OPTIMIZATION OF FERMENTATION CONDITIONS OF NATURALLY PROCESSED ARABICA COFFEE *(Coffea arabica)* IN UGANDA

BY

KHASSIM ZULFAT

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SEPT, 2024

DECLARATION

I Khassim Zulfat, declare that this dissertation, to the best of my knowledge, is my original work and has never been submitted for consideration for a degree award from any university or other higher education entity.

. ……………………………… ……………………………..

Signature Date

APPROVAL

This certifies that the work that has been submitted here is the student's original work, completed under our supervision and direction, and is now prepared for submission to the Kyambogo University Board of Examiners.

1. Nakyinsige Khadijah (Ph. D)

Department of Food Science and Technology,

Faculty of Science,

Kyambogo University.

Signature………………………………. Date……………………………

2. Mutambuka Martin (Ph. D)

Department of Food Science and Technology,

Faculty of Science,

Kyambogo University.

Signature………………………………. Date……………………………

DEDICATION

I dedicate this book to my spouse, Kazibwe M. Bashir, my children, Alma.B. Kazibwe and Abdallah. B. Kazibwe, my parents Mr. Khassim Said and Ms. Mutesi Sofia for the magnificent support throughout this academic journey. I also dedicate it to Prof. Mahmud Mpezamihigo, who encouraged and supported me to embark on this journey.

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ABSTRACT

Coffee is very popular due to the unique sensory characteristics of its brew. This research aimed to determine the best fermentation method and duration for Arabica coffee with respect to its volatile, physicochemical, and sensory components.

Gas chromatography/mass spectrophotometry (GCMS) was used to examine volatile chemicals that had been extracted using solid-phase microextraction (SPME). Descriptive sensory evaluation with 8 trained panelists was used to assess the cup quality. Response Surface Methodology and Principle Component Analysis were used for data analysis. Significant models were generated to describe the relationship between fermentation time, fermentation type, and the physicochemical, and sensory properties as well as volatile compounds of Arabica coffee. Both fermentation type and time had an impact on sensory qualities such as fragrance/aroma, flavor, sweetness, acidity, body, balance, aftertaste, uniformity, and cleanliness. The optimum fermentation time and fermentation type were 16.8 hours and spontaneous fermentation respectively. Principle component analysis was performed to relate sensory attributes with physicochemical properties and volatile compounds. Furaneol, coffee temperature, 3-ethyl-2-hydroxy-2-cyclopenten-1-one, and acetaldehyde were positively correlated to each other. 1-(1H-pyrrol-2-yl)-ethanone, furan were related, 3-hydroxy-2-butanone, acetic acid, brix, 2,3-pentanedione and ethyl isovalerate, 2,3-butanedione (diacetyl), 2-methylbutanal, 3-methylbutanal were positively correlated, and there was a positive correlation between 2-butanone, pH, pyrazines.

CHAPTER 1

INTRODUCTION

1.1.Background

Coffee is distinctly the oldest and most pivotal commercial agricultural commodity, serving as a significant contributor to Uganda's foreign exchange earnings. World coffee production was 168.5 million bags in the coffee year 2021/2022, of which Africa contributed 19.4 million bags (ICO, 2023). With 1% of the world's Arabica coffee and 6% of the world's Robusta coffee produced worldwide in 2021, Uganda ranked second in Africa for coffee production after Ethiopia (FAOSTAT, 2021). Coffee has continuously added an average of 15% to the nation's total income from exports during the last ten years (ICO, 2019). Uganda exported 4.45 million 60kg bags of coffee worth $492 million (UCDA, 2018). The total coffee export volume of Uganda amounted to 301,366 tons of “green” coffee, forming the second largest commodity export for Uganda, contributing about 12.4% to the country’s total exports (Ategeka, 2024).

Global coffee consumption increased by 4.2% (ICO, 2023) and there is currently a "latte revolution" in the coffee business, with an estimated 1.5 billion cups of coffee consumed daily worldwide (Hertz, 2020). Moreover, coffee also provides as a key source of income, especially for rural smallholder farmers. In 112 districts of Uganda, coffee is grown by around 1.5 million people, and for about 500,000 of these households, it represents their primary source of income (UCDA, 2020). If coffee is grown effectively, it may increase the productivity of smallholders and promote regional development in the nations that produce it (FAO, 2012).

Despite the uniqueness of Uganda’s coffee, Uganda is not known as a producer of high-end coffee (Bamwesigye & Hlavackova, 2019). Specialty coffee is the fastest growing segment of the global coffee market. Coffee's unique sensory qualities account for its diverse appeal.

Postharvest processes, like fermentation, significantly impact the coffee beverage quality and are important in illustrating how consumers perceive the beverage's quality (Huch & Franz (2015). Pectin is the main carbohydrate polymer in coffee mucilage, and fermentation is essential to its breakdown and sensory qualities. This process results in the release of fundamental sugars and important compounds such as ethanol, acetaldehyde, and acetic acid, alongside a broad array of smaller molecules like esters, higher alcohols, aldehydes, ketones, and terpenoids. These components interact to provide the unique flavor and aromatic characteristics that make coffee unique (Kim et al., 2016).

Roasting is the major step in which volatile compounds are generated (Baggenstoss et al., 2008) through chemical reactions like streckers degradation, Maillard reaction, and nonenzymatic reactions (Farah, 2012). However, fermentation improves the flavor of coffee by producing precursors for the formation of volatiles during roasting (de Melo Pereira et al., 2015). The role of microorganisms i.e. bacteria and yeasts, in altering the coffee cup quality during anaerobic processing has been a topic of discussion for decades. Moreover, constant monitoring of fermentation process conditions like temperature, time, sugar concentration, and pH among others, is crucial in specialty coffee processing (Flórez-Delgado, 2020).

The consumption of sugar by microorganisms involved in fermentation results in the production of several enzymes, alcohols, and acids that can affect the sweetness, acidity, and salt content of coffee beans (Barbosa et al., 2019; Silva et al.,2000). Yeasts, bacteria, and filamentous fungi are among the native types of microorganisms that cause fermentation; they first appear as process contaminants. According to surveys, *Pichia kluyveri*, *Pichia anomala*, *Hanseniaspora uvarum*, *Saccharomyces cerevisiae*, *Debaryomyces hansenii*, and *Torulaspora delbrueckii* are the most commonly found yeast species during the fermentation of coffee (Silva et al., 2008; Vilela et al., 2010). *Saccharomyces cerevisiae* has widely been studied and is known to produce high concentration of pectinase which help in degradation of the muscilage (Klingel et al., 2020).

1.2. Problem Statement

Despite the increased coffee production index, Uganda still earns less from the coffee exports due to low value addition amongst other challenges facing its coffee industry like climate change, wilt disease and Price instability among others (Hertz, 2020).

To produce high value specialty coffee, it is highly imperative to evaluate how postharvest processes like fermentation can be controlled to improve coffee sensory quality (Bressani, Martinez, Evangelista, & et al, 2018). For decades, Ugandan coffee has been processed commonly using dry or wet processes with fermentation facilitated by naturally occuring microorganisms in the coffee (Nakendo, Musoli, Kananura, & Wagoire, 2018).

While fermentation plays a major role in determining the coffee's sensory profile, little is known about inoculating Ugandan coffee with starting culture and how this affects the coffee sensory quality. Moreover, there has been no study done to optimize fermentation conditions of Ugandan Arabica coffee, to determine the optimal fermentation conditions that would result into high quality coffee sensory profile. This research, thus intended to optimize the fermentation conditions i.e. temperature, pH, brix and fermentation time of naturally processed Arabica coffee in Uganda.

1.3. Objectives

1.3.1 General Objective

- To optimize fermentation conditions of naturally processed Arabica coffee in Uganda with respect to physicochemical compounds, volatile compounds and sensory attributes of Arabica coffee.

1.3.2 Specific Objectives:

1. To optimize fermentation time and fermentation type of naturally processed Arabica coffee with respect to physicochemical and sensory properties as well as volatile compounds.
2. To determine the relationship beween physicochemical parameters (temperature, pH, volatile compounds, processing parameters (fermentation time, temperature, brix, pH and starter culture) and sensory atributes of Arabica coffee.

1.4 Hypotheses

1. There is no relationship between fermentation type, fermentation time and physicochemical properties, sensory properties and volatile compounds in Arabica coffee.
2. There is no relationship between processing parameters, physicochemical parameters and sensory quality of Arabica coffee.

1.5. Justification

Enhancing the quality of coffee boosts its commodity value on the international market, raising the standard of life for many Ugandans participating in the coffee value chain and contributing to the nation's income (economy). Therefore, if Uganda continues to export low quality coffee, it will lead to more revenue losses and consquently, demoralization of small holder farmers due to low income that leads to higher poverty levels among their families.

One of the ways of improving coffee quality is by controlling the key quality determinant postharvest processes like fermentation and drying, that greatly contribute to the coffee cup quality. This research intended to achieve this by optimizing fermentation conditions of naturally processed Arabica coffee in Uganda.

CHAPTER 2

LITERATURE REVIEW

2.1. Coffee

2.1.1. Physical and nutrient composition

Coffee is classified within the Coffea genus and the Rubiaceae family, encompassing approximately 103 tropical tree and shrub species. Coffee beans, essentially the plant's seeds, are stored in the cherries that the coffee plant bears. The coffee fruit has several distinct layers: an outer layer that ranges from ripe orange-red to deep red (known as the exocarp), a juicy inner layer consisting of yellowish-white pulp and mucilage (referred to as the mesocarp), and encasing the seeds, there's a simple yellow parchment layer (known as the endocarp) and a silvery skin called the integument (Ferrao & et al, 2015).

The outer layer of the fruit, known as the exocarp, offers external protection due to its single-cell layer covered by a waxy layer. While in their immature state, the fruits exhibit a green coloration, which transitions in shades from red violet to deep red then to yellow or orange during ripening process, varying based on the specific genetic makeup. The inner pulp known as the mesocarp is both juicy and fibrous, delivering a sweet taste. It has significant amounts of proteins, lipids, lipid minerals, tannins, polyphenols, and caffeine in addition to being high in carbs including glucose, fructose, and pectin (Janissen & Huynh, 2018; Murthy & Naidu, 2012). The parchment layer, referred to as the endocarp, is a delicate, yellowish, and brittle component resembling paper. It's primarily made up of alpha-cellulose, lignin, hemicellulose, and ash, which constitute its composition (Esquivel & Jimenez, 2012). Polysaccharides, notably cellulose and hemicelluloses, make up the majority of the silver skin. It also consists of proteins, polyphenols, monosaccharides, and a number of other insignificant ingredients (Farah & dos Santos, 2015).

This stratum contains substantial amounts of dietary fibers and phenolic compounds, which give it impressive antioxidant properties (Esquivel & Jimenez, 2012). The silver skin wraps around the endosperm and embryos, enclosing them within the two elliptical seed halves (Esquivel & Jimenez, 2012).

2.1.2. Overview of Uganda’s coffee sector

Uganda is an East African country situated approximately 800 kilometers inland from the Indian Ocean, on the other side of the Equator. Its latitude is between 10 29' and 40 12' North, and its longitude is between 290 34' and 350 0' East. There are 200,523 km2 of land on its 241,551 km^2 total area. Uganda is expected to have 41.6 million people living there in 2020 (UBOS, 2020).

Coffee holds significant importance within the non-arid tropics as a vital agricultural commodity. It is recognized as a valuable cash crop, cultivated across more than 50 nations. Its cultivation catalyzes enduring social advancement by creating substantial rural employment opportunities, fostering business growth, and yielding environmental advantages. The wide variety of globally appreciated coffee variations and qualities contribute to high-value specialty or gourmet coffee offerings, further enhancing its significance (Hameed et al., 2018).

Furthermore, coffee holds the distinction of being the most extensively traded tropical agricultural commodity globally. In the 2009/2010 period, approximately 93.4 million bags were shipped, resulting in estimated exports valued at US$ 15.4 billion, as outlined by the International Coffee Organization (ICO, 2013). Approximately 25 million people worldwide are dependent on the coffee produced by small-scale farmers. The International Coffee Organization (ICO) reports that more than 70 countries throughout the world cultivate coffee. According to ICO (2013), South America, Africa, and Southeast Asia are the main producers of coffee in the world.

Top coffee-producing countries in the world include the Dominican Republic, Ecuador, El Salvador, Ethiopia, Guatemala, Honduras, India, Indonesia, Java, Ivory Coast, Mexico, Nicaragua, Papua New Guinea, Peru, Tanzania, Thailand, Uganda, Vietnam, Angola, Cameroon, Cuba, and the Democratic Republic of the Congo (ICO, 2016).

Coffee stands as Uganda's oldest and most crucial commercial agricultural produce, serving as the main source of revenue in foreign currency. It has consistently contributed 15% of the nation's total export revenue over the past decade (ICO, 2019). The primary destination for Uganda's coffee exports is the European Union, constituting more than 70% of the total export volume. Following closely is Sudan, which imports slightly over 10% of Ugandan coffee, while the USA accounts for approximately 3% of Uganda's coffee exports (UCDA, 2011). Uganda's export market, on the other hand, is highly diverse, with 16 importing countries. Twenty-nine national and multinational businesses control the majority of the export market, accounting for almost 85% of it. 15% of coffee exports were under the exclusive control of Ugacof (U), Ltd. in 2011 (UCDA, 2011). In 2011, 73.4% of the market was possessed by the top ten importing companies.

2.1.3. Coffee Varieties

In Uganda, approximately 1.7 million households are engaged in coffee cultivation, with an average plot size of under one acre. In Uganda, coffee cultivation primarily involves two main types: Arabica and Robusta. Although arabica dominates the world with about 85% of the production and robusta contributes only 15%, its the reverse in Uganda with robusta largely grown (85%) and arabica at 15%. Arabica originated from Ethiopia in 1912 and is mainly cultivated in Highland areas (1500-2,300 m above sea level), whilst robusta grows at lower altitudes (1200m above sea level). Arabica is therefore mostly grown in the West Nile (Nebbi and Okoro districts), the East, on the Rwenzori Mountain, and in the South Western regions (Kisoro and Kabale). The brands "Wugar" (Washed Uganda Arabica) and "Drugar" (Dry Uganda Arabica) are used to sell Arabica coffee. Due to its greater quality, Arabica coffee has a higher competitive edge in the global market. In Uganda, a diverse range of Arabica coffee strains are cultivated, encompassing SL 28, ideal for high-altitude environments, SL 14, well-suited to medium elevations, KP 423, also thriving at medium altitudes, and the ancient Nyasaland variety, prevalent in regions like Mountain Elgon, Rwenzori, and the Zeu mountains within Zombo District of the Northwestern sub-Region. The Nyasaland strain was introduced to Uganda from Malawi (previously known as Nyasaland) during the early 1920s (UNDP, 2012).

2.1.4. Importances of Coffee

In addition to being a major source of income, coffee has been shown to be effective in treating Alzheimer's disease, migraines, and asthma. Coffee is a stimulant, therefore it can keep you from sleeping, which makes you work more hours. Coffee is also known to have about 700 different compounds, which makes it useful in a variety of industrial applications, especially in the chemical industry (Miranda, Steluti, Fisberg, & Marchioni, 2017).

2.1.5. Coffee Agro-ecological zones in Uganda

The cultivation of this commodity spans various regions within the country, encompassing both highland and lowland areas. Notably, it thrives along the slopes of Mountain Elgon that borders Kenya, as well as the slopes of the iconic Mountain Rwenzori bordering DR Congo. Additionally, Arabica coffee flourishes on the slopes of Mount Muhabura in Kisoro, located in the southwestern part of Uganda, while robusta coffee is cultivated in the central region, including districts like Wakiso, Mpigi, Masaka, and Rakai. Western and southwestern districts host both robusta and arabica varieties, and the West Nile region (northwestern Uganda) boasts cultivation of both arabica and robusta coffee (Ategeka, 2024). Altitude tends to cause a variation in the Arabica coffee characteristics i.e. Arabica coffee at altitude <1800m above sea level tends to be different from that at altitude >2000m above level with better cup profiles (FNC-Cenicaf'e, 2013).

The Robusta coffee variety is cultivated in the Busoga and Northern regions. Within the realm of Robusta, two distinct varieties exist: Nganda and Erecta. To enhance productivity, high-yielding Clonal Robusta Coffee is being introduced, boasting nearly fourfold yields compared to traditional strains. This initiative seeks to replace aging and diseased tres (UCDA, 2020).

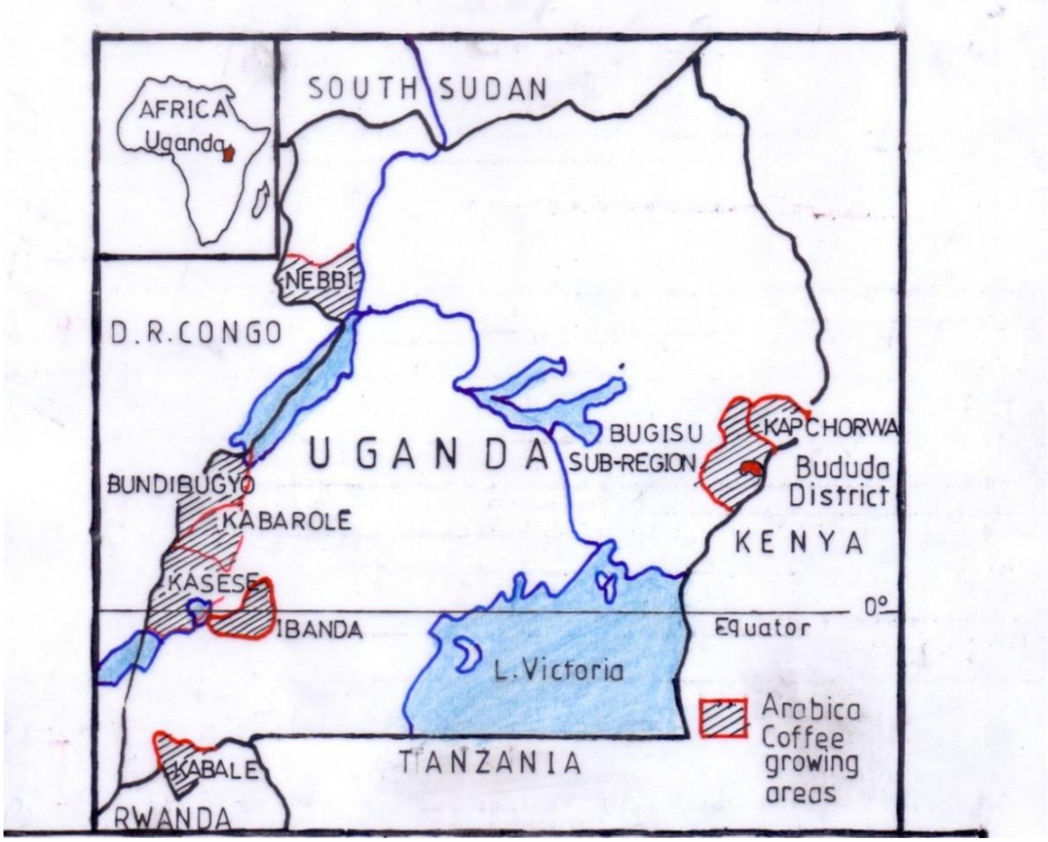

Figure 2. 1. Map showing the Arabica coffee growing areas of Uganda
Source: Mugerwa (2020)

2.1.6. Coffee Production

Uganda's coffee cultivation engages approximately 1.7 million farmers distributed across 108 districtsUganda's coffee production mainly comprises two primary types: Robusta and Arabica, with Robusta being the dominant variety, outnumbering Arabica by a ratio of 4 to 1. The distribution of coffee acreage, tree count, and coffee-growing households by region is presented in Table 2.1. In 2014, Uganda boasted a coffee tree population of 295 million, spread across an extensive area of 353,907 hectares. Presently, Uganda is actively undertaking a registration initiative for coffee farmers to precisely assess the real extent of coffee acreage and the density of coffee trees (ICO, 2019).

