IPMP, 0.4 - 6.5 ng/L for IBMP and 0.4 - 6.5 ng/L for a blend of IPMP-IBMP. When the compounds were spiked in coffee, the BET values were estimated between 16.2 - 1581 ng/L for IPMP, 40.5 - 3953 ng/L for IBMP and 6.5 - 632.5 ng/L for a blend of IPMP-IBMP. However, the average BET values for a panel of cuppers were 0.7 ng/L for IPMP, 1.3 ng/L for IBMP, 1.4 ng/L for a blend of IPMP-IBMP when measured in water; and 110 ng/L for IPMP, 384 ng/L for IBMP and 66.7 ng/L for a blend of IPMP-IBMP when measured in coffee

beverage. Generally, the cuppers demonstrated higher sensory sensitivity to IPMP compared to IBMP in both water and coffee.

The observed higher thresholds in coffee than water indicated a loss of sensory properties when IPMP and IBMP were added in coffee beverage. This has demonstrated an effect of flavor compounds to mask the PTD-associated compounds to a certain extent. Generally, the cuppers demonstrated variations in their sensory sensitivity to IPMP and IBMP. Since IPMP and IBMP were reported as the precursors of PTD, the results of this study can be extrapolated to PTD. On this basis, the PTD is still problematic in coffee business since cupping is not efficient enough to detect PTD-associated compounds at low concentrations. Although some cuppers have demonstrated powerful sensory sensitivity to PTD compounds, regular refresher trainings on cupping with emphasis on PTD identification are recommended. Future research should further investigate recognition thresholds for PTD compounds to confirm our conclusion.

CHAPTER 6. IMPACT OF ROASTING PROFILE ON IPMP AND IBMP CONTENTS IN COFFEE BEANS

ABSTRACT

Roasting of coffee is a core operation in the development of pleasant flavors when green coffee beans are subjected to suitable temperatures. The temperature and time of roasting play a major role in producing roasted coffee with balanced flavor. Among various volatile compounds produced during roasting, pyrazines contribute to either pleasant or unpleasant flavors. For example, 2-isopropyl-3-methoxypyrazine (IPMP) and 2-isobutyl-3-methothoxypyrazine (IBMP) were reported to contribute to the off-flavor of coffee known as potato taste defect (PTD). This study was conducted to investigate the effect of roasting on the development of IPMP and IBMP in coffee and thereby improve our understanding of factors potentially impacting PTD occurrence. Four samples of Arabica coffee were collected and roasted at various temperatures ranging from 50 to 200°C for 8 minutes. Concentrations of IPMP and IBMP were measured in each sample using gas chromatography - mass spectrometry (GCMS). The study demonstrated two main phases of change in IPMP and IBMP contents of coffee beans upon roasting, following a non-linear relationship. The first phase was described by a decline of concentration at temperatures below 100°C, followed by a phase transition at around 120°C, and finally a second phase of rise in concentrations when roasting temperatures were above 120°C. The optimum concentration rise of IPMP and IBMP was observed from 130 to 150°C and 170 to 200°C, respectively. As the coffee was exposed to elevated temperatures, small lightness (L*) color values of beans were recorded as a result of development of characteristic dark brown color. The present study also demonstrated a negative correlation between L* color values and the concentrations of IPMP and IBMP of roasted coffee beans. Hence, the change in beans color upon roasting can be a potential predictor of IPMP

and IBMP development in coffee beans. More research is recommended to determine the occurrence of PTD upon roasting at various profiles of temperature and time.

INTRODUCTION

The happiness hidden in a cup of coffee beverage originates from the pleasant aroma and taste developed during coffee roasting. When green beans are exposed to high roasting temperatures, physical and chemical reactions take place inside and outside the beans to form volatile and non-volatile compounds (Schenker and Rothgeb, 2017), which in turn give rise to perceived sensorial qualities of coffee. Since considerable research interest has focused on coffee flavor, previous studies have classified the main volatile compounds of roast coffee as aldehydes, alcohols, acids, furans, ketones, phenols, pyrazines, thiols and sulfides among others (Seninde and Chambers, 2020; Toci and Boldrin, 2018; Handayani, 2016; Ku Madihah et al., 2012). This is an indicator of coffee flavor complexity as was also indicated by researchers (Poisson et al., 2017; Poltronieri and Rossi, 2016, and Donfrancesco et al., 2014) who reported that hundreds of flavor compounds were isolated from roasted coffee. However, equilibrium of these compounds is required for a balanced quality of coffee beverage. Since these compounds are developed during roasting, the profile of a suitable temperature and time should be applied. Accordingly, Schenker and Rothgeb (2017) and Ku Madihah et al. (2013) have recommended a medium roasting profile for a balanced coffee flavor. There is a difference between each of the light, medium and dark roast as a result of different corresponding roasting profiles that vary from one roaster to another. However, according to literature, typical coffee roasting temperatures vary from 160 to 250°C with time ranging between 3 and 35 minutes (Schenker and Rothgeb, 2017; Ku Madihah et al., 2013; Mendes et al., 2001; Parliment, 2000). Various research studies have investigated the development of volatile

compounds in coffee since they are the pillars of coffee flavor. It was reported that the intensities of certain flavor compounds in coffee beans increase as a result of chemical reactions (Seninde and Chambers, 2020; Handayani, 2016) while others decrease at extreme temperatures (Franca et al., 2009). For example, the caramel flavors were reported to develop due to caramelization and Maillard reactions (Schenker and Rothgeb, 2017; Sunarharum et al., 2014).

The importance of pyrazines in coffee has been noted, most importantly the potential of pyrazines to contribute to both pleasant and off-flavors (Seninde and Chambers, 2020; Schenker et al., 2002; Koehler et al., 1971). Pyrazines are monocyclic aromatic rings with two nitrogen atoms (Müller and Rappert, 2010; Ji and Bernhard, 1992) and are naturally found in green beans of coffee, but the majority is formed during roasting (Mutarutwa et al., 2020; Seninde and Chambers, 2020; Jackels et al., 2014; Murray and Whitfield, 1975). Previous studies were carried out to investigate the formation of pyrazines in various model systems. Hence, it has been previously determined that pyrazines are produced when the amino group of amino acids and carbonyl group of sugars react in the presence of heat (Ji and Bernhard, 1992; Misharina et al., 1992; Shibamoto and Bernhard, 1976; Murray and Whitfield, 1975; Maga and Sizer, 1973; Wang and Odell, 1973; Koehler and Odell, 1970). The increase of pyrazine concentrations was also reported in roasted coffee as a result of Maillard and Strecker's degradation reactions (Franca et al., 2009). On the other hand, thermal degradation of pyrazines has been reported by Schenker and Rothgeb (2017) at extreme temperatures. Another study revealed a rise of methylpyrazines when coffee beans were heated at 205°C, and a decrease at temperatures beyond 205°C (Hashim and Chaveron, 1996).

Although the formation of pyrazines in coffee roasting has been investigated and documented, IPMP and IBMP also are pyrazines of concern in coffee which have not been sufficiently studied. The presence of these undesirable methoxypyrazines was reported to generate green or pea-like

aroma known as PTD in coffee beverages (Poisson et al., 2017; Gueule et al., 2015; Jackels et al., 2014; Gueule et al., 2013; Czerny and Grosch, 2000). Mutarutwa et al. (2020) analyzed green coffee beans collected from different locations and found IPMP and IBMP contents of 1.09 - 3.2 ng/g and 9.92 - 138.49 ng/g, respectively. The purpose of their study was to discriminate PTD and non-PTD green coffee beans based on chemical composition. Unfortunately, the roasted beans were not examined. Moreover, previous studies were carried out to examine the response of pyrazines when exposed to high temperatures. However, most of the experiments were conducted in model systems of nitrogen-containing compounds and sugars (Ji and Bernhard, 1992; Misharina et al., 1992; Shibamoto and Bernhard, 1976; Murray and Whitfield, 1975; Maga and Sizer, 1973; Wang and Odell, 1973; Koehler and Odell, 1970). Thus far, the methoxypyrazines have not been studied in isolation to understand their formation. In addition, none of the studies has used coffee beans to evaluate the development of IPMP and IBMP during roasting. Hence, it is not known how PTD-associated compounds are developed during coffee roasting. Accordingly, the present study was conducted to assess how roasting temperature influences the development of IPMP and IBMP in coffee beans. The findings will provide guidance on the optimum roasting profile to control PTD-associated compounds and PTD in roasted coffee.

MATERIALS AND METHODS

Chemicals

Standards of IPMP and IBMP (99% purity, Sigma-Aldrich, Saint Louis, USA) were purchased and used to identify and quantify similar compounds in coffee samples. 5-Bromo-2-methoxypyridine (BMP; 95% purity, Fisher Scientific, USA) was added as an internal standard to take into account the variability of GCMS analysis. Ethyl acetate (99.9% purity, Fisher Scientific, USA) was used to extract organic compounds from coffee samples. The Milli-Q water was purified with E-pure model D4641 (Barnstead International, Iowa, USA). Acetone (99.6%; Fisher Scientific, USA), ethyl acetate (99.9%; Fisher Scientific, USA) and methanol (99.8%; Sigma-Aldrich, Saint Louis, USA) were used to clean the sample injector syringe.

Description of coffee samples

Four samples of green beans of Arabica coffee (*Coffea arabica*) were selected. The sample labeled as "sample 1" consisted of defective coffee beans, including insect damaged beans. Defective beans were collected after a sorting process that separated physically damaged (defective) from intact green beans. The intact green beans from this sorting were labeled as "sample 2". The sample labeled as "sample 3" consisted of floats that were obtained after the floating step of the wet processing method. Floating involves immersing fresh coffee cherries in water to separate unripe and defective cherries which float, from ripe cherries which sink. The fourth sample was unsorted and contained good, defective and float beans. It was described as "sample 4". All of the collected samples were previously processed by the wet method of coffee processing.

Roasting of green coffee beans

Aliquots of green coffee beans were roasted using different roasting profiles. To control the roasting parameters, an "IKAWA" pro digital micro roaster (IKAWA Ltd; London, United Kingdom) was used to roast the samples. Although the "IKAWA" roaster maximum capacity is 60 g of beans, it was preferred for this research since it allows setting and adjusting the desired roasting profile to produce consistent roasting conditions. Sample 1 was exposed to nine roasting profiles while samples 2, 3 and 4 were each roasted using 11 different profiles (Table 6.1). The fan parameters of the roaster are presented in Table 6.2. In both tables, profile 1 consists of green beans that were not roasted for comparison purposes. Due to limited sample size for sample 1, the roasting profiles of 75 and 170°C were not completed. Roasting was carried out in three replications.

