Quality of Cauca coffee (*Coffea arabica* L.) under different agricultural management practices

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PUBLISHED CONTENT

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- This work is included verbatim in the thesis as Chapter 2

CONTENT

INTRODUCTION

Coffee is the second most consumed beverage worldwide, and one of the most important agricultural products for the Colombian economy, being the country with the third highest annual production in the world. Particularly, the coffee produced in the department of Cauca has become increasingly important at the national level, producing a very good quality export coffee that bears the denomination of origin Café de Cauca, granted by the Superintendency of Industry and Commerce (SIC).

The quality of coffee does not have a universal standard of measurement in the market. Its rating depends on the producing country and, although it generally focuses on the characteristics of the bean and cup sensory tests, there are multiple ways to measure quality and the factors that affect it. Therefore, the search to improve the quality of coffee comes from many fronts, and it becomes crucial to identify, throughout the coffee production chain, which are the aspects that most affect the quality of the final product, since this determines which coffee sells best and at which price, in a competitive market where supply exceeds demand.

Because cup quality and sensory attributes of coffee are affected by many factors such as coffee species and variety, growing environment, and processing, the study of these factors, their intrinsic properties, and the relationships between them become imperative. Environmental factors (i.e., climate, altitude, soil) are those that exert the greatest influence on the chemical composition of coffee, mainly because they influence fruit set and development. For example, the nutritional composition of the soil has been found to be important in determining the organoleptic attributes of the cup (Aluka et al., 2016; Yadessa et al., 2019). Thus, considering that the physicochemical properties of the soil determine its nutritional composition, the study of these properties becomes of great importance for the production of quality coffee.

At a chemical level, numerous studies have found that certain metabolites are responsible for providing coffee its organoleptic properties, and that their concentration affects the quality of the drink. Examples of them are caffeine, chlorogenic acids, and trigonelline, which are involved both in the bitterness of coffee and in the formation of other compounds, such as furans, pyrazines, pyrroles, and pyridines, which will define the flavor and aroma of the coffee drink (Farah et al., 2006). Due to this relationship, the content of some metabolites has been proposed as an additional tool to evaluate the quality of coffee, as a criterion for the selection of grains, and as an indicator of various factors such as coffee variety and geographical origin.

The quality of coffee is also influenced by the phytosanitary management of the crop. Since the crop is sensitive to pests and diseases, the use of pesticides is necessary to sustain coffee production, especially in a country whose economy greatly benefits from it. However, compliance with pesticide residue limits is key for guaranteeing both the health of consumers and the production of quality coffees with export potential. Additionally, there is a considerable commercial value of coffees grown under organic systems or qualified as specialty coffees, compared to those produced traditionally.

Therefore, an understanding of the effects of agronomic management practices on quality and chemical changes in the beverage is required. For Colombia, however, there is no information available on the chemical composition of coffees grown under different management practices, thus preventing their characterization and differentiation.

Considering the above, this work focuses, within the coffee production chain, on the pre-harvest, and within this, on the agronomic management of crops, specifically on organic, specialty and traditional management. Three aspects were chosen to evaluate each of these management practices: the physicochemical properties of the soil, the content of pesticides and of metabolites. Finally, the object of study was the species *Coffea arabica* L., cultivated in the department of Cauca, Colombia.

1. Objectives

GENERAL OBJECTIVE

Establish the impact that three types of agronomic management (organic, traditional and specialty) have on the quality of coffee (*Coffea arabica* L.).

SPECIFIC OBJECTIVES

- Identify the attributes that are the most used in scientific literature for assessing the coffee quality throughout the different stages of the production-to-consumer chain, as well as the most relevant topic areas of research in coffee quality, through a systematic mapping.

- Study and compare the soil quality of the coffee crops from the agronomic practices organic, traditional and specialty

- Measure and compare the quality of coffee from the food safety perspective of the agronomic practices organic, traditional and specialty through the compliment of the MRLs.

- Stablish and compare the metabolite content in coffee beans cultivated using organic, traditional and specialty coffee management practices

2. Document structure

This document has the following structure:

- Chapter one presents the basic concepts for this study
- Chapter two presents a systematic mapping around the concept of coffee quality
- Chapter three present the physicochemical characterization of the soils from different management practices
- Chapter four presents the measurement of pesticides and soils from coffee crops with different management practices
- Chapter five presents the measurement of metabolites by HPLC, infrared spectroscopy and purge and trap GC-MS methods in coffee from different management practices
- Chapter six presents the conclusions

3. References

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CHAPTER 1: Background

1. Coffee in Colombia and in the Cauca department

The coffee plant (**Figure 1**) belongs to the family Rubiaceae and to the genus *Coffea*. Of the more than 100 species of the genus *Coffea*, four are widely cultivated and constitute commercial coffees: *Coffea arabica* L. (arabic or arabic coffee); *C. canephora* Pierre ex A. Froehner (Robusta coffee); *C. liberica* W. Bull ex Hiern (Liberian or Liberian coffee); and *C. excelsa* A. Chev. (excelso coffee). The principal types of coffee consumed throughout the world are the ones from the Arabica and Robusta species: Arabica represents 80% of the coffee consumed in the world, coming mainly from South America, Central America and Central Africa; while the Robusta species is mostly cultivated in Africa, Asia and Brazil (Jürgen Pohlan & Janssens, 2010; Puerta-Quintero, 2008).

Coffea arabica is species cultivated in Colombia. As all species within the genus, it is of tropical African origin, although its natural populations are restricted to the montane forests of southwestern Ethiopia. In its natural state, *C. arabica* can measure between 4–6 meters, albeit in cultivation it does not exceed 2 meters due to pruning in order to keep manageable heights (Anzueto et al., 2005).

Figure 1. Coffee plant illustration (Köhler, 1887) cited in (Stueber, 2003)

Coffee has specific requirements for its production, such as special conditions of soil, temperature, precipitation, and altitude above sea level. The ideal conditions for cultivation are between 1200 and 1800 meters above sea level, temperatures between 17 and 23°C and average precipitation around 2.000 millimeters distributed throughout the year. Although, it is also possible to produce an exceptional coffee in zones with different temperatures, precipitation frequency and at upper heights (FNC, n.d.-c). However, in cold climates with a temperature lower than 19°C, the development of the varieties decreases, affecting production. Moreover, in hot climates with an average temperature above 21.5°C, the productive life is shorter, the harvest time comes earlier and it has a shorter period of time (Montes Rojas et al., 2012).

The history of coffee as the second most popular beverage in the world began centuries ago in Ethiopia, Africa, where coffee was consumed by chewing its leaves or in infusions. Its expansion throughout the world began in the 16th century at the hands of the Arabs, arriving in Turkey in 1554.

Later, in the 17th century, it entered Europe through the port of Venice and spread across the continent, finally reaching America around the 18th century. (FNC, n.d.-d). In Colombia, coffee farming has a long tradition that dates back to the 18th century. Around 1732, it is believed that the Jesuits were the first to bring and produce coffee crops at the Minor Seminary of Popayán (Cauca). There is also data around 1741 on the presence of coffee crops in Riohacha (La Guajira) and in Santa Marta (Magdalena). Nevertheless, it was only until a hundred years later that coffee became one of the most important instruments of socioeconomic development in the country (FNC, 2011).

Colombia offers a great diversity of flavors due to multiple factors that affect coffee production as are geography, clime and the processing (FNC, n.d.-b). 95% of coffee-growers are families that cultivate coffee farms or plots, with coffee crops that on average do not exceed two hectares, where the harvesting and post-harvest tasks are generally carried out by the coffee growers themselves, making coffee an almost artisanal product. Associated with Colombian coffee quality is the large number of producers in the country and the dispersion of the crop throughout the varied national geography. Coffee crops spread between the Sierra Nevada and the Andes Mountains (Figure 2), in whose coffee is generally grown between 1,200 and 1,800 meters above sea level, constituting the perfect environment for the cultivation of Colombian mild coffee. Thus, coffee is the product that has come to be an integrator of the Colombian nationality. This agricultural activity has generated income and social development in the rural areas of Colombia, meaning rural development, income redistribution and peace building (FNC, 2011).

Figure 2. Coffee regions in Colombia (FNC, n.d.-c)

"Colombia produces a mild Arabica coffee that is widely sought by the cognoscenti, contrasting strongly with coffee from non-mild Arabica producers elsewhere" (Oberthür et al., 2011). Colombian coffee has been recognized in the international market for its quality and smoothness. Currently, 30% of Colombian coffee exports are marketed as specialty coffee in any of its sustainable, origin or preparation categories (Puerta-Quintero & Pabón Usaquén, 2018).

The different varieties of *Coffea arabica* cultivated in Colombia depend on the specific conditions of each climatic zone or region (Cenicafé, 2004). The coffee that Colombia exports as green coffee beans is usually a mixture of the main varieties grown in the coffee regions, including Caturra, Colombia,

Tipica, Borbón and Tabi; also including the Castillo® variety and the regional Castillo® varieties. This is the coffee marketed with the designation of origin Café de Colombia (Puerta-Quintero, 2008).

For instance, on August 10, 2011, the agency Superintendence of Industry and Commerce (SIC) awarded the "Café de Cauca" designation of origin, defining it as "a coffee with a very strong and caramelized fragrance and aromas, and in the cup, it presents high acidity, medium body, a balanced, clean, smooth overall impression with some sweet and floral notes". The designation of origin allows to guarantee the origin of a product and protect it, in addition to supporting its quality (FNC, 2012).

The department of Cauca has great potential to produce high quality coffees. It has an unbeatable environmental offer with its diverse climate and topography due to the Western and Eastern mountain ranges that cross it, making it the 4th coffee producer nationwide and the first in number of coffee families. Around 90,000 thousand families are in charge 93,000 hectares of coffee of the varieties Castillo, Colombia, Caturra, Tipica, Borbón and Tabí in 34 municipalities, which provides an important contribution to the economic development of the department and the national industry (FNC, 2017, n.d.-a).

In this work, all samples of coffee were collected from 5 plots form 3 neighboring coffee farms located in the municipality of Cajibío (Cauca). This municipality, along with 10 others, form the so-called Central Coffee Region of the department of Cauca. This region is made up of 43,000 coffee-growing families belonging to indigenous Nasas and Misak, afro-descendant communities and peasants. Here, the about 44,500 hectares of coffee cultivated are located at 1,700 meters above sea level mainly in the Plateau of Popayán, characterized for its volcanic ash soils. The National Federation of Coffee Growers of Colombia describes its cup profile as characterized by its medium high acidity, balanced cup, medium body, having a pronounced fragrance and aroma with caramel and floral notes (FNC, n.d.-a). Although this could change depending on the processing and varietals.

2. Coffee cherry and Coffee processing

Coffee cherry (**Figure 3**) is formed by one or two beans or seeds (endosperm) covered, consecutively, by four layers: the silver skin (integument), parchment (endocarp), mucilage (pectin layer or inner mesocarp), pulp (outer mesocarp) and skin (exocarp). These layers are removed during the postharvest processing along with enough water to obtain coffee green beans with a range between 10 and 12% of moisture (G. V. de M. Pereira et al., 2019).

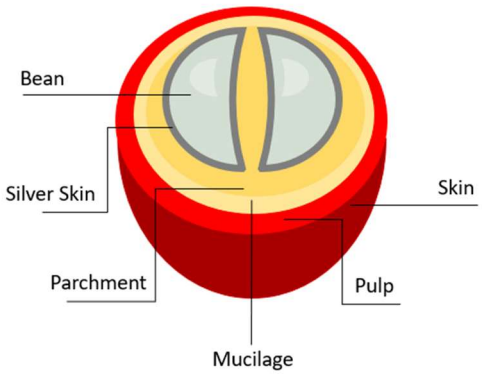

Figure 3. Cherry coffee bean

The exocarp or skin, the outermost layer, is as thin as ~0.5 mm and has a tough, smooth surface. It provides the fruit external resistance. When the bean matures goes from green to either red, yellow, or orange, depending on the coffee variety. Then, there is the outer mesocarp or the fruit's flesh. It is a yellowish-white, fleshy, juicy and sweet substance. The skin and the fruit's flesh (the pulp) are composed mainly of carbohydrates (35–85%), soluble fibers (30.8%), minerals (3–11%) and proteins (5–11%). However they also contain organic compounds such as caffeine, chlorogenic acid and tannins (Iriondo-DeHond et al., 2019; Janissen & Huynh, 2018). The next layer is inner mesocarp or the mucilage, which surrounds and binds the coffee beans together and it is removed either through fermentation or mechanically (Anzueto et al., 2005). It is mainly composed of water, protein, sugar, pectin, and ash (Esquivel & Jiménez, 2012). The fourth layer is the endocarp or parchment, which covers both coffee seeds and separates them from each other. It is strong and fibrous, mainly composed of (α-)cellulose, hemicellulose, lignin, and ash. When mechanically removed, it is called the coffee hull, and dehulling is the process of removing it (Iriondo-DeHond et al., 2019). The innermost layer covering the green coffee (endosperm) is the integument or silver skin, a thin, delicate, closely held layer that is mainly composed of fiber, polysaccharides, protein, fat, and ash. This layer is removed by turning into chaff during the roasting process (Iriondo-DeHond et al., 2019; Rotta et al., 2021).

After the harvesting, coffee processing must begin immediately to prevent fermentation and deterioration of the pulp. Different post-harvest methods play an important role in maintaining and improving the underlying quality of coffee. The main post-harvest processes are the wet, mostly applied to Arabica, semi-dry, and dry or natural methods mostly applied to Robusta coffee (Bee et al., 2005; Hamdouche et al., 2016; Hameed et al., 2018)

The dry method is the cheapest, simplest and oldest method and makes the so called 'natural' coffees (Hamdouche et al., 2016). First, the harvested cherry coffee beans are washed, sorted and then and dried (sun-dried or machine-dried) until moisture content reaches 10–12% (De Bruyn et al., 2017). The drying step is the most important one of this process, since it impacts the final quality of green coffee that is obtained a step further after husking (Hamdouche et al., 2016).

The wet process involves several steps. First, the cherries are mechanically depulped, this way removing the skin (exocarp) and the pulp (outer mesocarp). Then, they are fermented by the microbial degradation of the mucilage layer, washed several times and dried (Hameed et al., 2018; L. L. Pereira et al., 2020). This method delivers a fully washed parchment coffee (parchment devoid of mucilage) and is reported to help preserve the inherent qualities of the bean, producing homogeneous coffee beans with less defects. Coffee treated by this process is commonly considered of a high quality and mostly ends being sold as higher price than natural coffee (Hamdouche et al., 2016). In Colombia and in the department of Cauca this is the most common method.

Semi-dry processing is an intermediate method of the wet and dry processing where coffee cherries are depulped and then dried (G. V. de M. Pereira et al., 2017).

3. Management Practices

4. Organic coffee

Organic crops, whose boom began in the 60s of the last century, are characterized by the total absence of synthetic chemicals and fertilizers in all production processes, thereby contributing to the conservation of the environment and human health (FAO, 2013). However, even though organic coffee has a considerably high price on the international market (Monteiro et al., 2018) and that consumer demand for organically grown plant-based foods has increased dramatically, only the 3% of world coffee production is grown organically (Badmos et al., 2020).

According to IFOAM - Organics International (International Federation of Organic Agriculture Movements) (2021), organic agriculture revolves around the principles of: protection and improvement of the health of living beings and the environment; Protection and use of ecological systems and cycles aiming at sustainability; creating fair relationships accounting for real environmental and social costs; and protection and care of the environment and future generations.

Accordingly, organic agriculture not only restricts the use of synthetic products in crop protection and nutrition, including harvest and postharvest (storage), also ensures soil health, responsible water use and treatment; and gives great importance to the seeds' origin, giving priority to native or endemic ones, (Gomez & Thivant, 2015). Moreover, no GMOs (genetically modified organisms) are allowed and depending on the certification agency there is also the omitting of the use of sewage sludge as fertilizers and plant hormones (Badmos et al., 2020).

Organic farming is regulated and certifiable to ensure that consumers buy authentic products and protects them from the misuse of the term. In Colombia, those responsible for this are the Resolution No. 036 of 2007 of the Ministry of Agriculture and Rural Development, which modifies Resolution 148 of 2004 for the use of ecological land, and the Resolution 199 of 2016 of also the Ministry of Agriculture and Rural Development, which partially modifies the regulation for organic production adopted by Resolution 187 of 2006.

5. Conventional or traditional farming

According to the "Regulations for the primary production, processing, packaging, labeling, storage, certification, import and marketing of Organic Agricultural Products" of the Colombian Ministry of Agriculture and Rural Development (2006), conventional farming is defined as an "agricultural production method in which synthetic chemicals are used in whole or in part".

6. Specialty coffee

On the other hand, there are also specialty coffees. According to the international Q Coffee System protocols and the Specialty Coffee Association (SCA) specialty coffee typically refers to coffees that can be distinguished on the basis of quality and uniqueness of origin (Carvalho et al., 2020; Lingle & Menon, 2017; Velásquez et al., 2019). The label of Specialty coffee is given by certifying organizations, which assesses coffee through 'cupping' protocols and hedonic evaluation. The cupping protocol, dictated by the Specialty Coffee Association, the most significant specialty coffee trade organization, employs a 100-point scale for total quality. Specialty coffees are those with a cumulative of 80 points or better (Specialty Coffee Association, 2021).

4. Coffee Quality

The quality of the coffee is evaluated by the appearance, color and smell of the parchment, green or roasted coffee beans, as well as the organoleptic qualities of the drink (Buenaventura-Serrano & Castaño-Castrillón, 2002). The quality of coffee beverages is influenced by both of pre-harvest factors such as climate, geographic location, genotype, and agronomic practices, and post-harvest factors including primary processing, drying, roasting and storage conditions (G. V. de M. Pereira et al., 2017). Nevertheless, there is no guideline in the coffee market to evaluate the quality of coffee green beans and every producing country has its own standards for trade and export (Rodrigues et al., 2009).

Cupping or sensory evaluation is the method used to know the aroma, flavor and quality of coffee. These help to determine the influence of the various factors and processing conditions on the quality characteristics of the product, and therefore, to know the aspects that contribute to the quality of a good cup of coffee. In the same way, defects present in the drink can be identified (Puerta-Quintero, 2009). In a sensory evaluation of coffee, it is important to describe, evaluate and analyze the intensity of sensory characteristics such as fragrance, aroma, acidity, bitterness, body, sweetness, residual flavor, global impression, granulometry, roasting, etc. (Jaimes et al., 2015; Puerta-quintero, 1996).

Sensory analysis is one of the most important criteria for evaluating the final product; however green coffee is not evaluated by taste criteria as it is almost impossible to determine them based on the appearance of the bean. Thus, each producing country has its own classifying criteria for purposes of trade and export. Overall, the green beans are graded based on the number of defects (e.g., Brazil, Jamaica, Ethiopia), the size of the beans (e.g., Brazil, Colombia, Tanzania), and the altitude of the cultivation area (e.g., El Salvador, Guatemala, Costa Rica) (Iwasa et al., 2015). In Colombia, where coffee is sold in the state of dry parchment, there are qualifications from Federation type to Pasilla, according to the percentages of defects such as the so-called peeled grain, guavas and half face (Marín López et al., 2004).

Another way to measure the quality of coffee is based on the relationship established with the chemical composition of the bean. The flavor and taste of coffee are directly influenced by genetic and environmental factors, the latter being the ones that exert the greatest influence on its chemical composition, largely since they affect maturation (Leroy et al., 2006; Marín-López et al., 2003). In fact, the relationship between the maturity of the grain and certain amino acids, carbohydrates, volatile and non-volatile compounds, and phenolic acids has been reported. Also other factors such as storage and growth conditions also affect this chemical composition (Choi et al., 2010).

The differences in the composition of the green coffee, the roasting conditions and the extraction procedures for the preparation of the drink, lead to a great diversity in the chemical composition of the final product (Borelli et al., 2002).

From a chemical point of view coffee roasting ends up being a very complex process, as hundreds of reactions take place simultaneously. Sugars and trigonelline act as flavor precursors, originating various substances (furans, pyrazines, pyrroles, pyridines, etc.) that will affect the taste and aroma of the drink. Bitterness is partly due to the phenolic substances that result from the thermal degradation of chlorogenic acids. Although caffeine is not involved in any reaction, it also contributes to bitterness (Franca et al., 2005). So, the highest quality coffees are the ones with reduced levels of chlorogenic

acids and caffeine and an increase of sucrose, amino acids, trigonelline, lipids content (Marquetti et al., 2016).

Another important criterion for coffee quality is food safety. Plant-based foods can contain chemical contaminants as a result of various factors, such as the use of agrochemicals; from exposure to contaminated environmental sources such as water, soil or air; by cross contamination or formation during its processing; migration from packaging materials; due to the presence of natural toxins from fungi; or by the use of prohibited additives and / or adulterants (Brandl, 2012).

In Colombia, food safety is controlled by different entities that are in charge of different areas (Ortiz Amaya & Martínez Martínez, 2011), such as The Ministry of Health and Social Protection, that regulates the health aspects of food quality and safety through the National Institute of Drugs and Food (INVIMA) and the National Institute of Health (INS); The Ministry of Agriculture and Rural Development and the Colombian Agricultural Institute (ICA), which oversee agricultural health protection and regulation; The Ministry of Commerce, Industry and Tourism and the Superintendency of Industry and Commerce, that establish the framework of action for the official entities that make up the National System of Standardization, Certification and Metrology; and The Colombian Institute of Technical Standards and Certification (ICONTEC), which is in charge of technical standards.

Also, Colombia is within the group of countries that use the maximum residue limits recommended by the *Codex Alimentarius*. Traces left by pesticides in treated products, or traces left by veterinary drugs in animals, are called “residues”. In the case of agricultural products and pesticides, a maximum residue limit (MRL) is the maximum level of residues of a pesticide that is legally allowed in food or feed (both inside and on the surface) when pesticides are applied correctly in accordance with good agricultural practices (FAO/WHO, 2021b). The Codex Alimentarius, or "Food Code" is a collection of standards, guidelines and codes of practice adopted by the Codex Alimentarius Commission (FAO/WHO, 2021a). The Codex Alimentarius Commission, created in 1963 and managed by FAO and WHO, establishes science-based standards, guidelines and codes of food practice that ensure food safety and quality by addressing contaminants, hygienic practices, labeling, additives, inspection and certification, nutrition and residues of veterinary drugs and pesticides (FAO/WHO, 2020). Currently, the Codex Alimentarius Commission is made up of 189 Codex Members, 188 Member Nations and 1 Member Organization (European Union) (FAO/WHO, 2021c).

The head of the Codex Alimentarius Commission in Colombia is the National Codex Alimentarius Committee, which is created by Decree 0977 of 1998 of the Ministry of Public Health. Its purpose is “to be a consultative body of the National Government for the formulation of the country's policies in relation to the standardization processes, analysis of principles and procedures that can be carried out in the Joint FAO/WHO Commission, its Executive Committee and its Auxiliary Bodies" (Ministerio de Comercio Industria y Turismo, 2022).

However, countries are sovereign to modify said values for each food to national standards, adopt more strict values than Codex due to consumption habits or not tolerate prohibited substances when toxicological information has not been established. Even certain countries limit the number of active ingredients present to three, regardless of the MRL values (Moreno & Machado, 2019). For example, the European commission has pesticides and their residues regulated under Regulation (EC) No

1107/20093 and Regulation (EC) No 396/2005. When governments adopt international standards, farmers and producers can meet consumer demands for safe food while gaining access to the global food market (FAO/WHO, 2020).

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CHAPTER 2: A systematic mapping study of coffee quality throughout the production-to-consumer chain

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Abstract

Coffee is one of the most consumed beverages in the world and is crucial in the economy of many developing countries. The search to improve coffee quality comes from many fronts, as do the many ways to measure quality and the factors that affect it. Several techniques are used to measure the different metrics to assess coffee quality, across different types of coffee samples and species, and throughout the entire process from farm to cup. In this work, we conducted a systematic mapping study of 1,470 papers to identify the aspects of quality that are the most important in the scientific literature to evaluate coffee throughout the processing chain. The study revealed that cup quality and biochemical composition are the most researched quality attributes. The main objective in the reviewed studies is the correlation between different quality measurements. The most used techniques are the analytical chemistry methods. The most studied species is Coffea arabica. The most used sample presentation is green coffee. The post-harvest stage is the most researched, in which quality control receives more attention. In the pre-harvest stage, management practices stand out. Finally, the most used type of research was the evaluation research.

KEYWORDS: Coffee quality, pre-harvest, post-harvest, coffee processing

7. Introduction

Coffee is a highly popular beverage, it has been consumed over 1000 years and today is consumed by about one-third of the world's population (Damatta et al., 2018; Mussatto et al., 2011). Is the second most consumed beverage after water and the most widely traded tropical product (Duong et al., 2020; FAO, 2018). Global coffee consumption reached a record 168.1 million bags in coffee year 2018/19 (ICO, 2020) and is currently grown in about 80 countries of four continents *(Bicho et al., 2013)*. By order of importance, the main producing countries are Brazil, Vietnam, Colombia, Indonesia, Honduras and Guatemala (ICO, 2021) and as such it plays a crucial role in the economies of several tropical countries as an important source of income, employment and local development in the producing or processing regions. Also, smallholder coffee producers are responsible for 80% of global coffee production, which makes the activity extremely important in maintaining rural lifestyles, providing better incomes, and wealth distribution (DaMatta & Cochicho Ramalho, 2006; Vellema et al., 2015)

Despite the back-ground of global inflation, continued price fluctuations and restrictions on trade, global coffee consumption continues increasing each year (ICO, 2020). This raise in coffee consumption is related to its unique organoleptic attributes, as well as to the beneficial effects that has demonstrated its long-term intake (Diaz-De-Cerio et al., 2019) and therefore, increases the demand for high quality coffee and specialty coffees (Liu et al., 2019).

The criteria used to define the coffee quality differ throughout the different stages of the production-to-consumer chain. According to Leroy et al. (2006), for the farmer, the quality of coffee depends on the easiness of the crop management and harvest, the production yield and price on the market. For the exporter or importer, coffee quality is related to bean quality, absence of defects, consistency of supply, the quantity at hand, physical characteristics as well as price. For the roaster, it depends on stability of characteristics, moisture and biochemical content, organoleptic features, origin and price. At the consumer level, whose preferences differ depending on the country, the quality of coffee is associated with price, taste and flavor, health and alertness, origin, environmental and sociological traits like fair trade and organic farming, etc.

On the other hand, coffee science has a different approach to quality and the factors influenced by genotype (coffee size and shape, color, chemical composition and flavor); environmental factors as climate, altitude, water availability, soils; cultivation practices that encases farming and post-harvest operations like fertilization, shade, crop management, coffee processing, storage, etc. (Wintgens, 2004).

Traditionally bean quality is assessed by their shape and size, color, the proportion of defective beans, and taste of the beverage produced after the roasting of the beans. The flavor of the coffee cup is related to the chemical composition of the bean which in turn is determined by the cultivar, the farming practices and post-harvest processing conditions such as fermentation, drying and roasting (Batista & Chalfoun, 2015). On the other hand, molecular science approach is to examine the molecular composition of coffee from each step of the process and stablish how is impacted; and determine the effect that every molecule has on the final beverage (Folmer, 2014). Furthermore, the coffee must be a safe product and present no risk to consumer health. The main issues of safety are contamination with pesticide residues and fungal mycotoxins, and international standards that prescribe permissible levels of these substances must be followed. Despite scientific advances that has the goal to standardize descriptions of coffee quality, it is still questioned, debated, and negotiated (Batista & Chalfoun, 2015).

Due to the economic impact of coffee around the world, research on coffee quality has become essential. Many studies have focused their efforts on understanding and improving it, and collectively they have shown that the variables that affect coffee quality and the approaches to measuring it are quite diverse. While there are several surveys and literature reviews that compile some of these studies, there is no literature that systematically summarizes and categorizes these aspects, making necessary a systematic mapping study that provides an overview of this research topic.

Systematic mapping is a methodology that is frequently used in medical research and software engineering, but less in the exact sciences and, as far as we know, never in coffee quality research. This is arguably due to limited knowledge of the method. As Petersen et al. (2008) indicated, a systematic mapping study provides a structure of the type of research reports and results that have been published in a research topic. This is conducted by categorizing them and presenting a visual brief of the results, i.e., the map. It often requires less effort while providing a more coarse-grained overview, identifying research gaps by graphing, showing in which topic areas and for which research types there is a shortage of publications. Additionally, it gives indications for lack of evaluation or validation research in certain areas. Systematic maps are therefore primarily concerned with structuring a research area (Petersen et al., 2015).

In this study a manual systematic mapping of 1,470 articles was performed with the goal of identify the studies most relevant to the research area of coffee quality and to investigate which quality attributes in coffee are the most popular in scientific literature for assessing the different stages of the production-to-consumer chain.

Specifically, the contributions of this paper are as follows:

- Identification of the quality measures used the most in coffee science.
- Identification of the principal objectives in the study of coffee.
- Identification of the methodologies that are the most common in coffee quality research.
- Identification of the species of coffee most investigated.
- Identification of the most common type of coffee samples used in coffee quality research.
- Identification of the stages of the production to consumer chain most studied by researchers for coffee quality evaluation.

8. Method

2.1 SYSTEMATIC MAPPING PLANNING

The mapping was conducted following the guidelines of Petersen et al. (2008). (i) Definition of the research questions, (ii) search for relevant papers using appropriate databases like Scopus and the search strings defined by the research questions, (iii) screening of the papers to see which are relevant applying inclusion and exclusion criteria, (iv) keywording using the abstracts to obtain a classification scheme consisting of facets based on the research questions, (v) data extraction after the sorting of the abstracts into the classification scheme; lastly, the results are analyzed based on the research questions. The general process followed is summarized in the flowchart shown in Figure 1. It includes the number of papers found after the search, the number of total papers used for the systematic mapping (relevant papers) and the number of facets in the classification scheme.

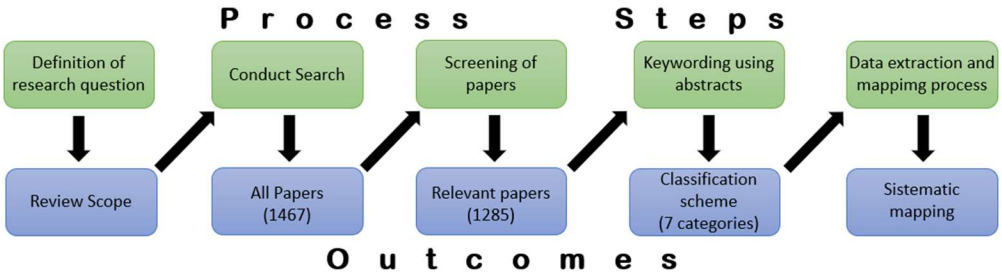

Figure 1. The systematic mapping process. Adapted from (Petersen et al., 2008)

2.1.1 Definition of research questions

The research questions is the most important part of any systematic study (Kitchenham & Charters, 2007). The research questions are the base for the entire methodology and cover the next aspects:

- The search process must identify primary studies that address the research questions.
- The data extraction process must extract the data items needed to answer the questions.
- The data analysis process must use the data in such a way that the questions can be answered.

The goal of this systematic study was focused to find the coffee quality studies and measures in relation with the production-to-consumer chain. The research questions involved as the search was conducted, the scope type of the samples and species of studies coffee and mainly, the coffee quality.

RQ1: What types of research papers have been published in coffee quality?
RQ2: What quality attributes have been considered for coffee using experimental data?
RQ3: What is the main objective of the research in terms of coffee quality?
RQ4: What kind of techniques have been used for measuring the coffee quality?
RQ5: What species of coffee are being investigated?
RQ6: What kind of samples are used in the measurement of quality?
RQ7: What stages of the coffee chain production are being investigated?

2.1.2 Definition of the scope

The search is conducted by creating appropriate search strings and using them on scientific databases to identify the primary studies (Petersen et al., 2008). As was broadly mentioned before, the quality of coffee is a complex subject, and it is measured by multiple ways. Therefore, this was reflected by the multiple search strings applied in the process. The search strings were changing as the search was producing results as more keywords were added. First, from all the research question were formulated the groups of keywords that are shown in **Table 1**.

Table 1: Keywords based on the research questions

RQ	Keywords
2	Coffee, quality, coffee quality, cup quality, beverage quality, metabolites, flavor, bean quality
3	Correlation, discrimination, improve, measure, properties
4	Infrared, chromatography, PCR, RMN, sensory, chemical, antioxidant, mycotoxins, ochratoxin, pesticide, fungicide, yeast, caffeine, trigonelline, chlorogenic, sugars, lipids, metabolites, metals
5	Arabica, Canephora, Liberica
6	Roasted coffee, green coffee, brew, coffee cherries, espresso.
7	Pre-harvest, Post-harvest, rust, pest, harvest, post-harvest, dehulling, depuping, drying, fermentation, *hampei*, organic, storage, soil, washing, defects, origin, specialty coffee

The search strings were structured using the PICO framework: Population, Intervention, Comparison and Outcomes Kitchenham and Charters (2007). This method is applied to identify keywords and formulate search strings from the research questions. This method was used to better manage the keywords presented before.

Population refers to the scope of the study. Based on the study aim, the population is empirical research in coffee quality. Therefore, two basic keywords of "coffee" and "quality" were extracted from the population part.
Intervention refers to a methodology, tool, technology, or procedure that addresses a specific issue. In the context of this study, it includes terms like sensory evaluation and chromatography.
Comparison indicates the methodology/tool/technology/procedure which the intervention is compared. In this study different type of samples used in the intervention are compared as green coffee, roasted coffee, arabica coffee, etc.
Outcomes implies the factors of importance to practitioners as improved reliability, reduced production costs, and reduced time to market. In this case the different objectives for measuring coffee quality were investigated by means of identifying the different strategies that have been used.

Keywords for the search string can be taken from each aspect of the PICO structure. The identified keywords were grouped into sets and their synonyms were considered to formulate the search string.

Set 1: Scoping the search for coffee quality, i.e., ''coffee quality''.
Set 2: Search terms directly related to the intervention, e.g., ''sensory evaluation'', ''chromatography'', "infrared"
Set 3: Search terms related to the comparison, e.g., "green coffee", "roasted coffee", "coffee arabica".
Set 4: Search terms related with the outcomes, e.g., "discrimination", "improvement", "correlation".
Set 5: Search terms related to the process of coffee, e.g., "drying", "fermentation", "storage", "harvest".

2.1.3 Establishment of the search strategy

The Scopus scientific database was used in this study due accessibility provided by authors, in addition Scopus is considered the largest and most complete scientific databases for conducting literature reviews, relevance in science and the most relevant electronic databases in food science (Salama et

al., 2017). This database supports nested Boolean operators and searching for titles, abstracts, and keywords. The search in the database was conducted between July 2020 and January 2021.

2.2 SEARCH EXECUTION

The resulting keywords from the PICO method were used in the Scopus databases, as mentioned before. These strings were formulated using a logical OR between synonyms and with a logical AND between the sets; for example: "cup quality" AND "infrared" OR "sensory evaluation" AND "roasted coffee" OR "green coffee" AND "discrimination" AND "fermentation". Due to the high number of keywords associated, the search strings are more complex generating less results, for example: coffee quality AND (green coffee OR roasted coffee) AND (discrimination OR improvement) AND (infrared OR chromatography OR chlorogenic) AND (fermentation OR drying OR harvest) got 3 results, while coffee quality AND (green coffee OR roasted coffee) OR (discrimination OR improvement) AND (infrared OR chromatography OR chlorogenic) OR (fermentation OR drying OR harvest) got 320 results. In this sense, shorter strings were used combining about two or three keyword sets, e.g., "coffee quality" AND ("mycotoxin" OR "ochratoxin") OR "Chromatography" AND ("green Coffee" OR "roasted coffee"). Applying the different search strings in these databases resulted in 1,467 hits in total.

All the bibliographic data, i.e. full texts and abstracts, were exported and stored using the reference management system Mendeley by Elsevier, for further analysis. Then, a sheet list with all the references was created, for further classification purposes.