Table 2. 1. Coffee cultivation across regions

Regions	Area harvested (ha)	Total, Trees	Trees that produce	Household number (hh)	Trees per hh	Percentage
western Uganda	79,773	94,155,423	61,342,735	265,144	231	23
Eastern Uganda	77,709	86,332,784	73,305,543	486,079	151	22
Northern Uganda	19,886	27,498,178	20,519,580	92,336	222	6
Central Uganda	136,247	151,082,141	109,993,307	611,782	180	38
South-West Uganda	40,292	49,549,569	29,430,081	258,182	114	11
Total	353,907	408,618,095	294,591,246	1,713,523	238	100

Source: UCDA,2019.

2.1.7. Volume of Coffee Production

Coffee is one of the most traded agricultural crop in the world. World wde production of coffee is about 10.8 metric tonnes with Brazil and Viet Nam being the first and sencond largest producers contributing about 30.2% and 18.6% global production respectively (FAOSTAT, 2021). Africa production is estimated to be around 1.4 metric tonnes contributing about 13.4% of global production with Ethiopia and Uganda being the leading and second leading producers producing 456,000 and 374,760 tonnes respectively in 2021 (FAOSTAT, 2021). Uganda Africas second leading producer with most production being from Greater Masaka, Busoga región, South-West Uganda And Westinile (UCDA, 2021). In contrast, global coffee consumption surged by a projected 4.2% to 175.6 million bags in the 2021–2022 coffee year, up from 0.6% the year before (ICO, 2023). Even though consumption exceeded production, there was still an accumulated surplus of about 8 million bags over the two preceding seasons.

2.1.8. Volume and Value of Uganda coffee export

In 2019, the coffee export volume reached 463,709 bags, each weighing 60 kilograms, with a total value of $45.26 million. This included 386,904 bags of Robusta coffee worth $36.70 million, and 76,805 bags of Arabica coffee valued at $8.56 million. These statistics indicated growth in both export quantity and value, reflecting a 17.99% increase in quantity and an 11.56% increase in value when compared to the corresponding month of the preceding year. Table 2.2 displays Uganda's coffee exports during the previous decade (ICO, Country Coffee Profile: Uganda, 2019).

Table 2. 2. Export quantity and value (measured in 60kg bags and US$)

Years	**Robusta coffee**		**Arabica coffee**	**Total**		
	Amount	Price	Amount	Price	Amount	Price
2008/2009	2,405,137	212,848,980	648,551	78,912,759	3,053,688	291,761,739
2009/2010	1,957,400	163,484,690	711,571	103,230,931	2,668,971	266,715,621
2010/2011	2,484,013	294,606,045	665,410	154,284,625	3,149,423	448,890,669
2011/2012	1,904,176	223,976,023	822,073	168,722,105	2,726,249	392,698,138
2012/2013	2,781,478	317,728,861	801,151	114,965,197	3,582,629	432,694,059
2013/2014	2,735,020	285,614,846	764,809	108,307,489	3,499,829	393,922,335
2014/2015	2,722,636	288,389,791	733,216	122,160,149	3,455,852	410,549,941
2015/2016	2,435,160	223,655,972	880,407	103,020,278	3,315,567	326,676,251
2016/2017	3,618,631	404,858,546	986,527	139,729,082	4,605,158	544,587,628
2017/2018	3,202,881	325,032,630	1,102,716	137,796,277	4,305,597	462,828,907

Source: ICO, 2019.

2.1.9. Uganda Coffee Export based on destination

The distribution of coffee exports of Uganda in 2019 is illustrated in Figure 2.2, with further details provided in Annex 1. Italy emerged as the leading recipient, capturing the largest market share at 32.90% (compared to 33.01% in the previous month). Germany followed closely with a share of 13.69% (11.71% previously), while Sudan secured 12.23% (up from 6.04%). India accounted for 5.97% (compared to 9.61%), and Switzerland held 4.68% (a rise from 2.64%). Notably, coffee shipments to African nations reached about 82 thousand bags, corresponding to a 17.71% of the market, in comparison with the previous month's 45,497 bags and 13.32% share. Uganda's principal coffee export market (UCDA, July, 2019).

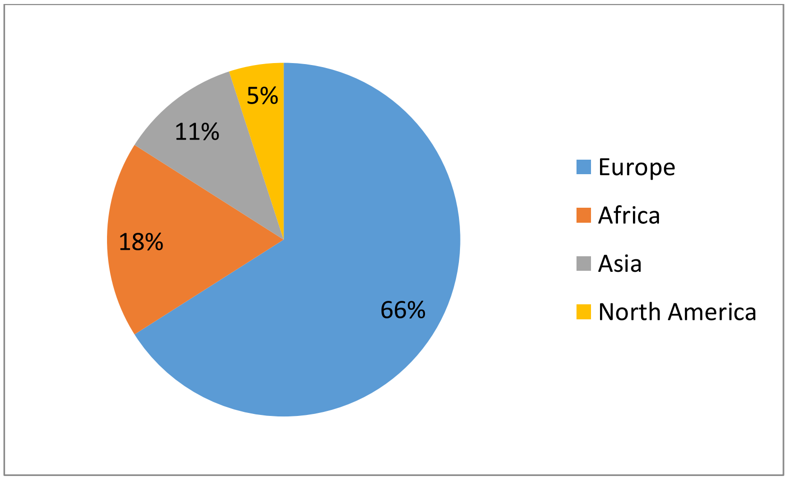

Figure 2. 2. Pie-chart illustrating the primary destinations of Ugandan coffee by quantity in 60 kg bags during July 2019

Source UCDA, 2019

Table 2.3 presents in 2019, exports of Arabica coffee were analyzed based on their type, grade, and unit price. Arabica coffee attained an average price of US$ 1.86 per kilogram, reflecting an increase of 4 cents compared to the price in 2019, which was US$ 1.82 per kilogram. Among the various coffee types and grades, Organic Drugar commanded the highest price of US$ 2.57 per kilogram. This price reflected a premium of 28 cents compared to Bugisu AA. Mt. Elgon A+ was close behind, commanding a unit price of US$ 2.40 per kilogram, an 11-cent premium above Bugisu AA.

Table 2. 3: The exportation of Arabica coffee was classified based on its type, grade, and unit price.

Type of Coffee	Amount 60kg bag	Percentage quantity	Value in US $	Percentage value	Unit cost US$/kg
Organic Druger	1,048	1.36	161,823	1.89	2.57
Organic Okoro	700	0.91	78,917	0.92	1.88
Arabica Bugisu A+	3,440	4.48	454,479	5.31	2.20
Arabica Bugisu AA	3,410	4.44	469,056	5.48	2.29
Arabica Bugisu AB	465	0.61	61,760	0.72	2.21
Arabica Bugisu PB	335	0.44	44,445	0.52	2.21
Mt Elgon A+	4,858	6.33	700,786	8.18	2.40
Mixed Arabica	2,560	3.33	470,488	5.49	3.06
Arabica Wugar	3,663	4.77	458,757	5.36	2.09
Arabica Druger	47,426	61.75	4,892,921	57.14	1.72
Other Arabicas	8,900	11.59	770,069	8.99	1.44
Total Arabica	76,805	100.00	8,563,500	100.00	1.86

Source: UCDA, 2019.

2.1.10. Coffee growth and Harvesting seasons

Coffee farming is a perennial activity; in Uganda, there are normally two main coffee harvest seasons; one from March to June for Arabica type and another from September to November for Robusta species. Most planting takes place between March and May during the first rainy season and between September and November during the second rainy season. In Northern Uganda, a single extended rainy season prevails from March to August (ICO, 2019). The alternation between main and minor harvesting periods is influenced by the region's location in either the North or South of equator (Table 2.4).

Table 2. 4: Coffee harvesting seasons in Uganda differ depending on the region.

Region in Uganda	October	November	December	January	February	March	April	May	June	July	August	September
Central región		Major crop (Robusta)						Minor Crop (Robusta)				
Masaka región	Minor crop (Robusta)							Major crop (Robusta)				
Eastern región		Major crop (Robusta)						Minor crop (Robusta)				
Bugisu/ Sebei región		Major crop (Arabica)						Minor crop (Arabica)				
Western region	Minor Crop (Robusta)						Major crop (Robusta)					
	Minor crop (Arabica)						Major crop (Arabica)					
West Nile region	Major crop (Arabica)						Minor crop (Arabica)					

Source: ICO, 2019

After maturing for three to four years, coffee trees bear fruit in clusters or lines along their branches. When the fruit is ready to be harvested, it becomes red or "berry-like." It takes six to eleven months for the cherries to ripen, depending on the variety of coffee plants. Arabica coffee's pH and sugar content typically ranges from 5.0 to 6.5 and 12 to 20, respectively (FAO, 2010).

2.2. Coffee processing

The initial stage of postharvest coffee processing involves gathering coffee fruits, typically done through either careful manual handpicking or by strategically placing sheets under the coffee trees and shaking them to collect the ripe fruits. However, there has been a significant global increase in the adoption of mechanical harvesters that employ branch vibration techniques. Autonomous vehicles, transportable equipment, and devices made to remove coffee beans from branches are some examples of these mechanical harvesters (Bee et al., 2005). The selected technique has a profound influence on the subsequent quality of the harvested fruit, which in turn affects its suitability for further on-farm processing. While manual handpicking permits the careful selection of coffee cherries at their optimal ripeness stage, this approach requires significant resources and labor investment. Many growers choose to respond by using techniques like mechanical harvesting or stripping, followed by sorting to remove the immature beans. During the sorting process, water is sometimes utilized to separate chaff and unripe cherries. Careful assessment of the water's microbial quality is imperative, as it can potentially influence the fermentation process of coffee, subsequently impacting the resulting cup quality (Huch & Franz, 2015).

Following the harvest, it is imperative to initiate coffee processing promptly to avert fruit deterioration due to undesirable fermentation or mold growth (Bee et al., 2005). The coffee fruit's exterior layers, which include the pulp and peel, are easily separated. The parchment, silver epiderm, and mucilage are tightly connected to the coffee beans (De Bruyn et al., 2017). Three distinct methods are utilized in coffee processing, namely dry, semi-dry, and wet processes, which are delineated as follows;

Coffee cherries are dried by air or exposure to the sun until their moisture content reaches approximately 10%–12% in the dry processing method, also known as natural processing. The pulp and dry skin are then extracted using cleaning and dehulling techniques. This yields the final product known as 'unwashed' or 'natural' coffee. 'Naturals' are characterized by their rich body and a smooth, sweet, and intricate cup profile (Poltronieri & Rossi, 2016). The dry/natural process boasts several advantages over alternative processing methods, including its simplicity, cost-effectiveness, ease of execution, reduced labor intensity, swift drying rate, elevated levels of stored proteins and hexose sugar that is glucose and fructose in the brewed coffee, and its environmentally friendly nature (Ategeka, 2024).

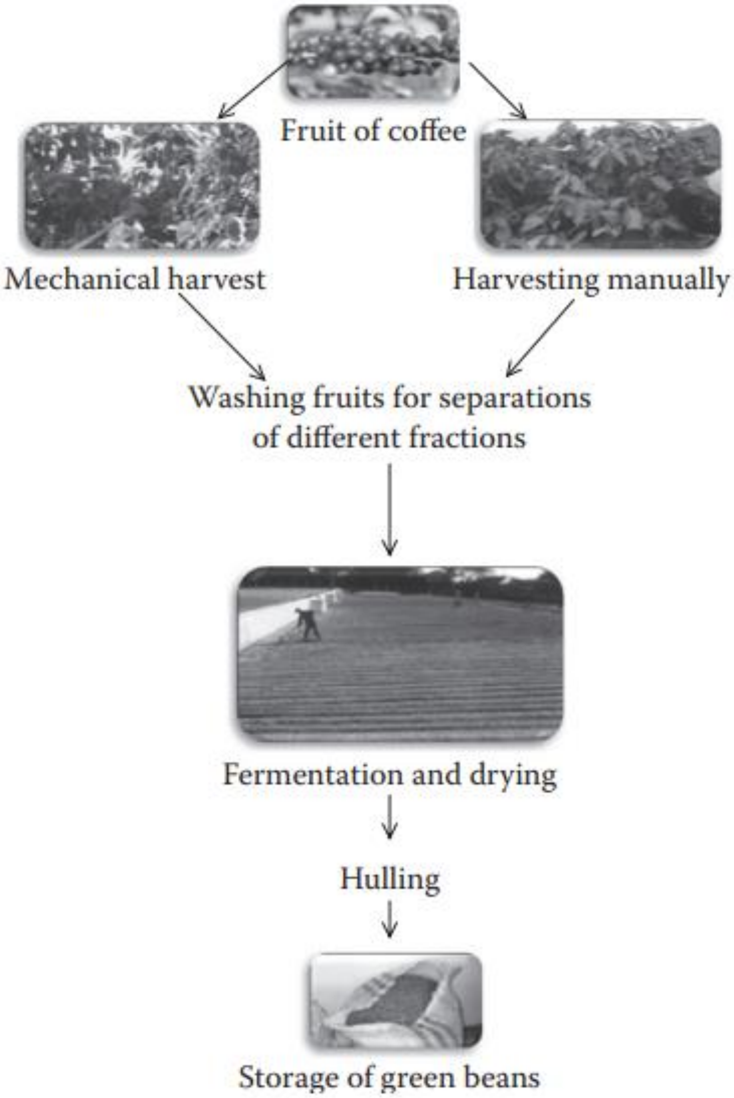

Figure 2. 3: The primary stages involved in the dry or natural coffee processing method (Rosane , Cristina , & Luis , 2012).

In contrast, the wet processing method, commonly employed for Arabica coffee, involves a more intricate sequence of stages. This method involves mechanically extracting the coffee exocarp and pulp, followed by microbial breakdown of the mucilage layer via fermentation. Ultimately, it finishes with the process of sun drying to eliminate excess moisture. This approach curtails the drying period (reducing it from 3 to 5 weeks to 8–10 days) and the necessary drying area for beans compared to dry processing (Bee et al., 2005). When using this method instead of dry processing, beans require less drying space and a shorter drying period—from three to five weeks to eight to ten days. (Bee et al., 2005). Several mechanical dryers, including forced air, round, static, and column dryers, or sun exposure can be used to complete the drying phase. The drying technique selected is determined by the processing strategy selected as well as by economic considerations (Pereira et al., 2016). Since the harvested fruits initially contain a moisture content of approximately 70%, retaining the exocarp and mesocarp prolongs the duration needed to achieve the ideal moisture levels during the dry processing method.

Consequently, employing mechanical dryers is discouraged, as it would entail significant and avoidable expenses. Nonetheless, because mechanical dryers can shorten drying times and lessen microbial deterioration, their usage in wet coffee processing has grown in popularity. (Kleinwächter et al., 2015). The outcome derived from the wet process is known as 'washed' or parchment coffee.

The semi-dry technique is a compromise between the wet and dry methods. This method skips the fermentation stage involved in removing the mucilage by depulping the cherries and drying the beans while they are still covered in it. This method produces coffee that is known as "pulped natural." 'Washed' coffees are considered to be of higher quality than 'unwashed' coffees, with a lighter body, higher acidity, and a richer scent. Conversely, 'pulped natural' coffees offer an intermediary profile between washed and unwashed variants. Furthermore, pulped naturals find strong favor in blends crafted for espresso coffee (Duarte et al., 2010).

2.3. Coffee Fermentation

2.3.1. Purpose of fermentation

Following their harvest and pulping, the coffee beans are either stretched out on a terrace for the semi-dry process or fermented in a wet process to aid in the breaking down and removing of mucilage.

2.3.2. Microorganisms employed in fermentation

The sugars found in the mucilage create an optimal environment for the proliferation of microorganisms, notably yeasts like *Pichia guilliermondii, P. anomala, Kluvyeromyces marxianus,* and *Saccharomyces cerevisiae*, in addition to lactic acid bacteria (LAB) such as *Leuconostoc mesenteroides, Lactobacillus plantarum, and Lb. brevis* (Evangelista et al., 2014; Leong et al., 2014). The development of these microbes yields a range of byproducts that can infiltrate coffee seeds and impact the coffee beans' ultimate quality (Silva et al., 2013).

In this instance, yeasts are essential for shaping the aromatic profile by generating various aroma-affecting compounds through metabolism carbon and nitrogen at the centre. Furthermore, multiple researches have indicated that yeast species commonly associated with coffee, including *Debaryomyces hansenii*, *Candida parapsilosis, Kluyveromyces marxianus, Pichia guilliermondii, Pichia. kluyveri, Pichia. fermentans, and Saccharomyces cerevisiae*, possess the ability to facilitate the breakdown of pectin which is the primary carbohydrates polymer found in muscilage of coffee. Several hydrolytic enzymes, including pectin lyase, pectin methyl esterase, and polygalacturonase, are involved in this breakdown process (de Melo Pereira et al., 2014; Masoud & Jespersen, 2006).