Once the roasting profile was configured, the process of roasting started with pre-heating to the initial temperature of the profile. Once the initial temperature was attained, an aliquot of 50 g of coffee was introduced in the heating chamber of the roaster. After 8 min of roasting, the roaster cooled down automatically to less than 50°C. During roasting, the relative humidity (47 - 60%) and temperature (20 – 24°C) of inlet air to the roaster were recorded. To determine the impact of extended roasting time, samples 2 and 3 were further roasted using additional profiles of fixed temperature and varying times. These profiles involved a temperature of 200°C for 6, 8, 10 and 12 min of roasting.

Table 6.1: Roasting temperature (measured with temperature sensor inside roasting chamber) and time applied to coffee samples, performed with "IKAWA" roaster. The profile "ID-1" consisted of non-roasted green beans that were kept at room temperature of 20°C. n.a.= Not available.

Profile ID	Temperature (°C) of roasting chamber	Time (min)
1	20 °C (Room temperature)	0
2	50	8
3 (n.a. for sample 1)	75	8
4	100	8
5	120	8
6	130	8
7	140	8
8	150	8
9	160	8
10 (n.a. for sample 1)	170	8
11	180	8
12	200	8

Table 6.2: Time and fan speed of "IKAWA" roaster applied to roasting profiles

Time (min)	Fan speed (%)
00.00	76
01.19	76
02:09	76
03:00	72
03:18	60
07:56	65
10:00	65

Extraction of pyrazines for GCMS analysis

A sample of 10 g of roasted coffee beans was ground with a Krups burr grinder GVX212 (Krups, USA) to coarse particles that passed through a Nº 20 US standard sieve size. To extract coffee compounds, 1 g of ground coffee was weighed with a precision balance model PG503-S (Mettler Toledo LLC, Ohio, USA) and dissolved in 2 mL of Milli-Q water. After mixing, the solution was heated at 50°C for 2 min in a dry bath incubator (Fisher Scientific, USA). The solution was immediately cooled down on ice for 1 min, then 1 mL of ethyl acetate was added and mixed for

10 min at 300 rpm in a multi-tier environmental shaker (Innova 4900, New Brunswick Scientific CO, Inc, New Jersey, USA). The supernatant from each sample was pipetted into 1.7 mL microcentrifuge tubes, and centrifuged in AccuSpin micro centrifuge (Fisher Scientific, Germany) at 16,060 × g for 5 min to separate the supernatant from the precipitate. From the upper layer supernatant, 90 µL were pipetted into a GC vial, then 10 µL of 100 µM BMP standard solution was added to obtain a final concentration of 10 µM BMP.

GCMS analysis

The samples were analyzed with gas chromatography (Agilent 6890N; Agilent Technologies, Inc.) coupled with an inert mass selective detector (Agilent 5973; Agilent Technologies, Inc.). The analysis started with automatic injection of 1 µL samples into the GCMS machine using splitless mode at 250°C, purge flow of 10 mL/min, purge time of 0.75 min and total flow of 13.8 mL/min. Once in the GCMS system, the chemical compounds of coffee were separated through a capillary column Rtx 5MS; Model Restek 12623; Crossbond 5% diPhe 95% DiMe Polysilox; with length of 30 m, 250 µm diameter and 0.25 µm film thickness (Restek corporation, Bellefonte, USA). The column was set at constant flow mode with initial flow of 1 mL/min. The oven initial temperature was 40°C. The temperature was increased to 100°C at 25°C/min, then to 150°C at 5°C/min, and finally increased at 40°C/min to 270°C and held for 5 min. The total run time was 20.4 min. Following separation in the column, the compounds ions were detected by MSD, quadrupole mass spectrometer with SIM mode. The masses set for SIM mode to identify the compounds were in order of m/z 137, 152, 124 for IPMP and m/z 124, 151, 94 for IBMP. The quantification masses were m/z 137 and 124 for IPMP and IBMP, respectively. The spectra were recorded with masses ranging between m/z 45 and 200.

Data collection and analysis

The measurement of L*a*b* values was performed on roasted beans with colorimeter PCE-CSM 5 (PCE Americas Inc., Jupiter, USA) to assess the color development upon roasting. GCMS data were handled and analyzed with Agilent MSD ChemStation G1701DA D.01.00 software (Agilent Technologies, Inc.) in combination with the spectral library NIST 02 MS and AMDIS 2.1 program. Peak areas for separate compounds were adjusted using the internal standard BMP by calculating the ratio between the peak area of each compound of interest in the coffee samples and that of the added internal standard. The concentrations of IPMP and IBMP in coffee were calculated from equations obtained from the standard curves of the same pure compounds. To prepare the standard curves, pure standards of IPMP and IBMP were diluted with ethyl acetate and analyzed with GCMS in a similar manner as was used for coffee samples. The retention times of IPMP and IBMP were then determined to facilitate identification of similar compounds in coffee samples. In addition, the chromatograms of coffee samples were matched to those of standards as another way to identify compounds of interest.

Curve fitting was performed for data using the software programs TableCurve 2D, version 2.0 (Jandel Scientific, San Rafael, CA) and MATLAB, version R2021a (MathWorks, Inc.). Statistical comparisons between coffee samples were performed with IBM SPSS statistics version 25, to determine the significant differences in concentrations of IPMP and IBMP. The change in concentrations (fall or rise) of IPMP and IBMP upon roasting was calculated by using equation 6.1.

$$\chi = \frac{(A-B)\times 100}{B} \tag{6.1}$$

χ= Percentage change of IPMP or IBMP concentration between two consecutive temperatures (°C)

A and B= IPMP or IBMP concentration (ng/g) of two consecutive temperatures (A is upper temperature and B is lower temperature)

RESULTS

Evolution of IPMP in coffee beans during roasting

Table 6.3 shows the results of concentrations of IPMP in coffee beans after roasting at various temperatures. The findings revealed two separate phases of change in IPMP content during roasting, consisting of an initial phase of slight loss of IPMP at lower temperatures and then a sudden rise of IPMP with increasing temperatures. Across the samples, a moderate loss of inherent IPMP (Murray and Whitfield, 1975) was observed at low temperatures up to 100°C or slightly more in some cases but not exceeding 120°C. However, this loss of IPMP should be studied further since, to our knowledge, no previous research has reported loss of IPMP in coffee roasted at low temperatures. However, it was previously reported that roasting coffee beans below 100°C induces endothermic reactions with drying phase, hence loss of water (Seninde and Chambers, 2020; Poisson et al., 2017; Schenker and Rothgeb, 2017; Gloess et al., 2014; Fabbria et al., 2011; Parliment, 2000). It can be assumed that a certain amount of IPMP likely vaporized with water since it has been reported that pyrazines evaporate easily as a result of low vapor pressure (Müller and Rappert, 2010).

The results in Table 6.3 show a shift from loss to formation of IPMP at temperatures around 120°C. This transition phase is in line with the results from the study conducted on synthesis of pyrazine in model reaction (Misharina et al., 1992) where the authors have reported a formation of pyrazines from 100°C (Shibamoto and Bernhard, 1976; Koehler and Odell, 1970) to 150°C.

A second phase associated with a rapid rise in IPMP concentration was observed at temperatures equal or above 130°C. The present study has revealed an optimum rise of IPMP concentration at roasting temperatures between 130 and 150°C (Figure 6.1). This is in line with the maximum temperatures of pyrazines formation of 130-140°C according to a previous study conducted by Misharina et al. (1992), and at 120°C in another study (Shibamoto and Bernhard, 1976). However, in the present study the IPMP concentration continued to increase at a low rate when the roasting temperature was above 150°C. The formation of pyrazines during coffee roasting has been attributed to Maillard reactions (Franca et al., 2009; Ji and Bernhard, 1992; Shibamoto and Bernhard, 1977) and Strecker's degradation reactions (Franca et al., 2009). In addition, according to Maga and Sizer (1973), the degradation of proteins at 190°C generates nitrogen residuals that are used in the synthesis of more pyrazines.

The present study did not investigate the effects of roasting temperatures beyond 200°C since the "IKAWA" roaster was sensitive to extended roasting temperatures. However, an additional experiment was carried out by roasting green beans at 200°C for 6, 8, 10 and 12 min to assess the behavior of IPMP when the roasting time was extended. Although the experiment was limited to samples 3 and 4 due to inadequate sample size, the results in Table 6.5 showed that the concentration of IPMP increased further with increasing roasting time. Similar results were also found when Wang and Odell (1973) studied the mechanism of pyrazine formation at 200°C for four hours. The authors reported increased pyrazine formation from amino-hydroxy compounds

in the presence of sugars and heat. It is important to point out that the concentration of IPMP started to slightly decline when the coffee was roasted at 200°C from 10 to 12 min (Table 6.5). A similar study was conducted by Hashim and Chaveron (1996), who also reported a loss of methylpyrazines in roasted Arabica coffee from Ivory Coast at temperatures above 205°C. However, the present study demonstrated fluctuations in concentrations presumably due to insufficient time points used in the experiment. On the other hand, the researchers (Shibamoto and Bernhard, 1976; Koehler and Odell, 1970) have reported variations in pyrazine concentrations when an experiment was carried out to produce pyrazines at temperatures above 150°C in a D-glucose and ammonia model. Hence, Koehler and Odell (1970) have suggested that the destruction of pyrazines is the cause of these fluctuations.

Despite the dissimilarities in quality grade of the starting green bean samples, there were relatively small differences in IPMP concentrations produced in these samples across roasting temperatures. However, there was a significant difference ($p < 0.05$) in IPMP concentration in unroasted green beans between sample 1 and the rest of samples (Table 6.3). The elevated levels of IPMP noticed in unroasted sample 1 was likely due to insect damaged beans (McPherson, 2018) as the sample consisted of defective green beans. When sample 1 was subjected to a modest roasting temperature of 50°C, an unexpected loss of almost 100% of IPMP was observed, while slight losses were observed in samples 2, 3 and 4 (Figure 6.1). Thus, the hypothesis that a single bean might contaminate a whole sample as described by other researchers (Mutarutwa et al., 2020; Jackels et al., 2014; Gueule et al.,2013) may explain the high level of IPMP in green beans of sample 1.

By comparing the samples at each of the roasting temperature levels (Table 6.3), there was no significant difference ($p > 0.05$) in concentrations of IPMP along the initial phase in samples 2, 3 and 4. The evolution in sample 1 was different from other samples, likely due to its elevated IPMP

concentration in green beans. The second phase corresponding to IPMP formation, was remarked by consistent changes ($p > 0.05$) in concentration of IPMP across all samples at roasting temperatures between 140°C and 170°C. This may indicate consistency of the mechanism of change in IPMP concentrations since the raw materials had different initial concentrations.

Table 6.3: Evolution of IPMP in coffee beans roasted at different profiles. Each sample of green coffee beans was exposed at different temperatures for 8 min in three replications. The results were expressed as concentration mean values ± SEM (ng/g of coffee) measured by GCMS. The values with different superscript letters within a row are significantly different ($p<0.05$).