2.3 SELECTION OF PRIMARY STUDIES

To get to the relevant papers from all the papers found during the search in the databases, it was needed to define inclusion and exclusion criteria. These criteria were used to exclude studies that are not relevant to answer the research questions and therefore are influenced by them. As Petersen et al. (2008) suggested it was also found important to add the exclusion criteria of papers that only refer to "Coffee quality" in the abstract introduction without addressing it any further or developing the idea. Following the inclusion and exclusion criteria, 182 papers were removed, remaining for the study 1,285 papers. Table 2 shows inclusion and exclusion criteria considered for the systematic mapping review.

Table 2: Inclusion and Exclusion Criteria

Inclusion criteria	Exclusion criteria
• Studies published online before 01/02/2021. • Studies evaluating and measuring coffee quality using at least one metric. • Papers with abstracts in English, Spanish, French and Portuguese. • Papers from the databases Scopus. • Books, papers, technical reports and grey literature describing coffee quality studies.	• The paper lies outside the coffee sciences domain (E.g., Economics). • Coffee quality is not part of the contribution of the paper, the terms related are only mentioned in the general introductory sentences of the abstract. • The paper presents no measurable outcome. • Literature that was only available in the form of abstracts and did not contain enough information to be considered for the classification scheme. • Duplicates.

2.4 DATA EXTRACTION AND CLASSIFICATION

The classification scheme is made based on keywords extracted from the abstracts to take the studies found into account. Following Petersen et al.'s (2008) criteria, it was made a pilot study on 200 papers were the keywording was made. The abstracts of this articles were read while looking for keywords and concepts key to the contribution of each paper. Then the keywords were combined in groups of concepts related to the research questions. Based on the pilot study it was constructed the mapping classifications for extracting data. After several iterations, the data extraction produced the nine classification groups shown in Table 3.

As mentioned before, in this study, nine main groups or facets were created. Each facet was associated with a topic area, for example quality metric, and was made up by the different categories derived from the keywording, in this case cup quality, bean quality, biochemical composition, soil quality, etc. And so forth goes the rest of the categories but the research type facet, which reflects the research approach used in the papers; this facet is general and independent from a specific focus area, allowing the comparison with other systematic mapping studies (Petersen et al., 2008).

The multiple category selections for each facet is stated in the last column. For instance, the value of the last column of RQ 7 (research type) is single. This shows that a paper could only be classified by a single research type. In contrast, the value of the last column of RQ 1 (quality metric) is multiple. This shows that one paper could have as quality metric more than one alternative (for example, cup quality and bean quality).

Table 3: Classification scheme

RQ	Facet	Categories	Multiple/Single
1	Research Type	Validation Research, Evaluation Research, Solution Proposal, Philosophical Papers, Opinion Papers, Experience Papers, Review Papers	Single
2	Quality Metric	Bean Quality, Cup Quality, Food Safety, Biochemical Composition, Soil Quality, Quality Perception, Crop Yield, Microbiological Composition, Physicochemical Properties, Crop Quality	Multiple
3	Quality Objective	Improve Quality, Measure Quality, Quality Correlation, Quality Discrimination	Multiple
4	Technique	Sensory Evaluation, Physical Evaluation, Analytical Chemistry Methods, Modeling, Microbiological Analysis, Molecular Biology Methods, New Technologies	Multiple
5	Coffee Species	Coffea arabica, *Coffea canephora*, *Coffea liberica*, Coffee, Hybrids, Blended Coffee, Not declared	Multiple
6	Coffee Sample	Green Coffee, Roasted Coffee, Instant Coffee, Brewed Coffee, Cherry Coffee, Coffee Plant	Multiple
7	Process' Stage	Pre-harvest Stage, Post-harvest Stage, Brewing Stage	Multiple
7	Pre-harvest Stage	Soil Conditions, Coffee Origin, Environmental Conditions, Crop Altitude, Shade, Genetic Traits, Coffee Breeding, Management Practices, Fruit Development	Multiple
7	Post-harvest Stage	Harvest, Processing, Drying, Sorting, Pretreatment & Additives, Storage, Packaging, Roasting, Grinding, Brewing, Quality Control	Multiple

From the classification scheme, all the abstracts were read, and the papers classified in the nine resulting groups. When abstracts do not contain detailed information required to properly classify an

article, the methodology section was read and, where necessary, the full paper was skimmed over. The facets used for the classification scheme in this study are explained in detail next:

2.4.1 Research Type

As Petersen et al. (2008) propose, it is taken into account that the general and topic-independent classifications allow to compare different systematic mapping studies from a similar perspective. The research type classification schema chosen for this work was the one proposed by (Wieringa et al., 2006) and summarized by Petersen et al. (2008), which consists of six research types. The research type called review papers was added to these six.

2.4.2 Quality Metric

Coffee quality can be defined in several ways; the definition depends on the step of the process the product is. So, the papers were classified considering all the measurements that can define coffee quality, e.g., bean quality, cup quality, biochemical composition.

2.4.3 Quality Objective

The papers were classified according to the objectives of the investigation in relation to coffee quality. This include improving quality as in coffee breeding; discriminate quality as the origin or the species of coffee; correlation of quality with different variables as different methods of fermentation; or measure quality as in sensory analysis or physicochemical properties of the coffee bean.

2.4.4 Technique

The papers were classified according to the kind of methodology used to research coffee quality. Papers with analytical methods that encases all processes requiring physicochemical measures; infrared and chromatography methods for example were encased in the instrumental chemistry classification; physical methods mainly includes the evaluation of coffee beans quality; sensory evaluation for the measure of the attributes of coffee beverage by tasters; modeling for papers that used multivariate methods or neural networks. Other classifications include studies that presented new technologies, microbiological analysis of coffee, molecular biology methods to study coffee quality.

2.4.5 Coffee Species

The coffee tree is a perennial plant belonging to the Rubiaceae family. The Coffea genus consists of 124 species (Davis et al., 2011), but commercially the most important are *Coffea arabica* (arabica coffee), *C. canephora* (robusta coffee), and *C. liberica* (Liberian or Liberica coffee, or excelsa coffee) that are used for beverage production, the two formers representing around 63% and 37% of the world production, respectively (Batista et al., 2016; Davis et al., 2006). The papers were classified by the species of coffee for which coffee quality was evaluated. This facet also includes hybrid varieties and coffee blends.

2.4.6 Coffee Sample

Studying the kind of samples used in the different studies of coffee quality and comparing how the results can vary is beneficial for decision-making for posing new studies, i.e., the measurement of

caffeine content in green coffee and in roasted coffee. The kind of sample also varies with the type of quality measurement, i.e., the sensory evaluation is only achieved in brewed coffee. The papers were classified according to the type of sample used for the study of coffee quality: cherry coffee, green coffee, roasted coffee, instant coffee, brewed coffee or coffee plant material.

2.4.7 Coffee processing stages

Musebe et al. (2007) reported that coffee quality is determined by 40% in the field, 40% at post-harvest primary processing and 20% at secondary processing and handling practices. That is, the tacit parameter is that 40% of the quality is due to pre-harvest factors and the remaining 60% by post-harvest procedures. While Folmer (2014) affirms that it is needed to view in a holistic way; like an orchestra where different players come together, and it is only by playing together that they can provide the highest quality. In addition, Louzada Pereira & Rizzo Moreira (2021) defend that this relationship does not exist and that the two lines are blurred, proposing a relationship of equality or multiple correlation between various phenomena. In this study, to better understand the focus of the papers, it was necessary to separate the different stages of coffee processing. Due to the significant number of possible classifications, the processing category was divided into pre-harvest and post-harvest stage. The pre-harvest stage category includes all variables of the crop handling, and post-harvest stage, starting at the harvest and continuing with all the primary and secondary processing and handling practices. The papers were classified according to the steps of the coffee processing in production-to-consumer chain involved in the study of coffee quality.

2.4.7.1 Pre-harvest. The interaction of pre-harvesting variables shape the overall quality attributes of coffee (Hameed et al., 2018). It is critical to coffee quality that the crop needs be met with the use of essential agricultural practices that influence production and productivity, as are phyto-technical practices, the use of improved cultivars, the control of pests and diseases, soil correction, fertilization, mineral nutrition and irrigation (Pereira De Oliveira et al., 2012). These factors are mostly related to agricultural variables ranging from the selection of a suitable geographical location to soil management and genetic material (Hameed et al., 2018; Wintgens, 2004). The papers were classified according to all the steps of the pre-harvest stage.

2.4.7.2 Post-harvest. The post-harvest practices are critical steps determining the coffee beverage quality, making the beans suitable for transport and roasting. These methods involve removal of the waste from the crop and taking off the outer layers of the beans (G. V. de M. Pereira et al., 2017). These steps have an important part in guaranteeing the changes of the perishable coffee cherries into more stable green coffee beans, with a moisture content of 10–12% to avoid undesired fermentation (Hameed et al., 2018). Post-harvest management activities conduct to obtain suitably dried coffee beans for roasting and significantly contribute to the quality of the coffee beverage (Haile & Hee Kang, 2020). This process changes the chemical composition of green coffee beans that directly or indirectly influences the quality and end products (Sunarharum et al., 2014; Wintgens, 2004). These activities involve a series of steps including cherry harvesting, de-pulping, fermenting, drying, storage, and others. The number of activities also varies according to the type of processing method (Haile & Hee Kang, 2020). Following post-harvest processing on farms, coffee beans can be transported to industrial plants, where semi-manufactured or finished products are obtained for

commercialization (G. V. de M. Pereira et al., 2017). The papers were classified according to all the steps of the post-harvest stage.

2.5 VALIDITY EVALUATION

To evaluate the validity of this mapping study, it were followed the suggestions by (Petersen et al., 2015) . Next it is discussed how the threats to the validity of this study were tackled.

Theoretical validity considers the quality of the sample of studies obtained from the population, and potential researcher bias in the study selection and data extraction and classification.

Study identification: it is possible that two mapping studies with the same subject select different sets of articles (Wohlin et al., 2013). To avoid that problem, it was used the PICO approach to systematically extract the keywords according to the objective of the research.

Data extraction and classification: the extraction of the data may introduce bias to the final results. To reduce this risk, it was performed over a fair number of papers reducing the risk of misclassified papers really affecting the final result. However, human judgment is generally prone to bias and cannot be completely eliminated.

Interpretive validity considers the validity of mapping study discussion and conclusions based on the results. This mapping study was solely relied on a quantitative analysis of the extracted data. The discussion and conclusions were drawn based uniquely on the quantitative results to reduce the problem of unclear conclusions.

Generalizability considers the degree to which the results can be generalized inside or outside of the studied population. By utilizing a systematic way to construct the search string, identify papers, and obtain results, besides the fact that this work provides a good population sample, it is considered that the results of this study can be generalized inside the population. However, since the study was designed for coffee quality using physical and chemical techniques, the results may not apply to other related populations, like health quality in general or medical research.

3 Results and discussion

In this section, the results of the classification of the selected studies are presented. Thus, this section is structured in terms of the classification provided in **Table 3**, which also corresponds to the answers to the research questions shown in section 2.1.1

3.1 RQ1: What types of research papers have been published in this area?

The RQ1 classified the primary studies according to the research methods categories that Wieringa et al. (2006) proposed, with an extra category added by the authors named review papers (**Figure 2**). The first category, with a 2.8%, is **validation research** and includes publications where the techniques investigated are novel and have not yet been implemented in practice. Techniques used are for example experiments, i.e., work done in the lab. E.g., a new technique to measure pesticides in roasted coffee (Da Silva Souza & Navickiene, 2019).

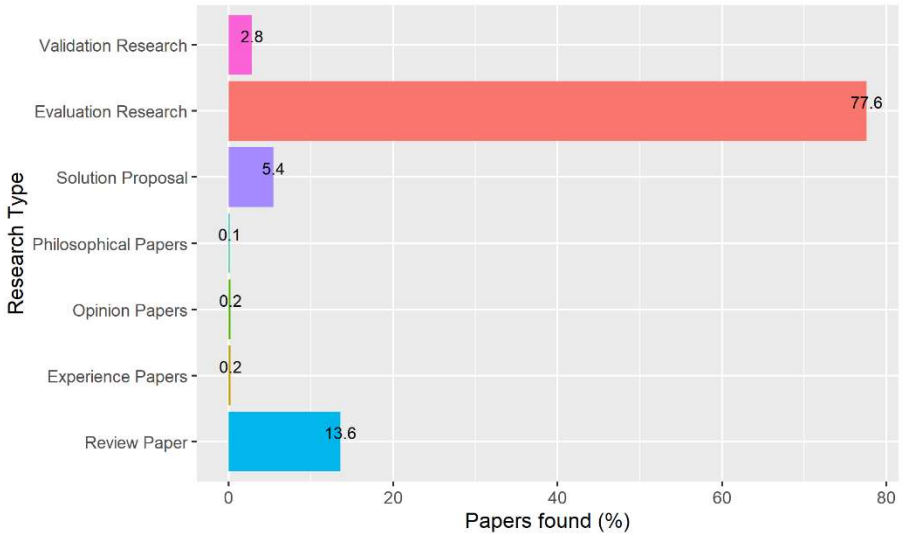

Figure 2. Percentage of the papers classified by research method

The majority of the primary studies (77.6%) are classified in the **evaluation research** category, consisting of papers that show techniques that are implemented in practice and an evaluation of the technique is conducted. That means, it is shown how the technique is implemented in practice (solution implementation) and what are the consequences of the implementation in terms of benefits and drawbacks (implementation evaluation). E.g., A spectroscopy technique to measure phenolic compounds in coffee and estimate sensory parameters (da Silva Araújo et al., 2021).

The **solution proposal** classification, with 5.4%, contains publications where a solution for a problem is proposed; the solution can be either novel or a significant extension of an existing technique. The potential benefits and the applicability of the solution is shown by a small example or a good line of argumentation. E.g., a new fermentation method to improve coffee quality evaluated by metabolomics (Aditiawati et al., 2020).

The next category, **philosophical papers**, includes papers that sketch a new way of looking at existing things by structuring the field in form of a taxonomy or conceptual framework. From the primary papers, only one (0.1%) fill the requirements: Development of a "living" lexicon for descriptive sensory analysis of brewed coffee (Chambers et al., 2016).

Only three papers (0.2%) where classified in the **opinion papers** category (Blanc, 2006; Folmer, 2014; Trench, 1936). These papers express the personal opinion of somebody whether a certain technique is good or bad, or how things should be done.

The **experience papers** category also contains only three papers (0.2%) (Aristizábal et al., 2012, 2017; Chalfoun, 2010). Experience papers explain on what and how something has been done in practice. It must be the personal experience of the author.

The last category is **review papers** with 13.6% of the primary studies. This category encases all papers and book chapters that do not present original research but that summarize the existing literature or knowledge on a given topic and that generally provide a critical evaluation.

3.2 RQ2: What quality attributes have been considered for coffee using experimental data?

To answer the RQ2 the quality metric facet was created and contains ten categories (**Figure 3**).

The **bean quality** category with 20.6% of all the papers, includes several ways of measuring the quality of the coffee beans. For cherry coffees, quality is evaluated based on the maturity of the fruit. For green beans it encases the physical quality of the coffee beans; aspects like size, density, color. But mainly it refers to the presence of defects found in certain coffee batches such as deviations in odor, color, size and shape of beans; and foreign bodies present in a relative amount of green coffee sample (Batista & Chalfoun, 2015).

The **cup quality** category, with a 39.3%, is a category that encases articles where the quality metric includes the attributes of coffee beverage that are distinguishable by senses and are usually assessed by professional coffee tasters. The results are expressed with a set of established terminologies like flavor, acidity, body and cup cleanness, etc. (Tolessa et al., 2016).

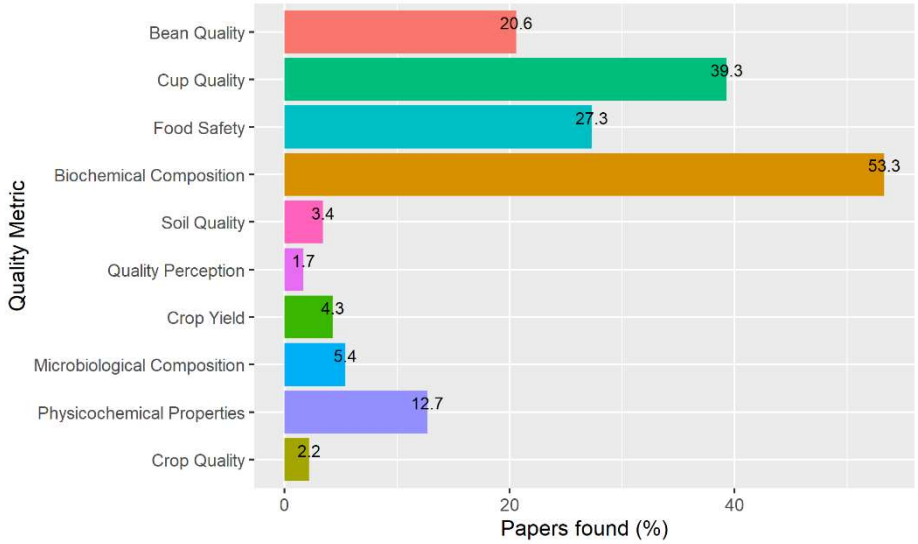

Figure 3. Percentage of the papers classified by coffee quality metric

The **food safety** category, with a 27.3%, includes papers that show the study or measurements of contaminants in coffee, whether they are originated from external sources like pesticides and mycotoxins or internal sources like acrylamide, that is produced during coffee roasting.

With most of the articles falling in this category, the **biochemical composition** category with a 53.3%, contains all papers that show research dedicated to the study or measurement of primary (sugars, lipids, etc.) and secondary metabolites (caffeine, chlorogenic acids, etc.) in coffee; also measurements of enzymatic and antioxidant activity.

The **soil quality** category, with 3.4 %, consist of publications that study or measure soil conditions and/or physicochemical properties from coffee crops.

In the category of **quality perception**, with 1.7%, are the papers that center on studies of how external input reflects on the cup quality perceived by consumers (design of the package, shape and color of the cup).

The **crop yield** category got 4.3% of the primary studies and includes those that use the coffee crop yield as a measure of quality; the harvest of a coffee plant represents the actual yield.

The next category is **microbiological composition** with 5.4% of the publications that have microbiological measurements in any stage of the coffee process (yeast, fungus, microbial communities).

With 12.7% the **physicochemical properties** category counts with papers that include physicochemical measurements or characterization in any stage of the coffee process (pH, moisture content, metals content).

Finally, around 2.2% of the **crop quality** category comprises publications that consider as a quality metric the health of the coffee tree.

3.3 RQ3: What is the main objective of the research in terms of coffee quality?

The RQ3 lead to the formation of the quality objective facet (**Figure 4**)

A 10.1% of the primary studies were classified in the **improve quality** category that includes all articles that have the objective of enhancing at least one measure of coffee quality, e.g., articles where the fermentation process variables are modified to improve coffee cup quality (Wang et al., 2020).
A 26.6% of the articles were classified in the **measure quality** category, in which the main objective of the research was the measuring of some quality metric. This includes articles with the characterizations of new coffee varieties, cultivars or genotypes (Lemos et al., 2020) or articles with new or modified techniques for the measuring of quality, e.g., New techniques for the measuring of ochratoxin A levels (Han et al., 2021).

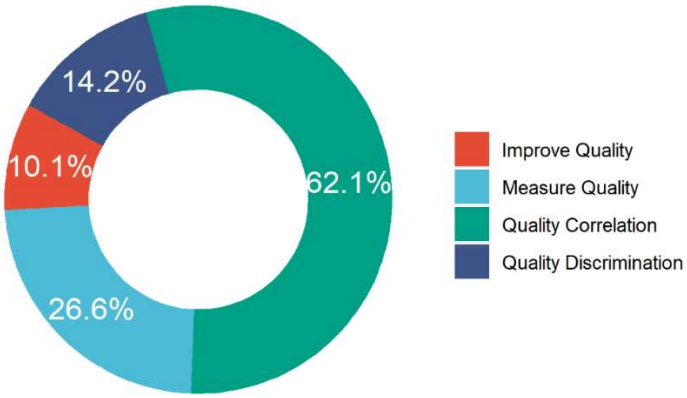

Figure 4. Percentage of the papers classified by coffee quality objective

Most of the articles were classified in the **quality correlation** category with 798 articles and 62.1%. The principal objective of these articles correspond to study the behavior of the different variables that affect coffee quality during all the processes it goes through, and the correlation between them,

e.g., how the biochemical content of coffee and therefore the coffee cup quality varies with the method of preparation of the coffee beverage (Bobková et al., 2021) or with the coffee roasting technique (Bolka & Emire, 2020). This category also includes how the implementation of new techniques in coffee processing affects different coffee quality measures, e.g., how different drying techniques affects biochemical composition (Dong et al., 2017).

Finally, the **quality discrimination** category stands with a 14.2 %. This category encloses all the articles based on different kinds of data and variables, where coffee samples can be discriminated by some quality metric, origin, type of coffee, etc. E.g., discriminating traditional or specialty coffee based on data from their IR spectrums and chromatographic measures (M. B. Abreu et al., 2020).

3.4 RQ4: What kind of techniques have been used for measuring the quality?

This facet, result of the RQ4, classified all the publications according to the type of technique used to study coffee and its quality (**Figure 5**).

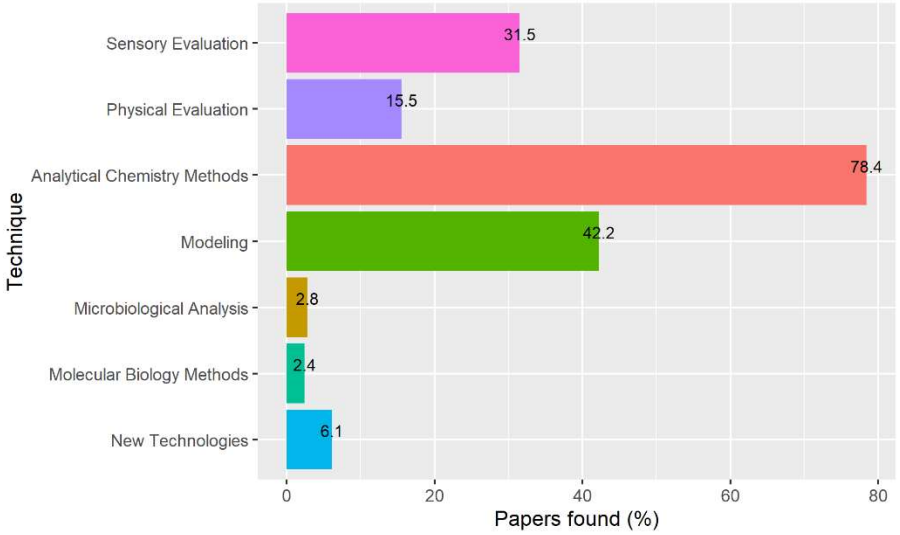

Figure 5. Percentage of the papers classified by technique of quality measure

Thus, the **sensory evaluation** category is comprised by publications where the technique used to measure coffee quality was the cupping method, and these amounted a 31.5% of the total. E.g., the evaluation of the sensory profile of the most cultivated Coffea canephora clones in the Western Amazon (Dalazen et al., 2020).

The category **physical evaluation** incorporates the 15.5 % of the primary studies in which the quality of coffee is measured by the assessment of the coffee grain, including techniques like sorting by size, color or defects, e.g., the effect of the shape and size of the bean on the cupping quality attributes of the beverage (Luna González et al., 2019).

From 1285 papers, 1007 (78.4%) are classified in the category of **analytical chemistry methods** indicating that in the investigation and measurement of coffee quality they were used instrumental chemistry techniques, as chromatography and spectroscopy, and/or laboratory analysis like acid-base

methods or potentiometry methods. As an example is the monitoring of coffee quality during storage using the raman spectroscopy technique (G. F. Abreu et al., 2019).

The **modeling category** is the second most important category in the techniques for studying coffee quality, with 42.2% of the total papers. It includes the use multivariate statistical methods, data mining, machine learning, and mathematical models in the data treatment juggling with the great number of possible variables directly involved in coffee quality. E.g., a computational model (based on users' tastes) recommends optimal coffee beans (De Berardinis et al., 2020).

The **Microbiological analysis** category has a 2.8% and involves papers that presents the use of techniques as microbial cultures, immunoassays, polymerase chain reactions (PCRs) for the identification, detection or enumeration of microorganisms in relation to coffee quality. E.g., the measurement of the microbiological characteristics of coffee inoculated with yeasts during the fermentation process (Da Silva et al., 2021).

The **molecular biology methods** category counts with 2.4% of the primary papers which involve techniques to explore the molecular basis of biological activity in relation to coffee quality; how molecules control cells, its processes and characteristics, activity and growth. E.g., the study of key galactomannan biosynthesis genes responsible for the accumulation of mannan storage polysaccharides on mature coffee seeds contributing to beverage quality (Ogutu et al., 2020).

The category **new technologies** includes 6.1% of papers that present innovative tools and techniques, a new app or machine, to measure coffee quality. E.g., the development of a sensor for temperature measurement in a coffee machine (Cosoli et al., 2020).

3.5 RQ5: What species of coffee are being investigated?

In order to answer RQ5 all the papers were also classified by the coffee species the research was centered on. **Figure 6** shows that 70.2% of papers study ***Coffea arabica***. ***Coffea canephora*** and **not declared** categories are closer together with 27.2% and 23.2%, respectively. The **not declared** category mainly contains articles where the species of the research are irrelevant and therefore not mentioned, e.g., the development of a method to measure pesticides contaminants in coffee.

Few papers carried on studies in **blended coffee** (around 4.2%) that is mixtures of varieties or species; **coffee hybrids** with 1.9% are genetically breeded coffee looking for a mix of characteristics, mainly from *Coffea arabica* and *Coffea canephora*.

Lastly, the coffee species ***Coffea liberica*** is the least studied with 1.1% of all the papers.

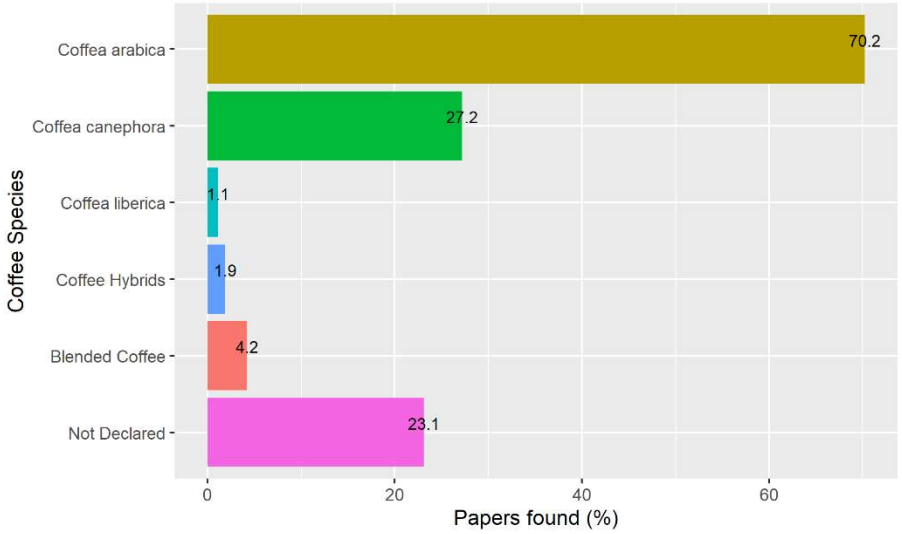

Figure 6. Percentage of the papers classified by the type of coffee species

3.6 RQ6: What kind of samples are used in the measurement of quality?

Answering what type of sample quality measurements are made on, six categories were produced (**Figure 7**). Coffee quality can be measured in the **coffee plant**, in the coffee fruit or **cherry coffee**, in the coffee seeds after processing the fruit that are called **green coffee**, in the roasted coffee seeds or **roasted coffee**, on processed roasted coffee producing **instant coffee**, or lastly on the beverage itself or **brewed coffee**. The most popular way to measure coffee quality in the reviewed publications is over **green coffee** (46.2%). This is followed by the **brewed coffee** and **roasted coffee** (40.0% and 37.9% respectively). The **cherry coffee** category encompasses 18.8% while **instant coffee** and **coffee plant** contain 6.6% and 6.5% respectively.

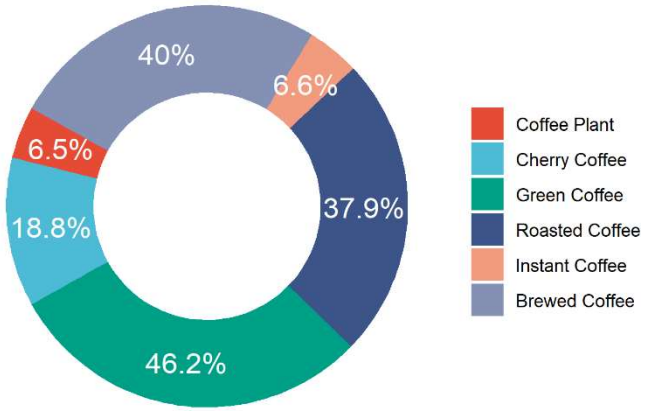

Figure 7. Percentage of the papers classified by type of samples used for quality measurements

3.7 RQ7: What stages of the coffee chain production are being investigated?

To answer this question three facets were created. The first one is **Process' stage**, that comprisses the **pre-harvest stage**, **post-harvest stage** and **brewing stage** categories (**Figure 8**). The first two categories, pre-harvest stage and post-harvest stage, became the other two facets, so each publication was classified in the specific step of the complex process that carries coffee from the farm to the cup.

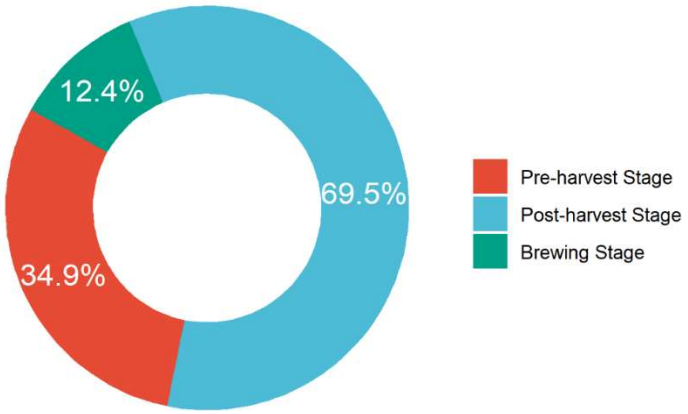

Figure 8. Percentage of the papers classified by the stage of the production chain

The first facet, process's stage, reveled that most of the publication are centered in researching the post-harvest stage with a 69.5% in this category. Then, pre-harvest stage with 34.9% and the brewing stage with 12.4%.

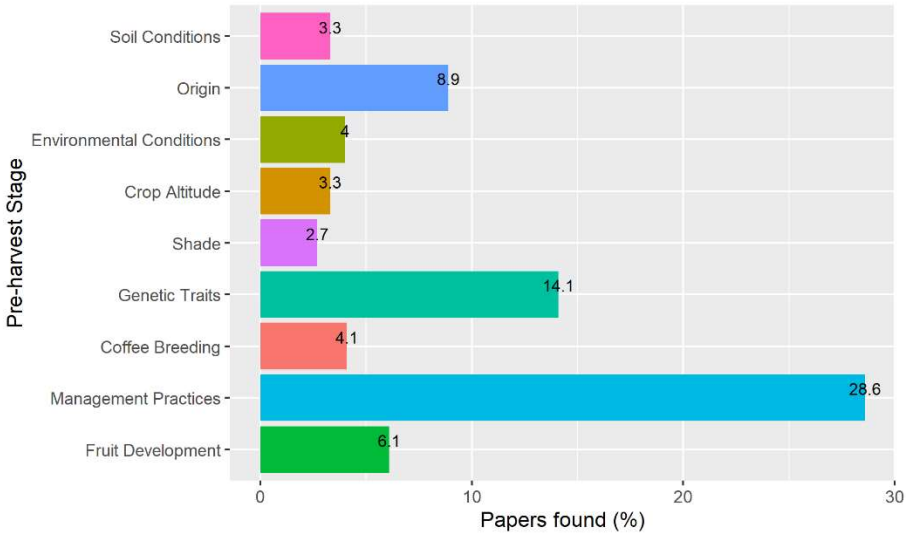

Figure 9. Percentage of the papers centered on the pre-harvest stage

The pre-harvest stage facet consists of nine categories that encompasses the processes that are prior to the harvest (**Figure 9**).

The first category, with 3.3%, is **soil conditions** and consist of papers that study the soil properties (Huyen et al., 2018), physicochemical attributes (Marcheafave et al., 2020), nutrition (Yadessa et al., 2019), fertility (Kouadio et al., 2018), microbiological conditions (Veloso et al., 2020), even soil color (do Carmo et al., 2016) and how it affects coffee quality.

The category **origin** (8.9%) includes publications that compare the origin of coffee with the respective quality (Badmos et al., 2020).

The **environmental conditions** category with 4% consist of publications that study how the climate (Rolim et al., 2020), the rainy and dry seasons (Yang et al., 2013), climate change (C. A. F. dos Santos

et al., 2015) and factors like relative humidity, temperature and water activity (Astoreca et al., 2010; Borém, Luz, et al., 2019; G. Oliveira et al., 2019) can affect the coffee quality.

Crop altitude (Girma et al., 2020), terrain aspect (P. V. Pereira, Silveira, et al., 2021) and slope (Avelino et al., 2005) and how this affects the coffee quality correspond to 3.3% of papers.

The shade category includes papers that investigate types of trees (Prado et al., 2018) and shade cover percentage of the coffee crop (Durand-Bessart et al., 2020) with 2.7%.

Genetic Traits (14.1%) is a category that includes all genetic characteristics of the coffee plant and fruits (Pérez-Molina et al., 2021), the comparison of coffee species (Wirani et al., 2016) and characterization of new varieties (Abubakar et al., 2019), genotypes (Barbosa et al., 2019) and cultivars (Barbosa et al., 2020).

The coffee breeding category (4.1%) consist of papers that study the development and resulting coffee quality of new varieties (van der Vossen et al., 2015), cultivars (Sera et al., 2020), genotypes (Barbosa et al., 2019) or germplasms (Venial et al., 2020).

Management practices is the category that contains most of the papers in the pre-harvest facet with 28.6%. This category involves all publications that study the management of the coffee crop and includes practices like fertilization (Dias et al., 2018), plague management and control (D. R. Pereira et al., 2021) as well as measurements of pesticides and mycotoxin contaminants (Reichert et al., 2018), agroforestry practices (Correia et al., 2020), and specifically the practices around the production of specialty coffees (Belchior et al., 2020), traditional coffees and organic coffees (M. B. Abreu et al., 2020).

Finally, the fruit development category (6.1%) contains of all the papers interested in the coffee maturation stages (de Oliveira Aparecido et al., 2018), including flowering (Masarirambi et al., 2009).

The post-harvest stage facet consists also of nine categories around the processes that occur after the harvest, including the harvest itself (Figure 10).
The first category correspond to harvest (2.9%) which relate it with coffee quality (Tolessa et al., 2017).

The processing category, the second in percentage with a 19.1%, includes all publications that centers on all the different steps that lead the coffee fruit or cherry coffee to the dry seed or green coffee. There are two main methods used around the world: the wet method and the dry method. In the wet method, the cherry coffee is mechanically depulped to remove the skin, fermented to remove the mucilage, and then dried. It is mainly used for Arabica coffees and coffees of higher quality (Alves et al., 2017). In the dry method, the cherry coffee is first dried and then mechanically dehusked. This process is used for most Brazilian, Ethiopian, and Haitian Arabica coffees, and for Robusta coffee in most parts of the world (Alves et al., 2017). This category includes papers centered on the processes of drying (Leobet et al., 2020), fermenting (Martinez et al., 2021), wet (E. C. da S. Oliveira et al., 2020) and dry processing (P. V. Pereira, Bravim, et al., 2021) as a whole.