2.3.3. Effect of fermentation on cup quality

The degradation of pectin results in the liberation of basic sugars like rhamnose, glucose, D-galacturonate, and L-arabinose, which serve as extra carbon sources for yeast metabolism and the generation of aromatic compounds (Kim et al., 2016).

During coffee mucilage fermentation, yeast primarily generates acetaldehyde, ethanol, and acetic acid as its key metabolic products. Notably, coffee yeasts tend to exhibit limited aldehyde dehydrogenase activity, leading to the absence of acetic acid production in certain single cultures. As part of the mucilage removal process, yeast cultures participating in fermentation produce a varied spectrum having small molecular weight taste components in addition to ethanol. These compounds encompass aldehydes, terpenoids, ketones, higher alcohols, and esters (Samsonowicz et al., 2019). Esters such as acetate and ethylesters are the most common type of volatile chemical formed among these molecules. They come into existence through the condensation reaction that occurs when fatty acids combine with alcohol molecules (Saerens et al., 2010).

Furthermore, because of their tight relationship to higher alcohols and precursor amino acids, the availability of a nitrogen supply has a significant impact on the production of esters (Dzialo et al., 2017). Esters are well-known for giving alcoholic beverages flowery and fruity scents (Procopio et al., 2011). Nonetheless, one recent and ongoing area of scientific interest is the use of yeast to enhance coffee quality. Many ongoing research endeavors are dedicated to pinpointing coffee yeasts capable of generating esters and elevating their presence in coffee beans.

Some of the yeast strains being explored for this purpose include, among others, *Pichia fermentans, Candida parapsilosis, P. guilliermondii, Saccharomyces cerevisiae, Yarrowia lipolytica*, and *Torulaspora delbrueckii* (Bressani et al., 2018a; Lee et al., 2017). Studies have shown that the synthesis of isoamyl (isopentyl) acetate, ethyl ethanoate (ethyl acetate), ethyl hexanoate, propyl acetate, and N-butyl acetate significantly contributes to creating appealing taste profiles in coffee beverages, evoking flavors that are reminiscent of descriptors like "banana raisin," "apricot," "nutty," "caramel," and "Sicilian lemon (de Melo Pereira et al., 2015; Evangelista et al., 2014).

As shown in Table 2.1, during coffee fermentation, yeast generates a diverse array of higher alcohols. These include isobutanol, isoamyl (isopentyl) alcohol, butane-2,3-diol, 1-butanol and 1-propanol, 2-methyl, n-butanol, 2-phenylethanol. The Ehrlich pathway of amino acid breakdown produces these compounds. The coffee mucilage contains elevated levels of amino acids that serve as substrates for Ehrlich pathway, which includes phenylalanine, valine, leucine, threonine, and isoleucine (de Melo Pereira et al., 2014). However, the exact connection between higher molecular weight alcohols produced by yeast and their influence on coffee quality remains unclear, underscoring the necessity for further research.

Coffee bean fermentation produces a variety of ketone molecules, including several related with yeast metabolism, most notably diacetyl (2,3-butanedione). The presence of 2,3-butanedione in roasted coffee beans has been connected to the development of a buttery aroma in coffee beverages (Evangelista et al., 2014). This compound is produced extracellularly as a result of yeast metabolism, where α-acetolactate undergoes chemical decarboxylation, leading to the formation of diacetyl (Hirst & Richter, 2016). It is critical to note that the conversion of alpha-acetolactate to diacetyl (2,3-butanedione) is a non-enzymatic process that could be accelerated by greater fermentation temperatures, resulting in enhanced synthesis of the powerful 2,3-butanedione (Kobayashi et al., 2005).

When coffee mucilage is removed by fermentation, aldehydes are generated. These aldehydes are essential because they serve as precursors to the synthesis of aromatic chemicals, such as higher alcohols and esters, which have a major effect on the final coffee's quality. These compounds undergo catabolism using alcohol dehydrogenase's function, which involves the conversion of alcohols into their corresponding aldehydes or ketones (Hirst & Richter, 2016). Furthermore, aroma-enhancing aldehydes such as 2-methyl-buten-2-al and ethanal (acetaldehyde) may be produced by yeast cells during mediated cell death and migrate inside coffee, influencing the fruity and floral fragrance of the resulting coffee beverage (Bressani et al., 2018b; Ribeiro et al., 2017).

The generation of terpenes during the removal of coffee mucilage through fermentation includes the formation of compounds like α-terpeniol, β-citronellol, β-citronellol, linalool, citronellol, and geraniol. These terpenes originate from glycoside precursors as a result of the activity of yeast β-glucosidase enzymes (Knopp et al., 2006; Mendes-Ferreira et al., 2009). Furthermore, certain yeast strains prevalent in coffee fermentation, such as *Saccharomyces cerevisiae, Hanseniaspora uvarum*, and *Torulaspora delbrueckii*, produces isoprene derivatives via the isopreniod pathway (Carrau et al., 2005). In addition, Silva et al. (2013) discovered linalool in coffee beans after roasting, which is generated by the yeasts Saccharomyces cerevisiae and Pichia guilliermondii. Linalool is well-known for giving numerous food products a fresh, lemony, and woody scent (Górska et al., 2017). Still, further investigation is required to fully comprehend the direct influence this compound has on the overall quality of coffee.

2.4. Coffee Quality

In general, coffee quality is a complicated attribute shaped by genetics, environmental conditions, farming practices, harvest methods, postharvest processes, drying techniques, storage conditions, industrial processes, roasting approaches, beverage preparation, and consumer preferences (Knopp et al., 2006; Laderach et al., 2010; Leroy et al., 2006).

2.4.1. Categories of coffee quality;

According to (ITC, 2002), coffee quality is classified into four types on the market, which include; Outstanding quality; this is a great coffee niche market with an amazing distinctive cup. These are of high grade and are available as straight estate or blended. They are typically produced in the same location or farm. They have an excellent cup of coffee, yet it is not as visually appealing as exemplary coffee. They account for a sizable portion of the speciality coffee market. Standard quality; this qualifies as Fair Average Quality (FAQ), but it does not provide an amazing cup. It does, however, account for the majority of global coffee consumption (85-90%), whereas exceptional and high quality account for barely 15%. Under grade or low grade coffees**;** In the US market, under grade coffees refer to those that fall below the GCA type grading, which entails 120 defects per 370 grams).

Research suggests that 40% of physical sensory and chemical attributes that differentiate coffee beans are influenced by preharvest factors, whereas postharvest processing plays a role in characterizing the remaining 60% of quality characteristics (Richard et al., 2007). Preharvest features primarily pertains a range of agricultural considerations, spanning from choosing an appropriate geographic location to the act of harvesting itself. Among the extensively examined agricultural aspects in coffee literature are altitude, latitude, land gradient, coffee cultivar, seedling quality, soil composition, fertilization practices, precipitation patterns, irrigation techniques, shade and sun environments, vulnerability to frost, climate shifts, prevalence of coffee pathogens, and harvesting methodologies (Hameed et al., 2020).

However, this research narrowed its scope to investigating the effect of postharvest variables, specifically fermentation and drying procedures in the context of natural processing, on the resultant quality of coffee.

2.4.2. Effect of drying and fermentation on quality of naturally processed coffee

In the realm of dry or natural coffee processing, where the entire fruit undergoes sun drying and fermentation, a coffee variety is born that boasts a rich body, diminished acidity, enhanced sweetness, tantalizing flavor complexity, and a smooth and pleasurable taste. This style of processing contrasts with the wet processing method, yielding a distinct flavor profile (Uman et al., 2016). Despite its lengthy duration, sun drying is a commonly employed technique. Notably, in countries such as Brazil, Indonesia, Paraguay, Ethiopia, Haiti, India, and Ecuador, a significant 95% of Arabica coffees are subjected to sun drying (Silva et al., 2008). In terms of raw quality, dry-processed beans have greater bean size and roast volume, but wet-processed beans have a higher moisture content (Tadesse et al., 2016).

Dry-processed coffee has a higher occurrence of damaged beans characterized by musty, earthy, and greenish hue coffee faults (Tadesse et al., 2016). Employing the natural processing method poses a considerable challenge in safeguarding and preserving the elevated quality of coffee. In this method, whole coffee cherries boasting intact outer fruit layers are subjected to sun drying, a process that takes place under direct sunlight on various surfaces such as flat terrains or raised beds. These surfaces encompass a range of options, including exposed earth, bamboo mats, cemented or brick floors, tables equipped with wire or plastic mesh, asphalt, or wooden platforms.

Robusta coffee is typically dried directly on the ground, whereas Arabica beans are dried on specifically designed cemented or bricked terraces, raised beds, and floor (FAO, 2010a).

Because of the interaction of increased temperatures and humidity in lowland locations, coffee dried on flat surfaces dries faster than coffee dried on elevated surfaces (Hameed et al., 2018). According to Jos et al. (2015), sun-drying rates on drying patios, considering temperatures ranging from 10 to 28°C and Relative Humidity between 34.5% and 61.2%, are 0.002 kg of water removed per kg of coffee per hour for both fully washed and natural coffee. Taveira and his colleagues argued that delayed drying (at temperatures of 60/40 °C) on heated air patios preserves exceptional cup quality more successfully than flat-ground surfaces (Tsegaye et al., 2014). Flat-ground surface dried coffee has worse physical and biological quality features (Tsegaye et al., 2014).

The decline in overall quality attributes can be attributed to factors such as interaction with foreign substances, the potential for re-moistening, which in turn leads to the development of mold, bacteria, and black beans due to insufficient air circulation. Conversely, employing wire mesh or bamboo mats has a dual function: it shields the coffee cherries from contaminants and inhibits re-moistening by facilitating improved air circulation (Selmar et al., 2006).

Moreover, excessive drying at higher temperatures can potentially harm coffee quality by causing impairment of the membranes of cells (Marques et al., 2008; Saath et al., 2010). Coffee membranes breakdown when moisture levels range between 20 and 30 percent at temperatures of 60 °C or above (Borém et al., 2013, 2014). Consequently, an effective strategy to preserve coffee quality involves initially subjecting it to a high drying temperature, followed by a lower temperature range. The treatment involving a transition from 60 °C to 40 °C using hot air, referred to as the '60/40 °C' treatment, leads to more positive outcomes. This includes lower values for natural and washed coffee germination, development, root protrusion, Emergence Speed Index (ESI), and open cotyledonary circumstances (Jos et al., 2015). Moderate rate of drying encourages the buildup of heavy weight sugars with reduced properties (Marques et al., 2008).

Furthermore, the final quality of the coffee in terms of its raw, cup, and general features is greatly influenced by the thickness of the coffee layer. According to Tsegaye et al. (2014), employing raised beds for sun-drying and maintaining a layer thickness of less than 20kg/m2 proves to be a highly efficient approach for achieving specialty-grade coffee with the desired intrinsic characteristics.

When there is a greater quantity of coffee cherries and an expansion of the drying area beyond 20 kg/m2, it leads to an increase in the percentage of defective beans. As indicated by research by Kouadio et al. (2012), approximately 80% of cherries exhibited defects when subjected to drying at a density of 40 kg/m^2. A thick covering of cherries that exceeds 20 kg/m^2 obstructs appropriate air circulation and causes uneven drying, necessitating longer fermentation durations. This accelerated fermentation process adds to cherry acidity and the degradation of chlorogenic acids. Furthermore, a prolonged fermentation period increases the chances of microbial growth, which could result in higher levels of the mycotoxin ochratoxin A being present (Suárez - Quiroz et al., 2004). When deteriorated, defective, or infected cherries are included in the processing, it leads to the creation of coffee with an undesirable aroma and off-putting flavors.

Furthermore, the variety and makeup of fermentative bacteria are greatly influenced by the environmental factors that prevail during fermentation. Six key factors are crucial in influencing the desired results of the fermentation process. These characteristics encompass water activity, sugar content, oxygen accessibility, acidity, temperature, and duration (Poltronieri & Rossi, 2016).

Understanding and regulating the interplay between microbial composition and diversity in respect to various environmental parameters becomes critical for efficiently managing the natural fermentation process. Their combined impact ultimately decides the entire grade of the coffee product (Kulapichitr et al., 2019).

2.6. Cup Quality

A coffee cup's overall quality is attained through a harmonic balance of artistic and scientific aspects throughout its creation. A superior cup lacks any unpleasant flavors. The postharvest processing steps significantly shape cup quality, dictating the existence, development, or depletion of key quality-defining volatile and non-volatile components. Cup quality is determined by the sensory qualities of the finished brewed coffee beverage, which include flavor, body, taste, acidity, and color. (Hameed et al., 2018).

The sensory qualities of coffee are essential for driving increased global coffee consumption and in distinguishing new specialty coffee varieties. The trade and enjoyment of coffee are intimately tied to its cup quality characteristics, which are molded by a wide array of factors encompassing both preharvest elements and postharvest practices. However, the focus of this study is directed towards investigating the influence of postharvest processing variables such as fermentation and drying on the sensory attributes of coffee. The goal is to create a specialty coffee product that stands out in the market.

Drying is a crucial step in the postharvest processing of coffee cherries and holds the potential to yield premium aromatic and flavor qualities, provided it is executed with care to minimize damage to delicate constituents such as proteins and enzymes (Taveira et al., 2012), to understand how various drying techniques affect the coffee cup's quality. Their research findings indicated that among different drying methods, coffee dried naturally in the sun (referred to as 'naturals') had the highest protein content, averaging 252 spots. In contrast, pulped coffee subjected to wet processing ('pulped') had a lower protein content, averaging 360 spots. This comparison was made between both naturally dried and pulped coffee that underwent drying processes in facilities operating at a temperature of 60 °C (Do Livramento, Alves, & Paiva, 2017).

A comparative examination of proteins revealed the presence of 18 to 22 distinct protein types in beans dried in open yards, exhibiting a significant increase in abundance ranging from 1.7- to 14-fold. These proteins include glyceradehyde-3-phosphate dehydrogenases and 11S globulin as well as proteins related with late embryogenesis and dehydrins. As a result, greater protein levels and specific free amino acids can be ascribed to the improved quality of coffee beans dried in open yards. Along with reducing sugars, these molecules strongly participate in Maillard reactions during the roasting process, increasing flavor and fragrance qualities (Selmar et al., 2008). The presence of free amino acids is also influenced by the enhanced production of storage proteins in yard-dried beans (Mondego et al., 2011).

The fermentation stage, a critical phase in coffee processing, engages in a nuanced and intricate relationship with the aroma and sensory attributes of coffee, ultimately shaping its cup quality (Lee et al., 2015). While the primary purpose of fermentation is to remove mucilage, it exerts a profound influence on coffee's aroma and sensory characteristics. Microorganisms present during fermentation release enzymes like pectinolytic, cellulose, and polygalacturonase. These enzymes specifically act on the fully esterified (methoxylated) pectin chains within the mucilage by breaking the 1,4-glycosidic bonds.

The use of nutrient-rich pulp and mucilage during the fermentation process results in the production of a variety of secondary metabolites produced by microorganisms, as well as precursors for aromatic compounds. These compounds migrate into coffee beans, resurfacing after roasting and brewing in the form of both volatile and non-volatile components, significantly impacting sensory and organoleptic properties. Significantly, volatile compounds, which play a critical function in determining the quality of fermented and roasted coffee beans, consist of a wide variety of chemical substances such as alcohols, ketones, aldehydes, carboxylic acids, esthers, pyrazines, pyrrols, pyridines, furans, sulfur compounds, oxazoles, phenols, furanones and others (Sunarharum et al., 2014).

Furans assume a prominent role as the primary volatile compounds, both in terms of their quantity and their substantial influences roasted coffee aroma (Akiyama et al., 2007). These substances primarily arise from transformation of acids, sugars and unsaturated fatty acids as a result of the roasting process, contributing malty and sweet aromas to the coffee. Among the most influential contributors to coffee flavor are thiols, each offering distinct flavor profiles. Within the realm of non-volatile compounds, essential components encompass carboxylic acids, lipids, gums, intricate polysaccharides, alkaloids, minerals, chlorogenic acids (CGA), proteins, and melanoidins (Buffo & Cardelli-Freire, 2004). For example, caffeine and trigonelline have a significant impact on the intensity, tartness, and the textura of coffee. Likewise, its secondary metabolic product CGA contributes to the astringency and bitterness of coffee beverages (Oestreich, 2010).

Microbial breakdown leads to production of acids from 6-carbon sugar and affect the perceived astringency or acidity in coffee drinks. Furthermore, these acids participate in interactions having chlorogenic and quinic acids as part of the roasting process, generating lactones that may also affect the coffee's aroma (Sunarharum et al., 2014). Carbohydrates and complex polysaccharides have specific functions: carbohydrates contribute to the sweetness of coffee, while complex polysaccharides capture volatile compounds, elevating the viscosity of the beverage to the desired extent. Lipids, forming the most significant part of coffee beans, play a crucial role in forming crema or cream emulsions.

Within “espresso” coffee, these combinations carry lipophillic flavors and vitamins. Amino acids are essential in the production of melanoidins, which are nitrogen or sulfur-containing heterocyclic molecules. These compounds are produced through either the non-enzymatic reaction or caramelization processes and have a substantial effect on the flavor of coffee. Minerals play a pivotal role as essential catalysts, driving a diverse array of biochemical reactions that ultimately give rise to a wide spectrum of aroma and flavor compounds (Oestreich, 2010).

However, it's important to understand that the degree of fermentation is critical in molding the concentrations of both volatile and non-volatile compounds found in coffee beverages. Regrettably, existing research on coffee fermentation has concurred on the challenge of controlling or preventing over-fermentation of coffee cherries, which leads to the formation of black, malodorous beans featuring defects in flavor such as alcoholic, floral, and sour notes (Jackels & Jackels, 2005). Curbing over-fermentation hinges largely on establishing a defined endpoint for the fermentation process. Determining this endpoint is a subjective process (Jackels & Jackels, 2005) and involves separation of the fermentation process and the fermenting mass. Their research unveiled that insufficient fermentation is characterized by incomplete mucilage breakdown, which may encourage microbial growth, while on the other hand, excessive fermentation.