Temperature (°C)	IPMP concentration (ng/g)			
	Sample 1	Sample 2	Sample 3	Sample 4
20 (Green beans)	991.7 ± 97.7^{b}	3.6 ± 0.1^{a}	8.6 ± 0.9^{a}	7 ± 1.9^{a}
50	14 ± 1.1^{b}	3.7 ± 0.3^{a}	8.1 ± 1^{a}	4.5 ± 1.1^{a}
75	n.a.	2.4 ± 0.3^{a}	6.2 ± 0.4^{a}	7 ± 2.7^{a}
100	14.1 ± 1.8^{b}	2.5 ± 0.7^{a}	5.5 ± 1.07^{a}	5.2 ± 1.3^{a}
120	3.9 ± 0.3^{a}	4.2 ± 0.7^{a}	6.4 ± 1.1^{ab}	9.9 ± 1.8^{b}
130	8.5 ± 1.3^{ab}	5.8 ± 0.5^{a}	5.7 ± 0.7^{a}	11.3 ± 0.6^{b}
140	29.7 ± 3^{a}	15.3 ± 1.9^{a}	23.7 ± 6^{a}	19.5 ± 2.1^{a}
150	46.6 ± 6.2^{a}	37 ± 8.3^{a}	55.9 ± 3.4^{a}	43.4 ± 5.9^{a}
160	75.2 ± 7.3^{a}	80.1 ± 6.7^{a}	82 ± 1.9^{a}	65.4 ± 4.8^{a}
170	n.a.	106.5 ± 0.7^{a}	189 ± 102.8^{a}	114.9 ± 7.8^{a}
180	88.9 ± 2.8^{a}	226.3 ± 32.1^{b}	228.9 ± 17.8^{b}	154.7 ± 18^{ab}
200	152.3 ± 6.5^{a}	340.5 ± 46.4^{b}	319 ± 29.4^{b}	309.3 ± 8.8^{b}

Evolution of IBMP in coffee beans during roasting

The four samples were similarly analyzed to study the effect of roasting on IBMP, and the results are presented in Table 6.4. The IBMP concentrations in the green beans of the four samples were not significantly different ($p > 0.05$). Similar to observations for IPMP, the findings in Table 6.4 showed that roasting temperatures contributed to development of IBMP in coffee beans in two phases. The initial phase or phase one corresponded to loss of IBMP up to a temperature of 100°C.

Again, the shift from phase one of loss of inherent IBMP to phase two corresponding to formation of IBMP, was observed at temperatures around 120°C. Similar to IPMP, the rise of IBMP concentration was generally observed when the roasting temperature was set at temperatures above 120°C. In contrast to IPMP, the maximal rise of IBMP was observed at roasting temperatures between 170°C and 200°C as shown in Figure 6.2. Similar to the findings for IPMP, the extension of roasting time promoted further increases of IBMP concentration as shown in Table 6.5. However, when sample 4 was roasted from 10 to 12 min, the concentration of IBMP declined slightly. The results of mean difference determinations (Table 6.4) between samples demonstrated variations in IBMP concentrations in all samples during phase one as well as at transition temperature from phase one to phase 2. However, the concentrations in all samples were not significantly different ($p > 0.05$) in phase two from 160°C to 180°C.

Table 6.4: Evolution of IBMP in coffee beans roasted at different profiles. Each sample of green coffee beans was exposed at different temperatures for 8 min in three replications. The results were expressed as concentration mean values ± SEM (ng/g of coffee) measured by GCMS. The values with different superscript letters within a row are significantly different ($p<0.05$).

Temperature (°C)	**IBMP concentration (ng/g)**			
	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**
N/A (Green beans)	78.3 ± 29.5[a]	67.7 ± 7.2[a]	101.4 ± 7[a]	57.7 ± 6.6[a]
50	71.5 ± 0.8[a]	60 ± 3.4[a]	113.6 ± 11.7[a]	66.7 ± 7.4[b]
75	n.a.	58.8 ± 6.5[a]	104.3 ± 5.7[a]	66.3 ± 10.8[b]
100	13.3 ± 2.9[a]	61 ± 5.7[b]	92 ± 14.4[b]	71.5 ± 2.7[b]
120	29.3 ± 15.1[a]	87.8 ± 6.3[b]	100.3 ± 9.9[b]	102.8 ± 7.2[b]
130	19.1 ± 1.6[a]	96 ± 1.1[b]	125.7 ± 13.8[b]	115.4 ± 6.6[b]
140	26 ± 1.3[a]	105.5 ± 5.9[b]	135.7 ± 3.9[c]	118.2 ± 7.8[bc]
150	74.4 ± 9.7[a]	109.4 ± 14.3[ab]	158.6 ± 17.5[b]	132 ± 3.6[b]
160	137.7 ± 16.4[a]	140.3 ± 17.3[a]	160.5 ± 14.2[a]	151 ± 11.5[a]
170	n.a.	209 ± 11.7[a]	196.3 ± 15[a]	228.6 ± 14.8[a]
180	398.6 ± 22[a]	341.2 ± 27.4[a]	355.9 ± 23.6[a]	331.4 ± 16.3[a]
200	756.1 ± 44[c]	487.8 ± 88.9[a]	531.6 ± 25.8[ac]	1052.3 ± 10.4[b]

Table 6.5: Evolution of IPMP and IBMP in coffee beans roasted at different profiles. Each sample of green coffee beans was roasted at 200°C for different durations of time in three replications. The results were expressed as concentration mean values ± SEM (ng/g of coffee) measured by GCMS.

Time (min)	**IPMP concentration (ng/g)**		**IBMP concentration (ng/g)**	
	Sample 3	**Sample 4**	**Sample 3**	**Sample 4**
N/A (Green beans)	8.6 ± 0.9	7 ± 1.9	101.4 ± 7	57.7 ± 6.6
6	375 ± 54.2	521 ± 4.2	476.8 ± 30.4	502 ± 25.1
8	332.6 ± 20.9	680.8 ± 22.9	531.6 ± 25.8	1052 ± 10.4
10	580 ± 65.9	829 ± 63.7	708 ± 171	1126 ± 100
12	551.5 ± 54.7	601.8 ± 24.8	824 ± 92.3	1038 ± 106.7

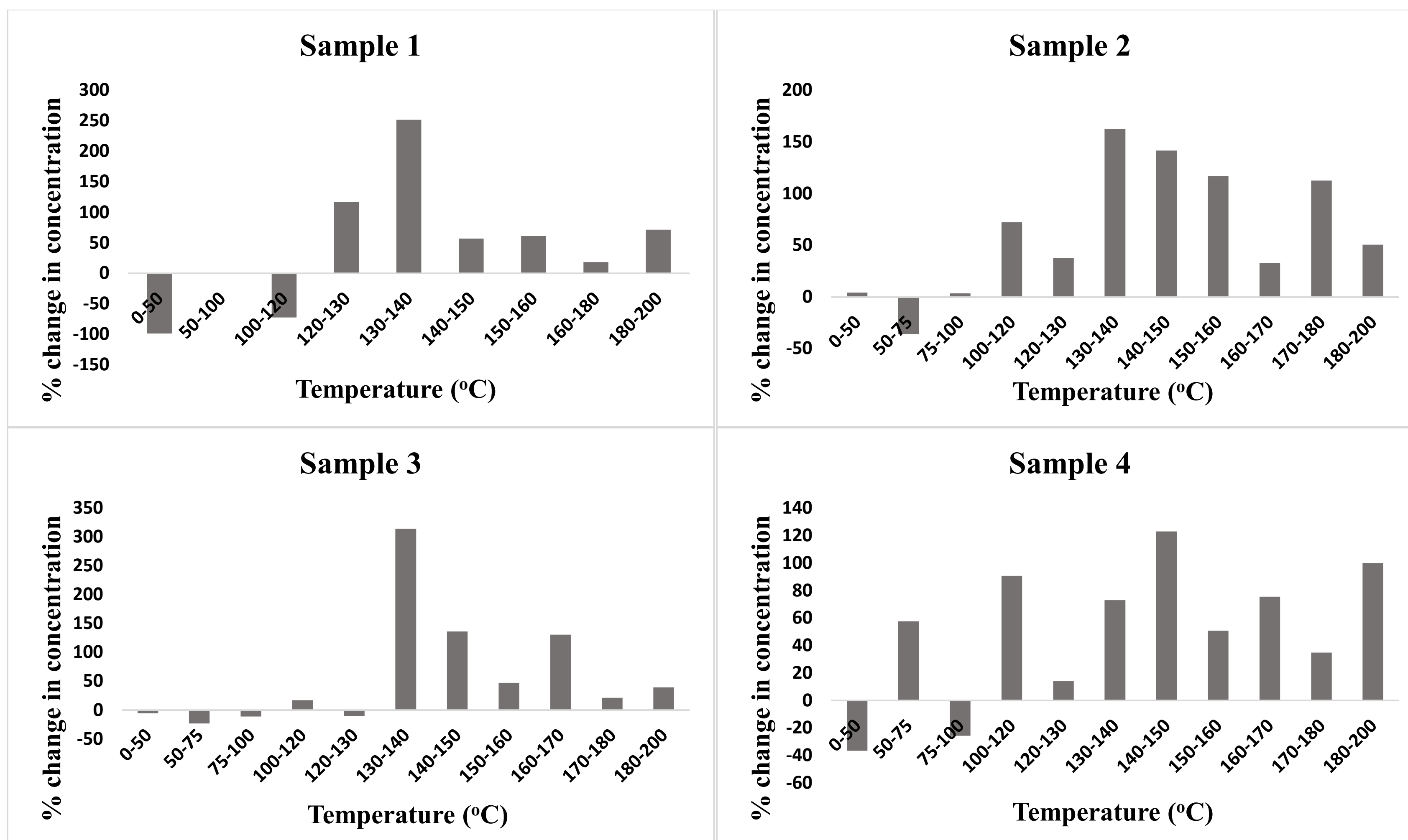


Figure 6.1: Percentage of change (fall or rise) in concentration (ng/g of coffee) of IPMP measured in coffee beans of four samples subjected at increasing temperatures for 8 min of roasting. The x-axis represents the transitions from lower to upper temperature (°C). The y-axis represents the change in percentage from lower to upper temperature. The chart columns above the x-axis line indicate a gain, while below the line indicate a loss in IPMP concentration.