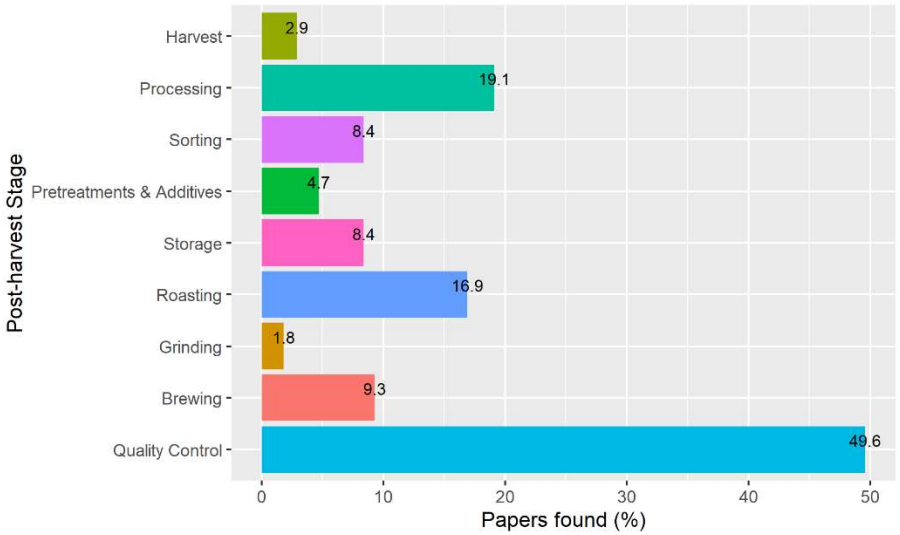

Figure 10. Percentage of the papers centered on the post-harvest stage

The **sorting** category (8.4%) focuses on papers that study coffee defects (Bigirimana et al., 2019), the differentiation of defective grains from non-defective (Habtamu & Belay, 2020), and grading, that is the physical classification of green beans using different sized holes meshes (Lingle & Menon, 2017).

The **pretreatment & additives** category (4.7%) includes processes over coffee to improve quality, like steaming the roasted coffee with defects (Kalschne et al., 2019), irradiation of green coffee to combat mycotoxins (Byun et al., 2020), the use of enzymes in the drying process (D. F. Santos et al., 2020) or to prevent the acrylamide contaminant produced during roasting (Corrêa et al., 2021), decaffeination process (Jeszka-Skowron et al., 2020), etc.

The **storage** category (8.2%) studies the different storage conditions and methods (Nadaleti et al., 2019), the different packaging methods and materials (Borém, Andrade, et al., 2019) and how they affect coffee quality.

The **roasting** category (16.9%) involves all the papers that relate the roasting methods and technologies (Bolka & Emire, 2020), roasting degree (Abubakar et al., 2020), speed (Toci et al., 2020), defects (Giacalone et al., 2019) and monitoring (Leme et al., 2019) and its effect on coffee composition (Williamson & Hatzakis, 2019) and quality.

The category **grinding** involves around 1.8% of the papers that study how the grinding grade (Lim et al., 2017) and particle size (Khamitova et al., 2020) affect the final quality and composition of roasted coffee.

The **brewing** category (9.3%) includes the different methods (Kwon et al., 2020) of brewing coffee like espresso (Apiletti et al., 2020), cold brew (Seninde et al., 2020), Turkish coffee (Ayseli et al., 2021), etc. and all the variables including temperature (Batali et al., 2020) and pressure (Ormaza Zapata et al., 2019) that affect the final quality of the beverage.

Finally, Quality control, the most populated category (49.6%) is transversal to all steps that occur after the harvest, from the cherry coffee to the brewed beverage. These publications include contaminant control (Chen et al., 2021), biochemical evaluation (Baqueta et al., 2021), sensory evaluation (L. F. B. Pereira et al., 2020), adulteration control (Milani et al., 2020), etc.

4 Conclusions

In this paper, we conducted a systematic mapping study on a body of literature that examines all aspects that affect coffee quality, in particular, the different measuring techniques, sample types, coffee species, and processing stages. First, the research questions were defined, from which a series of keywords strings were produced and then used in the Scopus database, resulting in 1470 studies. By applying inclusion and exclusion criteria, 1280 papers were selected for data extraction. As a result, it was found that the type of research papers published in the area is mainly of evaluation, where new techniques are implemented and evaluated. Furthermore, the most considered quality attributes using experimental data are biochemical composition, which involves the study and measurement of metabolites, followed by cup quality determined by cuppers. The main objective of the revised papers, in terms of coffee quality, is quality correlation, where different variables affecting coffee quality are contrasted. In addition, the most used techniques to measure quality were the analytical chemistry methods, in accordance with the main quality attributes measured, i.e., the biochemical composition. Likewise, the most investigated species of coffee is *Coffea arabica*, and the type of samples most used in the measurement of quality is the green coffee. Finally, among the three stages of the coffee production chain that are being investigated, the post-harvest stage is the more studied. From the pre-harvest stage, the most investigated processes are the management practices that include fertilization and pest control. From the post-harvest stage, the most researched process is quality control, which includes all the steps in every process dedicated to measuring the quality of coffee

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CHAPTER 3: Characterization of soils physicochemical properties from coffee crops with different agronomic practices

1. Introduction

The coffee cup quality and its sensory attributes depend on different factors such the genetic material the beans come from, the processing method of handling of the bean from harvest to the brew preparation, the environmental and soil conditions where the coffee plant is grown (Cheng et al., 2016; Yadessa et al., 2008). Coffee beans in ideal state of maturity, cultivated in soils of volcanic-origin and at high altitude are known to deliver the best sensory attributes in cup (Tolessa et al., 2017), however, it is not totally clear what is the real impact of each of these aspects and how they interact with each other to produce a certain cup quality profile (Morales-Ramos et al., 2020). Ideal soils for coffee should be deep, permeable, slightly acidic and porous (D'souza & Jayarama, 2006 cited in Yadessa et al., 2008). Also, literature show that volcanic soils often produce a cup of coffee with potent acidity and a good body, and a more balanced cup (Bertrand et al., 2006; Tolessa et al., 2017; Yadessa et al., 2008).

From a soil perspective, to achieve the optimum yield and quality of coffee, the nature and properties of the soil are of utmost importance (Tolessa et al., 2017). Soil fertility is a very dynamic process, affected by factors including organic matter content, the proportion of nutrients, cation exchange capacity (CEC), pH and soil texture and structure, etc. For example, soils with lower pH have a reduced number of binding sites for nutrients and are consequently generally less fertile. Or the higher the CEC the more fertile that soil may be, supplying nutrients to the plants and reducing soil erosion and protecting the soil against pH rapid changes. Furthermore, the soil organic matter (SOM) plays an important role for soil productivity and quality maintenance, promoting biological diversity, enhancing terrestrial ecosystems composition, acting as energy source, raising the soil's water holding capacity, aeration and infiltration, and if its content in the soil drops it can negatively affect soil fertility affecting the biological and physicochemical properties of (González-Ubierna et al., 2012; Kouadio et al., 2018; Martins et al., 2015).

On the other hand, plants generally require 16 or more essential nutrients for optimal growth and development. These nutrients are called essential because they have specific metabolic functions in plants. Macronutrients (N, P K, Ca, Mg, S) are needed in large quantities and are linked with their role in making up the bulk of the carbohydrates, proteins, and lipids of plant cells, whereas micronutrients (Fe, Cu, Mn, B, Mo, Zn, Cl) are required in small amounts and mostly participate in the enzyme activation process of the plant (Sadeghian Khalajabadi, 2008; Yadessa et al., 2019). Therefore, nutrients are important for vegetative growth of coffee trees and production of high quality beans, therefore nutrient imbalances can affect coffee quality (Njoroge, 1998). Also, the concentrations of microelements found in the soil generally determined the amounts in the leaf and in the green bean (Aluka et al., 2016). Deficiencies in nutrients have been related to lower quality coffees (Njoroge, 1998). Microelement concentrations and availability could be affected by the interaction between the various elements like soil texture and location, tree age and elevation, etc. (Aluka et al., 2016).

On the other hand, Rekik et al. (2019) concluded that coffee as well as the wine grapes could produce high-quality products with soils that not necessarily have high values of soil health indicators. Non

the less, for the production of quality coffee, the quality of the soil is still a very important factor, specifically the balance between the different nutrients due to its relation with the cup quality (Njoroge, 1998; Yadessa et al., 2008).

In this work, five coffee plots of volcanic soils, located in the Cajibío municipality, with coffee crops under different management practices (organic, traditional and special) are studied. The objective of this study is to compare the soil from coffee crops with different management practices through physicochemical variables (% OC, % MO, NH_4^+, NO_3^-, total N, P, pH, texture, % sand, % clay, % silt, Ca, K, Mg, Fe, Mn and CEC) in order to determine if they can be differentiated.

2. Methodology

2.1 SAMPLING SITE, SAMPLING AND STORAGE

The soil samples were collected at the municipality of Cajibío (Cauca) at 1700−1900 m above sea level. The location is about 14 km north of Popayán city (Cauca) with an average yearly temperature of 24 °C during the day and 14 °C at night and average yearly rainfall of 700 mm. Soil samples were collected in the harvest season of 2018. Five plots with different management practices: one for organic coffee (**P1**, 2°35'04.0"N 76°32'52.8"W), two for specialty coffee (**P2**, 2°35'10.5"N 76°33'14.5"W and **P3**, 2°35'11.0"N 76°33'02.7"W), and two for traditional coffee (**P4**, 2°35'02.2"N 76°32'49.3"W and **P5**, 2°35'16.2"N 76°33'03.7"W) were evaluated. The organic and specialty coffee´s plots were located at the same farm and the traditional coffee plots where from two neighboring farms (**Figure 1**). The coffee crops correspond to the *Coffea arabica* varieties *Bourbon* (P1)*, Tabi* (P2) and *Castillo* (P3, P4 and P5).

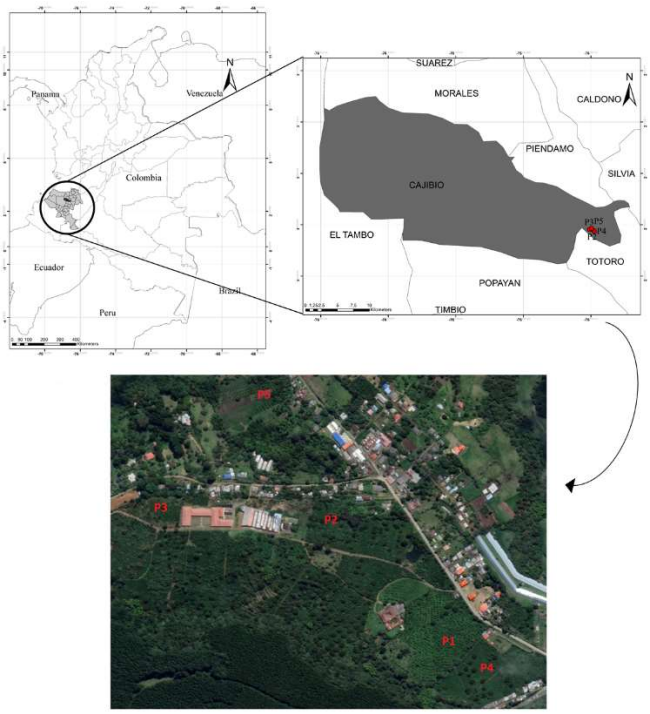


Figure 1. Plot's locations in the municipality of Cajibío (Cauca, Colombia)

The sampling plan for the soil consisted of dividing each of these plots into ten equal areas, and in each one of these ten areas, ten simple samples were collected to form a combined sample. Thus, there were a total of ten combined samples per plot. The procedures adopted for sampling consisted of taking soil samples at a depth of 0–20 cm within the projection of the plant canopy. The samples were dried at room temperature and away from sunlight by spreading them in carboard sheets. Then they were grinded and sieved (2 mm mesh). Finally, the samples were placed in plastic bags, which were kept at room temperature until analysis.

2.2 ORGANIC MATTER (OM) AND ORGANIC CARBON (OC) PERCENTAGES

The organic carbon and organic matter were determined by the Walkley – black method (Walkley & Black, 1934). 10 ml of 1 N potassium dichromate are slowly added to 0.5 g of soil (dry and sifted through a 2 mm mesh), stirring gently until a homogeneous mixture is obtained. Quickly, 20 ml of concentrated sulfuric acid are added, stirring vigorously for 30 seconds. The mixture is left to stand for 30 minutes. Then, it is diluted with distilled water to make up to 200 ml and allowed to cool. Next, 1 ml of phosphoric acid is added. A few drops of diphenylamine indicator are added to the resulting solution until a dark tone is obtained. Finally, it is titrated with a 0.5 N ferrous ammonium sulfate (FAS) solution.

The percentage of organic carbon is determined through the equation:

$$\%CO = \frac{(B - S) * N * 0.003}{W_t * 0.77}$$

Where,

B = volume of FAS used up for blank titration.

S = volume of FAS used for sample titration.

N = normality of FAS from blank titration

Wt = weight of soil sample

0.003 Stoichiometric factor, weight of a milliequivalent of carbon.

0.77 The recovery factor for this method is 77 percent, determined by Walkley

The percentage of organic matter is determined through the equation:

$$\%OM = \%OC * 1.724$$

2.3 AMMONIACAL NITROGEN (NH_4^+)

5 g of the soil (dried at 40°C and sieved through 2mm) were weighed in a falcon tube and 25 ml of KCl 2N were added. Next, the samples were shaken in a shaking machine Pall shaker, model PN 4821 (Portsmouth, United Kingdom), for one hour at 400 rpm and then filtered. 20 mL of the filtrate, 150 mL of water, a few drops of phenolphthalein and 0.1 g of calcined magnesium oxide are added to the distillation tube until the solution turns pink. The resulting solution was distilled using a K-355 Buchi distiller (Flawil, Switzerland) with 100% steam for 5 minutes. The distillate was collected in a wide-mouth container with 15 mL of a 4% w/v boric acid solution. Next, titration is performed with a 0.005N hydrochloric acid solution.

2.4 NITRATES (NO_3^-)

In a falcon tube, 1 g of soil sample (dried and sieved 2mm) and 20 ml of deionized water were added. The sample was homogenized and placed in an incubator at 40°C for one hour. Afterwards, the sample was centrifuged at 4500 rpm for 10 minutes in a Boeco centrifuge, model U-320R (Hamburg, Germany) and the supernatant was extracted from the falcon tube. The sample was allowed to acclimate to room temperature and was then filtered through a nylon acrodisc with a pore diameter of 0.45 µm.

The analyses were carried out on Dionex ICS-1000 Ion chromatograph, Thermo Fisher Scientific (Waltham, USA), consisting of an isocratic pump, an anion pre-column (Dionex AG14A, 4x50 mm), and anion separator column (Dionex AS14A, 4x250 mm) coupled with an anion self-regenerating suppressor (ASRS 300, 4 mm). The chromatographic separation was performed using 25.0 mmol/L KOH solutions as mobile phase at 0.25 mL/min. The operating parameters were: compartment temperature, 28°C; column oven temperature, 30°C; suppressant, KOH; conductivity of the mobile phase when stabilizing the equipment, <0.30 µS/cm; suppressor current, 19 mA; flow type, isocratic; analysis time, 18 min/sample.

2.5 TOTAL NITROGEN, KJELDAHL (TOTAL N)

0.5 g of dry and sieved soil (2 mm) were added in disintegration tubes. A Kjeldahl tablet and 8 mL of 98% sulfuric acid were added. The tubes were then heated up to 400 °C. The digestion end-point was defined when the samples turned green. The tubes were allowed to cool at room temperature. Then, the contents of the tubes were steam-distilled in a Buchi K-355 automatic distiller (Flawil, Switzerland), using 25 mL of a sodium hydroxide solution and 30 mL of water. The steam obtained from distillation was collected in an Erlenmeyer flask containing 100 mL of a boric acid solution and the Tashiro's indicator (methylene blue and methyl red). The distillation was carried out in an automatic distiller 848 Titrino plus Buchi (Flawil, Switzerland) with a solution of sulfuric acid 0.1 N.
Nitrogen contents in the samples were estimated through the equation:

$$Total\ N = \frac{(V1 - V2) * N * 0.014}{Ws}$$

Where,
V1 = Volume of sulfuric acid spent during sample titration (mL)
V2 = Volume of sulfuric acid spent during blank titration (mL)
N = Normality of the sulfuric acid used in the titration
Ws = Weight of the sample (g)
0.014 Nitrogen equivalent weight conversion factor (meq)

2.6 AVAILABLE PHOSPHORUS IN SOILS (P)

The Available phosphorus in soils was determined by the modified Bray II Method. 3 g of dry soil (homogenized and sieved through a 2 mm opening mesh) were weighed in a falcon tube. 20 mL of Bray II extracting solution (NH_4F 0.03 N + HCl 0.10 N) was added. Then, the sample shaken vigorously for 40 seconds and then filtered. An aliquot of 1 mL was taken from the soil extract and placed in an Erlenmeyer flask. Subsequently, 6 mL of distilled water and 2 mL of chloromolybdic acid were added and stirred. Then, 1 mL of diluted stannous chloride was added and stirred. After about 10 minutes the solution turns blue, the colorimetric intensity of which increases with the degree of

concentration. The samples were analyzed in an Evolution 300 uv/vis Spectrophotometer, Thermo Fisher Scientific (Waltham, USA), at 630 mm.

$$P\left(\frac{mg}{kg}\right) = \frac{(A-B)-\mathrm{I}}{M} * \frac{20}{W} * \frac{100+pw}{100} * Df$$

Where,
A = Sample absorbance
B = Blank absorbance
I = intercept of the calibration curve
M = Slope of the calibration curve
W = Weight of the soil in grams
Pw = Percentage of humidity in the dry soil at 105 °C
Df = dilution factor

2.7 SOIL PH

Soil pH was measured at a soil:water ratio of 1:1 (weight/ weight). 10 g of air-dry and sieved soil (2mm) and 10 mL of deionised water were shaken together for 30 sec and then left to settle for 30min. Then, the pH was determined with a pH meter WTW Inolab pH 7110 (Mexico city, Mexico).

2.8 SOIL TEXTURE

Soil texture was determined by the Bouyoucos Method. The determination of soil texture consists of the separation and quantification in percentage of particles with a diameter of less than 2mm in a soil sample, which can be separated into sand, clay and silt. Based on this result, the textural triangle is consulted to obtain the textural class of the sample. The FAO and the USDA classify the particle sizes as follows: the clay fraction is less than 0.002 mm, the silt between 0.002 and 0.05 mm and the sand between 0.05 and 2 mm (Corral-Pazos-de-Provens et al., 2022).

Every sample should be air dried and passed through a 2mm sieve prior to analysis. 60 g of soil was weighed into a beaker and 40 mL of hydrogen peroxide was added to remove organic matter. It was set aside to evaporate. This procedure was repeated until there was no more effervescence. After removing the organic matter and drying the soil sample, 50 g were weighed. Water was added to cover the surface and 40 mL of sodium hexametaphosphate 50g/L was added and allowed to set for 15 minutes. Mechanical stirring was then started for 5 minutes. The content was transferred to a 1 L graduated cylinder and distilled water was added to complete the liter, with an ASTM 152H Hydrometer, Gilson (Columbus, USA) inside the suspension. To make a reading, the hydrometer was removed, and the soil suspended with a hand shaker, shaking for one minute. Then, the hydrometer is placed back in the cylinder without disturbing the suspension and readings are taken at 40 seconds and at 2 hours after the manual dispersion has finished. The percentages of clay, silt and sand are determined through the next equations:

$$\%\, Clay + \%\, Silt = (reading\ at\ 40s) * 2$$
$$\%\, Sand = 100 - (\%\, Clay + \%\, Silt)$$
$$\%\, Clay = (reading\ at\ 2h)\ *\ 2$$

With this percentages the corresponding texture is determined in the texture triangle.

2.9 CATIONIC EXCHANGE CAPACITY (CEC)

The cationic exchange capacity was calculated by taking the final values in cmol/kg of the bases of the sample, analyzed by Atomic Emission Spectrophotometry. 5 grams of dry soil (sieved through a 2 mm mesh) were weighed and 40 ml of 1M ammonium acetate were added. It was left overnight. Next, it was vacuum filtered and the filtrate was transferred to a 200 ml flask where it was made up to volume with distilled water. Ca, Mg, Na and K were determined by microwave plasma induced Atomic Emission, MP-AES 4200 (Santa Clara, USA). The data was converted to cmol/kg by dividing the ppm of each base by the equivalents of each element. An equivalent of one of these bases results from dividing the atomic weight of the base by its valence (Juo et al., 1976).

$$\text{ppm of Mg}/120 = \text{Mg cmol}^{+}\cdot\text{kg}^{-1}$$
$$\text{ppm of Na}/230 = \text{Na cmol}^{+}\cdot\text{kg}^{-1}$$
$$\text{ppm of Ca}/200 = \text{Ca cmol}^{+}\cdot\text{kg}^{-1}$$
$$\text{ppm of K}/390 = \text{K cmol}^{+}\cdot\text{kg}^{-1}$$

2.10 MICRONUTRIENTS

Micronutrients were determined by the Mehlich-1 double acid procedure (Mehlich, 1953). Five grams of soil were added to 25 ml of Mehlich I solution (0.05N HCl + 0.025N H_2SO_4), which was stirred for 15 min and then filtered. The metals Cu, Fe, Mn and Zn were determined by a Microwave Plasma Atomic Emission Spectroscopy MP-AES 4200, Agilent (Santa Clara, USA).

2.11 STATISTICAL ANALYSIS

All data was analyzed by descriptive analysis to describe the data set. Each variable was evaluated with an ANOVA so all data was tested for normality with the Kolmogorov-Smirnov test, Levene's test for equality of variances and independence of residuals by a graphic method. Fisher's least significant difference (LSD) was used to identify which means were statistically different. For the analysis of all the data, principal component analysis (PCA) was conducted on all variables. Cluster analysis was performed with the Ward method and Euclidean distance. Statistical analyses were performed using the R-Project for Statistical Computing, R version 4.1.1 (Vienna, Austria) and Statgraphics XVI (The Plains, USA)

3. Results and discussion

Table 1 shows the means of all the soil properties measured and their standard deviations. To compare the different coffee plots for each one of the measured variables it was applied an analysis of variance ANOVA and where necessary, it was applied power transformation to assure that the required statistical assumptions were met: normality, heteroscedasticity, independence of residuals (See Table 2).

Table 1. Mean and standard deviations of the parameters measured in the soils of the coffee plots with organic, traditional and special management practices.

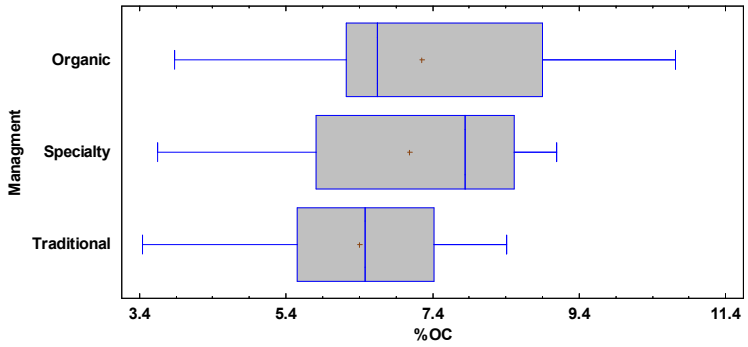
	Organic	Specialty	Traditional
%OC	7.25 (2.11)	7.08 (1.69)	6.40 (1.29)
%OM	12.50 (3.63)	12.21 (2.92)	11.03 (2.22)
NH_4^+ mg·kg^{-1}	29.16 (15.86)	36.69 (25.33)	34.93 (18.12)
NO_3^- mg·kg^{-1}	120.07 (10.51)	64.40 (17.05)	43.55 (9.06)
Total N g·kg^{-1}	0.59 (0.09)	0.53 (0.09)	0.53 (0.10)
P mg·kg^{-1}	14.50 (2.56)	12.33 (7.90)	19.05 (6.33)
pH	5.15 (0.17)	4.95 (0.20)	5.18 (0.37)
% Sand	72.59 (2.71)	69.82 (4.39)	69.66 (5.15)
% Clay	15.72 (2.76)	15.25 (4.72)	14.59 (3.50)
% Silt	11.63 (2.44)	15.52 (4.12)	15.98 (5.04)
Texture	Sandy loam	Sandy loam	Sandy loam
Ca cmol·kg^{-1}	2.30 (0.59)	2.58 (1.23)	4.68 (2.46)
K cmol·kg^{-1}	0.99 (0.24)	0.75 (0.15)	1.03 (0.26)
Mg cmol·kg^{-1}	1.38 (0.44)	0.93 (0.25)	1.42 (0.46)
Na cmol·kg^{-1}	< LOQ	< LOQ	< LOQ
CEC cmol·kg^{-1}	4.97 (1.10)	4.49 (1.34)	7.40 (3.02)
Zn cmol·kg^{-1}	< LOQ	< LOQ	< LOQ
Cu cmol·kg^{-1}	< LOQ	< LOQ	< LOQ
Fe mg·kg^{-1}	4.94 (1.45)	4.17 (1.76)	3.60 (1.58)
Mn mg·kg^{-1}	38.52 (16.30)	79.85 (49.76)	42.17 (28.33)

The method to determine which means are significantly different from others for each measured variable is Fisher's Least Significant Difference (LSD) procedure. With this method there is a 5.0% risk in saying that each pair of means is significantly different, when the true difference is equal to 0. This method is shown in tables with X's, where there are no statistically significant differences between those levels that share the same column of X's.

Table 2. p-values for the ANOVAs applied to all the soil properties measured, power transformations and the ANOVA's statistical assumptions: normality, heteroscedasticity, independence of residuals. * p-values < 0.05.

Variables	Power transformation	ANOVA (p-value)	kolmogorov-smirnov normality test (p-value)	Levene's test of homoscedasticity (p-value)	Independence of residuals
%OC		0.2924	0.979104	0.124338	Complies
%OM		0.2918	0.982596	0.12412	Complies
NH_4^+ mg·kg^{-1}	-0.5	0.6851	0.303142	0.438838	Complies
NO_3^- mg·kg^{-1}	0.5	0.0000*	0.146697	0.0674036	Complies
Total N g·kg^{-1}		0.1951	0.998916	0.934327	Complies
P mg·kg^{-1}	2	0.0161*	0.51465	0.118074	Complies
pH	-4.5	0.0372	0.894059	0.053889	Complies
% Sand		0.2052	0.054082	0.195692	Complies
% Clay		0.7362	0.214802	0.073953	Complies
% Silt		0.0304*	0.074964	0.088714	Complies
Ca cmol·kg^{-1}	-0.1	0.0004*	0.444485	0.105103	Complies
K cmol·kg^{-1}		0.0004*	0.801682	0.090032	Complies
Mg cmol·kg^{-1}		0.0004*	0.772844	0.082214	Complies
CEC cmol·kg^{-1}	-0.35	0.0003*	0.554593	0.056571	Complies
Fe mg·kg^{-1}		0.1136	0.474021	0.400472	Complies
Mn mg·kg^{-1}	-0.5	0.0647	0.596627	0.143711	Complies

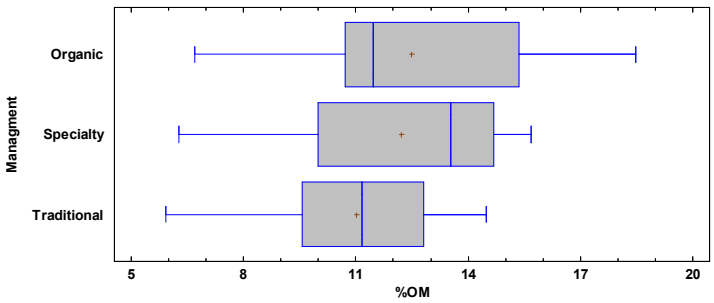
Soil organic carbon, the major component of soil organic matter, was high (> 6%) (Aksoy et al., 2016) for all the studied soils. The highest value (7.25 %) was found under organic management and the lowest value (6.40 %) was found under traditional management (**Table 1**). Management practices did not affect the organic carbon percentage (% OC). There is no statistically significant difference ($p < 0.05$) between the mean of % OC between one management and another (**Table 2**). Additionally, the % OC forms a homogeneous group, according to the alignment of the X's in one column (**Figure 2**). The highest variability was found for organic management.



Management	*Mean*	*Homogeneous groups*
Traditional	6.3965	X
Specialty	7.081	X
Organic	7.249	X

Figure 2. Box-plot and representation of LSD of the organic carbon percentage for the different management practices

So consecuently, the average soil organic matter was also high (>10%) for all the studied soils (Castro Franco & Gómez Sánchez, 2013). The highest value was 12.50 % for the organic management and the lowest 11.03 % for the traditional management (**Table 1**). The percentage of organic matter did not vary with the management practices with no statistically significant differences ($p > 0.05$) between the mean of the one management practice and another (**Table 2**). This was confirmed by the configuration of the X's, showing the means form a homogeneous group (**Figure 3**). The highest variability was found for organic management.

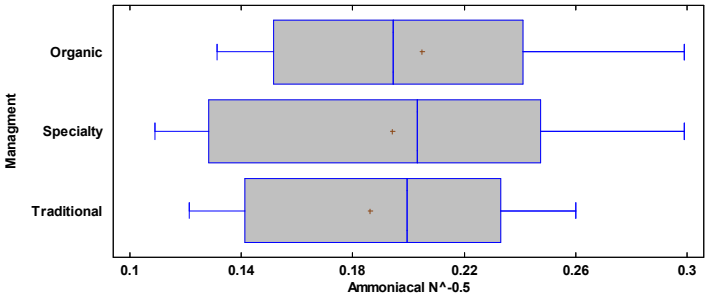


Management	*Mean*	*Homogeneous groups*
Traditional	11.027	X
Specialty	12.207	X
Organic	12.499	X

Figure 3. Box-plot and representation of LSD of the organic matter percentage for the different management practices

Ammoniacal-N is the form of nitrogen that is directly taken up by plants, but also is an important intermediate in the N reactions in soil (Chen, 1997). The highest concentration was from the soils of specialty coffee (36.69 mg/kg). The lowest concentration was from the soil of organic coffee (29.16 mg/kg) (**Table 1**). The NH_4^+ of the soils was not affected by the difference in management practices. There were no statistically significant differences ($p > 0.05$) between the means of the three

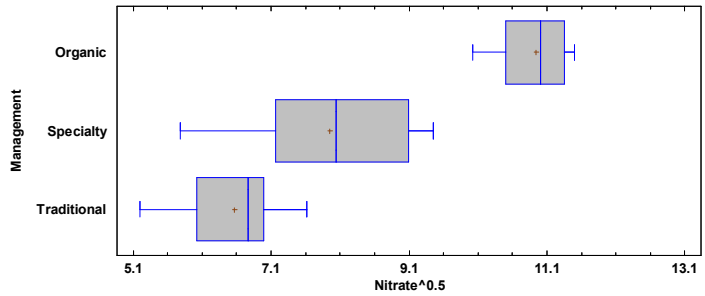
managements (**Table 2**) with the X's forming a homogeneous group (**Figure 4**). There was a high variability for this property for all three managements, especially for the specialty management.



Management	*Mean*	*Homogeneous groups*
Traditional	0.18632	X
Specialty	0.194208	X
Organic	0.20473	X

Figure 4. Box-plot and representation of LSD of the ammoniacal nitrogen for the different management practices

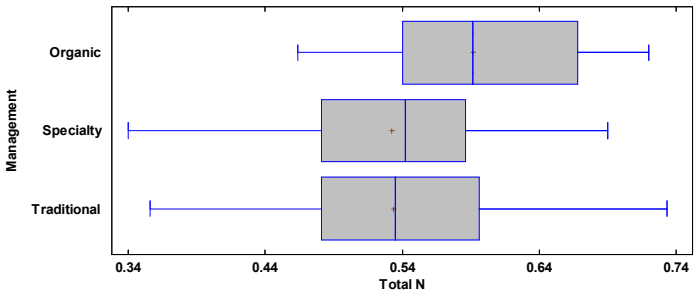
Nitrate-N is the other form of nitrogen which is available for plant uptake (Sullivan et al., 2011). The available nitrate was affected by the different management practices, with values of the same order as reported previously by Olaya et al., (2019) for soils from the same zone (68.77-105.02 mg·kg). Organic management soils had the highest nitrate concentration (120 mg·kg), while traditional management had the lowest value (43.55 mg·kg) (**Table 1**). Significant differences (p>0.05) were found when comparing the means (**Table 2**) and according to the alignment of the X's there were found three different groups, meaning that there is a statistically significant difference between the nitrate concentration in all three managements (**Figure 5**). There was also found a high variability in the specialty management data.



Management	*Mean*	*Homogeneous groups*
Traditional	6.56425	X
Specialty	7.95434	X
Organic	10.948	X

Figure 5. Box-plot and representation of LSD of the nitrate for the different management practices

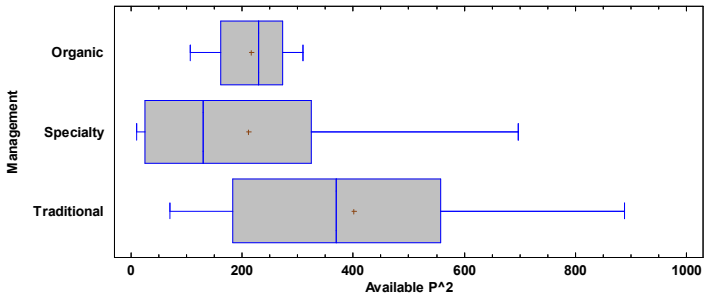
Total nitrogen analysis corresponding to N in all inorganic and organic forms (Sullivan et al., 2011). The total N content in soils at 0-30 cm is reported to be within a range of 0.15–2.18 g·kg^{-1} (Batjes, 2014). Cajibío (Cauca) soils are classified as andosols and lithosols (FAO & UNESCO, 2007; Rekik et al., 2018) with total N of 0.91 and 0.42 g·kg^{-1} respectively (Batjes, 2014). In this work the soils with the highest average of total N were form organic management with 0.59 g·kg^{-1} and the organic and traditional management soils have a tie with 0.53 g·kg^{-1} (**Table 1**). The total N content was not affected by the different management practices. There was no statistically significant difference (p>0.05) between the means (**Table 2**), and the X's form a homogeneous group being align in the same column (**Figure 6**).



Management	*Mean*	*Homogeneous groups*
Specialty	0.5326	X
Traditional	0.53345	X
Organic	0.5917	X

Figure 6. Box-plot and representation of LSD of the total nitrogen for the different management practices

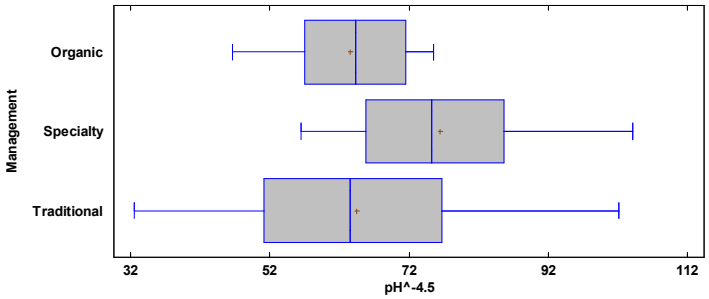
In relation to available P, the average values from the studied soils are low (10-20 $mg \cdot kg^{-1}$). Despite this, their values are within the edaphic levels (15-40 $mg \cdot kg^{-1}$) that correlate with the demands of coffee crops (Castro Franco & Gómez Sánchez, 2013). The management practices affected the P concentration, with the highest value of 19.05 $mg \cdot kg^{-1}$ for the traditional coffee plots and the lowest value of 12.33 $mg \cdot kg^{-1}$ for the specialty coffee plots (**Table 1**). The ANOVA showed that there is a statistically significant difference between the mean of Available P between one management level and another ($p < 0.05$) (**Table 2**). Available P in soils under traditional management differed significantly from organic and specialty coffee management, with the X's forming two homogeneous groups (**Figure 7**). The available P form the specialty coffee soils have the greatest variability.