Furthermore, the identification of the fermentation's conclusion was based on the continuous monitoring of the pH levels within the fermenting mixture. In this context, a relevant sign of the conclusion of coffee fermentation has been established, primarily relying on the pH levels falling within the range of 4 to 5.5 (Jackels & Jackels, 2005). Investigating the significance of the pH range and its consequences for coffee quality qualities, researchers discovered that a lower pH endpoint results in a decrease in cup quality due to over-fermentation. Recognizing the significance of coffee fermentation, FAO (2006) identified pH, duration, and acidity as essential elements in the process. In addition to managing pH levels, achieving greater control over the fermentation process can be achieved by implementing starter culture technology and introducing pectinolytic enzymes into the fermenting mixture. Moreover, the use of fungal strains that produce ochratoxin A (OTA) can also contribute to more precise fermentation management (Massawe & Lifa, 2010). Incorporation of cellulases in conjunction with an appropriate starter culture accomplishes several important outcomes. It notably reduces the fermentation duration to a mere 30 minutes, diminishes the presence of OTA-producing fungi, and amplifies the presence of precursors involved in the Maillard reaction and caramelization. These precursors include reducing sugars. This, in turn, enriches the coffee's sweetness, complemented by caramel and subtle acidic notes (Lin, 2010; Velmourougane, 2013).

2.7. Gas Chromatography – Mass Spectrophotometry

Undoubtedly, gas chromatography (GC) has long stood as the quintessential tool in coffee aroma analysis. It serves as an effective avenue for comprehending and reconstructing the intricate flavor profiles of coffee. Depending on the particular use case, gas chromatography (GC) can be equipped with different sample injection and detection mechanisms (Semmelroch & Grosch, 1996).

In the last thirty years, gas chromatography has been instrumental in clarifying, describing, and measuring the compounds that are essential for coffee aroma. The heart of every GC analysis relies on the capillary column used for chromatography. At the column's entrance, the sample is introduced as a tiny vapor plume. While moving through the column, which is lined with an organic solvent, the different compounds in the sample display differing affinities for the solvent, resulting in unique travel speeds (Lindinger et al., 2009).

As a result, while traversing the column, they segregate, causing individual compounds to reach the column's end at distinct intervals. At the end of the column, a detector identifies and ultimately measures these compounds as they appear one after another. This allows for the complete aroma profile to be reconstructed by combining the individual aroma-active compounds that are detected as they exit the column. The detector employed at the exit of the column is a mass spectrometer. This facilitates the recording of a mass spectrum to aid in identification. The recorded mass spectrum is compared against an extensive database of thousands of mass spectra, enabling the determination of the active flavor compounds (Yeretzian et al., 2010).

2.8. Use of RSM (Response Surface Methodology)

RSM models and analyzes issues in a form of response influenced by several variables by combining mathematical and statistical methodologies (Tedjo, Marlia, Sophi, Indra, & Mochammad, 2020). The objective is to attain the optimum systematic performance by optimizing the reaction through variable level optimization. Research involving several variables and three or five treatment levels can benefit from the application of this methodology. With the aid of statistical software, this RSM can provide a three-dimensional graph (Myers, Montgomery, & Anderson, 2009).

The RSM approach was used because it can investigate the relationship between numerous variables to determine the best conditions for bioprocess manufacturing and to forecast the outcome. (Chang, Lee, & Pan, 2006). In this study, RSM was used to optimize the fermentation time and fermentation type of naturally processed Arabica coffee in Uganda.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Coffee samples were acquired from smallholder farmers in Bulambuli. Mainly SL 14 and Nyasaland varieties were used, since they are the main Arabica coffee varieties grown in the area. Simple random sampling was utilized to select farms from which coffee samples were picked. Coffee beans from the selected farms were separated to be used in the study. The coffee cherry was hand picked at physiological maturity stage, where the entire surface of the fruit is a dark red color. Sugar content of the cherries was measured to guarantee and validate the state of maturity of the product; values higher than 15 ° Brix were accepted for the study. Yeast i.e. *Saccharomyces cerevisiae* (Young's brew super wine yeast) was obtained from Young's UBrew, Bilston, West Midlands, United Kingdom), 35kg Plastic Fermentation drums, were purchased from Nice house of plastics, Uganda.

3.2 Experimental Design

Table 3.1 shows the D-optimal design (with 14 runs) that was used to study effect of fermentation time and fermentation type on physicochemical and sensory properties as well as volatile compounds of Arabica coffee. Fermentation time ranging from 14 h to 38 h while fermentation type had 2 levels; spontaneous and innoculated fermantation. The study design was developed using Design Expert version 13. Fermentation time and Fermentation type were the independent variables whereas sensory quality of coffee was the dependent variable in this study.

Table 3. 1: D-Optimal design employed to investigate how fermentation time and fermentation method impacted the physical, chemical, and sensory attributes of Arabica coffee

Runs	Fermentation time (hrs)	Fermentation type
1	14	Innoculated
2	26	Spontaneous
3	38	Innoculated
4	14	Spontaneous
5	14	Spontaneous
6	14	Innoculated
7	26	Innoculated
8	14	Spontaneous
9	38	Innoculated
10	26	Spontaneous
11	14	Spontaneous
12	14	Spontaneous
13	26	Innoculated
14	38	Spontaneous

3.3 Coffee Processing

3.3.1. sample preparation

Green, underripe, and overripe coffee beans were hand-sorted out of the harvested crop. Subsequently, the cherries were separated from lower density objects such as leaves and sticks by floating and stirring the coffee in a drum of water. Finally, a sample of 100 cherries from the remaining coffee was taken to the quality evaluation board. The percentage of ripe cherries was determined by counting the ripe red cherries on the board and measuring the sugar content (°brix). Coffees with more than 98% ripe cherry were used in the study.

3.3.2. Fermentation

The coffee, in an optimal state of maturity, was placed in 35-kg capacity drums, which was sealed to make it impossible for oxygen to enter the drum during fermentation, for anaerobic fermentation. Each run constituted 30kg of cherries, and fermenation drums were fitted with air locks containing wáter, to allow release of excess carbondioxide, whilst preventing entry of oxygen. For innoculated fermentation runs, a starter culture of *Saccharomyces cerevisae* (30g per run) was used.

The drums were then taken to the fermentation area for the time defined for the treatment according to the experimental design. The ambient temperature and relative humidity of the fermentation area were measured, using the Sensor Push datalogger thermo-hygrometer (H5179 Wifi Govee thermo hygrometer, supplied by Goove, China). Once the established fermentation time was reached, the drums were opened, the sugar quantity of the beans was recorded, thereafter which were taken to the drying area.

3.3.3. Drying

Each coffee sample was dried separately in solar drying beds (Innovakit SAS, Coloumbia), which was built using timber, without any chemical treatment added that may affect the attributes of the product. These beds had a plastic mesh to hold the coffee, which allowed the aeration of the product and the safety of the process. Agroplast 6-gauge plastic (Innovakit SAS, Coloumbia) was used to protect the coffee from rain and retain thermal energy at night.

The bean temperature and moisture was measured daily with the Kett450 electronic humidity meter (Belt and Bearings Co., Ltd, Japan). Both within and outside the drying beds, the surrounding temperature and relative humidity was measured with the Push sensor device (Belt and Bearings Co., Ltd, Japan) that has datalogger function and remote data transmission.

The coffee was mixed at two (2) hour intervals with the Innovakit SAS plastic rake to guarantee uniform drying, increase aeration, and reduce the probability of fungal attack. When the coffee reached 10% humidity, it was packed in Grain pro bags (GrainPro, Inc, Washington, DC, USA) and taken to the warehouse and stored under desirable conditions that maintained the coffee quality. Samples for analysis were obtained from the lot at scheduled intervals.

3.4 physicochemical characterization of Arabica coffee

3.4.1 pH measurement

The pH of the coffee simples were determined in triplcate using a calibrated pH (Apera Instruments AI311 Premium Series PH60 Waterproof pH Pocket Tester Kit, Replaceable Probe, ±0.01 pH Accuracy) meter according to AOAC method 973.41 (1998).

3.4.2 Determination of ˚Brix

Coffee brix was measured according to ISO 2173:2003 using a digital refractometer Cole-Parmer RSA-BR32T Refractometer 0-32% with ATC (Cole-Parmer**,** Bunker Court Vernon Hills, Illinois, United States). For each measurement, 3-5 coffee beans were used, and the solid components were pressed against the sensor within the equipment until they fully filled the inner area. Brix was employed as an index of the beans' ripeness.

3.4.3 Determination of temperature

The temperature was measured using hand digital thermometer (HI 98509, HANNA instruments, USA).

3.5 Gas Chromatography – Mass Spectrophotometry

The analysis was conducted at Michael Qian’s Laboratory at Oregon State University, USA. Solid phase-microextraction gas chromatography mass spectrophotometry was employed to extract the volatile compounds in the coffee. The GC vial was filled with 10ml of sample. The internal standard quinoxaline (10L), alkane standard (20L), and magnetic stirring bar were all used. The simples were stirred for 40 min at 70˚C for volatile chemical adsorption. The fiber was then placed into the gas chromatograph. By altering an existing approach, gas chromatograph-mass spectrophotometry (GC-MS) analysis was performed utilizing a DB-WAX column with length, inner diameter, and passe thickness of 60m, 250m, and 0.25m, respectively (Lee et al., 2017). Helium was employed as a carrier gas at a flow rate of 1.0mL/min and in splitless mode (splitless time: 1 minute). The oven temperature was held at 44^{o}C for 5 min, then increased to 170^{o}C at 3^{o}C/min for 10 min before being raised to 240^{o}C at 8^{o}C/min.

3.6 Sensory evaluation

3.6.1 Preparation of samples for cupping

A representative sample of 1 kg of dried cherry was selected to be hulled and processed according to the Speciality Coffee Association of America (SCAA, 2015). The moisture content of the beans was also measured using a moisture analyzer (Belt and Bearings Co., Ltd, Japan). From the green coffee, a 100 g sample was obtained; the defects were eliminated and classified. The defect-free sample was classified by size, using meshes. The beans were classified and cupped as follows; beans over mesh sixteen (16), beans over mesh fifteen (15), and the beans that remained on the mesh fifteen (15), (A+, B+, and B as it is commercially known) were roasted separately and allowed to recuperate for a minimum of eight hours.

3.6.2 Cupping

A panel of 8 (eight) Q-cuppers was used for the cupping procedure, and three (3) cups per sample, mesh classification, and a ratio of 0.055 g of coffee per ml of water were used. The panel of cuppers identified the attributes, notes, and profiles of each one of the samples, based on the Speciality Coffee Association of America (SCAA) cupping protocol (SCAA, 2015). The cuppers registered the quantity of primary and secondary defects, attributes, notes and the profile of each of the samples cupped. Aroma, after taste, acidity, flavor, body, balance (a synergistic mix of flavor, acidity, and body), uniformity, clean cup, sweetness, and overall impression were the sensory aspects that were examined. The overall score was calculated as the sum of these assessed attributes.

3.7 Data Analysis

Data were analysed using response surface methodology (RSM) using Design Expert software version 13.0.5 (Stat-Ease Inc., Minneapolis, United States). Response Surface Methodology was used to optimize fermentation conditions (fermentation time and fermentation type) with respect to the sensory quality of Arabica coffee. The coefficient of determination (R^2) and analysis of variance (ANOVA) were used in selection of suitable models to describe the relationship between the factors and responses. Backward elimination regression with the p-value criterion ($p<0.05$) was used to refine models that have numerous insignificant terms. Principal Component Analysis was conducted using XLSTAT 2018 Version 20.1.49320 (Addinsoft, NY, U.S.A) to establish the relationship between physicochemical properties, volatile compounds and sensory quality.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Physicochemical properties of Arabica coffee during fermentation

Table 4.1 shows the significant models that relates fermentation time, fermentation type and physicochemical properties of Arabica coffee during fermentation.

Table 4.1: Significant models showing the relationship between fermentation time, fermentation type and physicochemical attributes during fermentation of Arabica Coffee

Responses	Significant Models	R^2
pH	$Y_1 = 4.81 - 0.09X_1 + 0.16X_1^2$	0.75
Brix	$Y_2 = 12.75 - 0.71X_1 - 0.81X_2$	0.64
Coffee Temperature	$Y_3 = 23.24 + 0.75X_2$	0.33
Ambient Temperature	$Y_4 = 22.44 - 0.38X_1 + 1.32X_1^2$	0.59

X_1=Fermentation time and X_2= Fermentation type. The model terms in the equations above are significant at $P<0.05$

R^2= Correlation coefficient.

4.1.1. pH

Table 4.1 shows the relationship between fermentation time, fermentation type and pH of the coffee. The pH of the coffee was described by quadratic model. The change in fermentation time and fermentation type was strongly correlated to change in the pH as depicted by R^2 (75%).

The pH of the fermented coffee decreased with increased fermentation time. The decrease in pH in spontaneous fermentation was mainly attributed to the degradation of complex organic substances (mucilage) into simpler sugars, which upon action by microorganisms yields the acid components in the fermenting coffee mass (Velmourougane, 2013).

4.1.2. Brix

Table 4.1 shows the relationship between fermentation time, fermentation type and brix of the coffee. The change in fermentation time and fermentation type was strongly correlated to change in brix as depicted by R^2 (64%). Both fermentation time and innoculated fermentation negatively affected total soluble solid content (˚brix) of the coffee. The negative effect of fermentation time may be as a result of utilization of sugar. The negative effect of fermentation type may be as a result of rapid conversion of sugar into alcohol by Saccharomyces cerevisiae (Mariyam et al., 2022).

4.1.3. Coffee temperature

Table 4.1 depicts the significant linear model showing relationship between fermentation type and coffee temperature. The change in fermentation type was moderately correlated to change in coffee temperature as depicted by R^2 (33%).

Innoculated fermentation had a positive effect on the coffee temperature. The increase in temperature could be attributed to heat discharged as a result of microbial growth and its enzymatic activities. (Velmourougane, 2013) reported increase in temperature during fermentation and attributed it to longer fermentation time required to degrade the thicker muscilage. Increase in temperature during fermentation has also been reported to be attributed to the degradation process and exothermic reaction during fermentation (Smith, 1985).

Generally, there was a positive correlation between the physicochemical parameters (pH, brix and coffee temperature) and the studied coffee fermentation time and types. Therefore, the coffee was then analyzed using gas chromatography/mass spectophotometry to determine the key volatile compounds that influence the coffee sensory profile.

4.2. Volatile compounds in Arabica coffee.

Table 4.2 shows significant models that relate fermentation time, fermentation type and volatile compounds.

Table 4. 2: Significant models relating fermentation time, fermentation type and volatile compounds in Arabica Coffee

Classes of volatiles	Responses	Significant Models	R^2
Volatile Acid	Acetic acid	$Y_{12} = 6.64x10^8 - 5.91x10^7X_1 + 3.96x10^7X_2$	0.52
Pyrazines	Ethylpyrazine	$Y_9 = 1.5x10^8 + 7.74x10^6X_1 - 9.89x10^6X_2$	0.58
	2-ethyl-6-methyl Pyrazine	$Y_{10} = 1.08x10^8 + 9.12x10^6X_1 + 1.65x10^7X_1^2$	0.76
	2-ethyl-3-methyl Pyrazine	$Y_{11} = 2.73x10^7 + 1.02x10^5X_1 - 2.06x10^6X_2$	0.59
Phenols	2-methoxy Phenol	$Y_{14} = 1.7x10^7 - 4.2x10^6X_1 + 3.58x10^6X_2$	0.67
Pyrrol	1-(1H-pyrrol-2-yl)-Ethanone	$Y_{16} = 4.42x10^7 - 6.32x10^6X_1 + 4.66x10^6X_2$	0.62
Aldehyde	Acetaldehyde	$Y_1 = 1.07x10^7 - 4.23x10^5X_2$	0.54
	2-Butanone	$Y_3 = 1.05x10^7 + 6.70x10^5X_1 + 2.24x10^5X_2$	0.54
	2-Methylbutanal	$Y_4 = 1.77x10^7 - 2.20x10^6X_1$	0.52
	3-Methylbutanal	$Y_5 = 2.01x10^7 + 3.67x10^6X_1 + 1.47x10^6X_2 + 5.99x10^6X_1X_2$	0.59
Furans	Furan	$Y_2 = 4.24x10^6 + 2.24x10^5X_2$	0.47
Furaneol	Furaneol	$Y_{13} = 4.23x10^7 + 3.06x10^5X_1 + 1.38x10^6X_2 + 2.64x10^6X_1X_2$	0.76
Ketones	2,3-Butanedione (Diacetyl)	$Y_6 = 4.34x10^7 + 3.74x10^6X_1 - 2.62x10^5X_2$	0.78
	2,3-Pentanedione and Ethyl isovalerate	$Y_7 = 8.37x10^7 + 1.58x10^7X_1 - 7.13x10^4X_2 + 2.2x10^7X_1X_2$	0.61
	3-hydroxy-2-Butanone	$Y_8 = 4.4x10^7 - 1.29x10^6X_1 + 1.25x10^6X_2$	0.50
	3-ethyl-2-hydroxy 2-cyclopenten-1-one	$Y_{15} = 2.22x10^7 - 3.24x10^6X_1 + 1.93x10^6X_2$	0.52

X_1=Fermentation time and X_2= Fermentation type. The model terms in the equations above are significant at P<0.05

R^2= Correlation coefficient.

4.2.1. Acetic acid

Acetic Acid is produced by biochemical synthesis from the oxidation of ethanol during the first stages of fermentation (Schwan & Wheals, 2004). Table 4.2 depicts the linear model showing relationship between fermentation time, fermentation type and acetic acid concentration. The change in fermentation time and fermentation type was moderately correlated to change in the concentration of acetic acid as depicted by R^2 (52%). Acetic acid contributes a pleasing fruity and wine-like flavor as well as a fermented aroma (Bressani et al., 2021). Acetic acid concentration decreased with increase in fermentation time and, innoculated fermentation positively influenced acetic acid concentration. The decrease of acetic acid content with fermentation time may be attributed to sugar during fermentation of coffee (Haile & Kang, 2019). Due to the acidic nature of the environment, there is increased utilization of the mucilage and a decrease in number of acetic acid bacteria during natural fermentation, leading to a more rapid rise in acidity in the medium (Puerta & Rios, 2011) reducing acetic acid production and substrate consumption (Galarza & Figueroa, 2022).