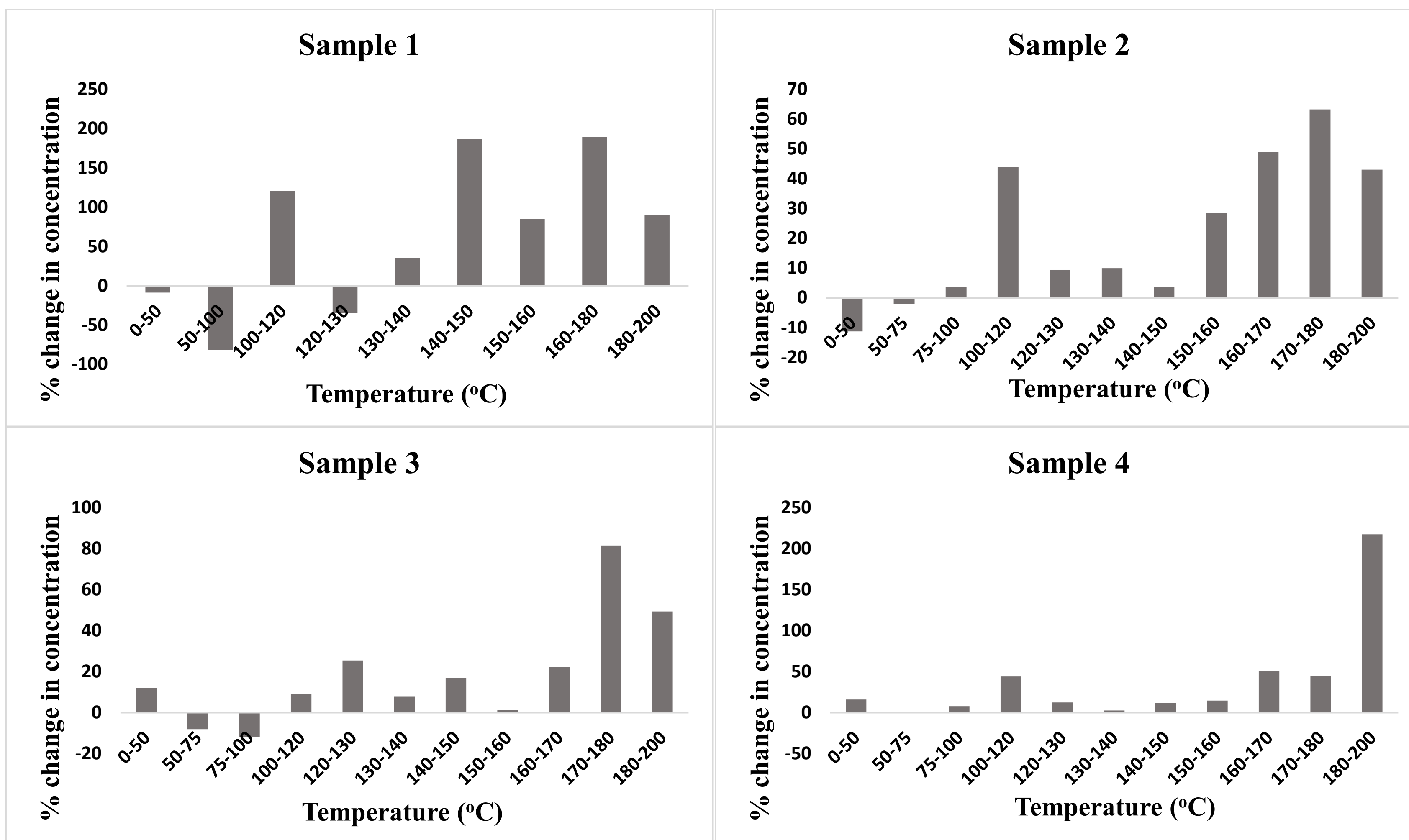


Figure 6.2: Percentage of change (fall or rise) in concentration (ng/g of coffee) of IBMP measured in coffee beans of four samples subjected at increasing temperatures for 8 min of roasting. The x-axis represents the transitions from lower to upper temperature (°C). The y-axis represents the change in percentage from lower to upper temperature. The chart columns above the x-axis line indicate a gain, while below the line indicate a loss in IBMP concentration.

Estimation of relationship between roast temperatures and concentrations of IPMP and IBMP in roasted coffee beans

Curve fitting was performed to find models that best fit the observed data. TableCurve software was used to fit the data of concentrations of IPMP and IBMP in coffee beans roasted at various temperatures. The fitting procedure resulted in a sum of two different models (equation 6.2) that fit the data of IPMP evolution in coffee beans during roasting. The two models described two phases of IPMP evolution in beans, with phase one described by an exponential decay regression model (equation 6.4) represented with the green fitted lines as shown in Figure 6.3. The second phase was described by a logistic dose response model (equation 6.5), represented by the blue fitted lines in Figure 6.3. When a model of the sum of the above two models was fitted to data of IBMP evolution obtained during roasting of coffee beans, the second phase corresponding to development of IBMP at temperatures above 100°C was not well described by a logistic dose response model. Hence, MATLAB software was used to fit the data, and resulted in a better fit power model. As a result, the evolution of concentrations of IBMP in coffee beans upon roasting was well described with a sum of two equations (equation 6.3) consisting of exponential decay regression model (equation 6.4) for phase one of loss of IBMP and a power model (equation 6.6) corresponding to the development of IBMP in coffee beans. On the other hand, the evolution of IBMP in beans during roasting did not fit well with an exponential decay regression model. Hence, curve fitting using MATLAB revealed an acceptable curve fit (Figure 6.4) with the power model (equation 6.6). The root mean squared error (RMSE) for each sample was reported and is presented in Table 6.6. The values of RMSE ranged between 96.77 and 254.67. Smaller values were obtained for IPMP compared to IBMP which had larger values (Table 6.6), indicating spreads of residuals from the fitting line as shown in Figure 6.3 and Figure 6.4. The same figures showed acceptable

good fit models that support the evolution of IPMP and IBMP in two main phases. It is clearly illustrated by the fitting curves that the evolution of IPMP and IBMP follows a non-linear model. This is in line with the findings of Ku Madihah et al. (2013) who found a good fitting non-linear (quadratic) model when pyrazine formation was studied in Robusta coffee roasted from 150°C to 180°C.

$$y_1 = y_3 + y_4 \quad (6.2)$$

$$y_2 = y_3 + y_5 \quad (6.3)$$

$$y3 = a + b * XP(c * \chi) \quad (6.4)$$

$$y4 = \frac{d}{1+(\frac{\chi}{e})^f} \quad (6.5)$$

$$y5 = g + (h * \chi^i) \quad (6.6)$$

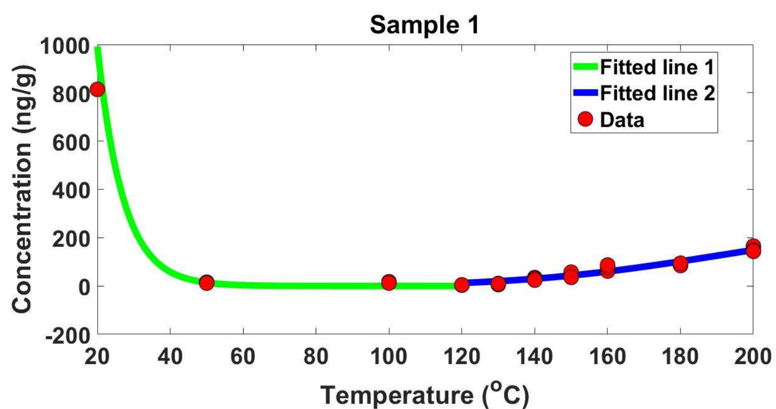
a, b, c, d, e, f, g, h, i: Model parameters (Table 6.6)

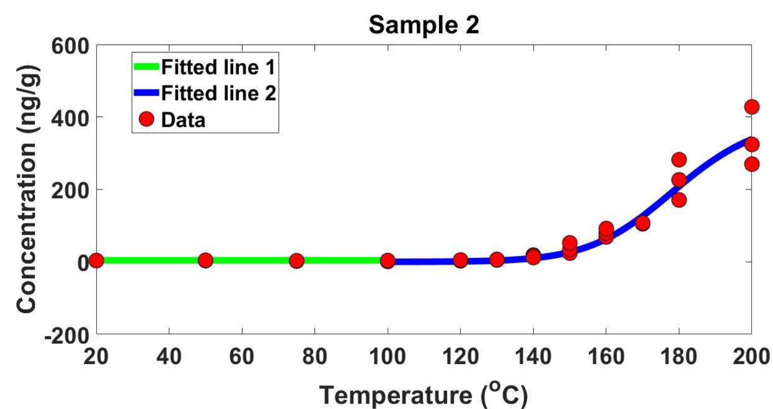
χ: Temperature (°C)

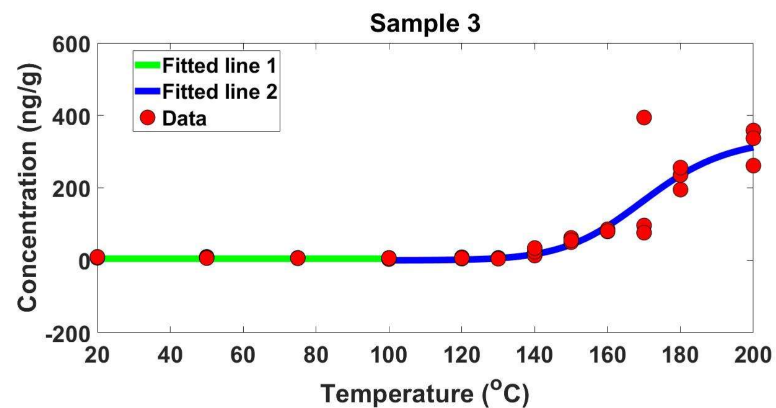
y: Fitted concentration of IPMP or IBMP (ng/g)

Table 6.6: Values of model parameters and root mean squared error (RMSE) obtained from model fitting performed with TableCurve and MATLAB software programs, to data of concentrations of IPMP and IBMP in coffee beans roasted at various temperatures (20-200°C).