Management	*Mean*	*Homogeneous groups*
Specialty	211.369	X
Organic	216.16	X
Traditional	400.996	X

Figure 7. Box-plot and representation of LSD of the available P for the different management practices

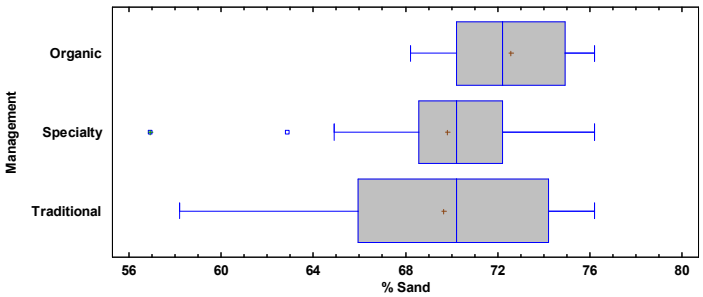
The values obtained of pH are classified between very strongly acidic (4.5-5) and strongly acidic (5.1-5.5) (Castro Franco & Gómez Sánchez, 2013). These also match to the ones reported from Rekik et al. (2018) of 4.8 and Olaya et al. (2019) of 4.92-5.16. Moreover, the pH values from the studied soils are within the edaphic levels (4.5 – 5.5) that correlate with the demands of the coffee crops (Castro Franco & Gómez Sánchez, 2013). The higher pH was from the traditional management with 5.18 and the lower pH was from the specialty management with 4.95 (**Table 1**). The pH was also affected by the management practices with the p-value of the ANOVA (**Table 2**) less than 0.05, showing that there was at least one mean statistically different. Also, the X's presented two groups with specialty management differing from the other two management types. Traditional management had the highest data variability (**Figure 8**).



Management	*Mean*	*Homogeneous groups*
Organic	0.000634919	X
Traditional	0.000644833	X
Specialty	0.00076451	X

Figure 8. Box-plot and representation of LSD of the pH for the different management practices

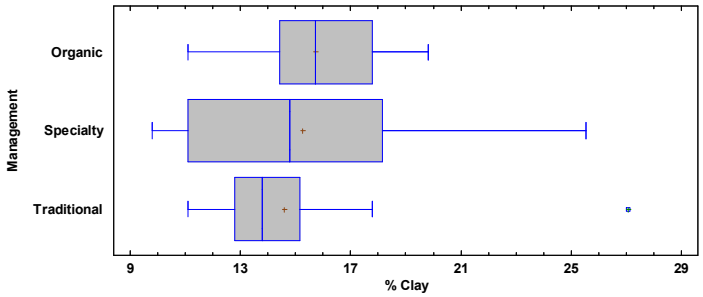
The soil texture of all the studied plots resulted as sandy loam with some patches of sandy clay-loam. Sand percentage was higher (72.59 %) for the organic coffee soils and lower (69.66 %) for the traditional management (**Table 1**) and was consistent with the values reported by Olaya et al. (2019) of 70.88 % - 72.90 % for soils of the same zone and use. It was found that the sand percentage did not have significant differences (p >0.05) between the management practices (**Table 2**) with a homogeneous group formed by the alignment of the X's in one column (**Figure 9**). The highest data variability was found for the traditional management.



Management	*Mean*	*Homogeneous groups*
Traditional	69.662	X
Specialty	69.824	X
Organic	72.594	X

Figure 9. Box-plot and representation of LSD of the sand percentage for the different management practices

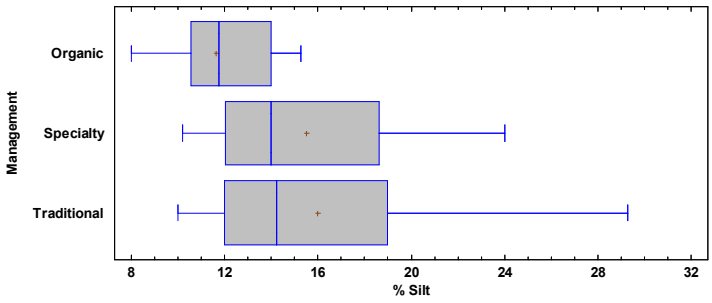
The clay percentage was higher for the organic coffee plot (15.72 %) and lower for the traditional management plots (14.59 %) (**Table 1**). No significative differences were found between coffee managements (p > 0.05) (**Table 2**) with the X's under the same column denoting a homogeneous group (**Figure 10**). The highest data variability was found for specialty management.



Management	*Mean*	*Homogeneous groups*
Traditional	14.587	X
Specialty	15.248	X
Organic	15.718	X

Figure 10. Box-plot and representation of LSD of the clay percentage for the different management practices

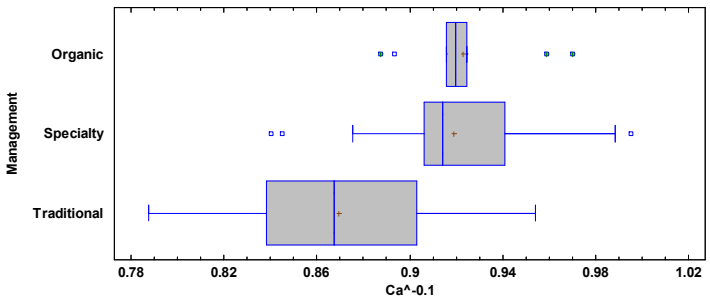
The silt percentage was higher for the traditional management with 15.98 % and the lowest value was for the organic management with 11.63 % (**Table 1**). The silt percentage had a p-value of the ANOVA less that 0.05, denoting a statistically difference between at least one of the means (**Table 2**). This was supported by the formation of two groups by the X's (**Figure 11**) with the organic management different form the other two managements. The highest data variability was found for traditional management.



Management	*Mean*	*Homogeneous groups*
Organic	11.631	X
Specialty	15.5155	X
Traditional	15.9815	X

Figure 11. Box-plot and representation of LSD of the silt percentage for the different management practices

For the calculation of the cation exchange capacity, the bases Na^{+}, Ca^{+2}, K^{+} and Mg^{+2} were measured. In case of the Na^{+} almost all the samples resulted in values smaller than the quantification limit of the method, so it could not be taken into account (**Table 1**). Calcium concentration for the three management practices was classified as low (2 – 3 cmol·kg) for the organic and traditional management soils and medium (3 - 6 cmol·kg) for the traditional management (Castro Franco & Gómez Sánchez, 2013). The highest value was 4.68 cmol·kg for the traditional management and the lowest 2.30 cmol·kg for the organic management (**Table 1**). The different management practices seemed to affect the Ca content. It was found that there were statistically significant differences ($p < 0.05$) between the means (**Table 2**) with the X's forming two groups, with the traditional management different from the other two managements (**Figure 12**). The higher variability was found for the traditional management.

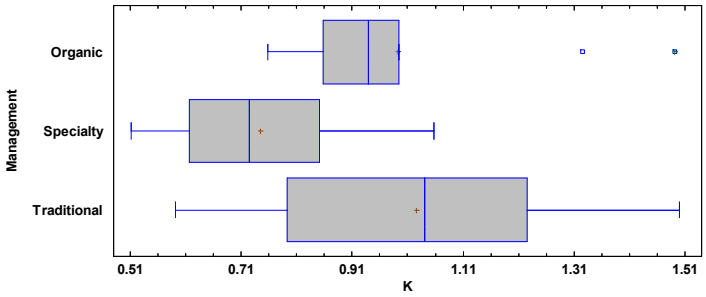


Management	*Mean*	*Homogeneous groups*
Traditional	0.8695	X
Specialty	0.919009	X
Organic	0.923149	X

Figure 12. Box-plot and representation of LSD of Ca for the different management practices

The concentration of potassium in the studied soils is classified as very high, being greater than 0.5 (Osorio, 2012). The highest value was for the soils of traditional managements with 1.02 cmol·kg and the lowest value was for specialty management with 0.75 cmol·kg (**Table 1**). The management practices affected the K content in the soils. There was a statistically significant difference ($p < 0.05$) between the mean of K between one management level and another (**Table 2**). The X's formed two

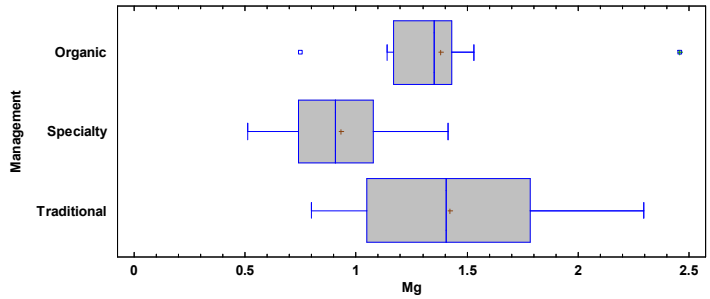
homogeneous groups with specialty management different from the other two managements (**Figure 13**). The traditional management has the highest variability.



Management	*Mean*	*Homogeneous groups*
Specialty	0.74515	X
Organic	0.9924	X
Traditional	1.0254	X

Figure 13. Box-plot and representation of LSD of K for the different management practices

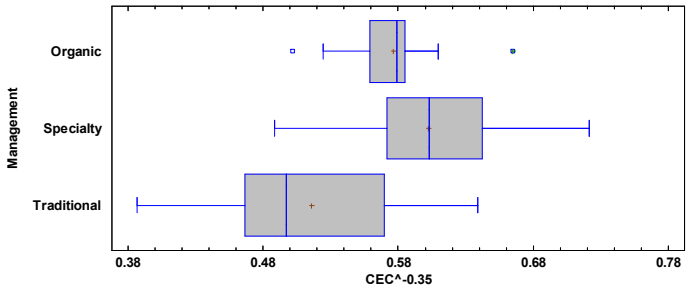
The levels of magnesium in the studied soils can be classified as low (0.5-1.2 cmol·kg), medium (1.2 – 1.8 cmol·kg) and high (>1.8 cmol·kg) (Castro Franco & Gómez Sánchez, 2013). The highest mean was for traditional management with 1.42 cmol·kg (**Table 1**), although this management type has the highest variability of the data, with samples with values that can be classified as high (**Figure 14**). The lowest mean is of the specialty management with 0.93 cmol·kg (**Table 1**). The concentration of magnesium is affected by the management practices. There is a statistically significant difference ($p < 0.05$) between the mean of one of the managements and another (**Table 2**). The X's form two groups with specialty management different from the other two (**Figure 14**).



Management	*Mean*	*Homogeneous groups*
Specialty	0.93135	X
Organic	1.3797	X
Traditional	1.42415	X

Figure 14. Box-plot and representation of LSD of Mg for the different management practices

The cationic exchange capacity calculated by the summation of the interchangeable bases Ca, Mg and K resulted in values considered very low (< 5 cmol·kg) and low (5 – 10 cmol·kg), with a few exception of samples from traditional management with values considered medium (10 – 20 cmol·kg) (Castro Franco & Gómez Sánchez, 2013).

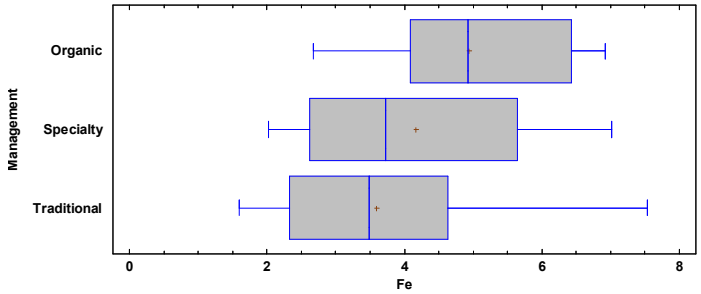


Management	*Mean*	*Homogeneous groups*
Traditional	0.515436	X
Organic	0.576446	X
Specialty	0.602157	X

Figure 15. Box-plot and representation of LSD of CEC for the different management practices

The highest value was from the traditional management soils with 7.40 cmol·kg and the lowest mean was from the specialty management with 4.49 cmol·kg (**Table 1**). As with the interchangeable bases, the CEC is affected by the management practices. There was a statistically significant difference ($p < 0.05$) between one of the means of one type of management and another (**Table 2**). Also, it was found that the alignment of the X's shows two groups with traditional management different from the other two (**Figure 15**). The highest variability was found in the traditional management.

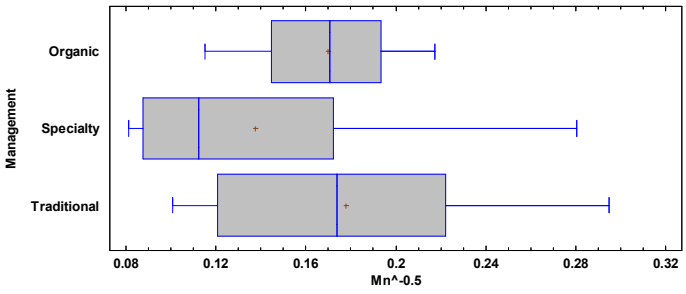
The levels of iron in the studied soils were found to be classified as very low (< 10 $mg \cdot kg^{-1}$) (Osorio, 2012), with the highest value of 4.93 $mg \cdot kg^{-1}$ for the organic management and the lowest value of 3.59 $mg \cdot kg^{-1}$ for the traditional management soils (**Table 1**). Management practices did not affect the Fe content. There was no statistically significant difference ($p > 0.05$) between the means of the three managements (**Table 2**) with the X's grouped together as one homogeneous group (**Figure 16**). The highest variability was found in the specialty management.



Management	*Mean*	*Homogeneous groups*
Traditional	3.5995	X
Specialty	4.166	X
Organic	4.939	X

Figure 16. Box-plot and representation of LSD of Fe for the different management practices

The manganese levels found in the studied soils is very high (> 20 $mg \cdot kg^{-1}$) (Osorio, 2012), with the highest values from the specialty coffee with a mean of 79.85 $mg \cdot kg^{-1}$ and the lowest mean form the organic management with 38.52 $mg \cdot kg^{-1}$ (**Table 1**). Even with very elevated levels of Mn, the soils are close to the range of recommended levels of Mn for coffee crops (between 5-50 $mg \cdot kg^{-1}$) (Castro Franco & Gómez Sánchez, 2013). Management practices did not affect the Mn content. It was found no significative difference ($p > 0.05$) between the means of the three managements (**Table 2**) and the X's form a homogeneous group (**Figure 17**). The highest variability was observed in the specialty management.



Management	*Mean*	*Homogeneous groups*
Specialty	0.137383	X
Organic	0.169897	X
Traditional	0.177777	X

Figure 17. Box-plot and representation of LSD of Mn for the different management practices

PCA, also known as analysis of principal components, was used to reduce the dimensions of the obtained data into a new set of independent variables of a smaller number that carry the variability of the original variables. These new variables or principal components can be used to explore if there are any patterns or groupings in relation to an external variable, in this case the management

practice. This class separation only occurs when the within class variations are smaller than the between-class variations (Li et al., 2020). In this work, a dimension reduction of the 16 variables, physicochemical properties measured in soils samples from coffee plots with different management practices (organic, specialty and traditional), was made by PCA.

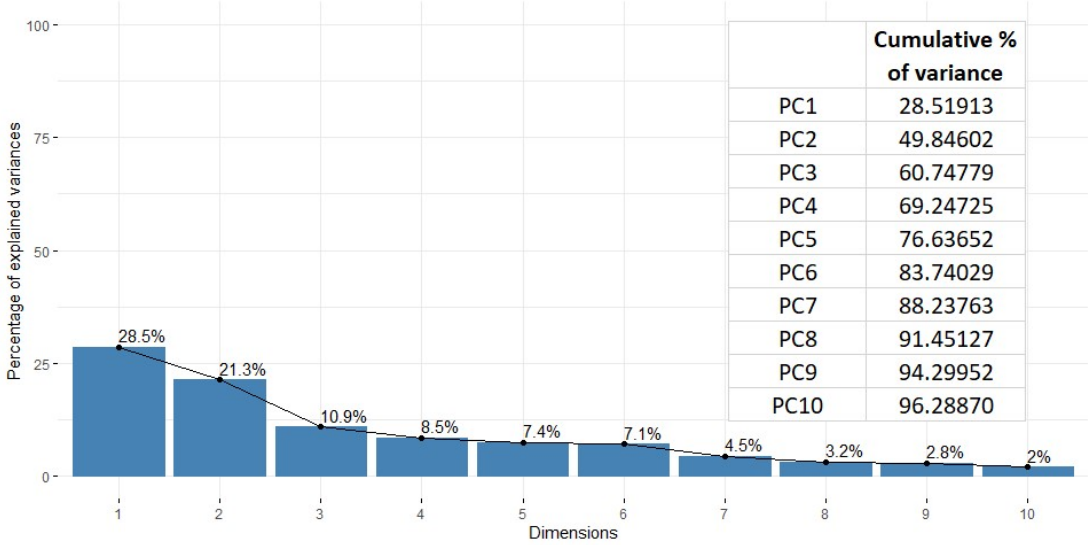


Figure 18. Scree plot of 10 principal components (Dimensions), the percentage of explained variance by each and the cumulative percentage of variance.

From 16 variables there were created 16 principal components with a 100 % of the explained variance. In the scree plot (**Figure 18**) is shown 10 principal components or dimensions and the variance explained by each one of them. It can be observed the decreasing rate at which the variance is explained by additional principal components. From the 16 physicochemical properties measured to the coffee crop soils, six were chosen, that explained 83.74 % of the total variance, for further analysis.

Table 3. Loadings of six principal components. The largest contribution of each variable is shown in yellow

	PC1	PC2	PC3	PC4	PC5	PC6
%OC	-0.206152	0.380633	0.072274	0.411449	0.121899	-0.079756
%OM	-0.206062	0.380696	0.072390	0.411278	0.122258	-0.079692
NH_4^+	0.059150	0.008606	0.442150	0.028859	0.059027	-0.589519
NO_3^-	-0.196950	0.119189	0.368818	-0.351228	0.227420	-0.283927
Total N	-0.140643	0.389115	-0.057799	0.078048	0.353151	0.180075
P	0.152018	0.267640	-0.284944	-0.039377	-0.320146	-0.406900
pH	-0.306876	0.106387	-0.059890	0.161327	-0.313745	0.059798
% Sand	-0.225849	0.213550	0.340016	-0.245515	-0.362534	0.324011
% Clay	-0.122112	-0.020733	-0.561875	-0.129197	0.448767	-0.129074
% Silt	0.332322	-0.153412	0.067965	0.378086	-0.058368	-0.231104
Ca	-0.360137	-0.243655	0.066695	0.001038	0.199577	-0.006041
K	0.220438	0.359985	-0.133236	-0.297709	0.025420	-0.104642
Mg	0.274354	0.314464	0.100906	-0.165195	0.123580	0.263474
Fe	-0.299744	0.066907	-0.096628	-0.400291	-0.071154	-0.288618
Mn	0.293710	-0.070950	0.301518	-0.061635	0.430804	0.135257
CEC	-0.360080	-0.307613	0.036414	0.061858	0.081918	-0.022788

Table 3 show the principal component loadings, which describe how much each variable contributes to a particular principal component. Large loadings, positive or negative, are related to a strong relationship between a variable and a principal component. The sign of a loading indicates if the correlation between variable and principal component is negative or positive. Next it is shown the variables that contributes the most to each principal component:

PC1= Ca, CEC

PC2= Total N, K, Mg

PC3= NO3, %Clay

PC4=%OC, %OM, %Silt, Fe

PC5= pH, % Sand, Mn

PC6= NH4, P

In Figure 19 is shown the biplot of PC1 and PC2 where it is possible to determine the distribution of the samples. These two dimensions are selected due to the percentage of variance that they represent together (49.84 %), containing between the two most of the information from the 16 measured variables.

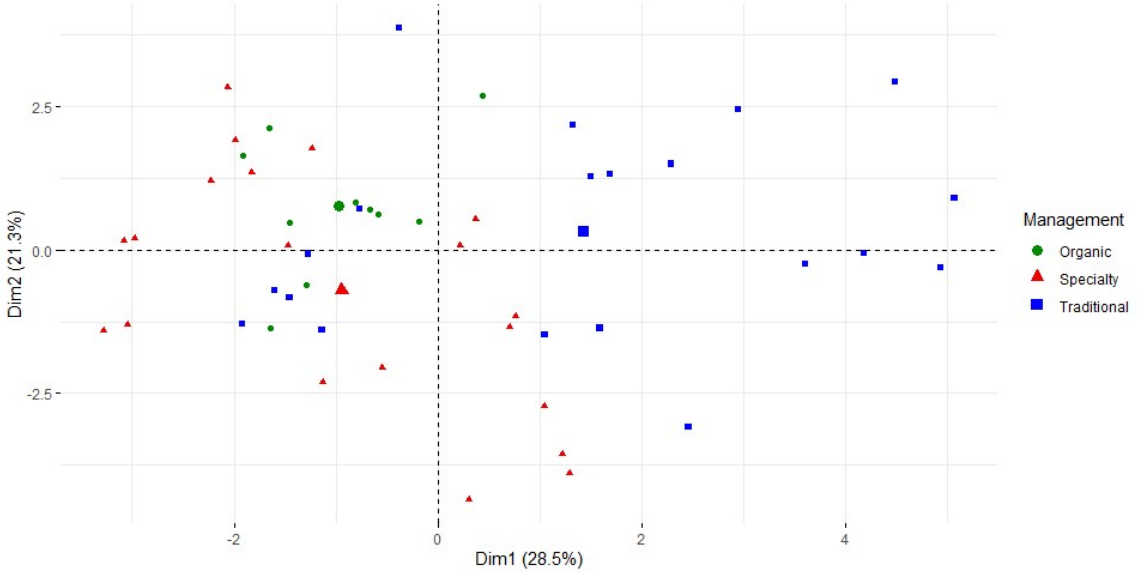


Figure 19. Scores plot of the two principal components of the principal component analysis model (PC1 and PC2). Colors for soils with coffee crops from different management practices: Organic (green), specialty coffee (red) and traditional (blue)

It is clearly observed that there is an overlapping between the three management practices, which is expected in soils of the same origin and with the same use: cultivated with coffee. But also, there is great variability within each type of management, so it is safe to say that the physicochemical properties are not evenly distributed in each plot, having samples that are clearly differentiated and unique for each practice, as it is with half of the traditional management soil samples (blue squares) and half of the specialty management soil samples (red triangles). Meanwhile the organic samples are overlapped completely by the organic and traditional managements.

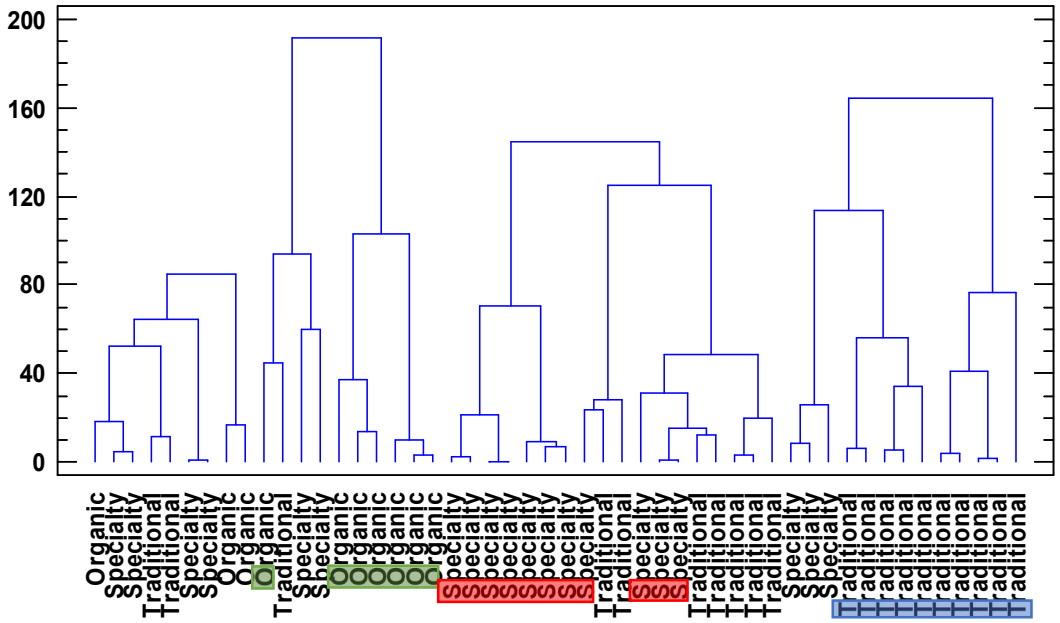


Figure 20. Dendrogram of the 6 principal components. Ward Method and Euclidian distance. Colors for soils with coffee crops from different management practices: Organic (green), specialty coffee (red) and traditional (blue)

Cluster analysis was applied to the 6 principal components, previously chosen from the reduction of dimensions, with the Ward method and Euclidean distance, resulting in the dendrogram of figure 20. This method has the advantage of taking all the available information, because the cluster is derived from the six principal components that explain the 83.74 % of the information while the grouping observed in figure 19 is based only in the first two principal components.

The resulting dendrogram can be divided in four groups or clusters. In the first cluster there are samples from each management practice mixed together. There is also a cluster where most of the samples that are grouped are from the organic management (green). Next, there is a cluster that groups mostly samples from the specialty management plots (red). Finally, there is a cluster that groups mainly samples from the traditional management plots (blue). Even when the groupings formed in this dendrogram show certain patterns of differentiation still there is not a total distinction between the three management practices.

The fact that both in the biplot of PC1 and PC2 and in the dendrogram a certain differentiation of management practices can be observed is due to the fact that, as presented before, several properties were affected by the type of management of the plots, with their means showing a statistically significant difference ($p < 0.05$) in the ANOVA. Even so, when all the variables are compared at the same time as in Figure 19 and Figure 20 there is some grouping by type of management, which would indicate that the soils of the studied plots have differentiation due to the different management practices, specially the traditional and specialty type.

4. Conclusions

In this work 16 physicochemical properties ((% OC, % MO, NH_4^+, NO_3^-, total N, P, pH, texture, % sand, % clay, % silt, Ca, K, Mg, Fe, Mn and CEC) were measured in order to compare three type of management practices used in five plots with coffee crops: organic coffee, traditional coffee and specialty coffee. A one-way ANOVA was conducted for every one of these variables where it was found that for the variables nitrate, available P, pH, %Silt, Ca, K, Mg and CEC there was a statistically significant difference ($p < 0.05$) between the mean of the physicochemical property between one management level and another. For the rest of the variables, % OC, % OM, ammoniacal N, total N, % sand, % clay, Fe, and Mn, their means have no statistically significant difference ($p > 0.05$). Furthermore, a reduction of dimensions was performed with a principal component analysis resulting in 6 new variables with an 83.74 % of cumulative percentage of variance. From the dispersion plot formed from the first two principal components, it was possible to discern that there was some differentiation between the management practices traditional and specialty. Lastly, using the 6 principal components it was performed a cluster analysis where the resulting dendrogram showed differentiation between the three management practices. This leads to conclude that although these soils maintain great similarities, the different management practices did affect the physicochemical properties of the soils.

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CHAPTER 4: Validation of a modified QuEChERS method in combination with LC-MS/MS to determine pesticides residues in coffee beans and coffee crop soils from different agronomic practices

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Abstract

Coffee production is crucial to the economy of many developing countries. However, the crop is susceptible to different pests and fungi, so the use of pesticides has become necessary to support the production of quality products. Consequently, pesticide residues may remain in the coffee products, so Maximum Residue Limits (MRLs) are established to protect the health of consumers. In this work, looking to know and compare the residue levels of 25 pesticides commonly used on coffee crops in the department of Cauca, Colombia in both green coffee and soils from different agricultural management practices, we (i) performed the optimization and validation of pesticide extraction using the QuEChERS method and liquid chromatography coupled to tandem mass spectrometry determination; and (ii) conducted a pesticide residue analysis on samples from traditional, organic and specialty coffee plots. The calibration curves in solvent and in matrix showed linearity with R^2 ≥0.995. The matrix effect was superior to 20 % for 48 % of the pesticides in coffee and for all the residues in soil. The precision and trueness of the method resulted in RSD no greater than 20 % and recoveries within the range of 70–120 % for both coffee and soil matrices. The MQL for all the pesticides was ≤10 $\mu g \cdot kg^{-1}$. Pesticide residues were found on samples from the traditional and organic plots, and not in the specialty coffee plots. The analyzed green coffee samples showed the presence of flutriafol, pyraclostrobin and chlorpyrifos, with flutriafol presenting the maximum concentration of 39.0 ± 9.40 $\mu g \cdot kg^{-1}$. Despite the presence of pesticide residues in some of the green coffee samples, these did not surpass the MRL. Additionally, carbendazim was found in the soil samples from the traditional plot, with a maximum value of 6.34 ± 2.09 $\mu g \cdot kg^{-1}$. Our results showed that the agricultural management practices did not influence the pesticide content, and that the correct use of pesticides results in an innocuous product with export potential.

Keywords: Green coffee, Andean soils, Organic Coffee, Traditional coffee, Specialty coffee

1. Introduction

Coffee is one of the most consumed beverages in the world. It is crucial to the economies and politics of many developing countries; its cultivation, processing, trading, transportation and marketing provide employment for millions of people worldwide (Pizzutti et al., 2012). However, coffee is a fairly

sensitive crop that can be affected by different pests and diseases (de Oliveira et al., 2016), with the coffee berry borer, coffee leaf rust and mycotoxin contamination being the most common and problematic (Barbosa et al., 2020; De Rezende & Taniwaki, 2020; Russell et al., 2014). Therefore, in order to sustain the production of a coffee country, the use of pesticides is inevitable (de Oliveira et al., 2016).

One of the consequences of pesticide use is that residues of active ingredients and metabolites may remain in the final products. These residue levels in food depend on a series of factors, such as the type of pesticide used (the chemical nature of the compounds), its use (formulations, doses, frequencies, mode of application, and withholding period), its chemical degradation (inside and outside the plant, and the metabolism in the plant tissues) and the climatic conditions (Moreno & Machado, 2019). Moreover, agricultural products can still become contaminated in other processes carried out in the farm, during national or international transportation, or during industrial processes (Puerta-Quintero, 2006).

Despite pesticides being designed to function with reasonable confidence and minimal risk to human health, their indiscriminate use and the unintentional exposure to them raise serious concerns about adverse health effects (Kim et al., 2017). While there is still a lack of knowledge about the risk posed by the presence of pesticides in food, and its long-term repercussions, it has been proven that, due to their toxicity, the consumption of pesticides alters lipid metabolism, the transport of vitamins and glucose, affects reproductive processes and immune response, and some are considered mutagenic, teratogenic or carcinogenic (Castilla Pinedo et al., 2012; Kim et al., 2017).

Looking to protect the health of consumers, Maximum Residue Limits (MRLs) are proposed to limit the amount of pesticide residues found on food products (FAO/WHO, 2022). These levels are established based on studies of the average daily intake of a particular agricultural product and the toxicity of the contaminant, and are set by each country, which means different countries may have different level values for the same pollutant in the same product (Farnsworth, 2012; Guerrero, 2003). These regulations have become essential in recent years with the increased use of pesticides in agriculture, as, for instance, between 2002 and 2013, 22% of the rejected food from Latin America and the Caribbean was due to the presence of pesticide residues, between 2009 and 2012, Japan rejected 5% of food due to pesticide contamination, almost half of which corresponded to coffee and cocoa, and in 2018 the European Commission rejected 5% of imported food due to pesticide residues (Rivers, 2013; Machado & Moreno, 2019). To ensure that residues in food do not exceed the MRLs, pesticides must be applied correctly in accordance with Good Agricultural management practices, and a withdrawal period (the interval between the last application and the harvest) must be established (Hernández et al., 2003; Moreno & Machado, 2019). Additionally, in the particular case of coffee, there are further regulations on the use of agrochemicals such as herbicides, fungicides, and insecticides, as well as Ochratoxin A content (Puerta-Quintero, 2006).

Coffee beans are a complex matrix of polyphenols, caffeine, and pigments (Chen et al., 2019). These soluble natural substances are present in high concentrations and can be easily extracted simultaneously, which, in consequence, can strongly interfere in the performance of analytical methods for exogenous compounds (Chen et al., 2019; OECD, 2007) Because of this, coffee is considered a difficult-to-analyze matrix and is classified in the group of "Difficult or unique

commodities" according to the “Guidance document on quality control and validation analysis procedures for the analysis of pesticide residues in food and feed” (SANTE/11312/2021, 2021). Consequently, extraction and quantification methods for pesticides are continuously optimized and modified to improve their performance as needed (Dias et al., 2013; Nielsen et al., 2015; Pizzutti et al., 2012), and “full validation data are generally called for to prove the suitability of the method” (OECD, 2007).

There is a limited number of published studies related to methods for measuring pesticides in coffee, in particular of those using liquid chromatography, as certain pesticides can only be analyzed by gas chromatography. Some of these studies include: Reichert et al. (2018), who presented the optimization and validation of an acetonitrile based method for the simultaneous extraction of 117 pesticides and 31 mycotoxins from green coffee beans, followed by liquid chromatography and electrospray ionization coupled to tandem mass spectrometry (LC-ESI-MS/MS) determination; de Oliveira et al. (2016), who validated a LC-MS/MS method using for the extraction and purification the QuEChERS technique, in order to detect and quantify the levels of flutriafol and pyraclostrobin residues in coffee beans; and Dias et al. (2013), who developed one of the most inclusive method to date, allowing the analysis of 123 pesticides (including isomers) in green coffee beans with a multi-residue LC-MS/MS method, using acetonitrile extraction and dSPE cleaning with C18 (modified QuEChERS).

In this study, looking to know and compare the residue levels of 25 pesticides commonly used on coffee crops in the department of Cauca, Colombia in both green coffee and soils from different agricultural management practices, we (i) performed the optimization and validation of pesticide extraction using the Reichert et al. (2018) modified QuEChERS method and liquid chromatography coupled to tandem mass spectrometry determination; and (ii) conducted a pesticide residue analysis on samples from traditional, organic and specialty coffee plots.

2. Material and methods

8.1 STANDARDS AND REAGENTS

Analytical standards of all the 25 pesticides studied (**Table 1**) at purities greater than 98% were purchased from Merck (Darmstadt, Germany). Individual pesticides stock standard solutions (1000 $mg \cdot L^{-1}$) were prepared in acetone and kept at -18 ºC, protected from light. A mix solution (10 $mg \cdot L^{-1}$) of the 25 target compounds was prepared by diluting an appropriate volume of each individual stock standard solution in acetonitrile. Working solutions of 1.0 $mg \cdot L^{-1}$ and 0.1 $mg \cdot L^{-1}$ were freshly prepared by dilution of the mixed pesticide standard solution in acetonitrile. The working mixture solution were also stored at -18 °C. These solutions were used as a spiking solution for recovery experiments and to obtain calibration curves in matrix and neat solvent by diluting them with blank coffee bean extract, blank soil extract or acetonitrile containing 1 % acetic acid, respectively. The standards prepared in matrix extracts were used also for the calculation of the matrix effect and recoveries. The calibration range used for quantification of the coffee and soil samples was 0.50-50 $\mu g \cdot L^{-1}$ (4.0-400 $\mu g \cdot kg^{-1}$).

Acetone, acetonitrile HPLC-grade, methanol LC-MS/MS grade, acetic acid and ammonium formate analytical grade were purchased from Merck (Darmstadt, Germany) and the QuEChERS extraction kit with 6 g of magnesium sulfate and 1.5 g of sodium acetate purchased from United Chemical

Technologies (UCT) (Bristol, USA). Ultrapure water was obtained from Milli-Q Gradient system, Millipore (Bedford, MA). The chemicals were used as received and without further purification.

8.2 SAMPLING SITE, SAMPLING AND STORAGE

Coffee and soil samples were collected at the municipality of Cajibío (Cauca, Colombia) at 1700−1900 m above sea level. The location is about 14 km north of Popayán city (Cauca) with an average yearly temperature of 24 °C during the day and 14 °C at night and average yearly rainfall of 700 mm. Soil and coffee samples were collected in the harvest season in June 2017, 2018, and 2019. Five plots with different agricultural management practices: one for organic coffee (P1, 2°35'04.0"N 76°32'52.8"W), two for specialty coffee (P2, 2°35'10.5"N 76°33'14.5"W and P3, 2°35'11.0"N 76°33'02.7"W), and two for traditional coffee (P4, 2°35'02.2"N 76°32'49.3"W and P5, 2°35'16.2"N 76°33'03.7"W) were evaluated by each year. The organic and specialty coffee plots were located at the same farm and the traditional coffee plots were from two neighboring farms.