4.2.2. Pyrazines

Pyrazines are fragrant chemicals that are unique to roasted coffee. Depending on the specific chemical groups attached to the pyrazine ring, pyrazine aromas can be described as nutty, roasted, corn-like, hazelnut-like, potato-like, or earthy. The pyrazines present were ethylpyrazine, 2-ethyl-6-methylpyrazine and 2-ethyl-3-methylpyrazine. Table 4.2 shows models showing relationship between fermentation time, fermentation type (natural and culture) and pyrazines. The relationship between fermentation time, fermentation type and 2-ethyl-6-methylpyrazine was described by quadratic model while those for ethylpyrazine and 2-ethyl-3-methylpyrazine were described by linear models. The change in fermentation time and fermentation type was moderately correlated to concentration of Ethylpyrazine, and 2-ethyl-3-methyl Pyrazine and strongly correlated to the concentration of 2-ethyl-6-methyl Pyrazine as depicted by R^2 (58%, 59% and 76%) respectively. Change in the Ethyl pyrazine concentration was positively influenced by fermentation time while negatively inflenced by innoculated fermetation. Increase in fermentation time increased 2-ethyl-6-methyl pyrazine concentration. This increase may be attributed to the impact of yeast on internal components like sugar and amino acids to produce alcohol, acids, esters, ketones and aldehydes which affect the synthesis of 2-ethyl-6-pyrazine (Elhalis et al., 2021). Fermentation time had a positive influence, while innoculated fermentation had negative influence on 2-ethyl-3-methyl pyrazine concentration. The positive influence of fermentation time may be as a result of yeast activity on sugar and amino acid. The decrease with innoculated fermentation may be related to degradation of complex carbohydrates into simple carbohydrates (Prakash et al., 2022) lowering the amount of substrate required for 2-ethyl-3-methyl pyrazine (Galarza & Figueroa, 2022).

4.2.3. 2-Methoxy phenol

The change in fermentation time and fermentation type was strongly correlated to change in the concentration of 2-methoxy Phenol as depicted by R^2 (67%). Table 4.2 shows linear relationship between fermentation time, fermentation type and 2-methoxy phenol. During roasting, phenol derivatives are generated by the heat destruction of chlorogenic acids, lignin, and the decarboxylation of phenolic acids (Flament, 2001). The concentration of 2-methoxy phenol dropped as fermentation time increased. The acidic conditions in the mucilage either removed molecules with the methoxy functional group, leading to a decrease in the content of 2-methoxy phenol in coffee beans (Vasanthy et al., 2021).

4.2.4. Pyrrol

Pyrrol chemicals are generated during the roasting process as a result of amino acid breakdown (Poltronieri & Rossi, 2016). Pyrroles are produced through the interaction of aldoses with alkylamines. This process includes the condensation of glucose with alanine, glucose with proline, or glucose with hydroxyproline (Toci et al., 2013). The pyrrole components add to the nutty, sweet, and burnt aromas (Zapata et al., 2018). In this study, the identified 1-(1-H pyrrol-2-yl) ethanone was negatively affected by fermentation time while cultured fermentation had positive influence. The change in fermentation time and fermentation type was strongly correlated to change in the concentration of 1-(1H-pyrrol-2-yl)- Ethanone as depicted by R^2 (62%).

4.2.5. Aldehydes

At high temperatures, aldehydes are created by the oxidative destruction of amino acids during their interaction with sugars, or at normal temperatures, aldehydes are formed by the interaction of amino acids and polyphenols in the presence of polyphenol oxidase (Hasbullah, 2021). The change in fermentation time and fermentation type was moderately correlated to change in the concentration of the aldehydes as shown by R^2 (50%).

Table 4.2 shows linear models representing the relationship between fermetation time, fermetation type and concentration of aldehydes studied. Acetaldehyde concentration was negatively affected by innoculated fermentation. This may be as a result of full conversion of acetaldehyde into alcohol by yeast in cultured coffee (Li & Mira de Orduña, 2017). Increased fermentation time decreased the concentration of 2-methylbutanal. This may be ascribed to the reduction and oxidation of 2-methylbutanal into 2-methylbutanol and 2-methylbutanoic acid respectively; and esters (Smit et al., 2009). Both fermentation time and cultured fermentation increased 3-methylbutanal concentration. The increase with fermentation time and cultured fermenation may be attributed to the decarboxylation of of Alpha-keto isocarproic acid to 3-methyl butanal by enzyme decarboxylase (Smit et al., 2009).

4.2.6. Furans

Furans are ring-shaped ethers primarily present in carbohydrate compounds that undergo browning reactions. Since green coffee beans contain a significant amount of sugars, mainly sucrose, these compounds are produced when coffee beans are subjected to the heat of the roasting process. This process entails the breakdown and rearrangement of carbohydrates, ascorbic acid, and unsaturated fatty acids (De Vivo et al., 2022; Sunarharum et al., 2014). The change in fermentation type was moderately correlated to change in the concentration of furans as depicted by R^2 (47%).

Innoculated fermentation had a positive impact on furan concentration. This may be due to the breakdown of amino acids through heat and the oxidative process of polyunsaturated fatty acids and ascorbic acid during the creation of furan compounds (Chaichi et al., 2015). Furans are regarded as a quality indicator in coffee fragrance, imparting a roasted candy aroma (Londoño-Hernandez et al., 2020).

4.2.7. Furaneol

Table 4.2 depicts a significant linear model demonstrating the association between fermentation time, fermentation method, and furaneol content. Furaneols belongs to furanone class and imparts caramel and sweet roasted aroma in coffee and is widely used in flavour industry because it has strawberry and pineapple flavour (Pinheiro et al., 2021). The change in fermentation time and fermentation type was strongly correlated to change in the concentration of Furaneol as depicted by R^2 (76%).

Furaneol concentration was positively influenced by both fermentation time and innoculated fermentation. This may be attributed to enzymatic release and/or acid hydrolysis of bound furaneol (Zhu et al., 2019). Furaneol has been observed to be enzymatically formed from fructose-1,6-bisphoshate in strawberry (Dahlen et al., 2001).

4.2.8. Ketones

Ketones are generated during the roasting process when the Maillard reaction causes the formation of diketones through the heating of glucose within the coffee beans. Ketones, often referred to as aliphatic volatiles, are created through the thermal interaction of glucose and cysteine (Knysak, 2017). The ketones identified in the sample were 2-butanone, 2,3-butanone (Diacetyl), 2,3-pentanedione and ethyl isovalerate, 3-hydroxy -2-butanone, 2-cyclopenten-1-one, 3-ethyl-2-hydroxy. Table 4.2 shows models that represents relationship between fermentation time, fermetation type and ketones. Butanone concentration increased with fermentation time and innoculated fermentation. The increase may be related to accumulation of 2-butanone since it requires secondary alcohol dehydrogenase for its conversion to 2-butanol (Russmayer et al., 2019).

Natural alcohol dehydrogenase works well with primary alcohols but is less effective with secondary alcohols (Russmayer et al., 2019). Increase in fermentation time increased the concentration of 2,3-butanedione while the concentration of 2,3-butanedione was higher in natural fermentation but lower in *Saccharomyces cerevisiae* innoculated fermentation. The lower concentration of 2,3-butanedione with *S. cerevisiae* innoculated fermentation is ascribed to activity of enzyme aldehyde reductase and alcohol dehydrogenase which generate 2,3-butanediol from 2,3-butanedione (Tang & Li, 2017).

Davey et al., (2022) reported generation of 2,3-butanediol during alcohol fermentation involving activity of *Saccharomyces cerevisiae* by the activity of butanediol dehydrogenase. Isovaleric acid and its esters contribute to jackfruit flavour. Fermentation time positively influenced 2,3-pentanedione and isovalerate concentration while use of culture negatively influences the concentration of 2,3-pentanedione and isovalerate concentration. The negative influence of fermentation type on concentration of 2,3-pentanedione may be attributed to activity of enzyme aldehyde reductase and alcohol dehydrogenase that could generate 2,3-pentanediol from 2,3-pentanedione (Tang & Li, 2017). 3-hydroxybutanone concentration decreased with fermentation time but increased with cutured fermentation. The decrease may be attributed to reduction of 3-hydroxy-2-butanone by Saccharomyces cerevisiae to 2,3-butandiol (Cheynier & Sarni-Manchado, 2010). Fermentation time had negative influence while innoculated fermentation had positive influence on 3-ethyl-2-cyclopenten-1-one. The adverse impact of fermentation time could be due to the reduction of 2-cyclopenten-1-one, 3-ethyl to 2-cyclopentanol, 3-ethyl. The change in fermentation time and fermentation type was strongly correlated to the change in concentration of 2,3-Butanedione (Diacetyl) and 2,3-Pentanedione and Ethyl isovalerate as depicted by R^2 78% and 61% respectively, whereas was moderately correlated to the change in concentration of 3-hydroxy-2-Butanone and 3-ethyl-2-hydroxy-2-cyclopenten-1-one as depicted by R^2 (50%) and (52%) respectively.

In summary, there was a positive correlation between the coffee volatile compounds and the studied coffee fermentation time and types. The formation of Acetic acid, aldehydes, furans, furaneol, ketones and pyrrols which are associated with pleasant aromatic notes in coffee was highly identified, while that of unpleasant notes like pyrazines was less. This was then followed by cupping of the coffee to determine the human perception of the coffee organoleptically.

4.3. Sensory properties of Arabica coffee

Table 4.3 show significant quadratic models showing relationship between fermentation time, fermentation type and average cup scores.

Table 4. 1: Significant models showing the relationship between fermentation time, fermentation type and sensory cup quality of Arabica Coffee

Responses	Significant Models	R^2
Average cup score	$Y_1 = 85.13 + 0.1198X_1 + 0.849X_1^2$	0.77

X_1=Fermentation time and X_2= Fermentation type. The model terms in the equations above are significant at $P<0.05$

R^2= Correlation coefficient.

Cup quality of the Arabica coffee was computed using the average all other sensory attributes assessed by individual cuppers. The attributes assessed were aroma, sweetness, flavour, after taste, acidity, body, balance, uniformity, clean cup, overall quality. The change in fermentation time and fermentation type was strongly correlated to contributed to change in the average cup score as depicted by R^2 (77%).

The average cup score was positively affected by fermentation time. This may probably be associated with increased acidity which can disbalance the overall cup preferences (Laukaleja and Kruma, 2019). The balance between acidity and sweetness may also contribute to increased cup score. Alex et al. (2016) found positive correlation between final cup quality and balance between flavor and sweetness.

In summary, fermentation time and fermentation type strongly influenced the average coffee cup score. This proved that fermentation, a postharvest process is highly crucial in coffee processing to produce high quality coffee.

4.4. Optimization

Optimization of ingredients was done using graphical and numerical techniques. Table 4.4 presents the different solutions from the optimization process. The variables were optimized using criteria of keeping fermentation time and fermentation type in range, maximizing average cup scores and brix, minimizing pH and keeping coffee temperature, ambient temperature and volatile compounds in the range (Appendix II) using numerical optimization. These conditions were selected based on desirable qualities of the coffee. Based on the desired criteria, four solutions were provided by the optimization software and the optimum solution with fermentation time 16.38hrs and spontaneous fermentation type that had higher desirability factor of 0.521 was selected.

Table 4. 2: Solutions from optimization

Attributes	**Relative Abundance**
Acetaldehyde	11139316.67
Furan	4015113
2-Butanone	9778186.305
2-Methylbutanal	19425397.59
3-Methylbutanal	20476122.6
2,3-Butanedione (Diacetyl)	40666880.44
2,3-Pentanedione and Ethyl isovalerate	88736357.37
2-Butanone,3-hydroxy-	43804466.45
Ethylpyrazine	153477865.2
Pyrazine,2-ethyl-6-methyl-	110945790.4
Pyrazine,2-ethyl-3-methyl-	29287971.59
Acetic acid	671260273.4
Furaneol	42823142.9
Phenol, 2-methoxy-	16764687.45
2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	22890248.7
Ethanone, 1-(1H-pyrrol-2-yl)-	44607359.69
Attributes	**Optimal Solution**
Fermentation time	16hrs
Fermentation type	Spontaneous
pH	4.982
˚Brix	14.13
Coffee temp	22.4˚C
Ambient temp	23.6 ˚C
Average cupper score	84.2%
Desirability	0.521

4.5. Principle component analysis

A principle component analysis was performed to identify the relationship between concentration of volatile compounds, cupper scores and physicochemical properties of coffee. Figure 4.1 presents PCA which explained 59.58% of the variability of data in the first and second components. A larger percentage (45.61%) of variance was described by PC1 and PC2 accounted for 13.97%. PC1 was largely characterized by brix, pH, 2-Butanone, 2-methylbutanal, 3-methylbutanal, 2,3-butanedione, 2,3-pentanedione and isovalerate, ethyl pyrazine. On the negative axis PC1 was described by coffee temperature, acetaldehyde, furan, 2-methoxy phenol, 2-2-hydroxy-2-cyclopenten-1-one, 1-(1H-pyrrole-2-yl) ethenone. The second principle component was largely characterized by acetic acid, furaneol and 3-hydroxy-2-butanone on positive axis while on negative axis it was characterized by 6-methylpyrazine, 2-ethyl, 3-methylpyrazine, 2-ethyl and average cup score.

Furaneol, coffee temperature, 3-ethyl-2-hydroxy-2-cyclopenten-1-one, acetaldehyde were positively correlated to each other. 1-(1H-pyrrol-2-yl)-ethanone, 2-methoxy phenol and furan were related. Pyrrols are formed from degradation of furan and amino acids derivatives as well as termal degradation of Amadori intermediates (Flament, 2001). 3-hydroxy-2-butanone, acetic acid, brix, 2,3-pentanedione and ethyl isovalerate, 2,3-butanedione (diacetyl), 2-methylbutanal, 3-methylbutanal were positively correlated. 2-butanone, pH, ethylpyrazine, 2-ethyl-6-methylpyrazine, and 2-ethyl-3-methylpyrazine all had a positive connection. This is most likely due to the fact that these compounds are produced from the same precussors. According to Lee et al. (2015) pyrazine chemicals are generated during caramelization during roasting from amino acid precussors.

Butanone, pH, ethyl pyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-3-methyl pyrazine, furans are positively related to the average cup score of Arabica coffee. This is explained by the fact that pyrazine and furans are the most abundant chemicals in coffee and are the primary families of molecules responsible for coffee aroma (Toci & Farah, 2014).

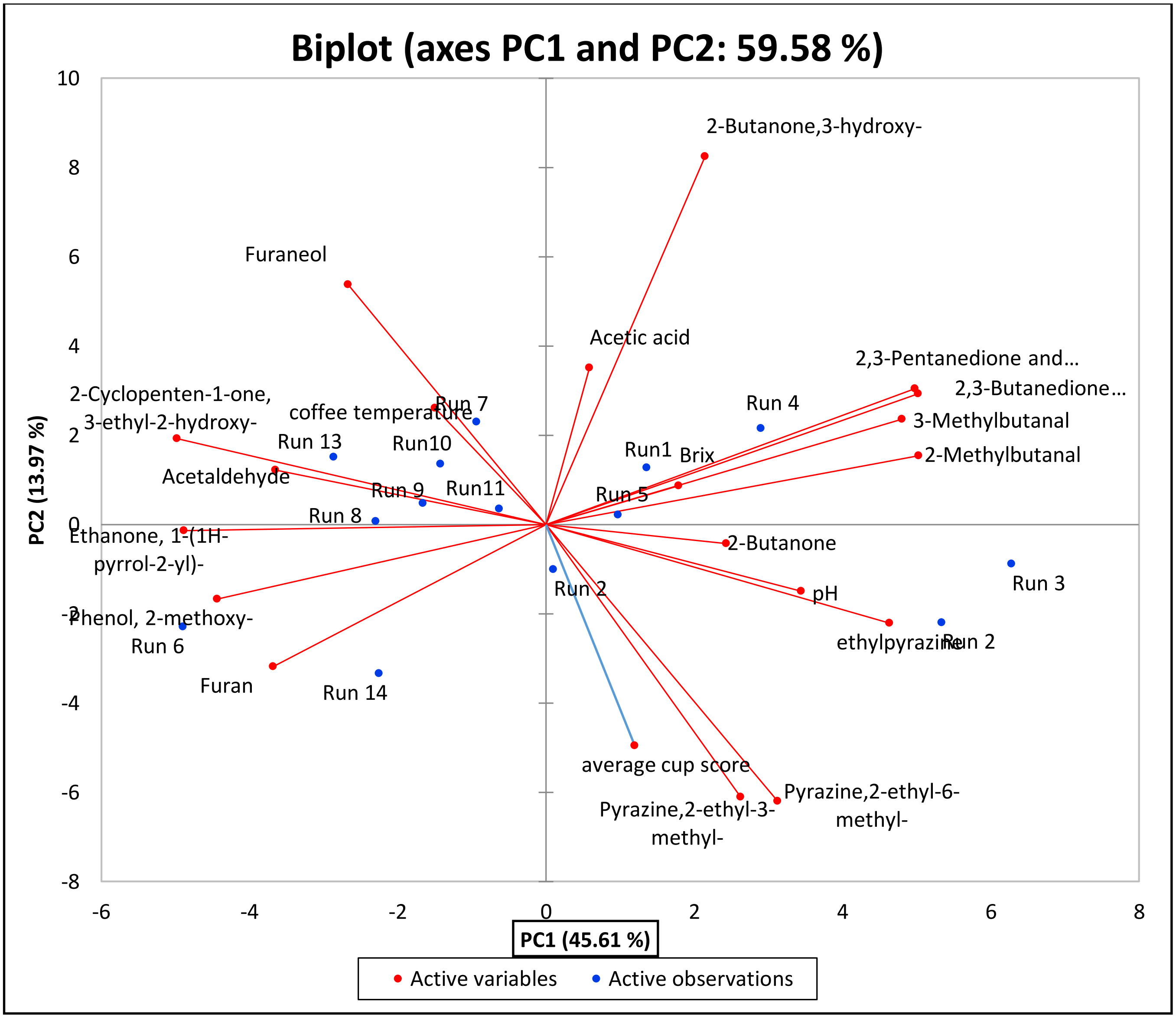

Figure 4. 1: A biplot showing average cup score, volatile compounds and physicochemical properties of Arabica coffee

CHAPTER 5

CONCLUSION AND RECOMENDATION

5.1. Conclusion

The response surface methodology was effectively employed to describe the relationship between fermentation time, fermentation type, and physicochemical, sensory, and volatile components in Arabica coffee. The fermentation time and fermentation type affected pH, brix, concentration volatile compounds and sensory properties of the coffee. Innocuated fermentation negatively influenced the concentration of acetaldehyde, 2,3-Butanedione, 2,3-pentanedione and ethyl isovalerate, ethylpyrazine, 2-ethyl-3-methyl pyrazine; while positively influenced the concentration of Furan, 2-butanone, 3-methyl butanal, 3-hydroxy-2-butanone, Acetic acid, Furaneol, 2-methoxy phenol, 3-ethyl-2-hydroxy-2-cyclopenten-1-one, 1-(1H-pyrrol-2-yl)-Ethanone. Increase in fermentation time positively influenced 2-butanone, 3-methylbutanal, 2,3-butanedione, 2,3-pentanedione and ethyl isovalerate, ethylpyrazine, 2-ethyl-6-methyl pyrazine, 2-methyl-3-methyl pyrazine, furaneol while 2-methylbutanal, 3-hydroxy-2-butanone, Acetic acid, 2-methoxy phenol, 3-ethyl-2-hydroxy-2-cyclopenten-1-one, 1-(1H-pyrrol-2-yl)-ethanone. Sensory attributes were influenced by fermentation time and fermentation type.