Model	Parameter	IPMP				IBMP			
		Sample 1	Sample 2	Sample 3	Sample 4	Sample 1	Sample 2	Sample 3	Sample 4
Exponential decay regression	a	-0.28	4.18	4.63	-0.20	-15.27	77.56	113.96	-
	b	16774.75	17416.08	-916.29	5.76	34.32	117478.87	15.18	-
	c	-0.14	-11.39	-2.14	-0.12	-0.05	-8.04	-163.62	-
Logistic dose response	d	309.73	406.85	341.81	739.35	-	-	-	-
	e	202.48	179.42	170.71	208.47	-	-	-	-
	f	-6.05	-14.77	-14.86	-8.86	-	-	-	-
Power	g	-	-	-	-	73.01	71.14	109.1	94.25
	h	-	-	-	-	1.998e-10	2.483e-11	5.654e-11	9.005e-11
	i	-	-	-	-	5.418	5.744	5.577	3.313
RMSE		324.06	116.66	122.76	96.77	254.67	146.54	140.13	277.78







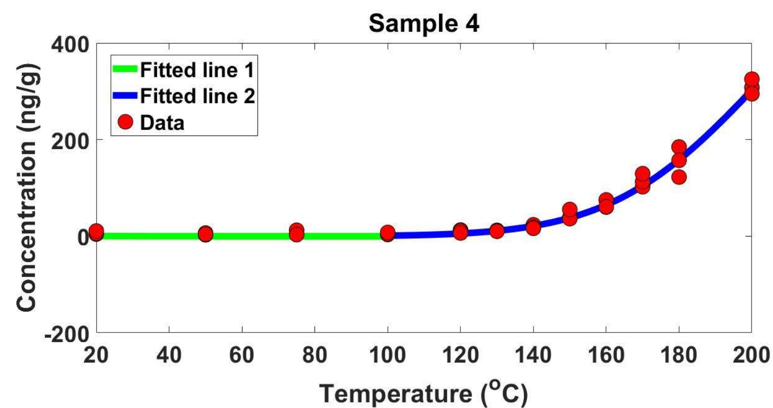
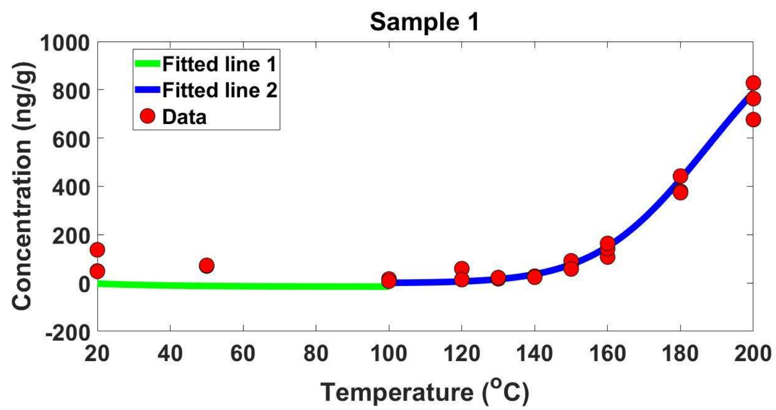
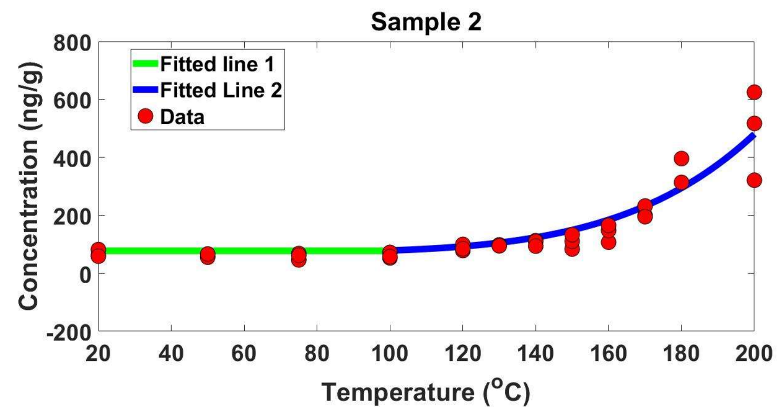
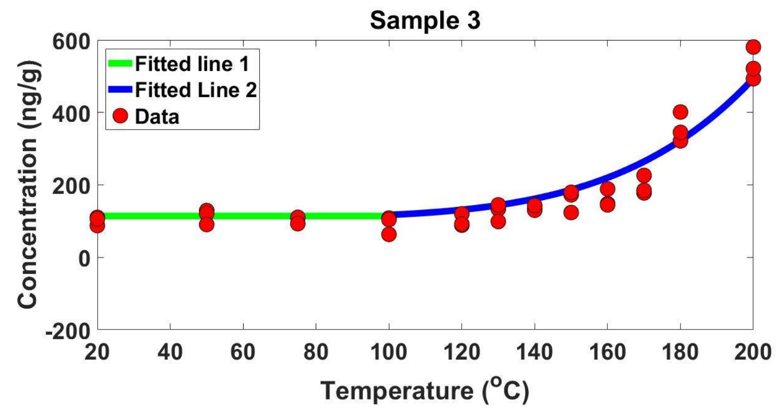


Figure 6.3: Non-linear relationship obtained from a sum of two model equations (exponential decay regression model fitted with line 1 and logistic dose response model fitted with line 2) between roasting temperature and IPMP content when four samples of coffee beans were roasted at various temperatures. The x-axis represents the temperature inside the roasting chamber, with 20°C standing for ambient temperature of non-roasted green beans. The y-axis represents the concentration of IPMP (ng/g of coffee).







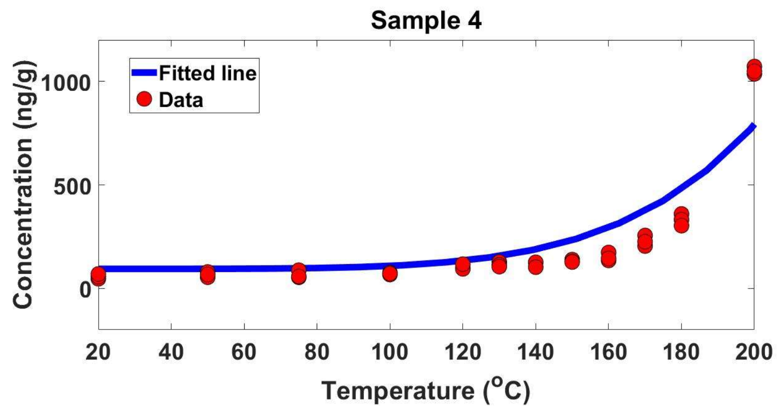


Figure 6.4: Non-linear relationship obtained from a sum of two model equations (exponential decay regression model fitted with line 1 and power model fitted with line 2) for samples 1, 2 and 3, and single power model for sample 4; between roasting temperature and IBMP content when four samples of coffee beans were roasted at various temperatures. The x-axis represents the temperature inside the roasting chamber, with 20°C standing for ambient temperature of non-roasted green beans. The y-axis represents the concentration of IBMP (ng/g of coffee).

Color development of coffee beans upon roasting

As expected, the findings showed that roasting induced a color change of coffee beans, and the intensity of color was proportional to the roasting temperatures (Baggenstoss et al., 2008). As the roasting temperature increases, the beans color changes (Figure 6.5) from light green to light brown, then dark brown color. Further increase of temperature leads to black colored beans (Seninde and Chambers, 2020). The measurement was performed with L*a*b* color scale, however the L* values were reported since they are directly related to the light color of green beans and dark color when roasted. The L* color values range from 0 (black/dark) to 100 (white/light) (Baggenstoss et al., 2008). The results presented in Table 6.7 demonstrated that from the green beans to the roasting temperature of 140°C, the L* values were still in the lightness region between 51 and 100. However, the L* values fluctuated likely due to tiny differences in lightness of color of beans as shown in Figure 6.5. As a result, the light color coupled with low roasting temperatures have indicated the early stages of roasting. As the roasting temperature increased, the L* values became smaller which is an indicator of development of typical brown color of coffee (Baggenstoss et al., 2008). The present study revealed small L* values of color measurement, indicating a development of dark color. The smallest L* values obtained were 20.9 ± 0.1, 18 ± 0.3, 18.1 ± 0.2 and 17.9 ± 0.3; measured on samples ID 1, 2, 3 and 4; respectively. These results were in accordance with L* values reported by Baggenstoss et al. (2008) when coffee beans were roasted at various extents of time and temperature. In addition, the lowest L* values were in accordance with the observed dark color of coffee beans roasted at 200°C for 8 min (Figure 6.5). An important finding of the present study is the shared temperature region (140 – 150°C) of transition from light to dark brown color with the optimum rise of IPMP in coffee beans. As described by Poisson et al. (2017), the change of color was attributed to the caramelization of sucrose in coffee beans. The

findings have therefore sparked interest for more data analysis to assess the possible correlation between L* values for color and the concentrations of IPMP as well as IBMP. Apart from sample 1 which had relatively elevated concentration of IPMP in green beans, the findings revealed negative strong correlation between the lightness values and the concentration of IPMP or IBMP (Table 6.8). As a result, the concentration of IPMP and IBMP increases with small lightness (L*) values. In other words, as the coffee beans turn brown in color due to roasting, their concentrations of IPMP and IBMP increase. A similar conclusion was also reported by Shibamoto and Bernhard (1977).

Figure 6.5: Coffee beans roasted at various temperatures

Table 6.7: Lightness (L) values (expressed as mean ± SEM) measured on roast coffee beans in three replications.*

Temperature (°C)	Sample 1	Sample 2	Sample 3	Sample 4
N/A (Green beans)	59.6 ± 0.5	58.5 ± 1.9	57.1 ± 1.9	55.4 ± 1.2
50	52.9 ± 0.1	56.8 ± 1.6	57.2 ± 0.3	56.3 ± 1.2
75	n.a.	61.1 ± 0.8	55.9 ± 3.3	58.3 ± 1
100	57.8 ± 0.3	62.3 ± 1.2	59.5 ± 2.3	56.7 ± 2.5
120	63.4 ± 0.1	58.4 ± 2.1	60.9 ± 2.4	58.9 ± 0.6
130	57.3 ± 0.6	54.7 ± 2	57.2 ± 2.8	58.1 ± 1.4
140	57.5 ± 0.7	52.1 ± 1	56.1 ± 2.2	51.3 ± 0.7
150	46.8 ± 0.1	41.9 ± 1.9	47.8 ± 2.1	43.7 ± 0.9
160	36.6 ± 0.1	34.6 ± 3.4	38.7 ± 2.5	33.1 ± 2.5
170	n.a.	27 ± 0.5	27.4 ± 0.5	26.9 ± 1.1
180	26 ± 0.1	23.1 ± 1	22.5 ± 0.5	21.6 ± 0.7
200	20.9 ± 0.1	18 ± 0.3	18.2 ± 0.2	17.9 ± 0.3

Table 6.8: Pearson correlation coefficients (R) of lightness (L) color of coffee beans upon roasting and the concentrations of IPMP and IBMP (N=30 for sample 1, N= 36 for samples 2, 3 and 4).*

Pyrazine	Sample	L*
IPMP	1	0.13
	2	-0.86
	3	-0.87
	4	-0.88
IBMP	1	-0.88
	2	-0.84
	3	-0.85
	4	-0.73

CONCLUSION

In conclusion, the findings of this study demonstrated that the concentrations of IPMP and IBMP in coffee beans increase with roasting temperature. However, the evolution of IPMP and IBMP upon roasting followed a non-linear model. The evolution process was described by two main phases of change in concentrations of IPMP and IBMP with increasing roasting temperature. The roasting process starts with slight decline of concentrations, described as initial phase from ambient temperature of green beans of 20°C to 100°C, followed with a phase transition at around 120°C described by the end of loss and the beginning of rise of concentrations. The final phase consisted of rise in concentrations of IPMP and IBMP from 130°C to 200°C. The optimum rise of concentrations was recorded at 130 – 150°C and 170 – 200°C for IPMP and IBMP, respectively. The extension of roasting time also resulted in further increases of IPMP and IBMP concentrations. Importantly, the present study has provided evidence of formation of IPMP and IBMP in coffee beans with increasing intensity of brown color. Finally, the results showed that the evolution of IPMP and IBMP during roasting was similar regardless of quality grade of green coffee beans. This study was limited by insufficient sample size and sensitivity of the roaster at high temperatures of roasting. Hence, more research should be conducted to investigate the development of pyrazines in coffee at extended roasting profiles. In addition, the occurrence of PTD in coffee beans roasted at various roasting profiles could be an interesting topic for future research.