The coffee samples correspond to the *Coffea arabica* varieties *Bourbon* (P1)*, Tabi* (P2) and *Castillo* (P3, P4 and P5). Samples were collected using simple random sampling, obtaining five combined samples, one for each plot, each year. The collected cherry coffee from the five different plots was then processed by the same person using same conditions in the wet method approach (Bee et al., 2005), as are the standard regional practices. The green coffee obtained was stored in a freezer at -20 °C until analysis.

The sampling for the soil consisted of collecting 30 simple random samples from each plot to form a combined sample per plot each year. The procedures adopted for sampling consisted of taking soil samples at a depth of 0–20 cm within the projection of the plant canopy where there is a greater chance to find the pesticides added to the plant. The soils from this work were of a sandy loam texture and had a 11.9 % as average of percentage of organic matter.

The samples of green coffee beans were grinded in a coffee processor Hamilton beach, 80393 model (Southern Pines, USA), then passed through a 2 mm sieve and kept at -18°C until analysis. The soil samples were dried at room temperature and away from sunlight by spreading them in carboard sheets. They were then grinded, homogenized and passed through a 2 mm sieve. Finally, the samples were placed in plastic bags, which were labeled and kept at -18°C until analysis.

8.3 SAMPLE EXTRACTION AND CLEAN-UP

In a 50 mL PTFE (Polytetrafluoroethylene) centrifuge tube 2.5 g of coffee or soil were slurried with 7.5 mL of water (ratio 1:4, *w/w*) and homogenized with an automatic shaking machine Pall shaker, model PN 4821 (Portsmouth, United Kingdom) for ten minutes at 750 rpm.

The pesticide extraction was based on the Reichert et al. (2018) modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) method. In brief, the method consisted in adding 10 mL of acetonitrile (1% acetic acid *v/v*) to the slurry samples and agitating for 10 minutes at 750 rpm using the automatic shaker. In order to remove water and induce phase separation, a QuEChERS extraction kit containing anhydrous magnesium sulfate and sodium acetate 6g/1.5g was added to the mixture and again agitated for 10 minutes at 750 rpm. Then, the tubes were centrifuged at 4000 rpm for 5 minutes in a Boeco centrifuge, model U-320R (Hamburg, Germany). Then, an aliquot of 0.5 ml of the

extract was transferred to a vial containing 0.5 ml of methanol. Lastly, the capped vials were taken to be analyzed at the UHPLC-MS/MS system.

8.4 ANALYZED PESTICIDES

The pesticides analyzed in this work were selected based on the following criteria: previous studies that indicate the most widely used in Colombia for the control of pests in coffee, such as those reported by Dallos and Guerrero (2005), Cruz-Cerón et al. (2011) and Moreno and Machado (2019); information acquired directly form the farms owners; and finally, that they were included in the Codex Alimentarius. Greater emphasis was placed on the pesticides most used in the department of Cauca over those listed in the Colombian legislation, as the ones legislated are not necessarily being used in coffee crops. In this sense, 25 pesticides were selected (see Table 1).

8.5 UHPLC-MS/MS SYSTEM AND OPERATING CONDITIONS

The equipment used was an ultra-high-performance liquid chromatography-tandem mass spectrometer UHPLC-MS/MS Waters (Milford, USA), Acquity H-Class system coupled to a Waters Xevo TQD, equipped with a Waters (Milford, USA) ESI ion source.

The chromatographic conditions for the coffee and soil samples were the same. 10 µL of each prepared sample was injected into the UHPLC-MS/MS system. Separation was carried out through a 2.1x50 mm Acquity BEH-C18 column of 1.7 µm particle size, Waters (Milford, USA). UHPLC conditions were as follows: 45 °C column temperature, 0.30 $mL \cdot min^{-1}$ flow and 17 min running time. Mobile phase A consisted in deionized water and methanol (95:5) with ammonium formate 5 mM, and mobile phase B was deionized water and methanol (5:95) with 5 mM ammonium formate. The gradient elution started with 0 % mobile phase B for 1.0 min, followed by a linear gradient to 70 % mobile phase B from 1.0 to 3.0 min. Then, a linear gradient until 100 % of B from 3.0 to 12.0 min and was held for 1.0 min. Lastly, 0 % of mobile phase B was reached in 0.1 minutes and held until 17 minutes.

Mass spectrometer parameters were 150 source temperature, 350 °C desolvation temperature; 650 and 50 $L \cdot h^{-1}$ were the desolvation and cone gas flow, respectively; finally, ESI(+) ionization mode and 3.5 kV capillarity voltage were used. All the Selected Reaction Monitoring (SRM) analyses were performed at 1.0 Da resolution for quadrupole one and 0.75 Da resolution for quadrupole two. Optimization of the precursor and product ions was achieved by infusion of the individual pesticide solutions directly into the mass spectrometer. Different fragmentation voltages were applied, and the optimal voltages ranged between 10 and 37 V. The collision energies were investigated and ranged from 10 to 68 V. The most intense transition was used for quantification, and the second most intense transition was used for confirmation. The mass spectrometry acquisition parameters for all the pesticides are shown in Table 1.

Table 1. Pesticides MRLs and mass spectrometry acquisition parameters

	MRLs (µg·kg^{-1})		Retention time	Transition quantification (T1)	Transition confirmation (T2)	Cone voltage (V)	Collision energy T1 (T2) (V)
	Codex Alimentarius	EU					
Azoxystrobin	30	30	5.82	404>372	404>329	25	25 (30)
Carbendazim	100	100	4.72	192>160	192>132	24	18 (28)
Carbofuran	1000	50	5.10	222>123	222>165	25	21 (16)
Chlorantraniliprole	50	20	5.69	484>453	482>284	25	15 (15)
Chlorpyrifos	50	200	9.68	352>97	352>200	26	32 (20)
Cyantraniliprole	50	30	5.23	475>286	475>112	26	12 (68)
Cypermethrin	50	100	10.9	433>191	435>193	22	16 (16)
Diazinon	*	50	7.45	305>169	305>97	20	22 (35)
Difenoconazole	10	50	8.13	406>251	406>111	37	26 (60)
Dimethoate	*	50	4.35	230>199	230>125	12	10 (20)
Flutriafol	150	150	5.44	302>70	302>123	30	16 (28)
Hexaconazole	*	50	7.65	314>70	314>159	31	22 (28)
Imidacloprid	1000	1000	4.35	256>209	256>175	20	16 (19)
Malathion	*	20	6.19	331>99	331>127	12	24 (12)
Methamidophos	*	50	1.81	142>94	142>125	30	15 (15)
Methomyl	*	100	4.01	163>88	163>106	10	10 (10)
Parathion	50	100	7.08	292>97	292>236	25	30 (14)
Permethrin	50	100	11.9	408>183	408>355	18	16 (8)
Phenthoate	*	10	7.07	321>247	321>163	22	10 (10)
Profenofos	40	50	8.77	375>305	375>347	32	18 (12)
Propiconazole	20	20	7.49	342>69	342>159	37	22 (34)
Pyraclostrobin	300	300	7.63	388>194	388>163	25	10 (25)
Thiamethoxam	200	200	4.08	292>211	292>132	25	10 (20)
Triadimefon	500	200	6.31	294>69	294>197	22	20 (15)
Triadimenol	500	200	6.51	296>70	296>99	12	10 (15)

* Not defined for green coffee

8.6 LINEARITY AND MATRIX EFFECT

Seven different concentrations from the working standard solutions were prepared by dilution with acetonitrile containing 1% acetic acid (*v/v*), and by dilution with blank coffee extract and blank soil extract. The resulting calibration curves were obtained: 0.5 1.0, 3.0, 5.0, 10, 20, 50 µg·L^{-1} corresponding to pesticide residue concentrations of 4.0, 8.0, 24, 40, 160 and 400 µg·kg^{-1} in coffee or soil. The regression hypothesis was tested through an analysis of variance (ANOVA) and the coefficients of determination (R^2) were calculated.

The matrix effect was calculated using the slope calibration curves in solvent in contrast to the ones in matrix (**Equation 1**) (Pizzutti et al., 2012).

$$\text{Matrix effect } (\%) = \left(\left(\frac{a}{b}\right) - 1\right) x100 \quad (1)$$

Where **a** is the slope of the calibration curve in matrix, and **b** is the slope of the calibration curve in solvent. The criteria for evaluation of the matrix effect for all pesticides is that it is not significative if the result falls between ±20 %.

8.7 PRECISION AND TRUENESS

Recovery experiments were performed to evaluate the method trueness and precision. Blank samples of coffee and soil were spiked with pesticides standard mixture solutions at three concentrations levels of 10, 20 and 50 µg·kg^{-1} with 10 replicates at each concentration.

The trueness (percentage extraction recovery, % recovery) was calculated by dividing the recovered concentrations by spiked concentration. Finally, the precision (relative standard deviation, RSD) was obtained by dividing the standard deviation by the average calculated concentration.

8.8 METHOD DETECTION LIMIT (MDL) AND METHOD QUANTIFICATION LIMIT (MQL)

The Method Detection Limit (MDL) and Method Quantification Limit (MQL) were statistically calculated based on the $t_{99}S_{LLMV}$ method (Corley, 2003). The standard deviation of seven replicate spiked at 10 µg·kg^{-1} was multiplied by 3.143 factor to calculate MDL. MQL was MDL multiplied by a factor of 3.

8.9 UNCERTAINTY

The relative expanded uncertanty (U_{exp}) was estimated by equation 2

$$U_{exp} = k \times \sqrt{{u_{Rw}}^2 + u_b^b} \times 100\% \quad (2)$$

Where **k** is the coverage factor, $\mathbf{u_{Rw}}$ is the within laboratory reproducibility, $\mathbf{u_b}$ is the method and laboratory bias. The coverage factor was k=2, ≈95%. The U_{exp} was estimated at 10, 20 and 50 µg·kg^{-1}.

8.10 QUALITY ASSURANCE AND QUALITY CONTROL

Once the methods were validated, the samples were analyzed. Within each 20 samples a method blank (soil blank matrix extract and coffee blank matrix extract) was analyzed to rectify that there was no cross-contamination during the extraction. Likewise, an analytical control of the method was done by fortifying a blank sample of coffee or soil at 10 or 20 µg·kg^{-1}. Also, two random samples were spiked at 10, 20 or 50 µg·kg^{-1} to evaluate the method recovery and precision during sample analysis. All the samples were measured in duplicate.

The recovery percentages for the analytical controls of the method in coffee were between 75 % (permethrin) and 120 % (cypermethrin) for the 10 µg·kg^{-1} control and 73 % (permethrin) and 120 % (thiamethoxam) for the 20 µg·kg^{-1} control. The recovery percentage of the spiked coffee samples was between 74 % (malathion) and 119 % (profenofos) for the 10·µg kg^{-1} concentration and 76% (azoxystrobin) and 117% (imidacloprid) for the 20 µg·kg^{-1}.

The recovery percentages for the analytical controls of the method in soils were between 70% (methomyl) and 118% (carbofuran) for the 10 µg·kg^{-1} control and 71% (malathion) and 114% (profenofos) for the 20 µg·kg^{-1} control. The recovery percentage of the spiked soil samples was between 71% (methomyl) and 114% (thiamethoxam) for the 10 µg·kg^{-1} concentration and 70% (imidacloprid) and 118% (chlorpyrifos) for the 20 µg·kg^{-1}.

3. Results and discussion

Based on the reported multi-residue technique using the QuEChERS approach by Reichert et al. (2018), it was developed a method for the extraction, purification, and analysis of pesticides in coffee and soil matrices, that was subsequently validated.

3.1 VALIDATION

All the validation results are presented in **Table 2** for coffee and **Table 3** for soil. The calibration curves were fitted to a linear function and the coefficients of determination (R^2) for all the pesticides were determined from standards in blank coffee or soil extract and in organic solvent. According to the data, the linearity was equally even for the calibration curves obtained from standards in solvent and those obtained from standards in both blank coffee and blank soil extracts. The R^2 for all the pesticides evaluated in coffee and soil were ≥0.995 in solvent and in matrix for both coffee and soil, which is suitable for good quantification purposes and shows the adequate linearity of the methods. Besides, the linear hypothesis for all pesticides both in solvent and in matrix were demonstrated in the range of 0.5-50 $\mu g \cdot L^{-1}$ (8.0-400 $\mu g \cdot kg^{-1}$) with an ANOVA.

The decision to use the calibration curve prepared from calibration standards in blank matrix extract or in organic solvent is based on the matrix effects. The values of the matrix effects for coffee are also presented in **Table 2** and for soil in **Table 3**. **Table 2** shows that the matrix effect for each individual pesticide evaluated in coffee is not significant for 12 (48 %) of the 25 pesticides. **Table 3** shows that the matrix effect for the pesticides evaluated in soil is significant for all the 25 pesticides. Due to the results, it was determined that because a great number of pesticides in coffee and all the pesticides in soil present a matrix effect, the quantification must be made using matrix-matched calibration standards in order to have a better quantification of the analytes.

Table 2. Pesticides in coffee: coefficients of determination (R^2) for curves in solvent and in matrix, matrix effects (ME, %), method detection limit (MDL) in $\mu g \cdot kg^{-1}$, method quantification limit (MQL) in $\mu g \cdot kg^{-1}$, average recoveries (Rec, %), RSD (%), n = 10 by level.

						Spike concentrations					
						10 $\mu g \cdot Kg^{-1}$		20 $\mu g \cdot Kg^{-1}$		50 $\mu g \cdot Kg^{-1}$	
	R^2 Solvent	R^2 Matrix	ME (%)	MDL	MQL	Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)
Azoxystrobin	0.997	0.998	-86.4	2.62	7.87	105	12.3	104	12.8	97.8	16.9
Carbendazim	0.997	0.998	-84.1	2.01	6.02	106	7.48	105	5.78	100	6.70
Carbofuran	0.996	0.997	-25.7	1.92	5.75	110	6.63	105	11.4	99.0	3.52
Chlorantraniliprole	0.997	0.998	-61.4	0.73	2.20	76.3	5.44	77.8	7.69	86.7	13.0
Chlorpyrifos	0.998	0.999	-20.9	0.30	0.90	117	1.27	106	7.44	107	8.67
Cyantraniliprole	0.998	0.997	-32.6	3.72	11.2	100	19.1	92.5	17.4	77.5	9.00
Cypermethrin	0.997	0.998	8.99	1.75	5.24	106	7.41	103	8.76	103	8.96
Diazinon	0.998	0.999	-52.4	0.67	2.00	116	2.06	114	3.70	109	6.67
Difenoconazole	0.998	0.997	-13.8	0.81	2.44	111	7.23	117	1.74	109	5.81
Dimethoate	0.999	0.998	7.59	2.37	7.11	104	9.89	91.9	8.92	91.7	9.09
Flutriafol	0.998	0.998	-21.2	1.34	4.01	109	7.04	110	5.07	108	6.43
Hexaconazole	0.997	0.998	5.29	2.26	6.77	104	10.8	114	2.76	116	2.20
Imidacloprid	0.998	0.998	2.04	3.13	9.39	92.0	15.7	99.2	15.0	101	7.17
Malathion	0.998	0.996	26.7	2.70	8.09	91.5	16.7	89.2	16.1	97.4	13.9
Methamidophos	0.996	0.997	80.5	2.22	6.67	91.4	9.99	88.5	6.55	91.0	6.09
Methomyl	0.997	0.997	21.8	1.27	3.82	102	6.45	85.2	13.6	72.2	2.89
Parathion	0.998	0.996	7.45	2.00	6.01	98.2	10.1	108	9.44	91.3	15.7
Permethrin	0.999	0.998	-9.67	1.65	4.94	104	5.70	99.0	9.90	93.7	12.2
Phenthoate	0.999	0.997	7.57	0.51	1.52	112	6.41	110	10.6	107	13.1
Profenofos	0.997	0.997	-11.0	2.21	6.64	110	4.80	110	6.59	104	8.11
Propiconazole	0.997	0.995	1.58	2.01	6.03	101	13.5	106	11.6	115	3.98
Pyraclostrobin	0.997	0.997	-24.3	1.17	3.52	115	3.57	116	1.88	110	5.20
Thiamethoxam	0.998	0.998	-3.69	3.12	9.37	99.2	13.6	98.1	12.4	101	9.83
Triadimefon	0.997	0.998	-54.8	3.44	10.3	97.2	17.5	103	10.4	109	4.18
Triadimenol	0.998	0.997	-6.32	0.74	2.22	112	6.14	115	2.38	108	5.52

Table 3. Pesticides in soil: coefficients of determination (R^2) for curves in solvent and in matrix, matrix effects (ME, %), method detection limit (MDL) in µg·kg^{-1}, method quantification limit (MQL) in µg·kg^{-1}, average recoveries (Rec, %), RSD (%), n = 10 by level.

						Spike concentrations					
						10 µg·Kg^{-1}		20 µg·Kg^{-1}		50 µg·Kg^{-1}	
	R^2 Solvent	**R^2 Matrix**	**ME (%)**	**MDL**	**MQL**	**Rec (%)**	**RSD (%)**	**Rec (%)**	**RSD (%)**	**Rec (%)**	**RSD (%)**
Azoxystrobin	0.997	0.996	-49.1	1.55	4.66	109	7.00	102	13.6	93.7	15.3
Carbendazim	0.997	0.997	-85.9	1.34	4.03	91.4	10.9	84.6	10.9	75.5	6.46
Carbofuran	0.996	0.996	-42.1	1.05	3.14	110	5.66	112	5.57	111	5.91
Chlorantraniliprole	0.997	0.996	-76.5	1.92	5.76	78.7	8.28	80.3	4.14	76.5	6.57
Chlorpyrifos	0.998	0.997	-48.4	2.20	6.61	93.7	13.3	82.8	7.31	83.4	7.86
Cyantraniliprole	0.998	0.995	-57.5	0.72	2.15	115	2.03	93.4	18.6	96.1	12.2
Cypermethrin	0.997	0.998	-67.7	1.35	4.05	115	2.89	108	5.46	98.4	8.54
Diazinon	0.998	0.996	-62.6	1.83	5.50	99.4	11.1	99.0	6.56	100	6.76
Difenoconazole	0.998	0.996	-83.1	1.70	5.10	106	10.9	98.3	4.34	96.7	4.41
Dimethoate	0.999	0.998	-68.5	1.47	4.41	111	4.37	112	4.64	110	7.76
Flutriafol	0.998	0.996	-74.0	0.71	2.12	112	3.33	103	9.37	97.7	15.1
Hexaconazole	0.997	0.997	-67.1	0.85	2.56	76.6	5.02	80.7	5.45	85.0	2.94
Imidacloprid	0.998	0.996	-74.7	0.96	2.89	89.2	12.2	90.9	7.32	91.4	8.85
Malathion	0.998	0.995	-68.7	3.36	10.09	96.2	18.2	101	10.3	102	11.0
Methamidophos	0.996	0.997	-66.6	1.25	3.74	86.6	6.31	94.0	9.03	88.1	5.64
Methomyl	0.997	0.996	-56.7	1.58	4.74	85.2	14.0	86.2	9.88	77.4	4.60
Parathion	0.998	0.999	-70.5	2.64	7.93	98.8	14.8	102	14.0	82.8	8.73
Permethrin	0.999	0.997	-69.8	1.13	3.39	107	6.28	100	4.10	93.9	11.9
Phenthoate	0.999	0.995	-73.1	2.21	6.64	100	9.84	87.3	4.85	89.9	13.5
Profenofos	0.997	0.995	-63.2	1.94	5.81	102	10.8	104	8.72	112	2.87
Propiconazole	0.997	0.997	-79.6	2.80	8.39	92.4	15.9	94.0	5.19	90.4	15.1
Pyraclostrobin	0.997	0.998	-65.1	1.06	3.17	103	6.70	96.9	5.35	97.9	9.74
Thiamethoxam	0.998	0.998	-67.0	1.92	5.75	102	9.17	105	7.45	103	5.66
Triadimefon	0.997	0.997	-75.0	1.09	3.27	79.1	5.93	88.2	7.92	91.5	14.5
Triadimenol	0.998	0.995	-60.6	2.00	6.00	97.1	10.8	94.5	10.9	94.3	10.9

The trueness of the method was evaluated as the average percentage recovery at the three spike levels and the precision that is expressed as intermediate precision (RSD %) for 10 replicates at each spiked level.

Table 2 and Table 3 show that from the 25 pesticides analyzed in this work all have agreeable results with a recovery within the 70–120% range and RSD ≤ 20% at the three spiked levels.

According to the $t_{99}S_{LLMV}$ method (Corley, 2003) the resulting MDL and the MQL are shown in Table 2 and Table 3. The resulting MQL are low enough so the method can be used to determine if these pesticides are below the MRL established for coffee by the Codex Alimentarius and the EU legislation (European Comission, 2022; FAO-WHO, 2022). Thus, the results presented show that the method and sample extraction present a high accuracy, especially with such a complicated matrix as is coffee and for the determination of pesticides of diverse chemical classes.

In Table 4 there are shown the estimated maximum values of U_{exp} for all pesticides studied in coffee and in soil. The U_{exp} results for coffee matrix ranged from 17–57% and for soil matrix from 21–56%. The resulting U_{exp} are consistent with the concentration levels that are being evaluated and the complexity of the matrices, producing random errors in the analytical methodology.

Table 4. U_{exp} (%) of the 25 pesticides in coffee and in soil

Pesticides	Coffee Max U_{exp}(%)	Soil Max U_{exp}(%)
Azoxystrobin	55	49
Carbendazim	25	33
Carbofuran	37	29
Chlorantraniliprole	40	32
Chlorpyrifos	27	35
Cyantraniliprole	57	56
Cypermethrin	31	27
Diazinon	22	29
Difenoconazole	22	26
Dimethoate	35	31
Flutriafol	24	37
Hexaconazole	30	21
Imidacloprid	45	44
Malathion	55	50
Methamidophos	29	28
Methomyl	45	49
Parathion	48	51
Permethrin	37	34
Phenthoate	40	38
Profenofos	28	29
Propiconazole	39	43
Pyraclostrobin	17	26
Thiamethoxam	42	34
Triadimefon	48	44
Triadimenol	20	35

3.2 PESTICIDES IN COFFEE

Fifteen samples by duplicate of green coffee from three different agricultural management practices: organic (3, P1), specialty (6, P2 and P3) and traditional coffee (6, P4 and P5) were analyzed. Pesticide residues were found on samples from the traditional and organic plots, and not in the specialty coffee plots. From these samples, two resulted with values over the quantification limit for flutriafol: 39.0 ± 9.40 $\mu g \cdot kg^{-1}$ for P5 (2019) and 9.83 ± 2.36 $\mu g \cdot kg^{-1}$for P1 (2018), and for pyraclostrobin: 22.8 ± 3.90 $\mu g \cdot kg^{-1}$ for P5 (2019) and 7.64 ± 1.30 $\mu g \cdot kg^{-1}$for P1 (2018). Two other samples resulted with residue values below the calculated quantification limit for flutriafol and chlorpyrifos (Table 5). It was found that the concentration of flutriafol and pyraclostrobin on the samples of traditional coffee and organic coffee are significantly below the MRLs of 150 and 300 $\mu g \cdot kg^{-1}$ respectively (see Table 1).

The lack of similar studies of pesticide presence in coffee for the region prevents a better comparison of the results of this study and their significance. However, flutriafol has been shown to be a persistent pesticide, even when respecting the withholding period, as indicated by Moreno and Machado (2019) who found concentrations of flutriafol residue between 4.00 and 15.0 $\mu g \cdot kg^{-1}$. Additionally, de Oliveira et al (2016) analyzed 10,297 samples of green coffee and found this pesticide with values above 5.00 $\mu g \cdot kg^{-1}$ in more than 30 % of the samples, and above 10.0 $\mu g \cdot kg^{-1}$ in over 10 % of the samples (1207 samples). These values are in the same order of magnitude as the ones found in this work and confirm the persistence of this residue in coffee.

The fact that all samples were below the MRLs implies that, even with the different agronomic management practices, the innocuity of the resulting coffee was guaranteed, making it safe for

consumption and potentially suitable for export, as it complies with the international regulations (Ferro et al., 2015). Furthermore, the results from this study show that the different agronomic management practices did not influence the pesticide content, as long as the pesticides are correctly used. Indeed, Moreno and Machado (2019) found that when used according to the Colombian government indications (i.e., the amount of commercial product per application, methods, equipment, and times of application including the withholding period), flutriafol, pyraclostrobin and chlorpyrifos do not exceed the MRLs established in the Codex Alimentarius. Thus, our results further highlight the importance of the awareness campaigns held by the Colombian government on the use of pesticides.

The presence of pesticides in organic coffee, even the in the low concentration presented, can be explained by the use of pesticides in neighboring plots, cross-contamination due to mismanagement of the pesticide products or the use of products that do not specify pesticide content (Gómez-Ramos et al., 2020; Walorczyk et al., 2013).

Table 5. Results of analysis of pesticides from the green coffee samples with values above MDL.

Sample	Flutriafol $\mu g \cdot kg^{-1}$	Chlorpyrifos $\mu g \cdot kg^{-1}$	Pyraclostrobin $\mu g \cdot kg^{-1}$
Traditional (P5, 2018)		< MQL	
Traditional (P5, 2019)	39.0 ± 9.40		22.8 ± 3.90
Organic (P1, 2017)	< MQL		
Organic (P1, 2018)	9.83 ± 2.36		7.64 ± 1.30

3.3 PESTICIDES IN SOIL

Fifteen samples by duplicate of soil from three different agricultural management practices: organic (3, P1), specialty (6, P2 and P3) and traditional coffee (6, P4 and P5) were analyzed. The 25 pesticides from **Table 1** were analyzed based on the validated method previously discussed. Of all the samples, two had values over the quantification limit (**Table 6**). These two samples come from one of the plots of traditional management (P4, 2018 and 2019) and present carbendazim residue levels of 5.93 ± 1.96 $\mu g \cdot kg^{-1}$ and 6.34 ± 2.09 $\mu g \cdot kg^{-1}$.

Table 6. Results of analysis of pesticides from the soil samples with values above MDL

Sample	Carbendazim $\mu g \cdot Kg^{-1}$
Traditional (P4, 2018)	5.93 ± 1.96
Traditional (P4, 2019)	6.34 ± 2.09

Although carbendazim is a persistent pesticide in soil, its low concentration found here, as well as the absence of other pesticide residues, can be explained by the high percentage of organic matter of the studied soils, as high levels contribute to the easier degradation of pesticides (Mohapatra and Lekha, 2016, Sadegh-Zadeh et al., 2017).

As the levels of pesticide residue in soil were low in the quantification range and did not correspond to the pesticides found in the coffee samples, it can be assumed that there was no relationship between the pesticides that persisted in both matrices. Additionally, as in the coffee samples, results show that the agricultural management practices did not influence the pesticide residue in the soil,

and that regardless of them, the use of this pesticide in the studied plots is adequate. Moreover, the fact that only one pesticide was found in the samples, and in such low concentrations, allows to conclude that there is little risk of contamination of groundwater and surface water (Tudi et al., 2021).

4. Conclusions

Twenty-five pesticides in coffee and in soil were monitored and quantified for the first time in the department of Cauca, Colombia. Validation of the QuEChERS method in combination with LC-MS/MS gave very satisfactory results for the analysis of the 25 pesticides in both matrices. The limit of quantification for each pesticide was substantially below the MRLs set in the Codex Alimentarius and by the European Union. All the pesticides have satisfactory recoveries for both coffee and soil matrices (within 70–120 %). The precision, expressed as %RSD, was below 20%. Frutiafol and piraclostrobin residues were detected in one of the coffee samples from traditional management and another from organic agriculture. There was also detected chlorpyrifos and flutriafol over the MDL but below the MQL in two samples from the organic and traditional plots. In all cases the pesticide residue concentrations were below the MRLs set in the Codex Alimentarius and by the European Union. Carbendazim residue was detected in two soil samples from traditional plots, having values just over the detection limit. No relationship was found between the pesticide residues found in green coffee with the one found in soil. The fact that of the 25 pesticides evaluated only three were detected and in very low concentrations and did not exceed the MRL established for green coffee indicates that the campaigns of awareness of the use of pesticides in the region are being fruitful, assuring the innocuity of the coffee produced. Lastly, our results showed that, as long as the pesticides are correctly used, the agricultural management practices did not influence the pesticide content and that it would result in a product with export potential.

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CHAPTER 5: The role of agronomic practices in metabolite content and sensory evaluation of roasted coffee

1. Introduction

The chemical composition of a cup of coffee is highly complex (Terra et al., 2013). There are over 1,500 volatile and over 1,000 non-volatile compounds in the coffee matrix, where more than 900 are generated during the roasting process (Folmer, 2014; Terra et al., 2013; Sualeh et al., 2020). Because these chemical components influence the quality of coffee, along with its taste and aroma, and some have an impact on human health, it has become necessary to characterize them and measure their constituent content in coffee, aiming to provide an authentic and high-quality product to the consumer (Safrizal et al., 2019; H. D. dos Santos & Boffo, 2021).

Among the non-volatile compounds, special attention has been placed on alkaloids caffeine (CAF) and trigonelline (TRG), as they have a major effect on the final quality of the coffee products (del Campo et al., 2010), and, along with chlorogenic acids (CGA), they have been used as a tool for the evaluation of coffee quality (Sualeh et al., 2020; H. D. dos Santos & Boffo, 2021). Namely, both CAF and TRG are involved in coffee bitterness (Del Campo et al., 2010; Sualeh et al., 2020). TRG is a precursor for the formation of various classes of volatile compounds during the coffee roasting, contributing to the aroma and taste of the brew (Wei & Tanokura, 2015; Sualeh et al., 2020). CGA are behind the aroma formation, acidity of the brew and coffee pigmentation (Farah et al., 2006), providing sweet, sour, and astringent tastes (Izawa et al., 2010). Accordingly, a positive correlation has been found of CAF content of both green and roasted coffee with astringency and bitterness, and of TRG and CGA contents of roasted coffee with flavor, body, and overall coffee cup quality, as well as with acidity in the case of CGA (Sualeh et al., 2020).

The characteristic aroma of coffee consists of a complex mixture of volatile compounds, including, among others, classes of pyrazines, aldehydes, furans, phenols, ketones, and sulfur compounds (Akiyama et al., 2005; Toci & Farah, 2014; Toledo et al., 2016). A delicate balance in the content of these compounds produces the desirable aroma of coffee, however, their composition in roasted coffee strongly depends on their composition in green beans, which, in turn, is strongly influenced by the coffee species and cultivars, growing conditions, postharvest processing, type of roast, storage, etc. (Toledo et al., 2016).

Because of the above, metabolite content has a discriminative and profiling potential, such that they have also been used to characterize coffees from different varieties and genetic groups (Sanz et al., 2002; Tran et al., 2017; Smrke et al., 2015), environmental conditions (Bertrand et al., 2012; Da Silva et al., 2005), pre-harvest and post-harvest processing (Gonzalez-Rios et al., 2007), geographic origin (Costa Freitas & Mosca, 1999), the presence of defective beans (J. R. Santos & Rodrigues, 2020; Toci & Farah, 2014; N. Yang et al., 2016) and ripening stage of the coffee cherry (Toledo et al., 2016; Velásquez et al., 2019). For instance, Abreu et al. (2020) were able to discriminate specialty and traditional roasted commercial coffees, being CGAs, TRG, lipids, organic and fatty acids the main metabolites responsible for the discriminations; Habtamu and Belay (2020) observed higher CAF and CGA content in defective coffee beans; Badmos et al. (2020) found variations in CGA composition in

coffees cultivated using organic, conventional, and biodynamic farming practices; and Toci & Farah (2014) found a higher number and concentration of volatile compounds in defective coffee beans, and identified some of them as potential markers for fingerprinting defective coffee seeds.

In this study, we aimed to identify if there is a difference in the metabolite content in coffee beans cultivated using organic, traditional and specialty coffee management practices. To this end, a targeted metabolomic approach through HPLC techniques was carried out to quantify CAF, TRG and the major CGA isomers found in coffee: 3-caffeoylquinic acid (3-CQA), 4-caffeoylquinic acid (4-CQA), 5-caffeoylquinic acid (5-CQA), 3,4-dicaffeoylquinic acid (3,4-diCQA), 3,5-dicaffeoylquinic acid (3,5-diCQA), and 4,5-dicaffeoylquinic acid (4,5-diCQA). Additionally, an untargeted metabolomic analysis was applied to the examination of all metabolites through a fingerprint study using a FT-IR technique, and to the study of the volatile profile using a purge and trap and GC-MS system. Finally, a sensory evaluation of the coffee was conducted.

2. Materials and methods

2.1 CHEMICALS

Caffeine, trigonelline, 3-caffeoylquinic acid (Neochlorogenic acid), 4-caffeoylquinic acid (Cryptochlorogenic acid), 5-caffeoylquinic acid (Chlorogenic acid), 4-bromo-fluorobenzene, 2-bromo-1-chloro-propane and silicon oxide of analytical grade were purchased from Merck (Darmstadt, Germany). Ultrapure water was obtained from Milli-Q Gradient system, Millipore (Bedford, MA). Acetonitrile HPLC-grade, methanol HPLC-grade, acetic acid and ammonium formate analytical grade were purchased from Merck (Darmstadt, Germany). The chemicals were used as received and without further purification.

2.2 STUDY AREA AND SAMPLE COLLECTION

All coffee samples were collected at the municipality of Cajibío (Cauca, Colombia) at 1700−1900 m above sea level. The location is about 14 km north of Popayán (Cauca, Colombia) with an average yearly temperature of 24°C during the day, 14°C at night, and average yearly rainfall of 700 mm.

The coffee samples correspond to the species *Coffea arabica* L varieties *Bourbon* (P1)*, Tabi* (P2) and *Castillo* (P3, P4 and P5). Three sampling campaigns were conducted during the harvest season in the months of June 2017, 2018, 2019. The sampling plan involved five plots with different management practices: one for organic coffee (P1, 2°35'04.0"N 76°32'52.8"W), two for specialty coffee (P2, 2°35'10.5"N 76°33'14.5"W and P3, 2°35'11.0"N 76°33'02.7"W), and two for traditional coffee (P4, 2°35'02.2"N 76°32'49.3"W and P5, 2°35'16.2"N 76°33'03.7"W). The organic and specialty coffee plots were located at Los Naranjos farm (2° 35′ 0.5″ N, 76° 32′ 57″ O), property of SUPRACAFÉ Company and "Parque Tecnicafe". The traditional coffee plots where from two neighboring farms, one of those owned by a rural school advised by Tecnicafé (P5). Samples were collected using simple random sampling, obtaining 5 combined samples, one for each plot.

2.3 COFFEE SAMPLE PREPARATION

The collected cherries from the five plots were processed at Los Naranjos farm; this process was conducted by the same person following the standard regional practices: using the wet method approach (see Bee et al., 2005) under the same conditions. Then, the husk and the parchment were

removed, followed by the removal of defective beans. Next, the size of the coffee beans was standardized by sieving the beans through a coffee sieve # 16.

A portion of the obtained green coffee samples from 2017 was set apart for immediate roasting and subsequent sensory analysis. The rest, as well as the complete green bean samples from 2018 and 2019, were stored at -20 °C until analysis or roasting.

The samples were roasted to a standard roast degree according to the SCA parameters at the Café Cielo Tostado roastery using a Diedrich IR-12 roaster. The samples for sensory analysis were sealed and reserved. The rest of the roasted coffee was stored in a freezer at -20 °C until analysis.