The optimal fermetation time and fermetation type for processing of the coffee is 16 hrs and spontaneous fermentation respectively. And from the PCA the average cup score is positively related to pyrazine, furan and butanone and pH of the coffee.

5.2. Recommendation

The study recommends;

I. Optimum conditions from this research should be utilized in industrial coffee processing to enhance coffee sensory quality.

II. Spontaneous fermentation should be utilized in industrial coffee processing rather than innoculated fermentation, because it yields better coffee cup quality. Moreover, spontaneous fermentation is an easier and more cost-effective type of fermentation to implement in industrial coffee processing.

III. Employment of better postharvest coffee processes to produce high-quality speciality coffee for high-end markets, because post harvest processing greatly impacts the final coffee cup quality.

IV. Fermentation of coffee for time longer than 38 hrs, since the longer coffee ferments, the more chemical derivatives are released that enhance the coffee cup profile, as long as coffee is not over fermented.

V. Further research in non-explored areas by this study for example;

   a) The impact of other postharvest procedures on the sensory quality of coffee.
   b) The impact of postharvest techniques on coffee chemical composition, since compounds like caffeine, trigonelline, melanoidin, and chlorogenic acids formed during coffee processing may affect human health.

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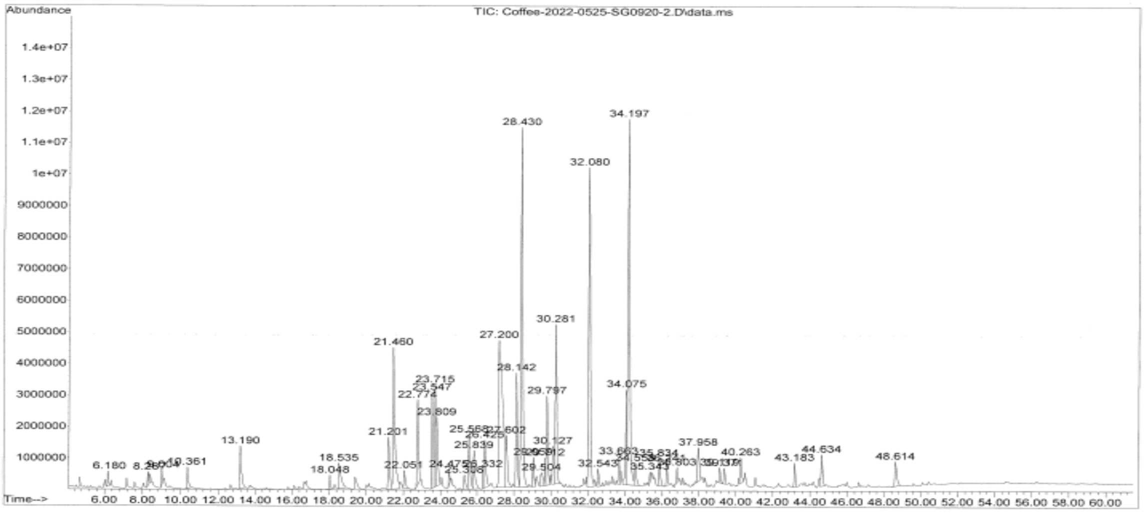
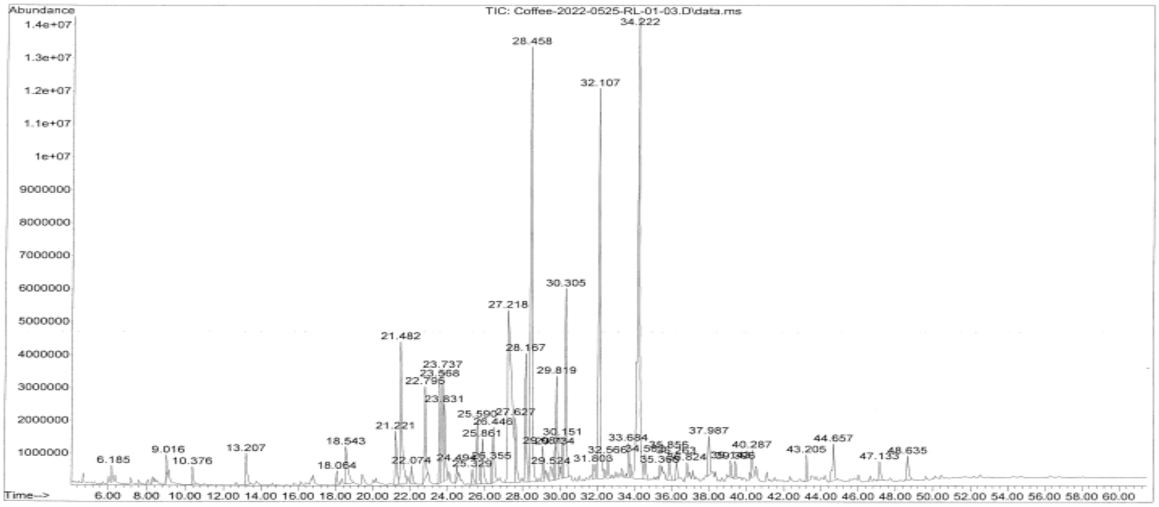
APPENDICES

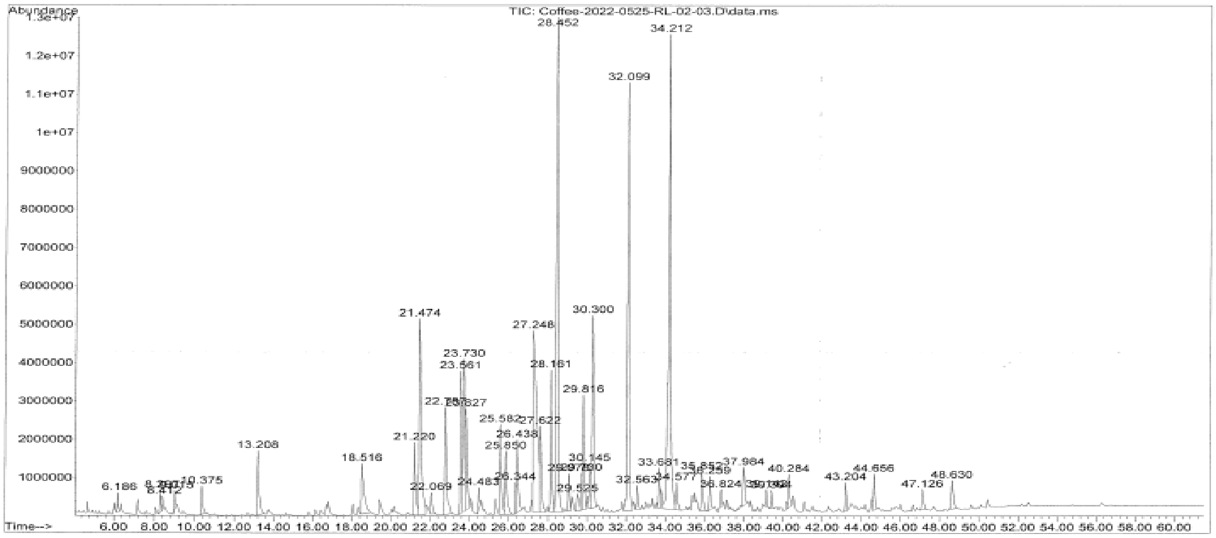
APPENDIX II: CRITERIA FOR OPTIMIZATION

Name	Goal	Lower Limit	Upper Limit
A:fermentation time	is in range	14	38
B:fermentation type	is in range	natural	culture
Acetaldehyde	is in range	9.95635E+06	1.16714E+07
Furan	is in range	3.66339E+06	4.68022E+06
2-Butanone	is in range	9.00887E+06	1.14647E+07
2-Methylbutanal	is in range	1.55007E+07	2.16904E+07
3-Methylbutanal	is in range	7.39275E+06	3.21978E+07
2,3-Butanedione (Diacetyl)	is in range	3.86007E+07	4.72103E+07
2,3-Pentanedione and Ethyl isovalerate	is in range	4.33565E+07	1.18884E+08
2-Butanone,3-hydroxy-	is in range	4.00936E+07	4.77301E+07
ethylpyrazine	is in range	1.25727E+08	1.78058E+08
Pyrazine,2-ethyl-6-methyl-	is in range	1.0027E+08	1.33729E+08
Pyrazine,2-ethyl-3-methyl-	is in range	2.38241E+07	3.18239E+07
Acetic acid	is in range	4.91836E+08	7.89864E+08
Furaneol	is in range	3.98691E+07	4.43063E+07
Phenol, 2-methoxy-	is in range	1.28808E+07	2.48734E+07
2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	is in range	1.71697E+07	2.6952E+07
Ethanone, 1-(1H-pyrrol-2-yl)-	is in range	3.69133E+07	5.61323E+07
pH	minimize	4.76667	5.16667
Brix	maximize	11	15.6667
Coffee temp	is in range	20.4	25.0667
Ambient temp	is in range	22.0667	24.8
Average cupper score	maximize	83.4375	85.125

GAS CHROMATOGRAPHY-MASS SPECTROPHOTOMETRY CHROMATOGRAMS



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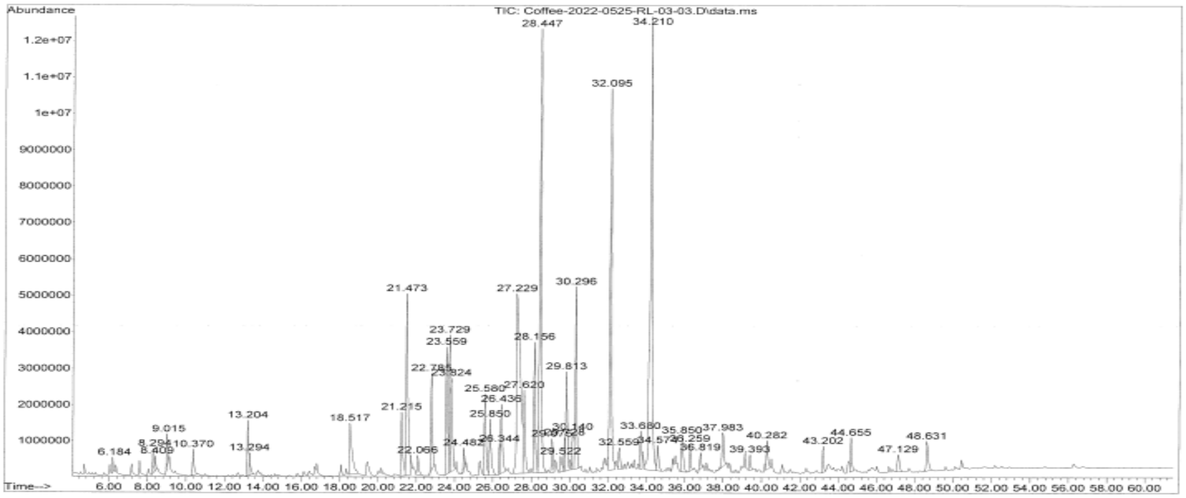
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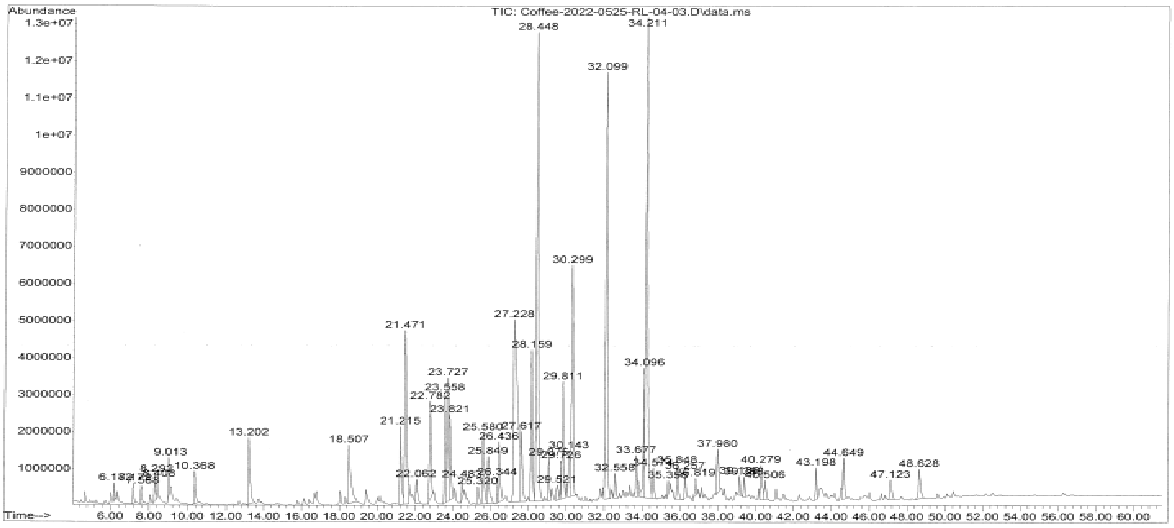
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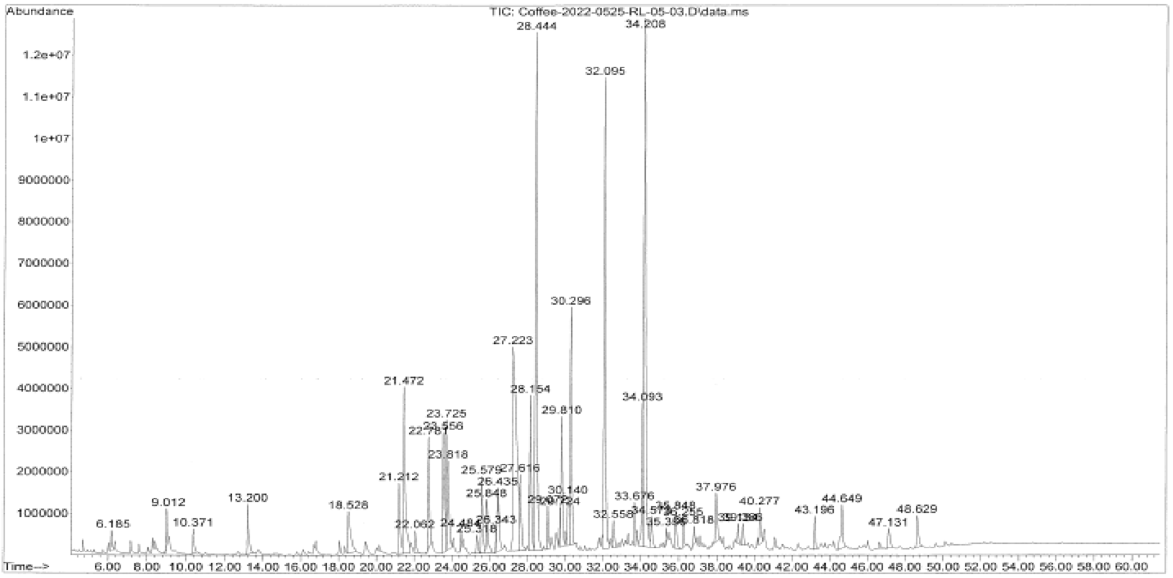
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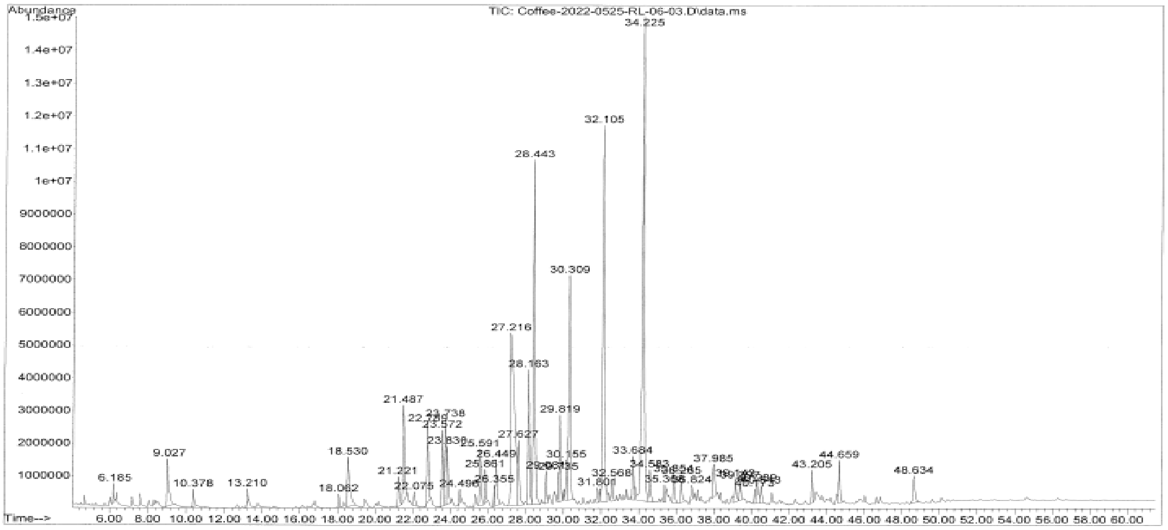
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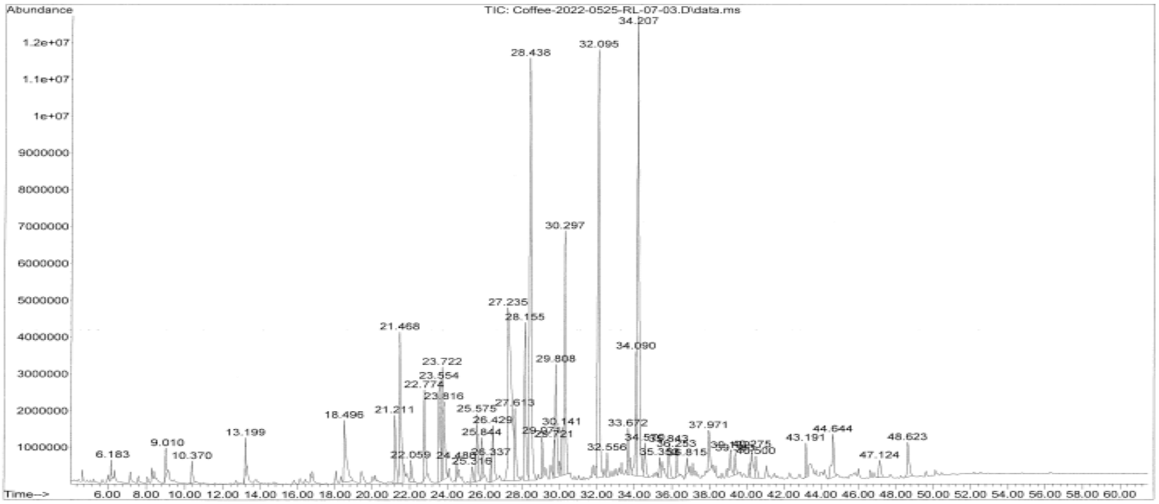
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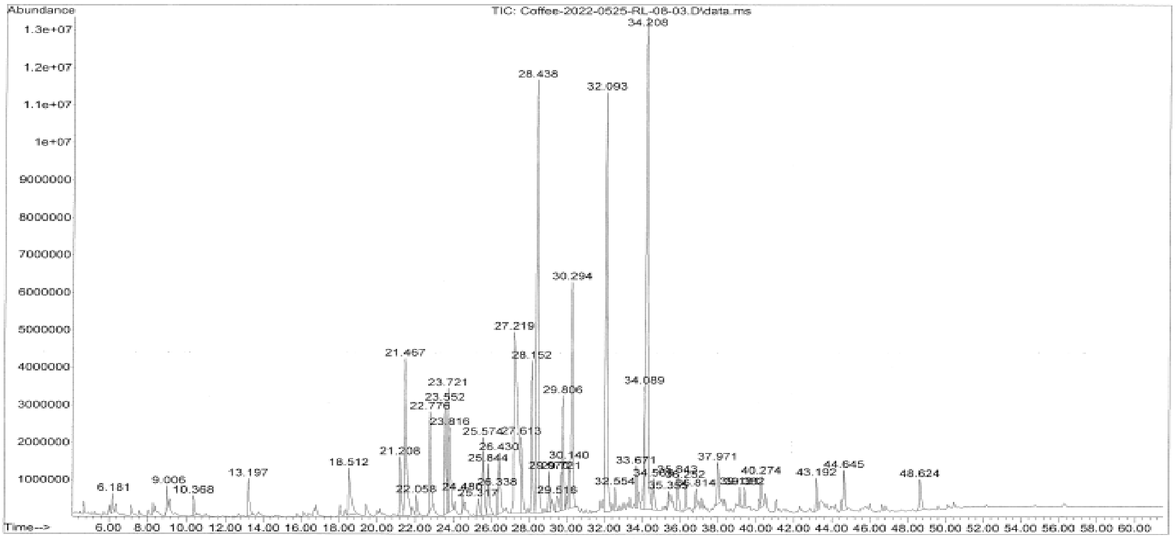
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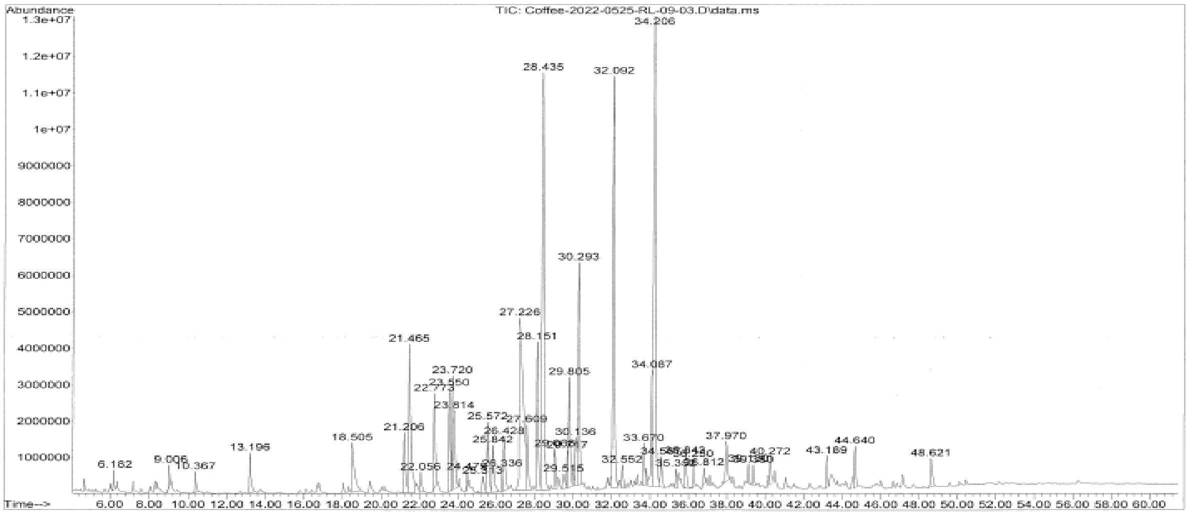
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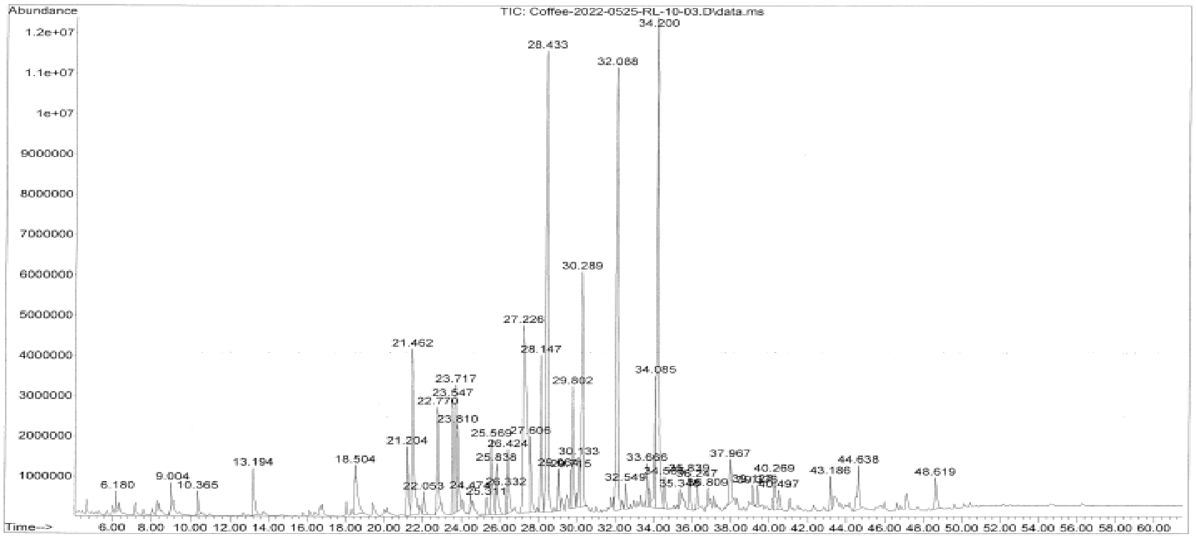