CHAPTER 7. CONCLUSION AND FUTURE STUDIES

CONCLUSION

This project has demonstrated that the occurrence of PTD in coffee is dependent on different considerations including the concentration of responsible pyrazines in coffee beans, the extent of roasting and the sensorial sensitivity of cuppers. Although IPMP and IBMP are considered as PTD-associated compounds, the findings have shown that the variations in distribution of IPMP and IBMP in coffee beans did not match with cupping results. However, the findings have shown potential evidence of relationship between PTD occurrence and IPMP in green beans. In addition, a relationship was found between the occurrence of PTD and the concentration ratio of IBMP to EDMP in roasted coffee.

Roasting of coffee significantly impacted the concentrations of IPMP and IBMP, which slightly decreased at roasting temperatures of less than 100°C, and markedly increased as the temperatures were increased. Since IPMP and IBMP generate PTD in roasted coffee and their concentrations differ with roasting temperature, the intensity of PTD in roast coffee is, therefore, anticipated to depend on roasting profile in addition to the inherent levels of the compounds in green beans. When the efficiency of cuppers was evaluated, the findings demonstrated that cuppers have exhibited high thresholds to detect IPMP and IBMP in coffee beverage. In addition, a disagreement among cuppers to detect PTD in coffee samples was discovered. However, the cuppers efficiently reproduced the cupping results when the samples were cupped in three replications as indicated by the consistent results between different cupping sessions. In addition, the loss of sensory properties when IPMP and IBMP were added in coffee beverages has demonstrated the potential of coffee volatile compounds to mask the PTD responsible compounds at a certain extent. In conclusion, the

effect of differences in sensory sensitivity among cuppers contributed to poor reliability to detect PTD. Based on these results, calibration of cuppers using PTD standard solutions prior to cupping sessions and regular refresher trainings are recommended to enhance the sensitivity of cuppers to PTD. To get reliable results of cupping, a panel of more than one cupper is recommended during quality evaluation since the cuppers have different sensory sensitivities.

FUTURE STUDIES

Due to limitations of time and sample size, we recommend future research to assess the effects of extended roasting profiles differing in temperature and duration on development of IPMP and IBMP during roasting. In addition, cupping of coffee roasted using various roasting profiles could be used to investigate the relationship between the evolution of pyrazines and detectable PTD. For more understanding of the contribution of IPMP and IBMP to PTD, it could be interesting to study how these compounds are affected when coffee cherries are processed to produce green beans. With reference to the hypothesis that a single defective bean might impact a whole sample of beans, further research on the transfer processes of IPMP and IBMP to or from coffee beans and beverages could further elaborate the inconsistent distribution of PTD in coffee beverage. This research could also address the potential of a single defective bean to result in perceivable PTD in coffee samples. Since previous research studies have investigated the formation of pyrazines in model systems to identify the reactants that are involved, future research should investigate the types of amino acids and sugars that are particularly involved in the formation of IPMP and IBMP. This may help to understand the contribution of amino acids and sugars of green beans to PTD. The study on thresholds of cuppers was only limited to detection threshold. However, our conclusion could be confirmed by recognition thresholds for evidence on the poor performance of

cuppers to detect PTD. It could also be interesting to investigate blending of IPMP and IBMP at different ratios to assess the influence of a single compound in a blend on perception of PTD.

APPENDICES

APPENDIX A. COFFEE CUPPING FORM (SCAA, 2003)

Specialty Coffee Association of America Coffee Cupping Form

Name: ______________________________

Date: ________________

Quality scale:			
6.00 - Good	7.00 - Very Good	8.00 - Excellent	9.00 - Outstanding
6.25	7.25	8.25	9.25
6.50	7.50	8.50	9.50
6.75	7.75	8.75	9.75

Sample #

Roast Level of Sample

Fragrance/Aroma — Score: 6 7 8 9 10 — Dry — Qualities: — Break

Flavor — Score: 6 7 8 9 10

Aftertaste — Score: 6 7 8 9 10

Acidity — Score: 6 7 8 9 10 — Intensity: High / Low

Body — Score: 6 7 8 9 10 — Level: Heavy / Thin

Uniformity — Score: ☐ ☐ ☐ ☐ ☐

Balance — Score: 6 7 8 9 10

Clean Cup — Score: ☐ ☐ ☐ ☐ ☐

Sweetness — Score: ☐ ☐ ☐ ☐ ☐

Overall — Score: 6 7 8 9 10

Defects (subtract) — Taint=2, Fault=4 — # cups ☐ X Intensity ☐ = ☐

Total Score ☐

Notes:

Final Score ☐

Sample #

Roast Level of Sample

Fragrance/Aroma — Score: 6 7 8 9 10 — Dry — Qualities: — Break

Flavor — Score: 6 7 8 9 10

Aftertaste — Score: 6 7 8 9 10

Acidity — Score: 6 7 8 9 10 — Intensity: High / Low

Body — Score: 6 7 8 9 10 — Level: Heavy / Thin

Uniformity — Score: ☐ ☐ ☐ ☐ ☐

Balance — Score: 6 7 8 9 10

Clean Cup — Score: ☐ ☐ ☐ ☐ ☐

Sweetness — Score: ☐ ☐ ☐ ☐ ☐

Overall — Score: 6 7 8 9 10

Defects (subtract) — Taint=2, Fault=4 — # cups ☐ X Intensity ☐ = ☐

Total Score ☐

Notes:

Final Score ☐

Sample #

Roast Level of Sample

Fragrance/Aroma — Score: 6 7 8 9 10 — Dry — Qualities: — Break

Flavor — Score: 6 7 8 9 10

Aftertaste — Score: 6 7 8 9 10

Acidity — Score: 6 7 8 9 10 — Intensity: High / Low

Body — Score: 6 7 8 9 10 — Level: Heavy / Thin

Uniformity — Score: ☐ ☐ ☐ ☐ ☐

Balance — Score: 6 7 8 9 10

Clean Cup — Score: ☐ ☐ ☐ ☐ ☐

Sweetness — Score: ☐ ☐ ☐ ☐ ☐

Overall — Score: 6 7 8 9 10

Defects (subtract) — Taint=2, Fault=4 — # cups ☐ X Intensity ☐ = ☐

Total Score ☐

Notes:

Final Score ☐

June 2003

APPENDIX B. CUPPING FORM TO DETERMINE THE THRESHOLD OF PTD IN WATER AND COFFEE

Name of cupper: ……………………………………………………………

Date: ……………………………

Cupping experience: ……

Instructions:

- You are provided with 3 coded samples
- Assess (by cupping) each sample in the order provided from left to right
- Determine which sample has PTD and record in the table below by encircling the right code based on your judgement

NB:

Taste the samples by leaving 30 seconds between samples after rinsing your mouth using the provided water

Sample code		
1		
2		
3		
4		
5		
6		
7		

APPENDIX C. CUPPING FORM TO ASSESS THE PERFORMANCE OF CUPPERS

Name of cupper: ……………………………………

Date: ………………..……

Cupping experience: …………….

Instructions:

- You are provided with coded samples
- Assess (by cupping) each sample
- Fill the questionnaire using the Liking scoring scale provided
- For "Yes or No" and "Low, Medium or High"; tick where applicable

NB: Taste the samples by leaving 30 seconds between samples after rinsing your mouth using the provided water

Liking scoring scale

Code	Degree of liking
7	Like very much
6	Like moderately
5	Like slightly
4	Neither like Nor dislike
3	Dislike slightly
2	Dislike moderately
1	Dislike very much

REFERENCES

REFERENCES

ACE. (2021). About COE auctions. (Alliance for coffee excellence) Retrieved 02 23, 2021, from https://allianceforcoffeeexcellence.org/about-coe-auctions/

Agwanda, C. O., Baradat, P., Eskes, A. V., & Charrier, A. (2003). Selection for Bean and Liquor Qualities Within Related Hybrids of Arabica Coffee in Multi- Local Field Trials. Euphytica, 131, 1-14.

Akiyama, M., Murakami, K., Ikeda, M., Iwatsuki, K., Wada, A., Tokuno, K., . . . Iwabuchi, H. (2007). Analysis of the Headspace Volatiles of Freshly Brewed Arabica Coffee Using Solid-Phase Microextraction. J Food Sci, 72, 388-396.

Alberts, P., Kidd, M., Stander, M. A., Nieuwoudt, H. H., Tredoux, A. G., & Villiers , D. A. (2013). Quantitative Survey of 3-Alkyl-2-Methoxypyrazines and First Confirmation of 3-Ethyl-2-Methoxypyrazine in South African Sauvignon Blanc Wines. S Afr J Enol Vitic, 34, 54-67.

Alves, R. C., Rodrigues, F., Nunes, M. A., Vinha, A. F., & Oliveira, B. M. (2017). State of the art in coffee processing by-products. In C. M. Galanakis (Ed.), Handbook of Coffee Processing By-Products-Sustainable Applications (pp. 1-26). London, UK: Academic Press.

Ameyu, M. A. (2017). Influence of harvesting and postharvest processing methods on the quality of arabica coffee (coffea arabica L.) in Eastern Ethiopia. J. Food Agric. Sci., 7(1), 1-9. doi:10.5897/ISABB-JFAS2016.0051

An, N., Xie, H., Gao, N., Deng, Y., Chu, W., & Jiang, J. (2012). Adsorption of Two Taste and Odor Compounds IPMP and IBMP by Granular Activated Carbon in Water. Clean-soil, air, water, 40(12), 1349-1356.

ASTM. (2004). Standard Practice E 679–04. Standard Practice for Determination of Odor and Taste Thresholds by A Forced-Choice Ascending Concentration Series Method of Limits. Philadelphia, PA: American Society for Testing and Materials.

Baggenstoss, J., Poisson, L., Kaegi, R., Perren, R., & Escher, F. (2008). Coffee Roasting and Aroma Formation: Application of Different Time-Temperature Conditions. J. Agric. Food Chem., 56, 5836–5846.