2.4 SENSORY ANALYSIS

The sensory analysis was performed by two groups of tasters: A group of experts from Cielo Tostado and a group from TECNiCAFÉ consisting of the two Q-Graders Javier Hoyos and Cesar Echeverry and another expert. For this, 7 samples of organic coffee, 10 of specialty coffee, and 17 of traditional coffee from the year 2017 were analyzed. These particular samples were immediately roasted after obtaining the green beans. Additionally, coffee notes and overall scores were also measured for the samples from the 2017, 2018 and 2019 harvests that had been stored at -20 °C (see section 2.3).

The sensory analysis followed the protocol of the Specialty Coffee Association (SCA, 2021). In this protocol, ten coffee attributes are evaluated (fragrance/aroma, flavor, aftertaste, acidity, body, uniformity, balance, clean cup, sweetness, and scoring) with a scale from 0 to 10, and the overall score of each coffee is the resulting value of the sum of these ten variables.

Coffee was cooled and sealed after roasting. 24 hours later, it was grinded for 1 minute to a fine medium/coarse size in a coffee processor Hamilton beach, 80393 model (Southern Pines, USA). Five cups per sample were prepared with 8.25 g of coffee and 150 mL of water at a temperature of 92–95°C. The evaluation of the coffee was started when it reached a temperature of 55 °C (SCA, 2021).

2.5 EXTRACTION AND ANALYSIS OF CHLOROGENIC ACIDS, CAFFEINE AND TRIGONELLINE

The samples of roasted coffee that were stored in the freezer at -18 °C were let to temper. Then, they were grinded for 1 minute to a fine grind size in a coffee processor Hamilton beach, 80393 model (Southern Pines, USA). Next, 0.5 grams of roasted ground coffee was placed on a falcon tube with 10 mL of distilled water and 10 mL of methanol. The falcon tubes were then heated in a water bath for 30 minutes at 80°C. Later, the falcon tubes were transferred to a Branson 2510R-DTH Ultrasonic Cleaner, Emerson (St. Louis, USA) where the extraction was carried out for 20 minutes. Then, the tubes were centrifuged at 4000 rpm for 5 minutes in a Boeco centrifuge, model U-320R (Hamburg, Germany). 125 µL of the extract were transferred to a vial containing 875 µL of water (1:40 dilution) to quantify the dicaffeoylquinic acids, and 25 µL of extract where also transferred to a vial containing 975 µL of water (1:8 dilution) to quantify the rest of the metabolites. The vials were taken to be analyzed at the HPLC-DAD system.

The instrumental analysis of the chlorogenic acids, caffeine and trigonelline was performed using an Agilent 1100 HPLC System with DAD Detector (Santa Clara, USA) equipped with a degasser, a quaternary pump, an autosampler, and a column compartment. Chemstation Agilent (Santa Clara, USA) was used for data acquisition. Separation was accomplished using a ZORBAX Eclipse XDB-C18 5

µm 4,6 mm x 150 mm column, Agilent (Santa Clara, USA) column with a column guard. HPLC conditions were as follows: 25 °C column temperature, 1.20 mL·min^{-1} flow and 34 min running time. Mobile phase A consisted in deionized water and acetic acid 0.5 % and mobile phase B was acetonitrile. The gradient elution started with 5 % mobile phase B for 5.00 min, followed by a linear gradient to 11 % mobile phase B from 5.00 to 15.00 min. Then, a linear gradient until 30 % of B from 15.00 to 20.0 min, until 90 % from 20.00 to 25.00 min and then was held for 3.00 min. Lastly, 95 % of mobile phase B was reached in 1.00 minute and held until 34.00 minutes. Detection wavelengths of 326 nm, 270 nm and 264 nm were used for the analysis of CGAs, caffeine and trigonelline respectively (**Figure 1**).

The identification and the quantification of the compounds was carried out with the metabolite standards. Linearity was demonstrated for each of the metabolites. The calibration curves were fitted to a linear function and the coefficients of determination (R^2) for all the metabolites resulted greater than 0.997. As quality assurance and control, every 10 samples a mix of the standards of 10 mg·L^{-1} or 6 mg·L^{-1} or 3 mg·L^{-1} was measured as the calibration curve verification control.

2.6 INFRARED ANALYSIS

The samples of green coffee beans were grinded in a coffee processor Hamilton beach, 80393 model (Southern Pines, USA), then passed through a 2 mm sieve and kept at -18°C until analysis. A PerkinElmer Fourier transform infrared spectrometer, Spectrum Two model (Waltham, USA) equipped with a DTGS sensor was used for infrared spectral acquisition. 15 samples of pulverized green coffee beans were applied onto the UATR diamond crystal, and a 100 force gauge was applied on top of the sample. Spectra were obtained over a wavenumber range of 4000– 600 cm^{-1} at 4 cm^{-1} spectral resolution with 16 scans for each spectral collection and a temperature of 22°C. Three spectra were collected for each sample and the samples were scanned in random order. Before using the acquired data, it was performed a normalization of the spectrums to avoid effects from the differences in mass between the samples used to prepare the pellets.

2.7 EXTRACTION AND ANALYSIS OF VOLATILE COMPOUNDS

The samples of roasted coffee that were stored in the freezer at -18 °C were left to temper. Then, they were grinded for 1 minute to a fine grind size in a coffee processor Hamilton beach, 80393 model (Southern Pines, USA). Immediately, 1 gram of ground coffee was transferred to pre-cleaned 20 mL glass vials with open-top screwcaps and Teflon-lined septa. Before capping the vials, 100 µL of IS solutions of 4-Bromofluorobenzene (10 µg·mL^{-1} in water) and 2-bromo-1-chloro-propane (20 µg·mL^{-1} in water) were added to each sample. Next, the vials were heated in a water bath for 30 minutes at 70°C. With a syringe, 10 ml of head space gas were collected and immediately injected to a Stratum purge & trap system and Aquatek 70 autosampler, Teledyne Tekmar (Manson, USA).

A classic purge-and-trap operating process was applied, including the three main steps: sample purging, analyte desorption, and baking. Analytes were purged out with a helium flowrate of 40 mL·min^{-1} and carried to a trap column #9 Trap (proprietary). Compounds were then desorbed by heating the trap to 260 °C for 4 min after being concentrated by opening the valves. Once the analytes were desorbed, the trap was cleaned at 260°C for 18 min to avoid potential memory effects of the tailing compounds. During desorption, the desorbed compounds were introduced into the helium carrier gas stream of an Agilent 7890A gas chromatograph (GC) (Santa Clara, USA) coupled with a mass

spectrometer Agilent 5975C (Santa Clara, USA). The purge-and-trap system was directly coupled to the gas chromatograph in a direct split interface (DSI) configuration, and, in order to avoid analyte condensation during the analyses, set at 230 °C. The end of the transfer line was directly inserted into the split injector of the GC. Full scan mode was used to detect the volatile compounds (mass range from 20 to 260 AMU). The experimental conditions for the chromatographic separation and the detection system are summarized in Table 1. All samples were analyzed in randomized order.

Table 1. Experimental conditions of the PT–GC–AED system

Purge and Trap Conditions	Sample Volume	10 mL
	Gas flow	40 ml·min^{-1} He
	Purge cycle	10 min at 20°C
	Desorb cycle	4 min at 260°C (preheat 245°C)
	Bake cycle	18 min at 260°C
	Trapping material	#9 (propietary)
GC conditions	Injection port	230 °C, split ratio 10:1
	Capillary column	DB-624 (30m x 250 um x1.4 um)
	Carrier gas	Helium, 1 ml·min^{-1}
	Oven program	200°C (20.5 min)
	Acquisition type	Scan
	Scan Time segments	Start mass 20 AMU, end mass 260 AMU

The relative abundance of each volatile compound present in the headspace was calculated by the GC peak height normalized to the peak height of the internal standard 2-bromo-1-chloro-propane. p-bromofluorobenzene internal standard was used to monitor the method repeatability. It was observed an RSD % of 16,81 (<20%) for the p-bromofluorobenzene, suggesting that the matrix effects were even across samples, and that semi-quantitative comparison of normalized peak areas was possible. Volatiles were identified by comparison of each mass spectrum with spectra in reference library NIST setting a score of minimum 80%.

2.8 STATISTICAL ANALYSES

A descriptive analysis was done on the data. Each variable, except for the infrared variables, was evaluated with an ANOVA so all data was tested for normality with the Kolmogorov-Smirnov test, Levene's test for equality of variances and independence of residuals by a graphic method. Fisher's least significant difference (LSD) was used to identify which means were statistically different. For the analysis of all the data, principal component analysis (PCA) was conducted on all variables in order to analyze all the data from each experiment (metabolites, volatiles and infrared variables) jointly, i.e., in two dimensions. The presence of structured groups from the resulting principal components was explored using hierarchical cluster analysis (HCA) and with discriminant analysis (DA). HCA was performed with the Ward method as the linkage rule and the square Euclidean distances. The DA was performed with the principal components so the statistical assumption of independence of variables was met. Statistical analyses were performed using the R-Project for Statistical Computing, R version 4.1.1 (Vienna, Austria) and Statgraphics XVI (The Plains, USA)

3. Results and discussion

3.1 SENSORY ANALYSIS

Table 2 shows the mean, standard deviations of the quality attributes measured by the sensory analysis of the roasted coffee from plots with organic, traditional and specialty management practices (2017 harvest). Since none of the cup quality attributes have a normal distribution, their comparison of the scores for the different management practices by analysis of variance was not possible. The Kruskal-Wallis test is a no parametric alternative to the unidirectional ANOVA, and an extension of the U test of Mann-Whitney that allows to compare two or more independent groups. This test is usually used when the statistical assumption of normality is not met (McKight & Najab, 2010). It was possible to apply an analysis of variance ANOVA to compare the coffees from different management practices with the sensory analysis overall score, as the required statistical assumptions for this variable were met: normality (p-value Kolmogorov-Smirnov test: 0.400141) heteroscedasticity (p-value Levene´s test: 0.524765) and independence of residuals. All the P-values for all the cup quality attributes were not less than 0.05, meaning that there is not a statistically significant difference between the means of the coffee attributes between one type of management and another, with a 95.0% confidence level (See Table 2).

Table 2. Mean, standard deviations of the quality attributes from the sensory analysis (2017 harvest). p-values from the Kruskal-Wallis test and ANOVA.

Management	Organic	Traditional	Specialty	p-value
Fragrance/ Aroma	7.93 (0.19)	7.87 (0.33)	8.08 (0.24)	0.2822
Flavor	7.79 (0.51)	7.87 (0.57)	7.88 (0.34)	0.7568
Aftertaste	7.64 (0.64)	7.79 (0.63)	7.83 (0.31)	0.7459
Acidity	7.71 (0.74)	8.07 (0.76)	7.80 (0.59)	0.3592
Body	7.61 (0.70)	7.93 (0.43)	7.85 (0.32)	0.2839
Uniformity	9.29 (0.49)	9.09 (0.51)	9.30 (0.67)	0.4925
Balance	8.29 (0.49)	8.44 (0.56)	8.50 (0.53)	0.7060
Cleanliness	9.00 (0.82)	8.88 (0.78)	8.90 (0.99)	0.9611
Sweetness	8.86 (0.90)	8.85 (0.70)	8.80 (1.03)	0.9188
Scoring	8.04 (0.74)	7.99 (0.59)	8.25 (0.42)	0.4219
Overall	82.14 (1.44)	82.78 (1.94)	83.18 (1.83)	0.5237

The resulting overall score classifies the coffee samples from the different management practices as specialty coffee, with a subclassification of *very good* (SCA, 2021). This score shows that even when the coffees come from different pre-harvest treatments, it is possible to obtain specialty coffees when the post-harvest processing is adequate.

Table 3 shows the overall score and cupping notes of the samples from the harvest of the years 2017, 2018 and 2019 that were stored at -20 °C before roasting. It can be seen how the overall scores were close to 80, while in the tasting trials of the 2017 harvest higher scores were obtained, which suggests the period and storage conditions affected the final score. Additionally, despite obtaining very similar overall scores, the tasting notes not only vary between the management practices, but also between plots and between years. Notes such as panela and dark chocolate are common in most coffees, which can be attributed to the fact that they come from the same area. But the differences in the notes of coffee from the same plot but from a different year of harvest could indicate how sensitive the coffee is to minimum variations, so it would be expected to find differences in the metabolomics tests.

Table 3. Notes from the coffee samples from three harvest periods (2017, 2018 and 2019) and different management practices.

Management (plot)	Year	Overall score	Notes
Organic Bourbon (P1)	2019	80.4	Panela, lemongrass, honey, sweet, cereal, eucalyptus, black tea
Organic Bourbon (P1)	2018	80.4	Panela, dark chocolate, caramel, molasses, citrus, lemon, cocoa
Organic Bourbon (P1)	2017	80.9	Panela, chocolate, lemon, orange, cocoa, sugar cane, lime
Specialty Tabi (P2)	2019	80.0	Panela, dark chocolate, raisins, fruity, caramel, orange, almonds
Specialty Tabi (P2)	2018	80.2	Orange, lemon, herbal residual, sugar cane, orange, herbal
Specialty Tabi (P2)	2017	80.5	Panela, lemongrass, herbal, cereal, fruity
Specialty Castillo (P3)	2019	80.0	Panela, wood, walnut, almond, lemongrass, chamomile
Specialty Castillo (P3)	2018	80.7	Panela, cocoa, raisins, orange, dark chocolate, tamarind
Specialty Castillo (P3)	2017	81.3	Molasses, fruity, almond, sour orange, cereal, molasses, walnut, fruity
Traditional Castillo (P4)	2019	80.5	Panela, caramel, cinnamon, wood, walnut, cloves, hazelnut
Traditional Castillo (P4)	2018	80.9	Panela, chocolate, dulce de leche, sweet, orange, sugar cane, caramel
Traditional Castillo (P4)	2017	81.3	Chocolate, raisin, fruity, sweet, caramel, wine, chamomile, lemon peel
Traditional Castillo (P5)	2019	80.3	Panela, dark chocolate, lemongrass, herbal, honey, cereal
Traditional Castillo (P5)	2018	80.3	Panela, dark chocolate, sugar cane, herbal, cereal, walnut, peanut, molasses, wood, citric
Traditional Castillo (P5)	2017	80.6	Panela, dark chocolate, sugar cane, brown sugar, molasses

Furthermore, sensory techniques, centered in quality assessment of the coffee and based on the specialty coffee association (SCA), are mainly used to determine if a coffee is special. However, in addition to cup quality, knowing other characteristics as origin and processing is becoming as important to the specialized and general consumer (Barrios-Rodríguez et al., 2021) so a more accurate measuring system is required.

3.2 CHLOROGENIC ACIDS, CAFFEINE AND TRIGONELLINE

Figure 1 shows the chromatogram of the separation of a standard solution mix of caffeine, chlorogenic acids and trigonelline at 10 $mg \cdot L^{-1}$. Even when the eight metabolites were perfectly separated in the chromatogram, the concentrations of the dicaffeoylquinic acid were very inferior to the rest of the metabolites, so it was necessary to make two different dilutions of the samples to be able to quantify all of them.

Table 3 shows the mean and standard deviation of the resulting concentrations of caffeine, trigonelline and chlorogenic acids, extracted and measured by liquid chromatography, for each management practice. The mayor component in coffee from the eight metabolites studied resulted to be the chlorogenic acid with values around 17 $mg \cdot g^{-1}$. The concentrations of caffeine and trigoneline were consistent with the values reported by Oberthür et al., (2011) of Arabica coffee from the Cauca region of 12.7 – 13.0 $mg \cdot g^{-1}$ and 9.2 $mg \cdot g^{-1}$ respectively. In the case of the chlorogenic acids, their degradation during the roasting of the coffee has been extensively reported, so their concentrations depend directly on the degree of roasting. In this work, as the goal was to compare the metabolite concentrations in roasted coffee with the same roasting conditions, this variation in

concentration has little consequence. Non the less, the results from the chromatographic analysis show values consistent with Arabica coffees with light roast (Moon et al., 2009; Perrone et al., 2008)

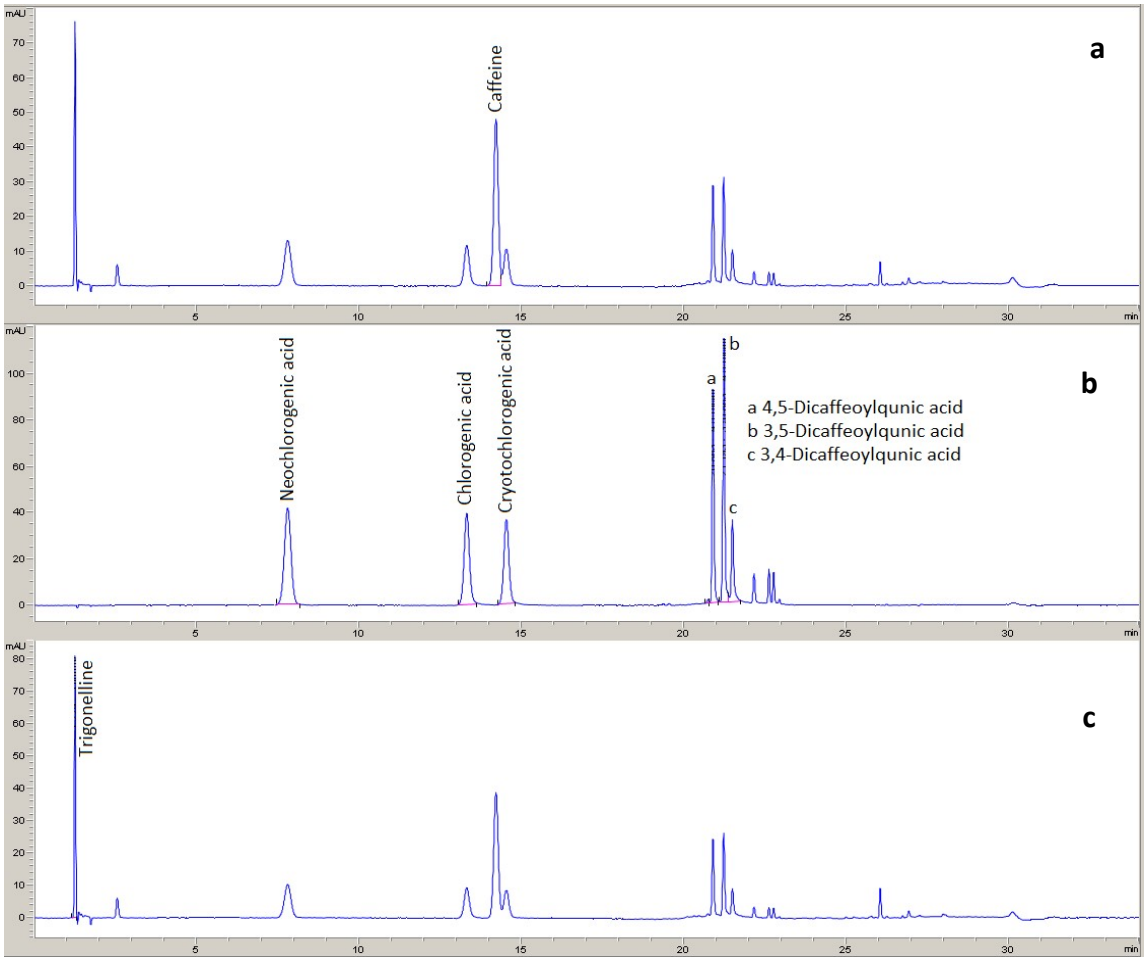


Figure 1. Chromatograms of the 10 mg·L^{-1} standard a caffeine at 270 nm; b Chlorogenic acids at 326 nm; c Trigonelline at 264 nm.

Table 3. Mean and standard deviations of the metabolite concentrations (mg·g^{-1} of coffee)

	Organic	Specialty	Traditional
Trigonelline	8.37 (0.92)	7.99 (1.44)	8.35 (1.32)
Caffeine	11.62 (1.21)	12.35 (2.06)	12.34 (1.46)
Neochlorogenic acid	5.87 (1.17)	5.91 (1.12)	6.17 (0.85)
Chlorogenic acid	16.93 (3.45)	16.86 (2.71)	17.82 (2.70)
Cryptochlorogenic acid	9.72 (2.03)	9.85 (2.23)	10.33 (1.56)
4,5-Dicaffeoylquinic acid	1.82 (0.33)	1.86 (0.24)	2.01 (0.16)
3,5-Dicaffeoylquinic acid	1.09 (0.21)	1.12 (0.17)	1.22 (0.10)
3,4-Dicaffeoylquinic acid	2.04 (0.40)	2.09 (0.34)	2.29 (0.20)

A comparison of each of the analyzed compounds in relation with the management practices studied was made through an ANOVA and all the statistical assumptions were checked (**Table 4**). Every metabolite has a normal distribution which was cheeked though a Kolmogorov-Smirnov test, with all of them with p-values greater than 0.05. Homoscedasticity was checked with Levene's test with p-

values larger than 0.05 for all variables and independence of residuals was also checked by graphic methods, complying for all metabolites. The results presented in **Table 4** show that the p-values of the ANOVA for each measured metabolite are larger than 0.05, meaning that there is not a statistically significant difference between the mean of said metabolite from one type of management and another, with a 95.0% confidence level. This means that the resulting concentrations of the eight metabolites measured did not vary between coffees from different management practices. In **Table 1** from the annexes, the results from the ANOVAs also taking into account the coffee varieties, show that there is still no difference in the metabolic content.

Table 4. p-values for the ANOVAs applied to all the metabolites studied, and the statistical assumptions: normality, homoscedasticity, independence of residuals.

	ANOVA (p-value)	Kolmogorov-Smirnov normality test (p-value)	Levene's test of homoscedasticity (p-value)	Independence of residuals
Trigonelline	0.7599	0.92954	0.71745	Complies
Caffeine	0.6487	0.95425	0.26962	Complies
Neochlorogenic acid	0.7726	0.87343	0.37965	Complies
Chlorogenic acid	0.6817	0.84757	0.68357	Complies
Cryptochlorogenic acid	0.7659	0.85131	0.46128	Complies
4,5-Dicaffeoylquinic acid	0.1886	0.44256	0.05647	Complies
3,5-Dicaffeoylquinic acid	0.1752	0.87252	0.06618	Complies
3,4-Dicaffeoylquinic acid	0.1676	0.83169	0.08313	Complies

A principal component analysis (PCA) was applied to the eight metabolites studied as variables in order analyze the data jointly to find out if there was any pattern of grouping in relation with the management practices. The scree plot of **Figure 2** shows how in the first two components there is a 90.3 % of the cumulative variance, meaning that the eight variables can be transformed in the first two components carrying out that percentage of the information.

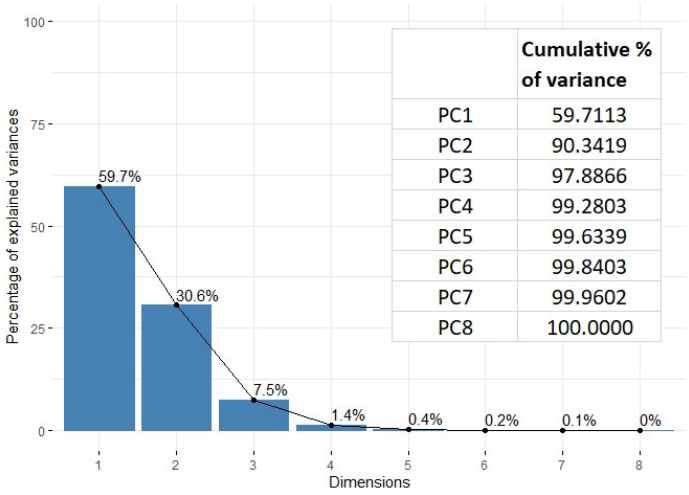


Figure 2. Scree plot of the principal components (Dimensions), the percentage of explained variance by each and the cumulative percentage of variance

In **Table 5** the loadings of the principal components 1 and 2 are shown, highlighted in yellow the largest contribution of a variable over a PC. The loadings show how the PC1 is formed principally with

the variables Neochlorogenic acid, Chlorogenic acid and Cryptoclorogenic acid. That is to say that the caffeoylquinic acids were the main formers of PC1. On the other hand, PC2 is formed of trigonelline, caffeine and the three dicaffeoylquinic acids.

Table 5. Loadings of two principal components. The largest contribution of each variable is shown in yellow

	PC1	PC2
Trigonelline	0.34584181	0.36354133
Caffeine	0.26403923	0.34784699
Neochlorogenic acid	0.42099202	0.18216292
Chlorogenic acid	0.42584556	0.11313999
Cryptochlorogenic acid	0.42255239	0.18937048
4,5-Dicaffeoylquinic acid	0.33181764	-0.4262125
3,5-Dicaffeoylquinic acid	0.28864107	-0.4890731
3,4-Dicaffeoylquinic acid	0.28307395	-0.4941107

Figure 3 is a scatterplot of the principal components 1 and 2. This figure permits to visualize the distribution of the samples in relation to the two new dimensions or principal components, with the scores or values of these two new variables describing the location of the samples. In the biplot of the PC1 y PC2 is observed that the coffee samples from organic management and from specialty management are really scattered, forming no grouping, while the traditional management most of the samples grouped together in the positive region of the PC2 (Dim2) that is most related with the trigonelline and caffeine concentrations (**Table 5**). Even as some differentiation is observed for the traditional management samples, it is still safe to conclude that this method cannot be used for samples classification.

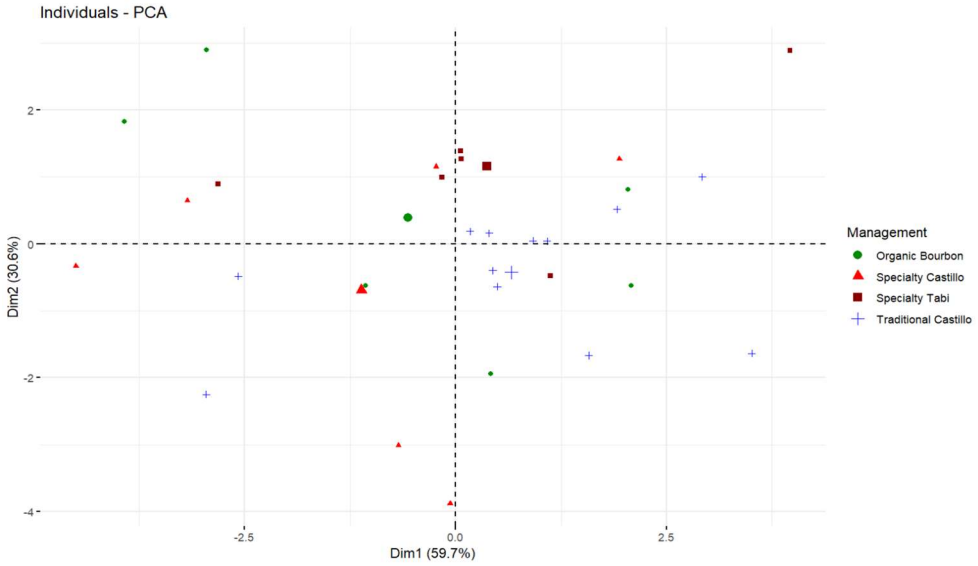


Figure 3. Scores plot of the two principal components of the PCA model (PC1 and PC2). Colors for coffee samples from different management practices: Organic (green), specialty coffee (red) and traditional (blue)

Hierarchical cluster analysis was applied with the goal of looking for groupings that could differentiate the samples for type of management. As shown in **Figure 4** the groups formed show no pattern and no differentiation between coffees from the three management practices or the different varieties.

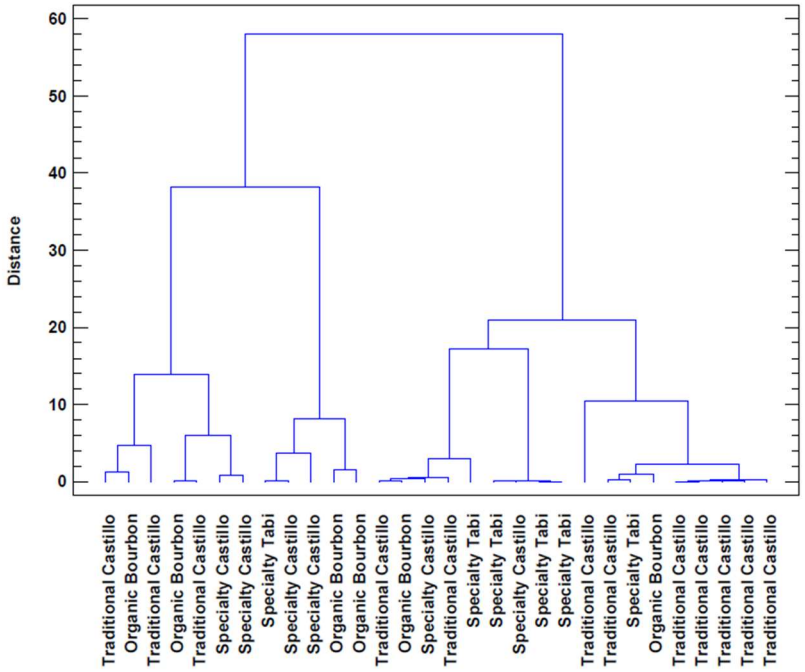


Figure 4. Dendrogram of the 2 principal components PC1 and PC2. Ward Method and square Euclidean distance.

Discriminant analysis (DA) is a supervised learning method, that is similar to the regression analysis, with the explained variable Y being categorical and the explanatory variables X's being continuous. Is generally used with the objective of classifying information obtaining a separation into groups or categories. It is a great method to evaluate if the explanatory variables can classify the observations into already known groups. This method requires that the variables met the statistical assumptions (**Table 6**) of normality, homoscedasticity and absence of multicollinearity (Johnson & Wichern, 2007). The independence of the variables was met using the principal components as the explanatory variables.

Table 6. Statistical assumptions for discriminant analysis

	Kolmogorov-Smirnov normality test (p-value)	Levene's test of homoscedasticity (p-value)
PC1	0.446822	0.558796
PC2	0.584709	0.153841

Discriminant analysis (DA) was used with the objective of evaluate how the caffeine, trigonelline and chlorogenic acids were able to classify the coffee samples into type of management groups (organic, specialty and traditional). The two principal components, resultant from the reduction of dimensions, were used to calculate the discriminant functions to classify the observations into the three management practices. First, the two components were evaluated so they met the statistical

assumptions (Table 6). Among the 30 observations or coffee samples used to fit the model, 10, or 33.33%, were correctly classified. In Table 7 is shown how from the 6 samples of organic coffee the model, based on the information from the measured metabolites, classified two samples as organic, one as specialty and three as traditional. Also, from the twelve specialty coffee samples cero were classified as such. The model, taking into account the varieties as well as the management practices predicted a somewhat better model with 15 samples (50.00 %) correctly classified (See Annexes, Table 2). Still not being nearly enough to be a model that could be used to differentiate samples.

Table 7. Classification table from the DA for the 30 samples of roasted coffee

Management	Group Size	Predicted Management		
		Organic	Specialty	Traditional
Organic	6	2	1	3
		(33.33%)	(16.67%)	(50.00%)
Specialty	12	7	0	5
		(58.33%)	(0.00%)	(41.67%)
Traditional	12	1	3	8
		(8.33%)	(25.00%)	(66.67%)

Percentage of cases correctly classified: 33.33%

There are three recent and related studies that have similarities to the present work. Abreu et al (2020) presented "for the first time the differentiation of traditional and specialty commercial coffee". They compared samples from an international fair of specialty coffees from different origin in Brazil and from coffees purchased in a supermarket. They used metabolic fingerprinting with the methods of HPLC-DAD, UV-vis and infrared spectroscopy. The HPLC-DAD method was used to analyze caffeine, trigonelline and chlorogenic acid. Furthermore, Badmos et al. (2020) found differences in chlorogenic acid content between organic and traditional (conventional) farming. They used the HPLC-ESI-TOF-MS chromatographic technique to analyze their samples and discriminate them with the chlorogenic acid content. Their samples came from different regions of Brazil and were from the species *C. arabica* and blends of *C. arabica* and *C. canephora.* Lastly, Górecki & Hallmann (2020) also found differences in caffeine and chlorogenic acid concentrations between coffees of organic and traditional managements, also from different origins in Peru.

Nonetheless, there are significant differences to this work that is important to highlight. In our case, except for the management practices, the following conditions of the coffee samples were kept uniform: origin, height, and microclimate, since all samples come from neighboring plots, species (*Coffea arabica* L.), post-harvest treatment, and roasting conditions.

3.3 INFRARED UNTARGETED METABOLOMICS

Figure 5 shows the infrared spectra of one of the studied samples. Full assignment of the spectral bands is a challenging problem, and it is not within the scope of this work. However, some generic characterization of the spectra can be made based on literature.

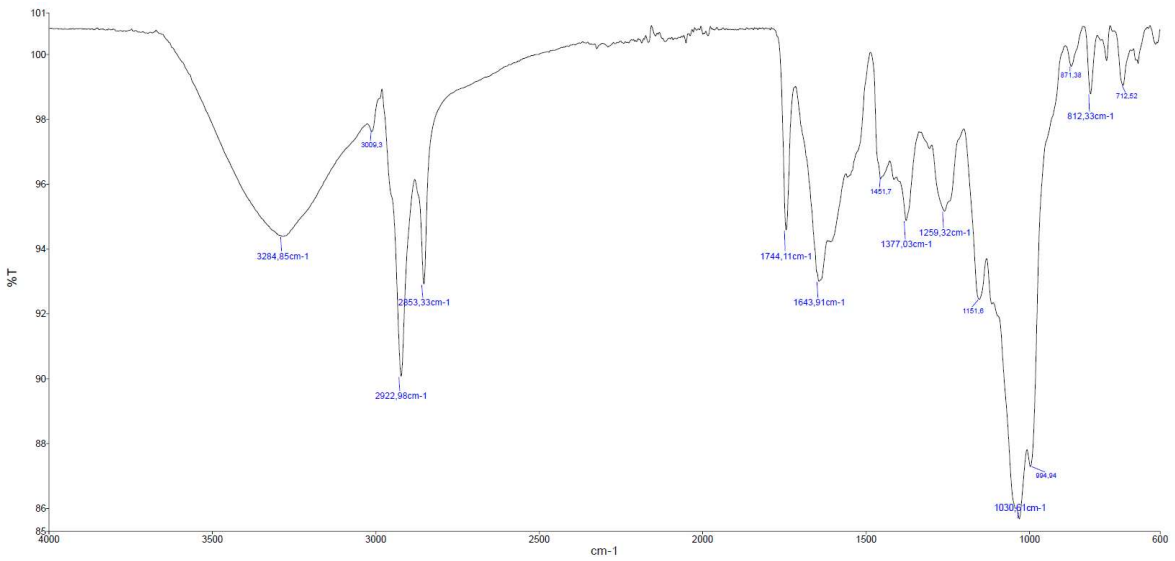


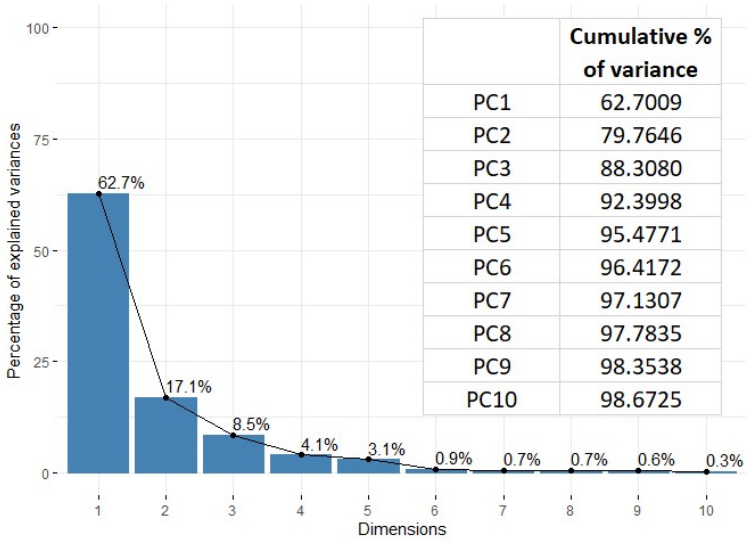
Figure 5. Infrared spectrum of a green coffee sample

Major peaks related to coffee components can be observed in the ranges from 3009 to 2850 cm^{-1}, and from the fingerprint region of 1800 to 600 cm^{-1} (Barrios-Rodríguez et al., 2021). The 3008 cm^{-1} band can be associated to the presence of lipids and can also be assigned to the symmetric stretching vibration of C-H cis-olefinic groups (=C-H cis in RHC=CHR) (H. Yang & Irudayaraj, 2001). The bands at 2925 and 2850 cm^{-1} are related with the CH_2 stretching of lipids (Craig et al., 2018). On the other hand, Craig et al. (2012) attributed the bands 2922, 2852 and 1658 cm^{-1} to caffeine by FTIR-ATR analysis of aqueous extracts of non-defective coffee spiked with caffeine. The transmittance at 1741-1726 cm^{-1} region is related to the axial symmetric C—O deformation of fatty acid esters (Link et al., 2014; N. Wang et al., 2011). This includes the band at 1743 cm^{-1}, also commonly observed in roasted coffee spectrums (Craig et al., 2012b) that Kemsley et al. (1995) associated to triglycerides while Lyman et al. (2003) attributed to aliphatic esters. The 1654 cm^{-1} and 1603 cm^{-1} regions are related to the caffeine carbonyl (J. Wang et al., 2009). The regions 1600-1300 cm^{-1} was related to the presence of trigonelline while the region 1450-1250 cm^{-1} to the presence of chlorogenic acids (Craig et al., 2012b). Carbohydrates are known to exhibit several absorption bands in the fingerprint region (1400–900 cm^{-1}) (Kemsley et al., 1995) while Chlorogenic acids also present peaks in the region of 1450–1000 cm^{-1} (Craig et al., 2012a; Kemsley et al., 1995; Lyman et al., 2003). Lastly, the peak at 1150 cm^{-1} has been related to C—O bonds of carbohydrates such as cellulose (Barrios-Rodríguez et al., 2021).