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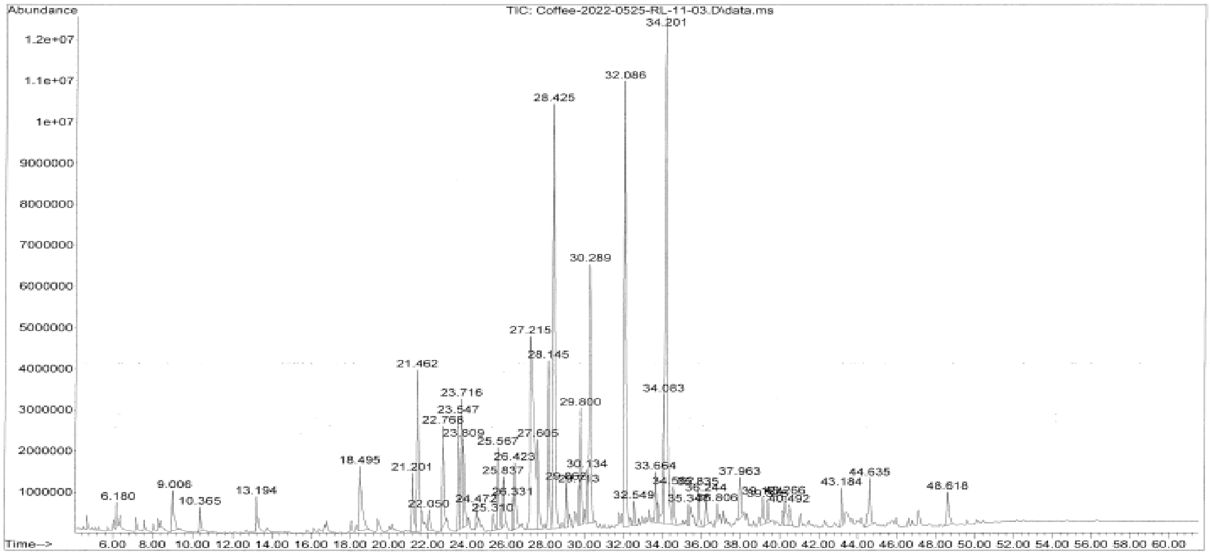

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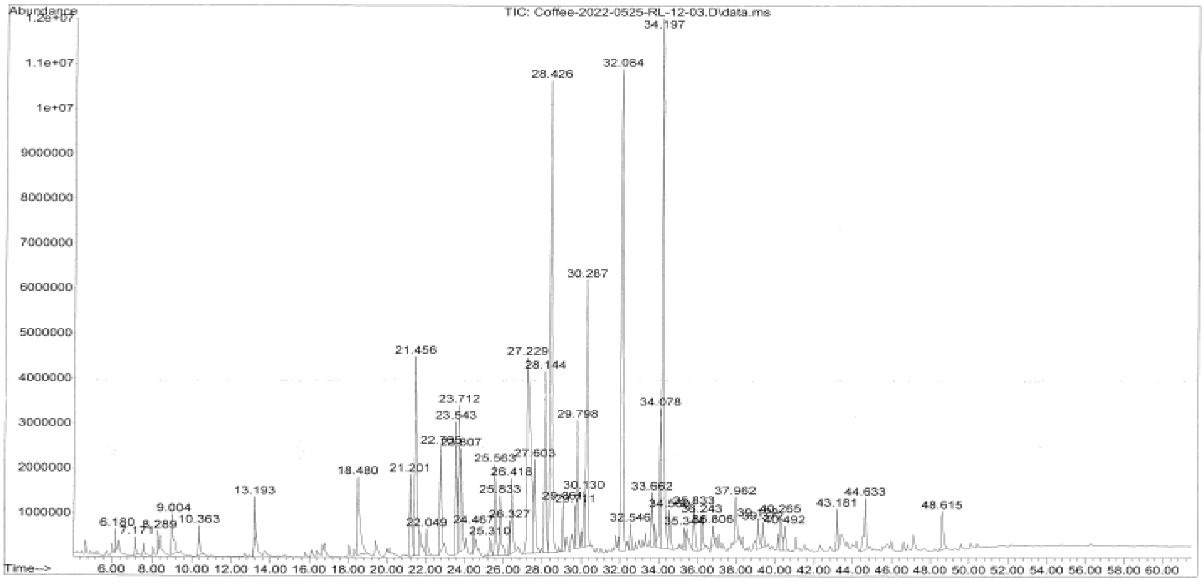

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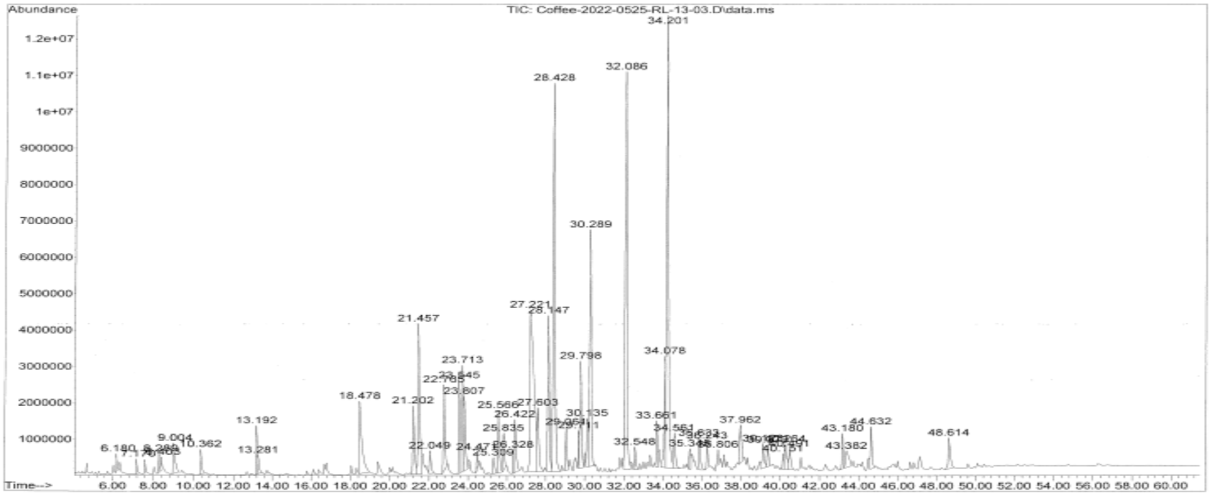

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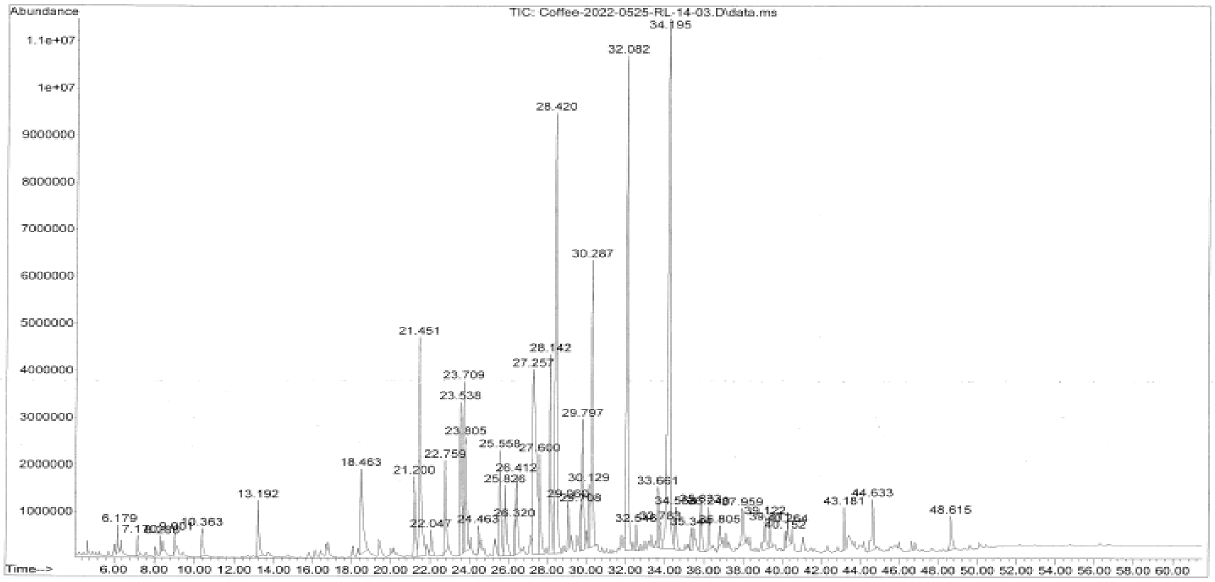
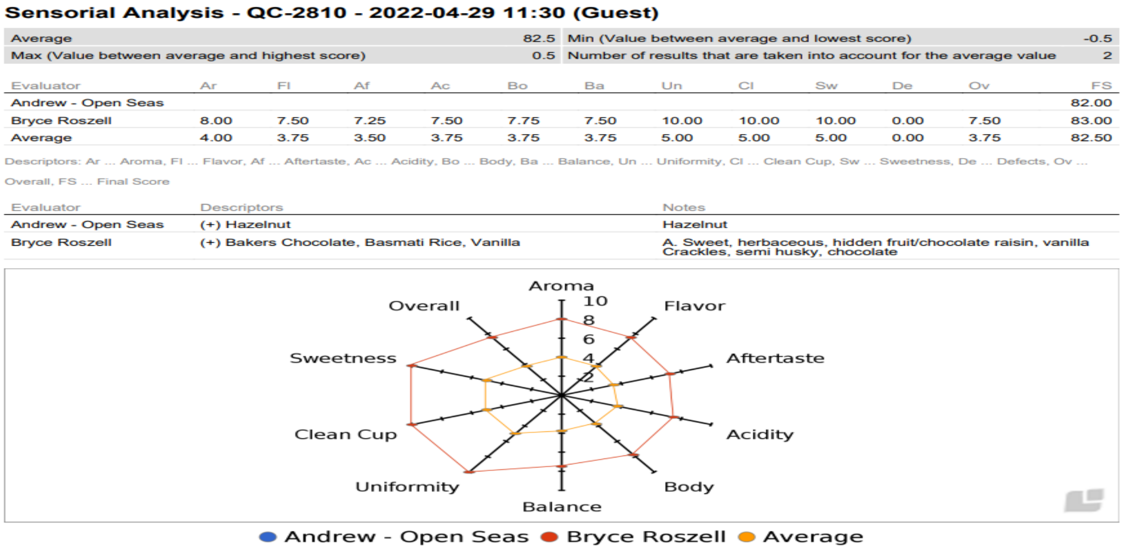