Barahona, I., Sanmiguel Jaimes, M. E., & Yang, J.-B. (2020). Sensory Attributes of Coffee Beverages and Their Relation to Price and Package Information: A Case Study of Colombian Customers' Preferences. Food Sci Nutr., 8, 1173–1186. doi:10.1002/fsn3.1404

Becker, R., Dohla, B., Nitz, S., & Vitzthum, O. (1987). Identification of the "Peasy" Off-Flavor Note in Central African Coffees. 12th International Scientific Colloquium of Coffee. Montreaux: Association Scientifique Internationale du Café.

Bi, J., & Ennis, D. M. (1998). Sensory Thresholds: Concepts and Methods. J. Sens. Stud., 13, 133-148.

Boudreaux , K. C. (2010). Economic Liberalization in Rwanda's Coffee Sector: A Better Brew for Success. In World Bank. Yes Africa Can: Success Stories from a Dynamic Continent.

Brennan, R. L. (1992). Generalizability Theory. Instructional Topics in Educational Measurement, 27-34.

Brennan, R. L. (2001). Multifacet universes of generalization and D study designs. In R. L. Brennan, Generalizability Theory. Statistics for Social and Behavioral Sciences. (pp. 95-139). New York, NY, USA: Springer.

Bressanello, D., Liberto, E., Cordero, C., Rubiolo, P., Pellegrino, G., Ruosi, R. M., & Bicchi, C. (2017). Coffee Aroma: Chemometric Comparison of the Chemical Information Provided by Three Different Samplings Combined With GC-MS to Describe the Sensory Properties in Cup. Food chem, 218-228.

Cheng, T. B., Reineccius, G. A., Bjorklund, J. A., & Leete, E. (1991). Biosynthesis of 2-Methoxy-3-Isopropylpyrazine in Pseudomonas Perolens. J Agric Food Chem, 39, 1009–1012.

Cho, J. S., Bae, B. K., & Moon, K. D. (2017). Qualitative Properties of Roasting Defect Beans and Development of its Classification Methods by Hyperspectral Imaging Technology. Food Chem, 220, 505-509.

Czerny, M., & Grosch , W. (2000). Potent odorants of Raw Arabica Coffee. Their Changes During Roasting. J Agric Food Chem, 48, 868-872.

Derndorfer, E., Baierl, A., Nimmervoll, E., & Sinkovits, E. (2005). A Panel Performance Procedure Implemented in R. J. Sens. Stud., 20, 217–227.

Donfrancesco, B. D., Guzman, G. N., & Chambers, E. (2014). Comparison of Results from Cupping and Descriptive Sensory Analysis of Colombian Brewed Coffee. J. Sens. Stud., 29, 301–311.

Eisele, T. A., & Semon, M. J. (2005). Best Estimated Aroma and Taste Detection Threshold for Guaiacol in Water and Apple Juice. J. Food Sci., 70(4).

Fabbria, A., Cevolia, C., Romanib, S., & Rosab, M. D. (2011). Numerical Model of Heat and Mass Transfer During Roasting Coffee Using 3D Digitised Geometry. Procedia Food Science, 1, 742 – 746.

Farah, A., Monteiro, M. C., Calado, V., Franca, A. S., & Trugo, L. C. (2006). Correlation Between Cup Quality and Chemical Attributes of Brazilian Coffee. Food Chem, 98, 373-380.

Franca, A. S., Oliveira, L. S., Oliveira, R. C., Pamela, C., & Augusti, R. (2009). Preliminary Evaluation of the Effect of Processing Temperature on Coffee Roasting Degree Assessment. J. Food Eng., 92, 345–352.

Franca, S. A., Oliveira, S. L., Oliveira, C. S., Agresti, M. C., & Augusti, R. (2009). A Preliminary Evaluation of the Effect of Processing Temperature on Coffee Roasting Degree Assessment. J Food Eng, 92, 345–352.

Ghosh, P., & Venkatachalapathy, N. (2014). Processing and Drying of Coffee – A Review. Int. J. Eng. Res. Technol., 3(12).

Giguère, J.-F., Piovesana, P., Proulx-Belhumeur, A., Doré, M., Sampaio, K., & Gallani, M.-C. (2016). Reliability of a Simple Method for Determining Salt Taste Detection and Recognition Thresholds. Chem. Senses, 41(3), 205-210. doi:10.1093/chemse/bjv080

Gloess, A. N., Vietri, A., Wieland, F., Smrke, S., Schönbächler, B., Sánchez-López, J. A., . . . Yeretzian, C. (2014). Evidence of Different Flavour Formation Dynamics by Roasting Coffee from Different Origins: On-line Analysis with PTR-ToF-MS. Int. J. Mass Spectrom, 365–366, 324–337.

Grosch, W. (2001). Review: Evaluation of Key Odorants of Foods by Dilution Experiments, Aroma Models And Omission. Chem. Senses, 26, 533-545.

Gueule, D., Fourny, G., Ageron, E., Mateos, A. L., Vandenbogaert, M., Grimont, P. A., & Cilas, C. (2015). Pantoea coffeiphila sp. nov., Cause of the ‘Potato Taste’ of Arabica Coffee from the African Great Lakes Region. Int J Syst Evol Microbiol, 65, 23–29.

Gueule, D., Gérard, F., Elisabeth, A., Lefleche, A., Grimont, P. A., & Cilas, C. (2013). Up-to-Date Knowledge on the "Potato Taste" of the Arabica Coffee Coming from African Great Lakes Region. 24th International Conference on Coffee Science. San José: Association Scientifique Internationale du Café.

Handayani, R. B. (2016). Coffee and its Flavor. Jurnal Ilmiah Rekayasa Pertanian dan Biosistem, 4(1), 222-227.

Harvey, L. (1986). Efficient Estimation of Sensory Thresholds. Behav. Res. Methods Instrum. Comput., 18(6), 623-632.

Hashim, L., & Chaveron, H. (1996). Use of Methylpyrazine Ratios to Monitor Coffee Roasting. Food Res Int, 28(6), 619-623.

Hjelmeland, A. K., Wylie, P. L., & Ebeler, S. E. (2016). A comparison of sorptive extraction techniques coupled to a new quantitative, sensitive, high throughput GC-MS/MS method for methoxypyrazine analysis in wine. Talanta, 148, 336-345. doi:10.1016/j.talanta.2015.10.086

Jackels, C. S., Marshall, E. E., Omaiye, G. A., Gianan, L. R., Lee, T. F., & Jackels, F. C. (2014). GCMS Investigation of Volatile Compounds in Green Coffee Affected by Potato Taste Defect And The Antestia Bug. J. Agric. Food Chem., 62, 10222−10229. doi:dx.doi.org/10.1021/jf5034416

Ji, H., & Bernhard, R. A. (1992). Effect of Microwave Heating on Pyrazine Formation in a Model System. J Sci Food Agric, 59, 283-289.

Kenneth, D. (2014). Rwanda and Central Africa: Sweet and a Hint of Savory. Retrieved 10 18, 2016, from http://www.coffeereview.com/rwanda-and-central-africa-2014/

Koehler, P. E., & Odell, G. V. (1970). Factors Affecting the Formation of Pyrazine Compounds in Sugar-Amine Reactions. J. Agr. Food Chem., 18(5).

Koehler, P. E., Mason, M. E., & Odell, G. V. (1971). Odor Threshold Levels of Pyrazine Compounds and Assessment of their Role in the Flavor of Roasted Foods. J. Food Sci., 36.

Ku Madihah , Y. K., Zaibunnisa , H. A., Norashikin, S., Rozita, O., & Misnawi, J. (2012). Optimization of Roasting Conditions for High-Quality Robusta Coffee. APCBEE Procedia, 4, 209 – 214.

Ku Madihah, Y. K., Zaibunnisa, H. A., Norashikin, S., Rozita, O., & Misnawi, J. (2013). Optimization of Roasting Conditions for High-Quality Arabica Coffee. Int. Food Res. J., 20(4), 1623-1627.

Laing, D. G. (1994). Perceptual Odour Interactions and Objective Mixture Analysis. Food Qual Prefer, 75-80.

Lawless, H. T. (2010). A Simple Alternative Analysis for Threshold Data Determined by Ascending Forced-Choice Methods of Limits. J. Sens. Stud., 25, 332-346. doi:10.1111/j.1745-459X.2009.00262.x

Lawless, T., & Heymann, H. (2010). Sensory Evaluation of Food Principles and Practices Harry, Second Edition. New York: Springer. doi:10.1007/978-1-4419-6488-5

Li, X., Lin, P., Wang, J., Liu, Y., Li, Y., Zhang, X., & Chen, C. (2016). Treatment Technologies and Mechanisms for Three Odorants at Trace Level: IPMP, IBMP and TCA. Environ Technol, 37(3), 308-315.

Lokker, B. (2017). Coffee Roasts from Light to Dark. Retrieved 11 18, 2018, from https://www.coffeecrossroads.com/coffee-101/coffee-roasts-from-light-to-dark

Maeztu, L., Sanz, C., Andueza, S., De-pena, M. P., Bello, J., & Cis, C. (2001). Characterization of Expresso Coffee Aroma by Static Headspace GC-MS and Sensory Flavor Profile. J Agric Food Chem, 49, 5437-5444.

Maga, J. A., & Sizer, C. E. (1973). Pyrazines in Foods: A Review. J. Agr. Food Chem., 21(1).

McPherson, J. E. (2018). Invasive Stink Bugs and Related Species (Pentatomoidea) Biology, Higher Systematics, Semiochemistry, and Management. Boca Raton, FL, USA: CRC Press.

Meilgaard, M. C., Civille, G. V., & Carr, T. B. (2016). Sensory Evaluation Techniques, 5th Edition. Boca Raton, FL, USA: CRC Press.

Mendes, L. C., de Menezes, H. C., Aparecida, M., & da Silva, P. A. (2001). Optimization of the Roasting of Robusta Coffee (C. canephora conillon) using Acceptability Tests and RSM. Food Qual Prefer, 12, 153 -162.

Michon, C., OSullivan, M. G., Delahunty, C. M., & Kerry, J. P. (2009). The Investigation of Gender-Related Sensitivity Differences in Food Perception. J. Sens. Stud., 24, 922–937. doi:10.1111/j.1745-459X.2009.00245.x

Miller, A., Scanlan, R. A., Lee, J. S., Libbey, L. M., & Morgan , M. E. (1973). Volatile Compounds Produced in Sterile Fish Muscle (Sebastes melanops) by Pseudomonas perolens. Appl Microbiol, 25, 257-261.

Miller, T. (2015). Methods and Compositions for Improving Coffee Flavor. US Patent No 2015054236.