In this work it was evaluated the spectrum region between 1900 and 601 cm^{-1} known as the fingerprint region that contains the information typically used to discriminate coffee samples (Link et al., 2014; J. Wang et al., 2009; N. Wang et al., 2011) as it has the absorption bands of the methylene angular symmetric deformation, the carbonyl axial symmetric deformation of esters, aldehydes, and ketones, and angular and axial symmetric deformations of the bond C—O from esters and alcohols (Link et al., 2014). The resulting data set from that wavelength range consisted in 1300 variables.

Principal component analysis was applied to the 1300 variables extracted from the infrared spectra in order to reduce dimensionality and to enable the visualization of the inherent structure of the data.

This method, as discussed before not only provides a more manageable data set, but it is a good method for the analysis of groupings. The scree plot in **Figure 6** shows how from 1301 can be reduced to the first three components with the 88.31 % of the total variance.



	Cumulative % of variance
PC1	62.7009
PC2	79.7646
PC3	88.3080
PC4	92.3998
PC5	95.4771
PC6	96.4172
PC7	97.1307
PC8	97.7835
PC9	98.3538
PC10	98.6725

Figure 6. Scree plot of the principal components (Dimensions), the percentage of explained variance by each and the cumulative percentage of variance

The large number of variables impede the ANOVA analysis for each one of them, but after the principal component analysis, this analysis of variance was applied to the first three PC (**Table 8**). With this analysis is clear that in every PC's case there is no statistical difference in relation with the management practices.

Table 8. p-values for the ANOVAs applied to the PC (power transformations), and the ANOVA's statistical assumptions: normality, heteroscedasticity, independence of residuals.
*Kruskal-Wallis test

PC	ANOVA (p-value)	kolmogorov-smirnov normality test (p-value)	Levene's test of homoscedasticity (p-value)	Independence of residuals
PC1	0.8436	0.8279	0.2178	Comply
PC2	0.2745*	Fail		
PC3	0.2448	0.9783	0.1758	Comply

In **Figure 7** is the biplot of the principal components PC1 and PC2 with 79.76 % of the cumulative variance. It can be observed that the samples from plots with different management practices overlapped, with no defined groupings. It also can be observed some differentiation of several samples from the traditional management from the rest of the samples in the negative region of the PC1. The negative loadings of the PC1 correspond to the peaks at 1756, 1733, 898, 839, 730 and 641 cm^{-1} (**Figure 8**). The bands at 1756 and 1733 are attributed by Lyman et al. (2003) to aliphatic esters (1755- 1740 cm^{-1}) and vinyl ester and lactones (1780-1762 cm^{-1}). The rest of the bands are in the fingerprint region where bands are associates with carbohydrates (Craig et al., 2012b) and chlorogenic acids (Liang et al., 2016).

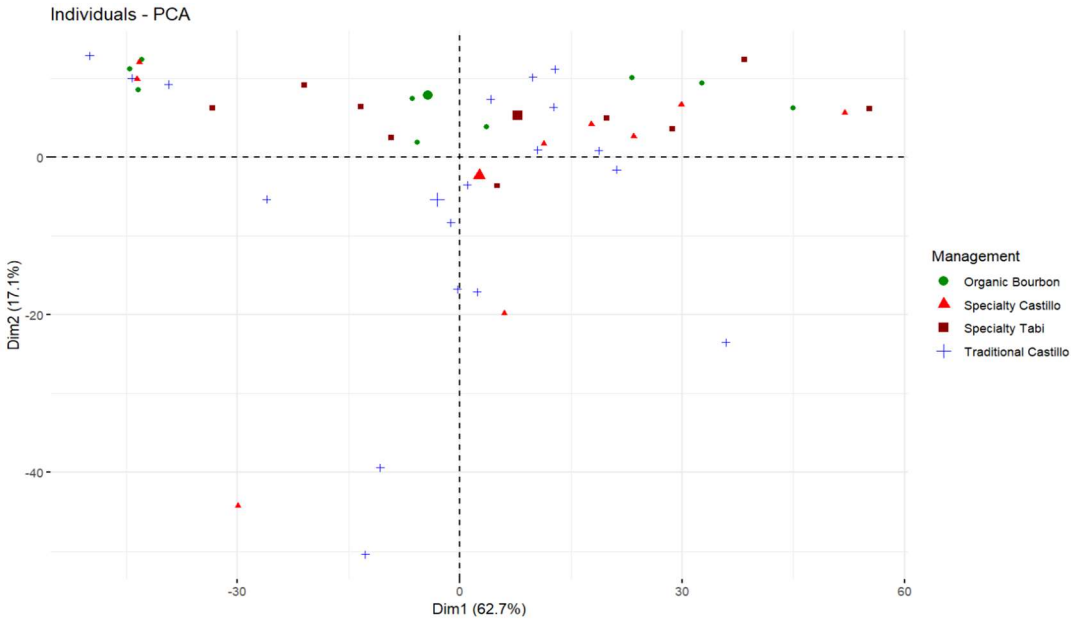


Figure 7. Scores plot of the two principal components of the PCA model (PC1 and PC2). Colors for coffee samples from different management practices: Organic (green), specialty coffee (red) and traditional (blue)

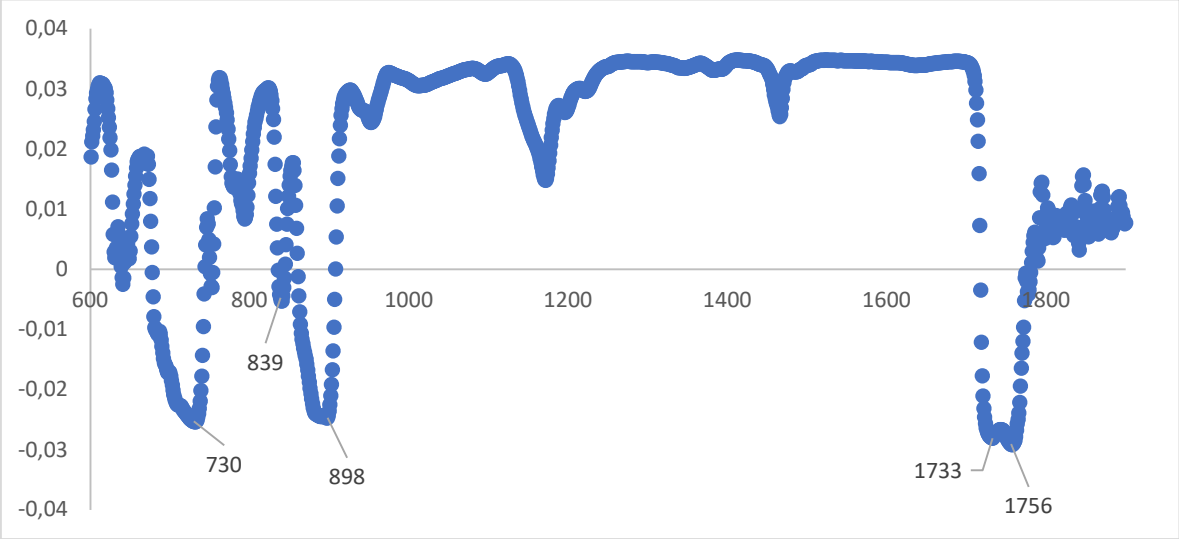


Figure 8 Loadings of the PC1 (Infrared)

The hierarchical cluster analysis was applied to the three first PCs from the reduction of dimensions resulting in the dendrogram from **Figure 9**. In the cluster it can be noted that the first group contains eight samples from the traditional management, as it is observed in **Figure 7**. The rest of the groupings formed contain samples from the three management practices studied mixed together, showing no other differentiation.

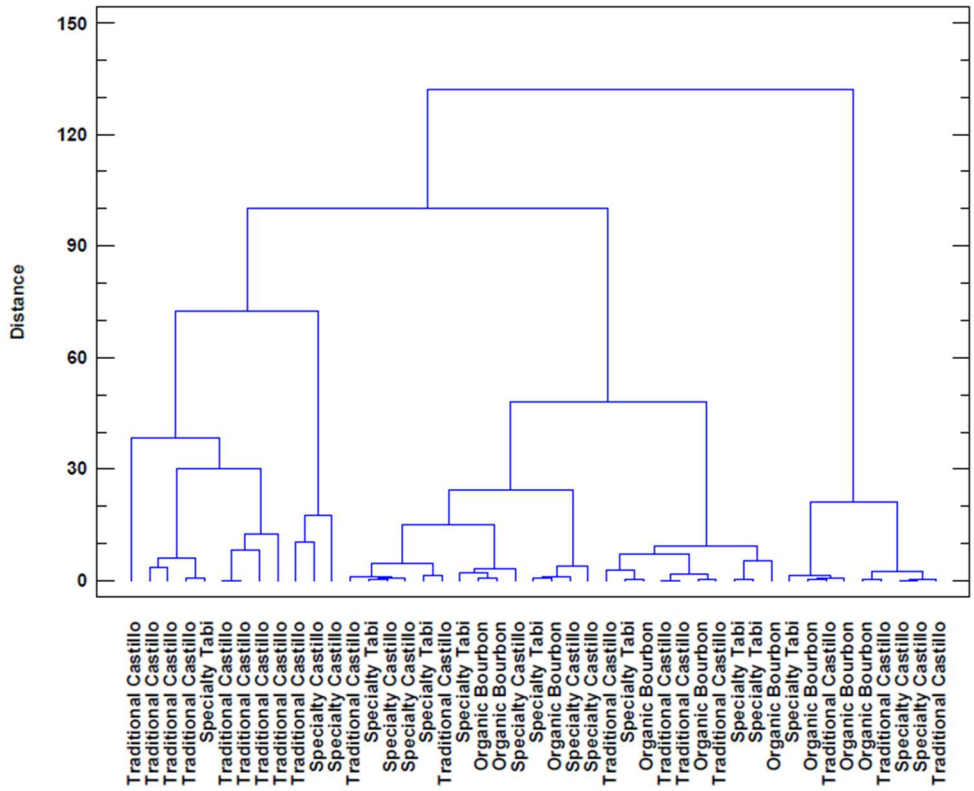


Figure 9. Dendrogram of the 3 principal components PC1, PC2 and PC3. Ward Method and square Euclidean distance.

The discriminant analysis was applied to the infrared principal components in order to know if with this data was possible to obtain a model that discriminated the samples into the studied management practices. The statistical assumptions for the DA were met with PC1 (62.7 % of variance) and PC3 (8.7 % of variance), leaving PC2 out of the model (See **Table 8**). With the two principal component the discriminant functions were calculated and used to classify all the observations into the three management practices. Among the 45 observations or coffee samples used to fit the model, 23, or 51.11 %, were correctly classified. The model, taking into account the variety as well as the management practices did not improve the possibility of classification of samples, with a 40.00 % of samples correctly classified (See Annexes, **Table 3**).

Table 9. Classification table from the DA for the 45 samples of green coffee

Management	Group Size	Predicted Management		
		Organic	Specialty	Traditional
Organic	9	3	3	3
		(33.33%)	(33.33%)	(33.33%)
Specialty	18	4	9	5
		(22.22%)	(50.00%)	(27.78%)
Traditional	18	1	6	11
		(5.56%)	(33.33%)	(61.11%)

Percentage of cases correctly classified: 51.11%

There is a recent study with similarities with this work. Monteiro et al. (2018) successfully used the techniques of proton-transfer reaction mass spectrometry (PTR-MS) and near-infrared spectrometry

(NIRS) for differentiation of farming systems (organic and traditional) using the chemometric tool PLS-DA. The samples that were classified vary in geographical origin, belonged to *Coffea arabica* and blends (*Coffea arabica/C. canephora* var. robusta) and were obtained from the manufacturer roasted at a medium roast.

As mentioned before, one significant difference with our work is that all conditions but management practice were kept as uniform as possible. Another important difference is that the samples used in this study for the IR analysis were of green coffee. Nonetheless, when comparing the IR spectra of green coffee and roasted coffee, the more prominent bands present similar levels of transmittance and is associated to the fact that the levels of caffeine and lipids do not change significantly during roasting (Craig et al., 2012a). This could be interpreted as that the IR spectrum is mainly formed by these compounds that are the most concentrated in coffee beans. As it could be observed on the previous section, the concentration of caffeine and of chlorogenic acids did not vary with the management practices, so it is to be expected that this will also be reflected in the IR spectra. And, with the results of this section, the same could be implied about the lipid content.

In addition, Abreu et al. (2020), whose work was discussed in the last section, found that infrared spectrometry had a lower discrimination between specialty and traditional coffees in comparison to the chromatographic separation and UV-vis spectrophotometry. In our case was the opposite, with 51.11 % of cases correctly classified for IR in comparison to the 33.33 % for the HPLC-DAD data.

3.4 GC-MS UNTARGETED METABOLOMICS. ROASTED COFFEE VOLATILE COMPOUNDS

It was carried out an untargeted metabolomic analysis of the volatile compounds (Figure 10). From all the volatile compounds detected in each chromatographic run, 28 where common for all the samples and available for comparison. The comparison was carried by the heigh of the chromatographic peak since no standards were used for quantification.

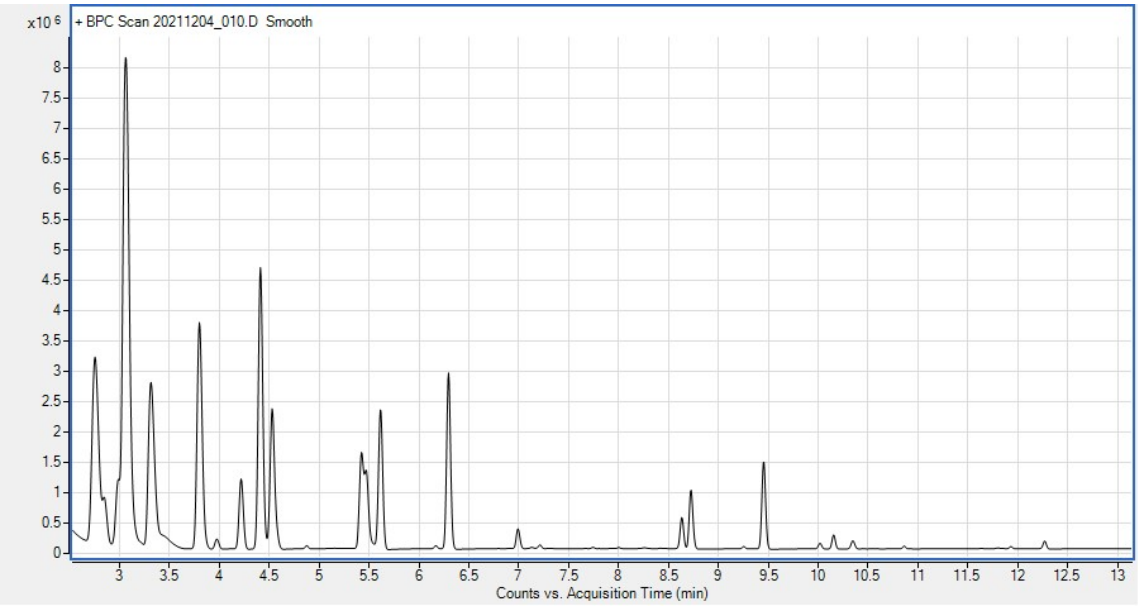


Figure 10. GC Chromatogram of a roasted coffee sample

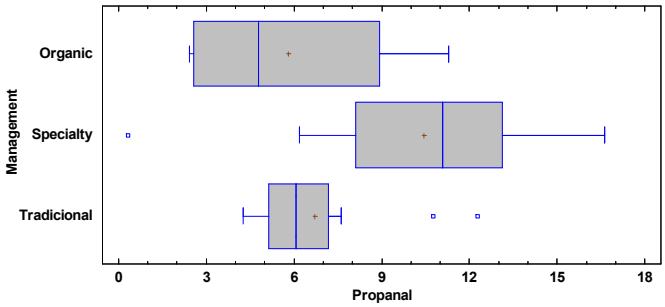
To compare the coffees from different management practices for each one of the 28 volatile compounds it was applied an analysis of variance ANOVA and where necessary (1-Methyl-1H-Pyrrole), it was applied power transformation to assure that the required statistical assumptions were met. When Levene's test fails, the p-value is calculated with Kruskal-Wallis test (Table 10). The volatile compounds that failed the Levene's test were unable to be compared individually (as in Figures 11-13). In Table 4 of the annexes, there is a comparison between the p-values with management practice, management practices & varieties, and plot as factors. From the 28 volatile compounds, 21 have p-values < 0.05 with management practice as a factor while 12 and 9 with management practices & varieties and plot as factors, respectively. In all cases, when variety was taken into account, there were no statistically significantly differences in the means between plots of the same management practice. As an example, there is the boxplot of the Furfural and the LSD X's tables in the annexes, Figure 3.

Table 10. Retention time (RT), p-values for the ANOVAs applied to all the volatile compounds respect the management practices and the ANOVA's statistical assumptions: normality, heteroscedasticity, independence of residuals. * p-values < 0.05.

Volatile Compounds (Power transformation)	RT	ANOVA (p-value)	kolmogorov-smirnov normality test (p-value)	Levene's test of homoscedasticity (p-value)	Independence of residuals
Acetaldehyde	2.097	0.0312*	0.5798	0.2241	Comply
Furan	2.847	0.0382*	0.7680	0.1502	Comply
Propanal	2.988	0.0180*	0.7178	0.2295	Comply
Dimethyl sulfide	3.107	0.0047*	0.3890	0.0777	Comply
Carbon disulfide	3.190	0.0306*	0.5291	0.6906	Comply
2-Methylpropanal	3.802	0.0221*	0.4870	0.3377	Comply
Methacrolein	3.978	0.4496	0.1078	0.4739	Comply
2-Methylfuran	4.219	0.0197*	0.4657	Fail	
3-Methylbutanal	5.475	0.0106*	0.3598	0.9126	Comply
2-Methylbutanal	5.617	0.0313*	0.4585	0.4714	Comply
2,5-Dimethylfuran	6.023	0.0316*	0.3364	Fail	
2-Pentanone	6.171	0.0567*	0.5646	0.2301	Comply
2-Vinylfuran	6.541	0.0316*	0.4680	Fail	
Pyrazine	6.991	0.0444*	0.3631	0.6935	Comply
Dimethyl disulfide	7.132	0.0493*	0.8874	0.7649	Comply
1-Methyl-1H-Pyrrole (0.1)	7.215	0.0894	0.8551	0.0534	Comply
3,4-Dihydro-2H-pyran	7.324	0.1987	0.3273	0.1313	Comply
3-Hexanone	7.744	0.0096*	0.1644	Fail	
2,3-Pentanedione	8.420	0.0018*	0.2651	0.1160	Comply
p-Cresol	8.586	0.2816	0.5616	0.6519	Comply
Dihydro-2-methyl-3(2H)-furanone	8.586	0.0246*	0.9024	Fail	
Methyl-pyrazine	8.725	0.0613	0.5409	0.5882	Comply
Volatile1	8.725	0.0094*	0.6203	Fail	
Volatile2	9.255	0.2188	0.5484	0.8521	Comply
Furfural	9.453	0.0073*	0.9704	0.2250	Comply
2-Furanmethanol	9.453	0.0652	0.7172	0.2960	Comply
1-(Acetyloxy)-2-propanone	10.015	0.0480*	0.4014	Fail	
2,5-Dimethylpyrazine	10.155	0.0217*	0.8985	0.5429	Comply

The method to determine which means are significantly different from others for each volatile compound is Fisher's Least Significant Difference (LSD) procedure. With this method there is a 5.0% risk in saying that each pair of means is significantly different, when the true difference is equal to 0.

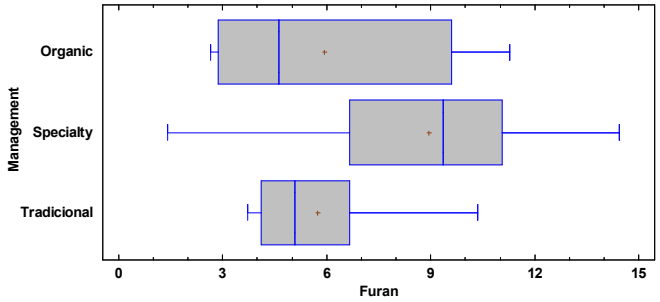
This method is shown in tables with X's, where there are no statistically significant differences between those levels that share the same column of X's (see annexes and **Figure 10**).
In the annexes are the boxplots of every comparable volatile compound from **Table 10** and the LSD X's tables. Even when out of 21 comparable compounds, 14 had a statistically difference concerning the management practices, in no case there was a differentiation between the three managements at the same time. In 6 cases the volatile compounds (propanal, dimethyl sulfide, 3-methylbutanal, 2-pentanone, 2,3-pentanedione and furfural) in the coffee from specialty management differed significantly from organic and traditional coffee management, with the X's forming two homogeneous groups. In **Figure 11** is shown the case of propanal.



Management	Mean	Homogeneous groups
Organic	5.79798	X
Traditional	6.70768	X
Specialty	10.4475	X

Figure 11. Box-plot and representation of LSD of the Propanal for the different management practices

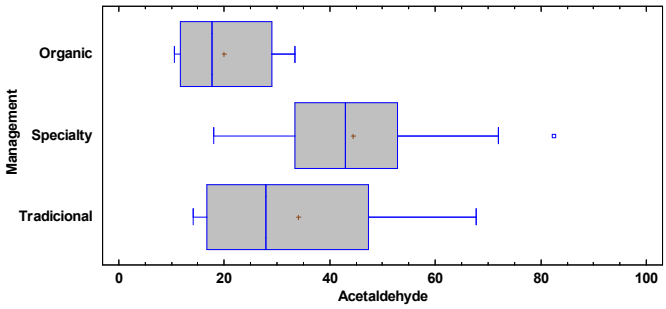
In two cases (Furan and dimethyl disulfide) the X's formed two groups with the traditional and organic coffees in one and the organic and specialty in the other group. Meaning that the concentration of furan and dimethyl disulfide are statistically different for the traditional and specialty coffees, but the organic coffee has concentrations that are statistically similar to both. In **Figure 12** it is shown the case of Furan.



Management	Mean	Homogeneous groups
Traditional	5.74785	X
Organic	5.93692	XX
Specialty	8.95013	X

Figure 12. Box-plot and representation of LSD of the Furan for the different management practices

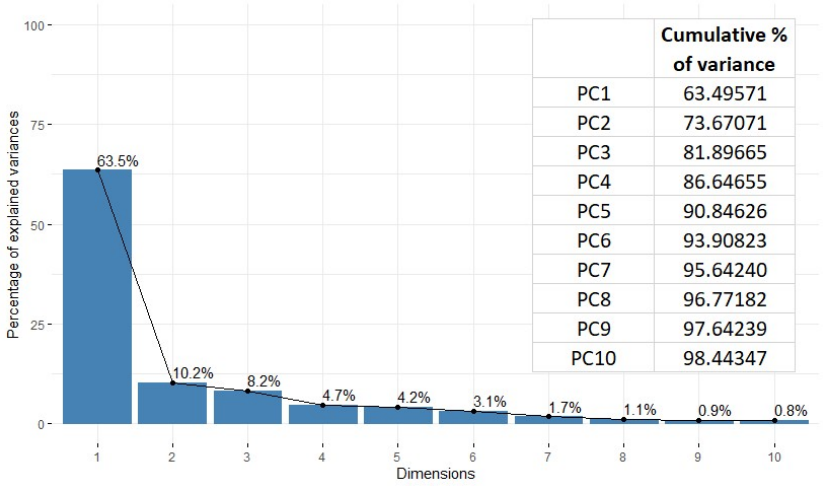
Lastly, in the other six cases (acetaldehyde, carbon disulfide, 2-methylpropanal, 2-methylbutanal, pyrazine and 2,5-dimethylpyrazine) the X's formed two groups with the organic and traditional management in one and the traditional and specialty in another. Meaning that the volatile compounds were statistically different for the organic and specialty coffees but the traditional is similar to both. In **Figure 13** it is shown the case of Acetaldehyde.



Management	Mean	Homogeneous groups
Organic	19.9908	X
Traditional	34.0983	XX
Specialty	44.4661	X

Figure 13. Box-plot and representation of LSD of the Acetaldehyde for the different management practices

Due to the variety and quantity of volatile compounds found in the roasted coffees statistical approaches were used to treat and interpret the experimental data. First a principal component analysis was applied to the raw data. As discussed before, this technique shortens the size of the data without the loss of information or variance by reducing the number of variables. In the scree plot (**Figure 14**) is shown 10 principal components or dimensions and the variance explained by each one of them. As shown in **Figure 14**, with the first three components there is 81.89 % of the variance from the 28 initial variables.



	Cumulative % of variance
PC1	63.49571
PC2	73.67071
PC3	81.89665
PC4	86.64655
PC5	90.84626
PC6	93.90823
PC7	95.64240
PC8	96.77182
PC9	97.64239
PC10	98.44347

Figure 13. Scree plot of 10 principal components (Dimensions), the percentage of explained variance by each and the cumulative percentage of variance.

In **Table 11** are shown the loadings of the principal components 1, 2 and 3 highlighting in yellow the largest contribution of a variable over a PC. The loadings show how the PC1 is formed with most of the volatile compounds while PC2 is formed acetaldehyde, 2-methylpropanal, 2,5-dimethylfuran, p-Cresol and 2,5-dimethylpyrazine. The PC3 is formed with methacrolein, dimethyl disulfide, 1-Methyl-1H-Pyrrole, 3,4-dihydro-2H-pyran and the unidentified volatile 2.

Table 11. Loadings of two principal components. The largest contribution of each variable is shown in yellow

Volatile Compounds	PC1	PC2	PC3
Acetaldehyde	0.08715455	0.4462373	-0.1025809
Furan	0.22409974	-0.0324854	-0.0621149
Propanal	0.21871947	0.04477174	-0.1007332
Dimethyl sulfide	0.15780459	0.07270102	0.06120052
Carbon disulfide	0.19574943	0.14632272	0.02094682
2-Methylpropanal	0.1712414	0.30723829	-0.2783812
Methacrolein	0.12188243	-0.1665689	-0.4664175
2-Methylfuran	0.22377665	-0.0958977	0.01883053
3-Methylbutanal	0.19998229	0.18228613	-0.2127512
2-Methylbutanal	0.17731903	0.31051317	-0.2258754
2,5-Dimethylfuran	0.19775067	-0.2200073	0.11992978
2-Pentanone	0.22768539	-0.0517511	-0.0606806
2-Vinylfuran	0.21295336	-0.1501865	0.13213002
Pyrazine	0.20937463	0.0155988	0.11458509
Dimethyl disulfide	0.13634257	-0.0230501	0.25025664
1-Methyl-1H-Pyrrole	0.209741	-0.0475641	0.22886558
3,4-Dihydro-2H-pyran	0.09708412	-0.3500961	-0.4058072
3-Hexanone	0.22672812	-0.0876727	0.09295346
2,3-Pentanedione	0.22911754	-0.0117822	0.03150715
p-Cresol	0.03359683	-0.3003743	0.2033517
Dihydro-2-methyl-3(2H)-furanone	0.22582376	-0.0303864	0.01161242
Methylpyrazine	0.20885431	0.09950185	0.09031297
Volatile1	0.22517009	-0.0162173	0.10972355
Volatile2	0.10388262	-0.3453655	-0.3626283
Furfural	0.20601658	0.14040295	0.0644774
2-Furanmethanol	0.19684512	-0.1231315	0.16400712
1-(acetyloxy)-2-propanone	0.22869365	-0.1092371	0.00914884
2,5-dimethylpyrazine	0.13662325	0.18886523	0.13158222

The biplot of PC1 and PC2 in Figure 15 shows the distribution of the samples. These two selected dimensions represent together 73.67 % of variance, containing most of the information from the 28 volatile compounds studied. In this plot it is seen that there are some groupings formed with respect to the management practices. There is some differentiation from most of the samples from specialty coffee, whose samples are in the positive region of PC2; the same the traditional coffee samples located in negative region of PC2. The organic coffee is more scattered. There is as well a lot of overlap between these groupings. The loadings from the positive region of PC2 indicate that the volatile compounds that mostly discriminate the specialty coffee samples are acetaldehyde, 2-methylpropanal, 2-methylbutanal and 2,5-dimethylpyrazine. As organic coffee is scattered between PC1 and PC2 it involves all the studied volatile compounds. Finally, the traditional management samples mostly are discriminated by the negative loadings of PC2 which involves the 2,5-dimethylfuran and p-cresol.

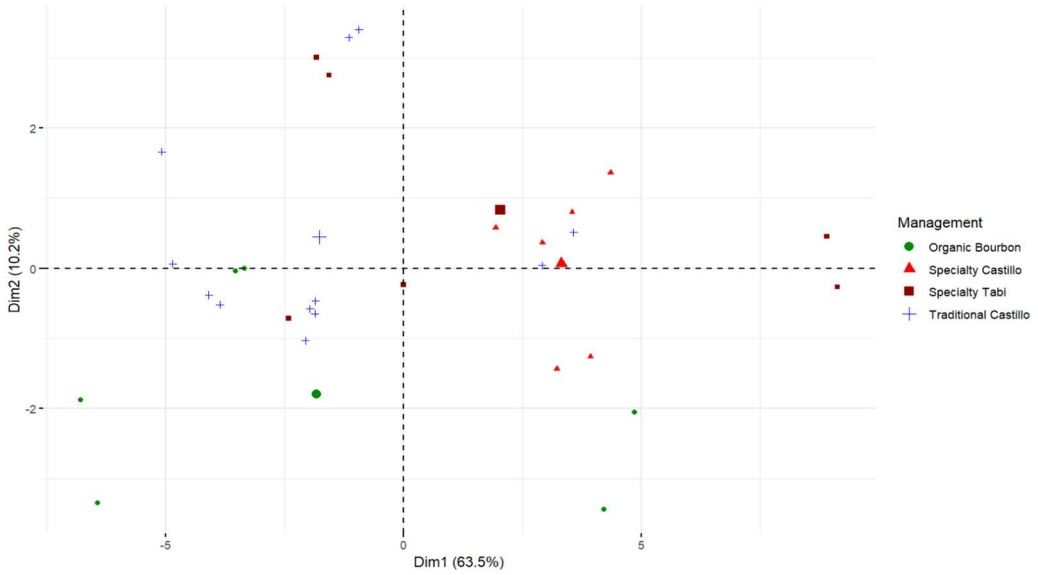


Figure 15. Scores plot of the two principal components of the principal component analysis model (PC1 and PC2). Colors for volatiles compounds form roasted coffee from different management practices: Organic (green), specialty coffee (red) and traditional (blue)

Cluster analysis was applied to the three principal components, previously chosen from the reduction of dimensions, with the Ward method and square Euclidean distance, resulting in the dendrogram of **Figure 16**. The resulting dendrogram, in disagreement with the results from the biplot, show that the samples do not get grouped by management practice. Neither by variety. It can also be seen that only the third cluster shows some differentiation as it is mainly formed with samples from the specialty management.

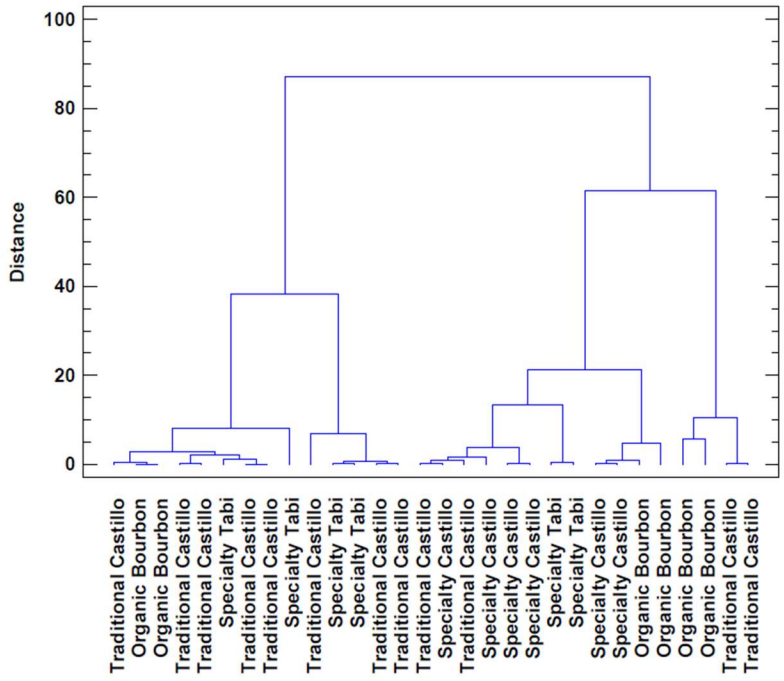


Figure 16. Dendrogram of the 3 principal components. Ward Method and square Euclidean distance.

The discriminant analysis was applied with the three principal components resulting from the reduction of dimensions. The statistical assumptions for three PC's for the DA were met (See **Table 12**).

Table 12. Statistical assumptions for discriminant analysis

PC	kolmogorov-smirnov normality test (p-value)	Levene's test of homoscedasticity (p-value)
PC1	0.4830	0.1032
PC2	0.6716	0.9136
PC3	0.7696	0.2883

With the three principal component the discriminant functions were calculated and used to classify all the observations into the management practices organic, specialty and traditional. Among the 30 observations or coffee samples used to fit the model, 20, or 66.67 %, were correctly classified (**Table 13**). This percentage, even if is not good enough to affirm that the model could be used to classify new coffee samples of unknown management practices, is it good enough to declare that there some differentiation of the volatile compounds concentrations between coffees produced with different management practices. This same model taking into account the varieties as well as the management practices amounted to a fit of 50.00% (See Annexes, **Table 5**). The fact that discriminant analysis was better than principal component analysis in differentiate the management practices was encountered before by Monteiro et al. (2018).

Table 13. Classification table from the DA for the 30 samples of roasted coffee

Management	Group Size	Predicted Management		
		Organic	Specialty	Traditional
Organic	6	3 (50.00%)	1 (16.67%)	2 (33.33%)
Specialty	12	0 (0.00%)	9 (75.00%)	3 (25.00%)
Traditional	12	2 (16.67%)	2 (16.67%)	8 (66.67%)

Percentage of cases correctly classified: 66.67%

Table 14 contains the 91 volatile compounds identified (NIST library, with a score of minimum 80%) in all the studied coffee samples. Of those 91, 49 are not common to the three agronomic managements. This leads to the conclusion that there are differences in the production of metabolites in coffee that can be related with the different agronomic management (organic, traditional and special) and/or the different varieties studied, a fact that is also highlighted in the differences in the notes found in coffee cupping (**Table 3**). Nonetheless, there are clear differences in the volatile presence between same varieties Castillo and agronomic management specialty and traditional.