COFFEE CUPPING SCORES



Sensorial Analysis - QC-2810 - 2022-04-29 11:30 (Guest)

Average	82.5	Min (Value between average and lowest score)	-0.5
Max (Value between average and highest score)	0.5	Number of results that are taken into account for the average value	2

Evaluator	Ar	Fl	Af	Ac	Bo	Ba	Un	Cl	Sw	De	Ov	FS
Andrew - Open Seas												82.00
Bryce Roszell	8.00	7.50	7.25	7.50	7.75	7.50	10.00	10.00	10.00	0.00	7.50	83.00
Average	4.00	3.75	3.50	3.75	3.75	3.75	5.00	5.00	5.00	0.00	3.75	82.50

Descriptors: Ar ... Aroma, Fl ... Flavor, Af ... Aftertaste, Ac ... Acidity, Bo ... Body, Ba ... Balance, Un ... Uniformity, Cl ... Clean Cup, Sw ... Sweetness, De ... Defects, Ov ... Overall, FS ... Final Score

Evaluator	Descriptors	Notes
Andrew - Open Seas	(+) Hazelnut	Hazelnut
Bryce Roszell	(+) Bakers Chocolate, Basmati Rice, Vanilla	A. Sweet, herbaceous, hidden fruit/chocolate raisin, vanilla Crackles, semi husky, chocolate

● Andrew - Open Seas ● Bryce Roszell ● Average

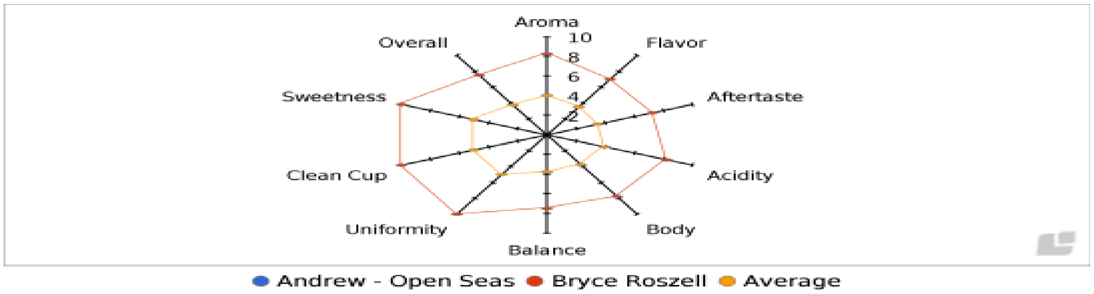
Sensorial Analysis - QC-2811 - 2022-04-29 11:30 (Guest)

Average	83.25	Min (Value between average and lowest score)	-0.25
Max (Value between average and highest score)	0.25	Number of results that are taken into account for the average value	2

Evaluator	Ar	Fl	Af	Ac	Bo	Ba	Un	Cl	Sw	De	Ov	FS
Andrew - Open Seas												83.00
Bryce Roszell	8.25	7.00	7.25	8.25	7.75	7.50	10.00	10.00	10.00	0.00	7.50	83.50
Average	4.00	3.50	3.50	4.00	3.75	3.75	5.00	5.00	5.00	0.00	3.75	83.25

Descriptors: Ar ... Aroma, Fl ... Flavor, Af ... Aftertaste, Ac ... Acidity, Bo ... Body, Ba ... Balance, Un ... Uniformity, Cl ... Clean Cup, Sw ... Sweetness, De ... Defects, Ov ... Overall, FS ... Final Score

Evaluator	Descriptors	Notes
Andrew - Open Seas	(+) Milk Chocolate, Peanuts	Chocolate, peanut butter
Bryce Roszell	(+) Bakers Chocolate, Sweet (-) Grapefruit	A. Cacao, sweet, sweet role, cinnamon Bitter, with grapefruit on long aftertaste



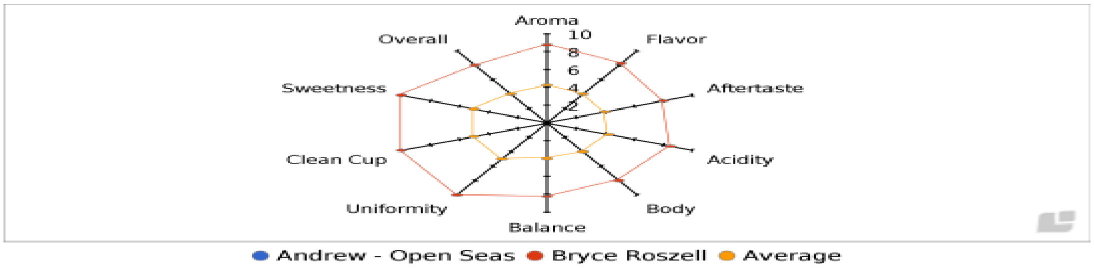
Sensorial Analysis - QC-2812 - 2022-04-29 11:30 (Guest)

Average	86.38	Min (Value between average and lowest score)	-1.38
Max (Value between average and highest score)	1.37	Number of results that are taken into account for the average value	2

Evaluator	Ar	Fl	Af	Ac	Bo	Ba	Un	Cl	Sw	De	Ov	FS
Andrew - Open Seas												85.00
Bryce Roszell	8.75	8.25	8.00	8.50	8.00	8.25	10.00	10.00	10.00	0.00	8.00	87.75
Average	4.25	4.00	4.00	4.25	4.00	4.00	5.00	5.00	5.00	0.00	4.00	86.38

Descriptors: Ar ... Aroma, Fl ... Flavor, Af ... Aftertaste, Ac ... Acidity, Bo ... Body, Ba ... Balance, Un ... Uniformity, Cl ... Clean Cup, Sw ... Sweetness, De ... Defects, Ov ... Overall, FS ... Final Score

Evaluator	Descriptors	Notes
Andrew - Open Seas	(+) Hazelnut, Strawberry	Candied hazelnut, strawberry
Bryce Roszell	(+) Strawberry, Cream, Dried Dates, Sweet Bread Pastry, Hazelnut	A. Reduced fruit, dates, strawberry jam, strawberry and cream, angle food cake Strawberry sweet.



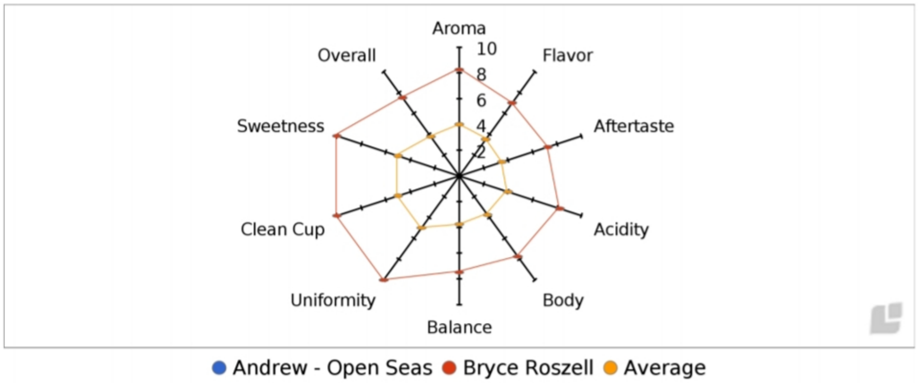
Sensorial Analysis - QC-2811 - 2022-04-29 11:30 (Guest)

Average	83.25	Min (Value between average and lowest score)	-0.25
Max (Value between average and highest score)	0.25	Number of results that are taken into account for the average value	2

Evaluator	Ar	Fl	Af	Ac	Bo	Ba	Un	Cl	Sw	De	Ov	FS
Andrew - Open Seas												83.00
Bryce Roszell	8.25	7.00	7.25	8.25	7.75	7.50	10.00	10.00	10.00	0.00	7.50	83.50
Average	4.00	3.50	3.50	4.00	3.75	3.75	5.00	5.00	5.00	0.00	3.75	83.25

Descriptors: Ar ... Aroma, Fl ... Flavor, Af ... Aftertaste, Ac ... Acidity, Bo ... Body, Ba ... Balance, Un ... Uniformity, Cl ... Clean Cup, Sw ... Sweetness, De ... Defects, Ov ... Overall, FS ... Final Score

Evaluator	Descriptors	Notes
Andrew - Open Seas	(+) Milk Chocolate, Peanuts	Chocolate, peanut butter
Bryce Roszell	(+) Bakers Chocolate, Sweet (-) Grapefruit	A. Cacao, sweet, sweet role, cinnamon Bitter, with grapefruit on long aftertaste



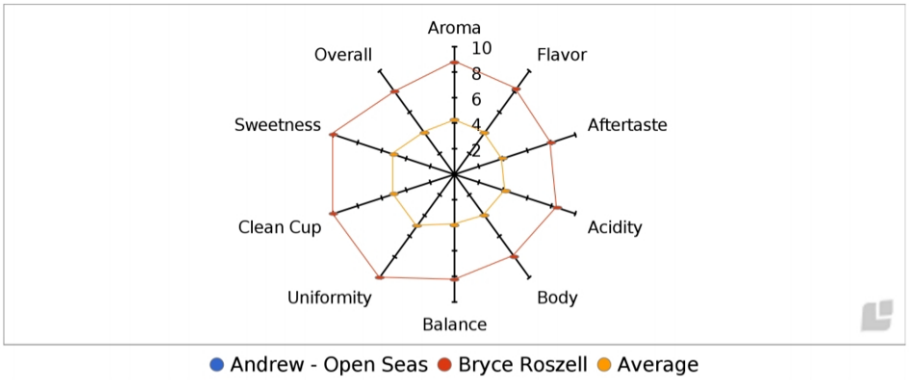
Sensorial Analysis - QC-2812 - 2022-04-29 11:30 (Guest)

Average	86.38	Min (Value between average and lowest score)	-1.38
Max (Value between average and highest score)	1.37	Number of results that are taken into account for the average value	2

Evaluator	Ar	Fl	Af	Ac	Bo	Ba	Un	Cl	Sw	De	Ov	FS
Andrew - Open Seas												85.00
Bryce Roszell	8.75	8.25	8.00	8.50	8.00	8.25	10.00	10.00	10.00	0.00	8.00	87.75
Average	4.25	4.00	4.00	4.25	4.00	4.00	5.00	5.00	5.00	0.00	4.00	86.38

Descriptors: Ar ... Aroma, Fl ... Flavor, Af ... Aftertaste, Ac ... Acidity, Bo ... Body, Ba ... Balance, Un ... Uniformity, Cl ... Clean Cup, Sw ... Sweetness, De ... Defects, Ov ... Overall, FS ... Final Score

Evaluator	Descriptors	Notes
Andrew - Open Seas	(+) Hazelnut, Strawberry	Candied hazelnut, strawberry
Bryce Roszell	(+) Strawberry, Cream, Dried Dates, Sweet Bread Pastry, Hazelnut	A. Reduced fruit, dates, strawberry jam, strawberry and cream, angle food cake Strawberry sweet,



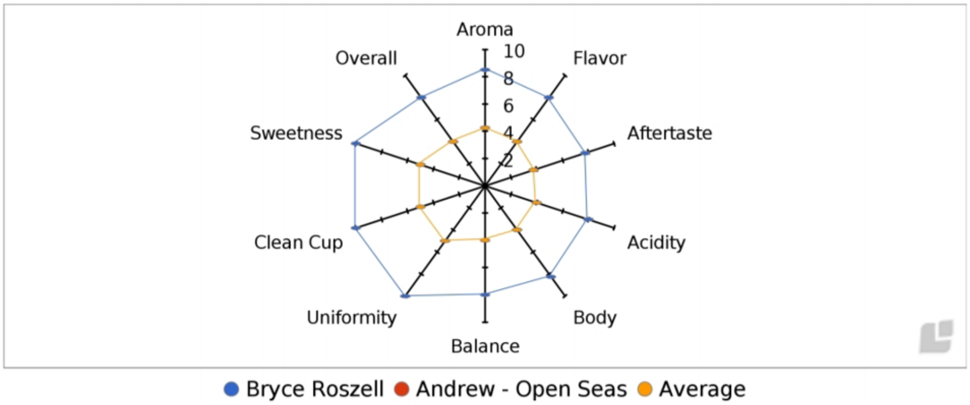
Sensorial Analysis - QC-2813 - 2022-04-29 11:30 (Guest)

Average	85.25	Min (Value between average and lowest score)	-1.25
Max (Value between average and highest score)	1.25	Number of results that are taken into account for the average value	2

Evaluator	Ar	Fl	Af	Ac	Bo	Ba	Un	Cl	Sw	De	Ov	FS
Bryce Roszell	8.50	8.00	7.75	8.00	8.25	8.00	10.00	10.00	10.00	0.00	8.00	86.50
Andrew - Open Seas												84.00
Average	4.25	4.00	3.75	4.00	4.00	4.00	5.00	5.00	5.00	0.00	4.00	85.25

Descriptors: Ar ... Aroma, Fl ... Flavor, Af ... Aftertaste, Ac ... Acidity, Bo ... Body, Ba ... Balance, Un ... Uniformity, Cl ... Clean Cup, Sw ... Sweetness, De ... Defects, Ov ... Overall, FS ... Final Score

Evaluator	Descriptors	Notes
Bryce Roszell	(+) Cinnamon, Butterscotch, Sweet Bread Pastry, Apple, Apricot	A. Sweet and mellow, candied nut, butterscotch, sweet breads, complex
Andrew - Open Seas	(+) Cinnamon, Sweet Bread Pastry, Apricot	Cinnamon Roll, apricot



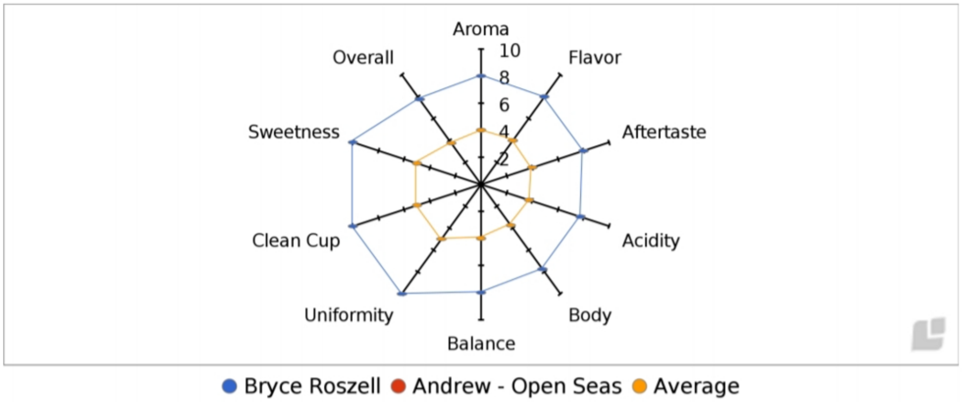
Sensorial Analysis - QC-2816 - 2022-04-29 11:30 (Guest)

Average	85.13	Min (Value between average and lowest score)	-0.13
Max (Value between average and highest score)	0.12	Number of results that are taken into account for the average value	2

Evaluator	Ar	Fl	Af	Ac	Bo	Ba	Un	Cl	Sw	De	Ov	FS
Bryce Roszell	8.00	8.00	8.00	7.75	7.75	8.00	10.00	10.00	10.00	0.00	7.75	85.25
Andrew - Open Seas												85.00
Average	4.00	4.00	4.00	3.75	3.75	4.00	5.00	5.00	5.00	0.00	3.75	85.12

Descriptors: Ar ... Aroma, Fl ... Flavor, Af ... Aftertaste, Ac ... Acidity, Bo ... Body, Ba ... Balance, Un ... Uniformity, Cl ... Clean Cup, Sw ... Sweetness, De ... Defects, Ov ... Overall, FS ... Final Score

Evaluator	Descriptors	Notes
Bryce Roszell	(+) Butterscotch, Vanilla, Apple, Cinnamon	A. Butterscotch candy! Citrus, vanilla Apple, vanilla,
Andrew - Open Seas	(+) Apple, Cinnamon, Sweet Bread Pastry	Apple, cinnamon, pastry



APPENDIX III: DATA COLLECTED DURING THE STUDY EXPERIMENTS

A: Physicochemical parameters

Sample	Brix	Ph	Coffee temp	Ambient temp	Drying time	Moisture content
1	12.3	5.2	25.1	24.1	31	10.1
2	13.7	5.2	21.5	23.3	31	10.2
3	12.3	5.0	23.5	24.6	31	10
4	15.3	5.1	22.3	24.7	32	10.3
5	15.3	5.0	22.1	24.7	31	10.5
6	12.3	5.0	22.9	24.8	32	10.2
7	12.0	4.8	24.8	22.2	30	9.3
8	12.7	4.7	25.8	23.7	31	10.5
9	11.0	4.8	23.8	22.8	29	9.6
10	13.0	4.8	24.1	22.2	30	9.9
11	15.7	5.0	22.8	23.6	31	10
12	13.3	5.0	20.4	23.5	31	9.8
13	11.7	4.8	23.9	22.1	31	10.1
14	12.3	4.9	24.3	22.7	30	10

B: Sensory Analysis

Sample	cupper 1	cupper 2	cupper 3	cupper 4	cupper 5	cupper 6	cupper 7	cupper 8	Mean Cup Score
1	84.25	85	83.5	84.75	84.25	86.25	82	83	84.1
2	85	84.75	83	**86.5**	85	**86.5**	84	**86.5**	85.2
3	84.5	84	83.25	85	84.5	84.75	85	86	84.6
4	85	85	83.5	85.75	85.5	85.25	83	84.5	84.7
5	84.25	84.25	83.5	86.5	84.75	86.25	84	85	84.8
6	81.75	84.25	84	**86**	85	**87.5**	**86**	**86.25**	85.1
7	85.25	84.75	82.5	83	83.75	80.5	85	**87.75**	84.1
8	84.5	85	83.25	85	84.5	86.5	85	85.25	84.9
9	83.75	83.75	83	85.75	84.75	**86**	**87**	**87**	85.1
10	82.75	84.25	83.75	86.5	85	77.25	85	85.75	83.8
11	84.75	84.25	83	85	84.25	84.5	83	83.5	84.0
12	83.75	84.5	83.5	85.5	85.5	85	83	84.5	84.4
13	82.25	84	83	85.5	84.5	79.25	84	85	83.4
14	84.5	84	84	84.5	83.75	85	84	85.75	84.4