Misharina, A. T., Golovnya, V. R., & Yakovleva, N. V. (1992). Effect of Time and Temperature on the Preparation of Pyrazines in Model Reactions of the Synthesis of Aroma-Forming Substances. Russ Chem Bull, 41, 1258–1263. doi:10.1007/BF00864192

Moreira, S. P., Nunes, M. F., Simoes, C., Maciel, E., Domingues, P., Domingues, M. R., & Coimbra, A. M. (2017). Data on Coffee Composition and Mass Spectrometry Analysis of Mixtures of Coffee Related Carbohydrates, Phenolic Compounds and Peptides. Data in Brief, 13, 145-161.

Moreno, D., Moreno, E., & Cadena, G. (1995). Bean Characteristics and Cup Quality of the Colombian Variety (Coffea arabica) as Judged by International Tasting Panels. 16th International Scientific Colloquium on Coffee. Kyoto: Association Scientifique Internationale du Café.

Müller, R., & Rappert, S. (2010). Pyrazines: Occurrence, Formation and Biodegradation. Appl Microbiol Biotechnol, 85, 1315–1320. doi:10.1007/s00253-009-2362-4

Murray, K. E., & Whitfield, F. B. (1975). The Occurrence of 3-Alkyl-2-Methoxypyrazines in Raw Vegetables. J. Sci. Fd Agric., 26, 973-986.

Mutarutwa, D., Navarini, L., Lonzarich, V., Compagnone, D., & Pittia, P. (2018). GCMS aroma characterization of vegetable matrices: Focus on 3-alkyl-2-methoxypyrazines. J. Mass Spectrom, 53, 871-881. doi:10.1002/jms.4271

Mutarutwa, D., Navarini, L., Lonzarich, V., Crisafulli, P., Compagnone, D., & Pittia, P. (2020). Determination of 3-Alkyl-2-methoxypyrazines in Green Coffee: A Study To Unravel Their Role on Coffee Quality. J. Agric. Food Chem., 68, 4743−4751. doi:10.1021/acs.jafc.9b07476

NISR. (2015). Integrated Household Living Conditions Survey: EICV 4, 2013/2014. Main Indicators Report. National Institute of Statistics of Rwanda. Kigali: Government of Rwanda.

Oden, G. (Undated). The Difference Between Light, Medium, and Dark Roast Coffee. Retrieved 11 18, 2018, from https://www.javapresse.com/blogs/buying-coffee/differences-between-light-medium-dark-roasted-coffee

Parliment, T. H. (2000). An Overview of Coffee Roasting. In T. H. Parliment, C. T. Ho, & P. Schieberle (Eds.), Caffeinated Beverages: Health Benefits, Physiological Effects and Chemistry (pp. 188–201). Washington, DC: American Chemical Society.

Peng, M., Jaeger, S. R., & Hautus, M. J. (2012). Determining Odour Detection Thresholds: Incorporating a Method-Independent Definition into the Implementation of ASTM E679. Food Qual Prefer, 25, 95–104.

Pereira, L. L., Cardoso, W. S., Guarçoni, R. C., da Fonseca, A. F., Moreira, T. R., & Caten, C. S. (2017). The consistency in the sensory analysis of coffees using Q-graders. Eur Food Res Technol, 243, 1545–1554. doi:10.1007/s00217-017-2863-9

Pereira, L. L., Guarçoni, R. C., de Souza, G. S., Junior, D. B., Moreira, T. R., & ten Caten, C. S. (2018). Propositions on the Optimal Number of Q-Graders and R-Graders. J. Food Qual. doi:10.1155/2018/3285452

Pickering, G. J., Karthik, A., Inglis, D., Sears, M., & Ker, K. (2007). Determination of Ortho- and Retronasal Detection Thresholds for 2-Isopropyl-3-Methoxypyrazine in Wine. J. Food Sci., 72(7).

Pickering, G. J., Karthik, A., Inglis, D., Sears, M., & Ker, K. (2008). Detection Thresholds for 2-Isopropyl-3-Methoxypyrazine in Concord and Niagara Grape Juice. J. Food Sci., 73(6). doi:10.1111/j.1750-3841.2008.00847.x

Poisson, L., Blank, I., Dunkel, A., & Hofmann, T. (2017). The Chemistry of Roasting- Decoding Flavor Formation. In B. Folmer, I. Blank, A. Farah, P. Giuliano, D. Sanders, & C. Wille (Eds.), The Craft and Science of Coffee (pp. 273-309). London, UK: Academic Press. doi:10.1016/B978-0-12-803520-7.00011-6

Poltronieri, P., & Rossi, F. (2016). Review: Challenges in Specialty Coffee Processing and Quality Assurance. Challenges, 7(19). doi:doi:10.3390/challe7020019

Raithatha, C. (2018). Panel Performance MeasuresSetting up Systems and Using Data and Feedback to Monitor Performance and Act on any Issues. In L. Rogers (Ed.), Sensory Panel Management- A Practical Handbook for Recruitment, Training and Performance (pp. 273-299). Woodhead Publishing. doi:10.1016/B978-0-08-101001-3.00007-0

Riberio, J. S., Augusto, F., Salva, T. J., Thomaziello, R. A., & Ferreira, M. M. (2009). Prediction of Sensory Properties of Brazilian Arabica Roasted Coffees by Headspace Solid-Phase Microextraction-Gas Chromatography and Partial Least Squares. Analytica Chimica Acta, 634, 172-179.

Samoggia, A., & Riedel, B. (2018). Coffee Consumption and Purchasing Behavior Review: Insights for Further Research. Appetite, 129, 70–81. doi:10.1016/j.appet.2018.07.002

Sanz , C., Maeztu, L., Zapelena, M. J., Bello, J., & Cid, C. (2002). Profiles of Volatile Compounds and Sensory Analysis of Three Blends of Coffee: Influence of Different Proportions of Arabica and Robusta and Influence of Roasting Coffee With Sugar. J Sci Food Agric, 82, 840-847.

Sanz-Uribe, J. R., Yusianto, Menon, S. N., Penuela, A., Oliveros, C., Husson, J., . . . Rodriguez, A. (2017). Postharvest Processing-Revealing the Green Bean. In B. Folmer, I. Blank, A. Farah, P. Giuliano, D. Sanders, & C. Wille (Eds.), The Craft and Science of Coffee (pp. 51-79). London, UK: Academic Press. doi:10.1016/B978-0-12-803520-7.00011-6

SCAA. (2003). Speciality Coffee Association of America Coffee Cupping Form. Retrieved 05 15, 2018, from http://www.falconcoffees.com/wp-content/uploads/2016/06/SCAA-score-sheet.pdf

SCAA. (2015). SCAA Protocols-Cupping Specialty Coffee. Retrieved 03 05, 2018, from http://www.scaa.org/PDF/resources/cupping-protocols.pdf

Scheidig, C., Czerny, M., & Schieberle, P. (2007). Changes in Key Odorants of Raw Coffee Beans During Storage under Defined Conditions. J Agric Food Chem, 55, 5768-5775.

Schenker, S., & Rothgeb, T. (2017). The Roast-Creating the Beans' Signature. In B. Folmer, I. Blank, A. Farah, P. Giuliano, D. Sanders, & C. Wille (Eds.), The Craft and Science of Coffee (pp. 245-271). London, UK: Academic Press. doi:10.1016/B978-0-12-803520-7.00011-6

Schenker, S., Heinemann, C., Huber, M., Pompizzi, R., Perren, R., & Escher, F. (2002). Impact of Roasting Conditions on the Formation of Aroma Compounds in Coffee Beans. J. Food Sci., 67(1).

Seninde, D. R., & Chambers, E. (2020). Coffee Flavor: A Review. Beverages, 6(44). doi:10.3390/beverages6030044

Shavelson , R. J., & Webb, N. M. (2005). Generalizability Theory. Retrieved 02 02, 2019, from https://web.stanford.edu/dept/SUSE/SEAL/Reports_Papers/methods_papers/G%20Theory%20AERA.pdf

Shibamoto, T., & Bernhard, R. A. (1976). Effect of Time, Temperature, and Reactant Ratio on Pyrazine Formation in Model Systems. J. Agric. Food Chem., 24(4).

Shibamoto, T., & Bernhard, R. A. (1977). Investigation of Pyrazine Formation Pathways in Sugar-Ammonia Model Systems. J. Agric. Food Chem, 25(3).

Spadone, J. C., Takeoka, G., & Liardon, R. (1990). Analytical Investigation of Rio Off-flavor in Green Coffee. J Agric Food Chem, 38(1).

Sualeh, A., Endris, S., & Mohammed, A. (2014). Processing Method, Variety And Roasting Effect on Cup Quality of Arabica Coffee (Coffea arabica L.). Discourse J Agric and Food Sc, 2, 70-75.

Sunarharum, B. W., Willias, J. D., & Smyth, E. H. (2014). Complexity of Coffee Flavor: A Compositional and Sensory Perspective. Food Res Int, 62, 315-325.

Talsma, P. (2016). Assessing Sensory Panel Performance Using Generalizability Theory. Food Qual Prefer, 47, 3–9.

Tempere, S., Cuzange, E., Malak, J., Bougeant, J., de Revel, G., & Sicard, G. (2011). The Training Level of Experts Influences their Detection Thresholds for Key Wine Compounds. Chem. Percept., 4, 99–115. doi:10.1007/s12078-011-9090-8

Thomas, E., Puget, S., Valentin, D., & Songer, P. (2017). Sensory Evaluation-Profiling and Preferences. In B. Folmer, I. Blank, A. Farah, P. Giuliano, D. Sanders, & C. Wille (Eds.), The Craft and Science of Coffee (pp. 419-456). London, UK: Academic Press. doi:10.1016/B978-0-12-803520-7.00011-6

Toci, T. A., & Boldrin, V. Z. (2018). Coffee beverages and their aroma compounds. In M. A. Grumezescu, & M. A. Holban (Eds.), Natural and artificial flavoring agents and food dyes, Handbook of food bioengineering, Vol 7 (pp. 398-425). London: Academic press.

Toci, T. A., & Farah, A. (2008). Volatile compounds as potential defective coffee beans' markers. Food Chem, 108, 1133-1141.

Tomic, O., Forde, C., Delahunty, C., & Næs, T. (2013). Performance Indices in Descriptive Sensory Analysis – A Complimentary Screening Tool for Assessor and Panel Performance. Food Qual Prefer, 28, 122–133. doi:10.1016/j.foodqual.2012.06.012

Van Gemert, L. J. (2011). Odour Thresholds: Compilations of Odour Threshold Values in Air, Water and Other Media, Second Edition. Utrect, Netherlands: Oliemans Punter & Partners BV.

Verhoef, A., Huijberts, G., & Vaessen, W. (2015). Introduction of a Quality Index, Based on Generalizability Theory, as a Measure of Reliability for Univariate- and Multivariate Sensory Descriptive Data. Food Qual Prefer, 40, 296–303.

Wang, P.-S., & Odell, G. V. (1973). Formation of Pyrazines from Thermal Treatment of Some Amino-Hydroxy Compounds. J. Agr. Food Chem., 21(5).