Table 14. Volatile compounds identified from the roasted coffee samples analyzed by GC-MS

Volatile	Compund cathegory	Organic Bourbon	Trad. Castillo	Specialty Castillo	Specialty Tabi	Odor description	Ref
3-methyl-butanoic acid	Acid		**x**		**x**	cheesy, sour, sweety, fermented	a
2-methyl-1-propanol	Alcohol	x	x	x	x	wine-like	h
2-methyl-3-buten-2-ol	Alcohol	x	x	x	x	herbaceus, fungus-like	j
3-Penten-2-ol	Alcohol	**x**	**x**		**x**	-	
(E)-2-methyl-2-butenal	Aldehyde	**x**	**x**	**x**		green, fresh, fruity	a
1H-Imidazole-4-carboxaldehyde	Aldehyde				**x**	-	
2,4-dimethylbenzaldehyde	Aldehyde	**x**	**x**	**x**		sweet, bitter, almond	g
2,5-dimethylbenzaldehyde	Aldehyde	**x**	**x**			almond-like	g
2,6-Dimethylbenzaldehyde	Aldehyde		**x**			-	
2-butenal	Aldehyde			**x**		pungent, suffocating	a
2-methyl-2-butenal	Aldehyde	**x**	**x**			old crop	k

Volatile	Compund cathegory	Organic Bourbon	Trad. Castillo	Specialty Castillo	Specialty Tabi	Odor description	Ref
2-Methylbutanal	Aldehyde	x	x	x	x	musty, cocoa, nutty, malty	a, b
2-Methylpropanal	Aldehyde	x	x	x	x	Fresh, floral, pungent	a
3-Methylbutanal	Aldehyde	x	x	x	x	fruity, sweet, malty, cocoa	a, b
5-methyl-2-furancarboxaldehyde	Aldehyde	x	x	x	x	Burnt	k
Acetaldehyde	Aldehyde	x	x	x	x	Fruity, fresh	a
Methacrolein	Aldehyde	x	x	x	x	sweet, pungent, hebaceous, orange	d
Propanal	Aldehyde	x	x	x	x	wine, earthy, whiskey, cocoa	c
2-methylbutane	Alkane				**x**	gasoline-like odor	g
(Z)-2-butene	Alkene	**x**	**x**		**x**	Slightly aromatic odor	g
(Z)-3-methyl-2-pentene	Alkene				**x**	-	
2-methyl-1-propene	Alkene	x	x	x	x	petroleum-like odor	g
2,5-dimethyl-2,5-cyclohexadiene-1,4-dione	Cyclics		**x**	**x**		-	
2,6-dimethyl-2,5-Cyclohexadiene-1,4-dione	Cyclics	x	x	x	x	-	
3,5,5-trimethyl-3-Cyclohexen-1-one	Cyclics		**x**			peppermint like odor	g
Cyclopentanone	Cyclics	x	x	x	x	peppermint like odor	g
2,3-dimethyl-2-Cyclopenten-1-one	Cyclopentene	**x**	**x**			-	
2-methyl-2-Cyclopenten-1-one	Cyclopentene	x	x	x	x	-	
Dihydro-2-methyl-3(2H)-furanone	Dihydrofuranone	x	x	x	x	nutty, bready, sweet, cocoa	a
2,4,5-trimethyl-1,3-Dioxolane	Dioxolane	**x**	**x**	**x**		alcohol	l
ethyl formate	Ester				**x**	fermented	m
2-(2-furanylmethyl)-5-methylfuran	Furan		**x**			burnt	k
2-(2-propenyl)-furan	Furan	**x**		**x**		-	
2,2'-methylenebis-furan	Furan		**x**			roasted	a
2,3,5-trimethylfuran	Furan	**x**	**x**		**x**	-	
2,3-dihydro-3-methylfuran	Furan	**x**				-	
2,3-dihydro-4-methylfuran	Furan	**x**	**x**		**x**	-	
2,3-dihydro-5-methylfuran	Furan		**x**	**x**		-	
2,5-dimethylfuran	Furan	x	x	x	x	meaty, roasted	a
2-ethylfuran	Furan	x	x	x	x	-	
2-Furanmethanol	Furan	x	x	x	x	caramel, coffee	a
2-Methylfuran	Furan	x	x	x	x	cocoa, nutty	a
2-Vinylfuran	Furan	x	x	x	x	phenolyc, coffee	a
3,4-dimethylfuran	Furan	x	x	x	x	-	
3-methylfuran	Furan	**x**	**x**	**x**		-	
Furan	Furan	x	x	x	x	cocoa, nutty	a
Furfural	Furan	x	x	x	x	sweet, woody, almond	a
5-methyl-2(3H)-furanone	Furanone	x	x	x	x	sweet, herbaceous, tobacco, earthy	h
1-(2-furanyl)-ethanone	Ketone	x	x	x	x	balsamic	n
1-(Acetyloxy)-2-propanone	Ketone	x	x	x	x	fruity, buttery, sour	h
1-cyclopentylethanone	Ketone				**x**	-	
1-cyclopropylethanone	Ketone	**x**	**x**			-	
1-Penten-3-one	Ketone	**x**				fresh, pungent	o
2,3-Butanedione	Ketone	x	x	x	x	caramel, buttery, creamy, sweet, vanilla	a, c
2,3-Hexanedione	Ketone		**x**	**x**	**x**	caramel, buttery, sweet	a
2,3-Pentanedione	Ketone	x	x	x	x	caramel, buttery	a
2,4-dimethyl-3-Pentanone	Ketone			**x**		-	
2-Butanone	Ketone	x	x	x	x	acetone, ethereal, sweet apricot	a, h
2-methyl-1-Penten-3-one	Ketone		**x**			-	
2-methyl-3-hexanone	Ketone	x	x	x	x	roasted	k
2-Pentanone	Ketone	x	x	x	x	sweet, fruity	b
3,3-dimethyl-2-butanone	Ketone				x	camphor-like odor	g
3,4-Hexanedione	Ketone	x	x	x	x	buttery, sweet, caramel	a
3-Hexanone	Ketone	x	x	x	x	fruity, sweet	a
3-Hydroxy-3-methyl-2-butanone	Ketone		**x**	**x**		-	
3-methyl-3-buten-2-one	Ketone	**x**	**x**			pungent	g
4-methyl-1-penten-3-one	Ketone	**x**				-	
3-methylphenol	Phenol	**x**		**x**	**x**	sweet, tarry	g
p-Cresol	Phenol	x	x	x	x	sweet, tarry	g
Phenol	Phenol	x	x	x	x	phenolic, plastic, rubbery	a
4-Methylpyridazine	Piridazine		**x**		**x**	obnoxious, sweetish odor	g
3,4-Dihydro-2H-pyran	Pyran	x	x	x	x	ethereal	g
2,5-Dimethylpyrazine	Pyrazine	x	x	x	x	cocoa, roasted, nutty	a
Methylpyrazine	Pyrazine	x	x	x	x	nutty, cocoa, roasted	a
Pyrazine	Pyrazine	x	x	x	x	nutty, roasted, cocoa	a
2,3-dimethylpyrazine	Pyrazine			**x**	**x**	nutty, roasted, cocoa	a
2,6-dimethylpyrazine	Pyrazine				**x**	nutty, roasted, cocoa	a
2-ethyl-5-methylpyrazine	Pyrazine			**x**		coffee, nutty	a
2-ethyl-6-methylpyrazine	Pyrazine			**x**	**x**	cocoa, roasted	a
ethylpyrazine	Pyrazine			**x**	**x**	nutty, roasted, cocoa	a
5-methyl-2-pyridinamine	Pyridine		**x**			-	
Pyridine	Pyridine			**x**		fishy, sour, fermented	a
2,5-Dimethylpyrimidine	Pyrimidine	**x**	**x**			nutty, roasted	i
1-Methyl-1H-Pyrrole	Pyrrole	x	x	x	x	woody, herbal, smoky	a

Volatile	Compund cathegory	Organic Bourbon	Trad. Castillo	Specialty Castillo	Specialty Tabi	Odor description	Ref
1-methyl-1H-Pyrrole-2-carboxaldehyde	Pyrrole				x	roasted, nutty	a
Pyrrole	Pyrrole			x	x	nutty, sweet	a
Carbon disulfide	Sulphur compound	x	x	x	x	sulfur, burnt	e
Dimethyl disulfide	Sulphur compound	x	x	x	x	off-flavor, garlic, putrid	f
Dimethyl sulfide	Sulphur compound	x	x	x	x	vegetable, tomato	b
3-Carene	Terpene	x				sweet, turpentine-like	g
3-methylthiophene	Thiophene	x		x	x	-	

Ref: a (Zakidou et al., 2021), b (Pereira et al., 2019), c (Smrke, 2020), d (Flament, 2002), e (Jo et al., 2019), f (Mcgorrin, 2011), g (PubChem, 2022), h (Lopes et al., 2021), i (Rattan et al., 2015), j (Api et al., 2015), k (Zapata et al., 2018), l (Gil et al., 2020), m (Lindinger et al., 2009), n (Ismarti et al., 2022), o (Mall et al., 2018).

4. Conclusions

In order to stablish if it was possible to classify coffee samples according to their agronomic management, in this work 15 samples of coffee from three different managements practices (organic, traditional and special) were analyzed, maintaining origin, type of soil, microclimate and postharvest treatment uniform across the samples. A sensory analysis was conducted; a targeted metabolomic analysis was performed, quantifying caffeine, trigonelline and chlorogenic acids; an untargeted metabolomic study was carried out by means of the analysis of infrared spectra of the green coffee samples; and finally, an untargeted metabolomic study was performed by measuring the volatile compounds of the roasted coffee samples.

With the sensory analysis that involved the evaluation of the coffee attributes fragrance/aroma, flavor, aftertaste, acidity, body, uniformity, balance, cleanliness, sweetness, scoring and overall, it was found that these did not differentiate the coffee samples in relation to the management practices. Additionally, the coffee from the three management practices was classified with the overall score as specialty coffee, with a *very good* classification with scores of 82.14, 82.78 and 83.18 for organic, traditional and specialty managements respectively. With these results, it is possible to assert that the specialty classification from the sensory analysis can be achieved, independently of the applied management practices, if the post-harvest treatment is appropriate. Furthermore, the sensory analysis of the other set of samples (2017, 2018, 2019 harvests) obtained very different cupping notes for the three management practices. A fact that suggests that there might be a differentiation in the metabolite content of coffees from different management practices.

On the other hand, an HPLC chromatographic analysis was carried out to evaluate the concentrations of caffeine, trigonelline, chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid, 4,5-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 3,4-dicaffeoylquinic acid, and use them to discriminate coffee samples from the organic, traditional and specialty managements. The ANOVA analysis showed that the concentrations of these metabolites had no statistically significant difference, and the PCA analysis also showed no differentiation between coffees from the three management practices. With the HCA and DA models (33.33 % of cases correctly classified) it was not possible to classify the samples into organic, traditional or specialty.

In addition, an untargeted metabolomic study was carried out by the infrared spectrometric technique of green coffee samples from plots with different agronomic management. Through an analysis of principal components, 1300 variables were turned to three, with 88.30 % of the variance, with which, through an ANOVA, it was found that there was no statistically significant difference with respect to the agronomic management of coffee. Even so, by PCA it was found that most of the coffee

samples from traditional management differed from the samples of organic and specialty coffee. Nonetheless, by the HCA and DA methods it was not possible to classify the samples by agronomic management, obtaining 51.11% of correctly classified samples for DA.

Another untargeted metabolomic study was carried out by a purge-and-trap and GC-MS analysis of volatile compounds in the roasted coffee samples from different agronomic managements. Of all the volatile compounds detected, 28 were common to all the samples analyzed. The analysis of variance for each of these volatiles revealed that, between one of the agronomic managements and another, there is a statistically significant difference in the means of 21 out of the 28 compounds. Although for none of these compounds the means from the three management practices were statistically different at the same time, e.g., the mean for the propanal from the specialty management is statistically different from the means of the organic and traditional management, which have no difference between them. On the other hand, the principal component analysis showed, despite an overlap of the three groups, that some differentiation was found between samples of the three agronomic managements. With the HCA method, no groupings were found by agronomic management, but the DA resulted in a model that correctly classified 66.67% of the cases. Although the model could be better, it verifies the fact that there is a certain differentiation, through the volatile compounds, of the three management practices. Additionally, there is the fact that of 91 volatile compounds identified, 49 of them are not common to the three agronomic managements (taking the varieties into account), which in turn is consistent with the very different cupping notes obtained for the coffee samples from organic, traditional and specialty management practices.

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CHAPTER 6: Conclusions

The quality of the coffee and the impact of these three studied managements was measured by sensory analysis, the characteristics of the soil, the pesticide traces and how it was reflected in the content of metabolites. Based on the work carried out and the results obtained, it is concluded that the objectives of the investigation were fulfilled.

First a systematic mapping was made in order to evaluate which aspects of quality are the most important in the scientific literature to evaluate coffee throughout the processing chain. As a result, the study revealed that biochemical composition and cup quality are the quality attributes that are most researched. The aim of most of these studies is correlate quality measurements while the most used techniques are analytical chemistry methods. The species with most studies resulted *Coffea arabica* and the presentation most common for the analysis is green coffee. The stage of the production chain more studied is the post-harvest, of which quality control has the most publications. From pre-harvest stage, however less studied, the management practices stand out in the literature. This last aspect, which arouses greater interest, has an important relationship with the quality of the coffee due to the use of pesticides, influence on the quality of the soil and on the metabolite content. Finally, the type of research that most revolves around coffee quality is the evaluation research.

Next was made a study of the impact on the soils' physicochemical properties of the three different types of agronomic managements. This to evaluate coffee quality from an agricultural point of view. 16 physicochemical soil properties (% OC, % MO, NH4+, NO3-, total N, P, pH, texture, % sand, % clay, % silt, Ca, K, Mg, Fe, Mn and CEC) were measured in organic, traditional and specialty coffee crop soils. The one-way ANOVA resulted that the variables nitrate, available P, pH, % Silt, Ca, K, Mg and CEC were statistically different ($p < 0.05$) between one management level and another. For the rest of the variables (% OC, % OM, ammoniacal N, total N, % sand, % clay, Fe, and Mn), no statistically significant difference ($p > 0.05$) was found. Furthermore, a principal component analysis was conducted and with it was possible to determine that there was differentiation between the specialty and traditional management practices. A cluster analysis was accomplished where the resulting dendrogram showed differentiation between the three management practices. This lead to conclude that although these soils maintain great similarities, the different management practices did affect their physicochemical properties.

Next, a validation of a pesticide extraction and quantification method was developed in order to evaluate the pesticide content in green coffee and soil samples from coffee crops under the three different management practices. The aim was to evaluate and compare the coffee quality from a food safety standpoint. The validation of the method had linearity with $R^2 \geq 0{,}995$, matrix effect for soil and coffee matrixes, RSD's no greater than 20 %, recoveries between 70-120 % and MQL ≤ 10 ug.kg. When analyzing the green coffee and soil samples, it was found traces of three out of 25 pesticides in the traditional and organic plots (flutriafol, pyraclostrobin and chlorpyrifos in the coffee samples and carbendazim in soil samples), but in no case they surpassed the maximum limits of residues. These results showed that despite some residues found in the samples, the management

practices did not influence the pesticide content. These results indicate that the correct use of the analyzed pesticides in the crops will not affect the product innocuity, which is an important factor in determining coffee quality.

Furthermore, the quality of the coffee produced by the three studied management practices was measured by sensory analysis and by the metabolite content. It also was determined if it was possible to classify the coffee samples according to the three agronomic management. Consequently, the samples were analyzed by sensory analysis; a targeted HPLC metabolomic analysis; an untargeted infrared metabolomic study and an untargeted GC-MS metabolomic study.

With the sensory analysis that involved the evaluation of the coffee attributes it was found that the management practices did not affect the resulting scores. It was also found very different cupping notes, which would indicate some differentiation in the metabolite content. Besides, the fact that all coffees classified as specialty coffees, with overall scores larger than 80, it can be concluded that that classification can be achieved, no matter the management practices, if the post-harvest treatment is appropriate.

The HPLC chromatographic analysis of the coffee samples to analyze of caffeine, trigonelline and chlorogenic acids was used to discriminate coffee samples from the organic, traditional and specialty managements resulting in no differentiation in their concentrations or in the models.

The untargeted metabolomic study of the infrared analysis of green coffee samples showed by PCA that most of the coffee samples from traditional management differed from the samples of organic and specialty coffee. Nonetheless, by the HCA and DA methods it was not possible to classify the samples by agronomic management.

The untargeted metabolomic study of GC-MS analysis of volatile compounds resulted in 28 common to all the samples analyzed. The ANOVA for each of these volatiles revealed that 21 compounds had statistically significant differences at least between two management practices. The PCA showed some differentiation between the samples of the three agronomic managements, even when overlapping occurred. Cluster analysis found no groupings by agronomic management, but the DA resulted in a model that correctly classified 66.67% of the cases. Furthermore, there is the fact that 49 volatile compounds were not common to the three agronomic managements, which is consistent with the very different cupping notes obtained in the sensory analysis.

To summarize, when contrasting the three agronomic managements it was found that:

- In the analysis of the soils, many similarities were found in their physicochemical properties due to the proximity of plots to each other, but differences were found in certain variables and a global classification was achieved by the cluster method. However, these differences are not reflected in the quality as seen in the overall score of the cupping analysis.
- The agronomic management that is done in the plots that were used for the collection of the samples in the field, does not reflect in the pesticide content, being below the MRL.

- In metabolite analysis there is a proportion of similarities, as is the case of metabolites by HPLC and by infrared, but there are also marked differences, as seen in the measurement of volatile compounds. These differences, which were not decisive for the quality measured in the cup (overall score), they could be introducing differences in the tasting notes.

Based on the outcomes of this research, we can derive broader inferences that have practical relevance to today's coffee sector, particularly from a producer's standpoint.

A specialty coffee is in fact a traditional or conventional coffee with an exceptional cup quality score. A coffee that, independently of the economic capacities of the producer, following a good post-harvest can achieve cupping notes that give it the added value of the specialty classification. The two plots of conventional coffee, which were analyzed in this study, serve as a clear example of a this. The P4 plot comes from a farm with very good resources while the P5 plot is owned by a low-income rural school where the students are taught the basics of coffee care. In both cases, cup scores above 80 were obtained, qualifying them as specialty coffees. Ultimately, management practices seem to primarily affect crop yield, rather than cup grading, which depends on the absence of physical and cup defects, i.e., bad flavors, mainly acquired through processing errors and sometimes excessive use of pesticides.

In organic coffee case, this work demonstrated that pesticides are not required to obtain a specialty coffee score. When buying organic, one invests in food safety and environmental care. It appeals to the conscience of the consumer who is becoming more and more reflexive about what type of products they consume and how these affect their health. The non-use of synthetic chemicals in fertilization, pest management and storage appeal to the consumer's need to take care of their health and the environment. However, the added value that is guaranteed is in terms of social and environmental quality, but not in terms of cup quality. There are many cases of organic coffees with numerous physical and cup defects.

In this work, in which it was ensured that the variability between the coffee crops under study was not related to origin, environmental conditions, altitude and post-harvest conditions, it was observed that there were no statistically significant differences in the measurements of cup quality. This leads to the conclusion that cup quality is more influenced by the post-harvest than by the pre-harvest, which is where organic agriculture mainly lies. Said cup quality, which by means of SCA scores, leads to the qualification of a coffee as a specialty coffee. This work provides a numerical and statistical proof of how necessary the correct care and methods are in each step of coffee processing, from the harvesting of the cherry coffee to the drying of the parchment coffee. As the National Federation of Coffee Growers has been saying for years: a good post-harvest makes all the difference in terms of cup quality.

Considering everything mentioned earlier, the great added value and marketing opportunity inherent to organic farming, along with a proper coffee processing that often leads to a high possibility of a cup evaluation with a Specialty Coffee qualification, would increase its price and visibility in the market. Furthermore, the embrace of organic management practices, centered around the utilization

of renewable resources produced on the farm itself, not only guarantees and encourages its self-sufficiency but also ensures the optimal maintenance of the environmental conditions for soil, water, and vegetation, thus allowing the uninterrupted continuation of productive activities.

ANNEXES

Table 1. p-values for the ANOVAs applied to all the metabolites studied respect the variety & management (Organic Bourbon, Specialty Castillo, Specialty Tabi, Traditional Castillo)

Variables	ANOVA (p-value)
Trigonelline	0.2261
Caffeine	0.0546
Neochlorogenic acid	0.4556
Chlorogenic acid	0.5669
Cryptochlorogenic acid	0.3977
4,5-Dicaffeoylqunic acid	0.3280
3,5-Dicaffeoylqunic acid	0.2834
3,4-Dicaffeoylqunic acid	0.2316

Table 2. Classification table from the Discriminant Analysis according to Variety and management

Management & Variety	Group Size	Predicted Management & Variety			
		Organic Bourbon	Specialty Castillo	Specialty Tabi	Traditional Castillo
Organic Bourbon	6	2 (33,33%)	2 (33,33%)	1 (16,67%)	1 (16,67%)
Specialty Castillo	6	1 (16,67%)	3 (50,00%)	2 (33,33%)	0 (0,00%)
Specialty Tabi	6	1 (16,67%)	0 (0,00%)	4 (66,67%)	1 (16,67%)
Traditional Castillo	12	1 (8,33%)	3 (25,00%)	2 (16,67%)	6 (50,00%)

Percent of cases correctly classified: 50,00%

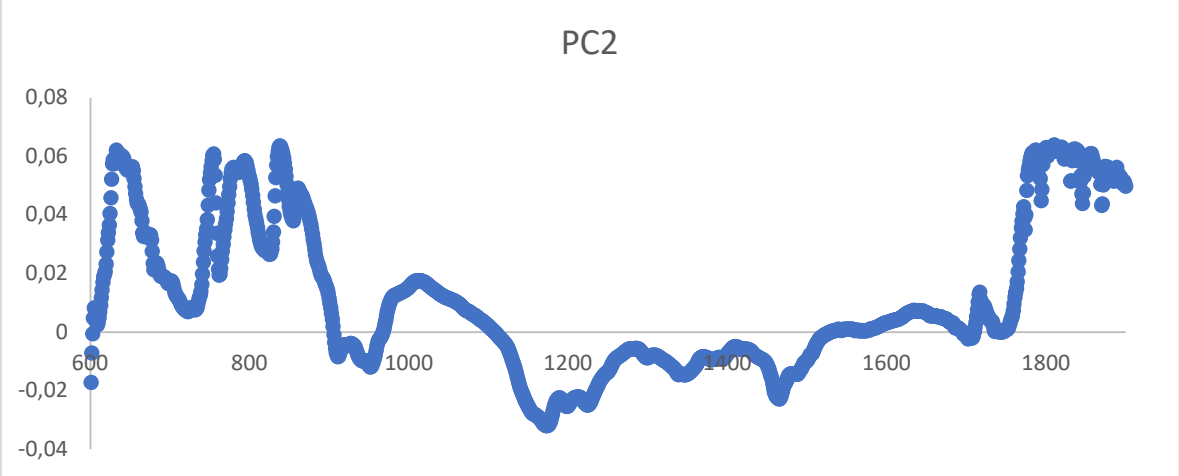


Figure 1 Loadings of the PC2 (Infrared)

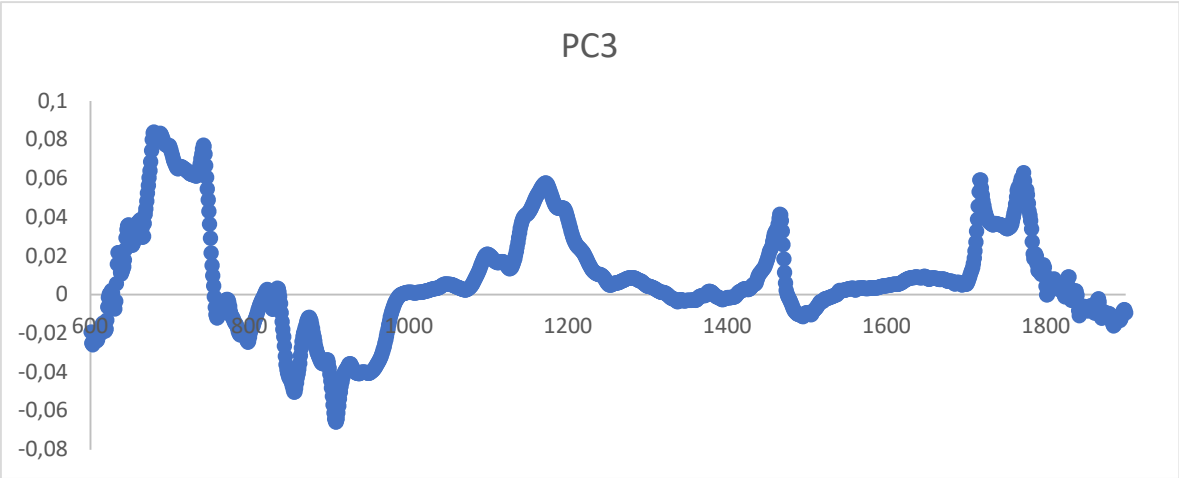


Figure 2 Loadings of the PC3 (Infrared)

Table 3. Classification table from the Discriminant Analysis according to Variety and management

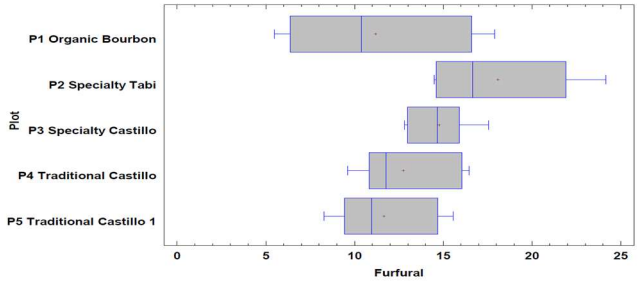
Management & Variety	Group Size	Predicted Management & Variety			
		Organic Bourbon	Specialty Castillo	Specialty Tabi	Traditional Castillo
Organic Bourbon	9	5 (55,56%)	0 (0,00%)	3 (33,33%)	1 (11,11%)
Specialty Castillo	9	2 (22,22%)	0 (0,00%)	5 (55,56%)	2 (22,22%)
Specialty Tabi	9	4 (44,44%)	0 (0,00%)	3 (33,33%)	2 (22,22%)
Traditional Castillo	18	5 (27,78%)	0 (0,00%)	3 (16,67%)	10 (55,56%)

Percent of cases correctly classified: 40,00%

Table 4. p-values for the ANOVAs applied to all the volatile compounds respect the management practices (**Table 10**, chapter 4); Management & Variety (Organic Bourbon, Specialty Castillo, Specialty Tabi, Traditional Castillo) and plot (P1, P2, P3, P4, P5). * p-values < 0.05. When Levene's test fails, the p-value is calculated with Kruskal-Wallis test (values in cursive)

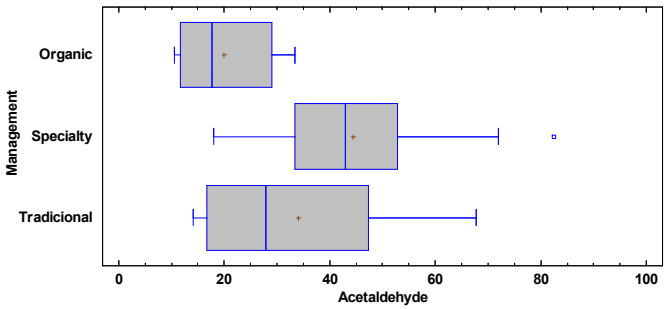
Volatile Compounds	p-value ANOVA/ Kruskal-Wallis test		
	Management	Variety & Management	Plots
Acetaldehyde	0.0312*	*0,0579*	*0,0782*
Furan	0.0382*	0,2044	0,2780
Propanal	0.0180*	0,0377*	0,0558
Dimethyl sulfide	0.0047*	0,0174*	0,0016*
Carbon disulfide	0.0306*	*0,0627*	*0,1134*
2-Methylpropanal	0.0221*	*0,0574*	0,0623
Methacrolein	0.4496	0,0925	0,1752
2-Methylfuran	*0.0197**	*0,0446**	*0,0774*
3-Methylbutanal	0.0106*	0,0055*	0,0144*
2-Methylbutanal	0.0313*	0,0728	0,1294
2,5-Dimethylfuran	*0.0316**	*0,0681*	*0,1193*
2-Pentanone	0.0567*	*0,1190*	*0,2103*
2-Vinylfuran	*0.0316**	*0,0137**	*0,0260**
Pyrazine	0.0444*	*0,1771*	*0,2836*
Dimethyl disulfide	0.0493*	0,0095*	0,0166*
1-Methyl-1H-Pyrrole	0.0894	*0,1298*	*0,1991*

Volatile Compounds	ANOVA (p-value)		
	Management	Variety & Management	Plots
3,4-Dihydro-2H-pyran	0.1987	*0,3291*	0,4855
3-Hexanone	*0.0096**	*0,0249**	*0,0456**
2,3-Pentanedione	0.0018*	*0,0033**	*0,0080**
p-Cresol	0.2816	0,1282	0,0781
Dihydro-2-methyl-3(2H)-furanone	*0.0246**	*0,0559*	*0,1081*
Methyl-pyrazine	0.0613	*0,1892*	*0,3113*
Volatile1	*0.0094**	*0,0206**	*0,0417**
Volatile2	0.2188	0,2261	0,2302
Furfural	0.0073*	0,0065*	0,0151*
2-Furanmethanol	0.0652	0,0216*	0,0429*
1-(Acetyloxy)-2-propanone	*0.0480**	*0,0896*	0,1461
2,5-Dimethylpyrazine	0.0217*	0,0334*	0,0647
Number of p-values < 0.05	21/28	12/28	9/28



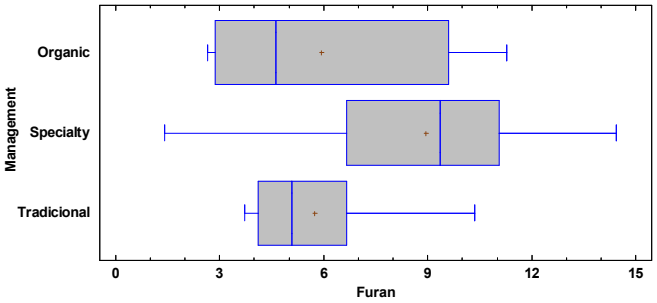
Plot	Mean	Homogeneous Groups
P1 Organic Bourbon	11,1826	X
P5 Traditional Castillo 1	11,6501	X
P4 Traditional Castillo	12,7454	X
P3 Specialty Castillo	14,7653	XX
P2 Specialty Tabi	18,0867	X

Figure 3. Box-plot and representation of LSD of the Furfural for the different plots



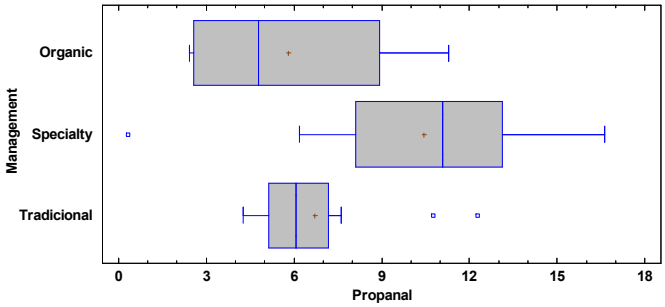
Management	Mean	Homogeneous groups
Organic	19.9908	X
Traditional	34.0983	XX
Specialty	44.4661	X

Figure 4. Box-plot and representation of LSD of the Acetaldehyde for the different management practices



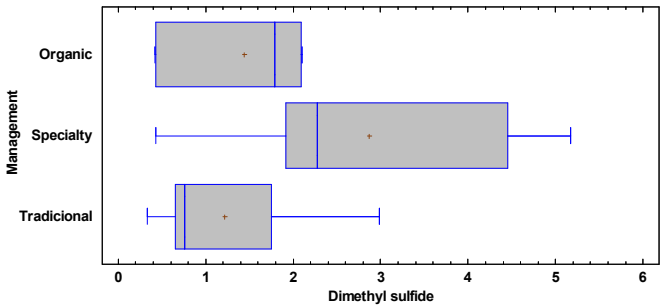
Management	Mean	Homogeneous groups
Traditional	5.74785	X
Organic	5.93692	XX
Specialty	8.95013	X

Figure 5. Box-plot and representation of LSD of the Furan for the different management practices



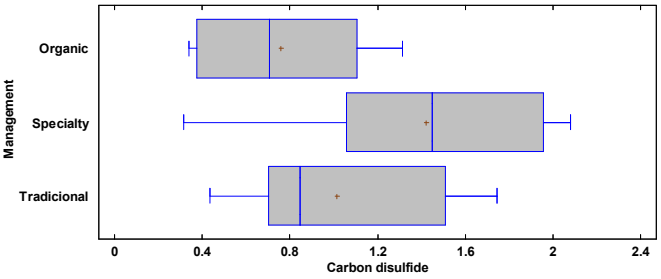
Management	Mean	Homogeneous groups
Organic	5.79798	X
Traditional	6.70768	X
Specialty	10.4475	X

Figure 6. Box-plot and representation of LSD of the Propanal for the different management practices



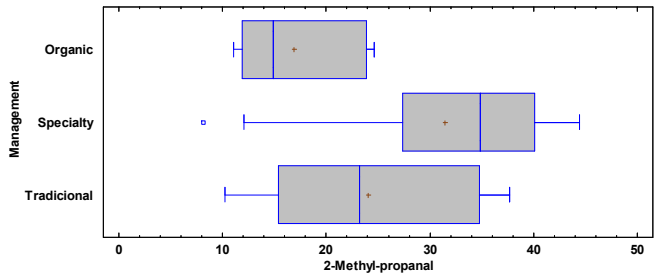
Management	Mean	Homogeneous groups
Traditional	1.21858	X
Organic	1.43917	X
Specialty	2.86828	X

Figure 7. Box-plot and representation of LSD of the Dimethyl sulfide for the different management practices



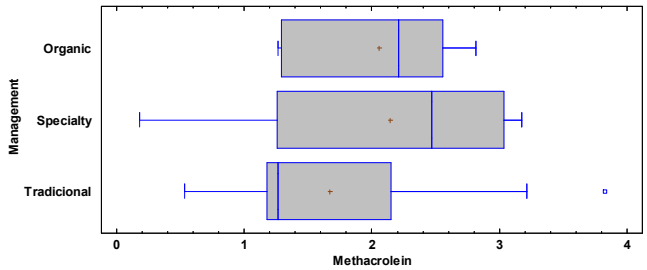
Management	Mean	Homogeneous groups
Organic	0.758883	X
Traditional	1.01523	XX
Specialty	1.42106	X

Figure 8. Box-plot and representation of LSD of the Carbon sulfide for the different management practices



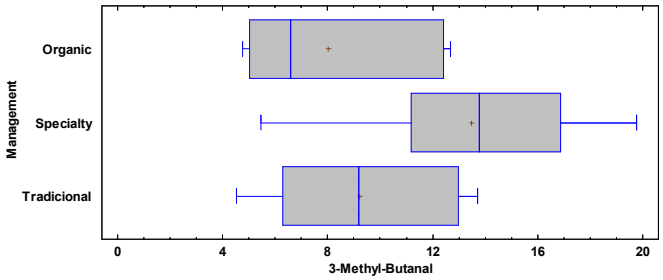
Management	Mean	Homogeneous groups
Organic	16.888	X
Traditional	24.0095	XX
Specialty	31.4777	X

Figure 9. Box-plot and representation of LSD of the 2-Methyl-propanal for the different management practices



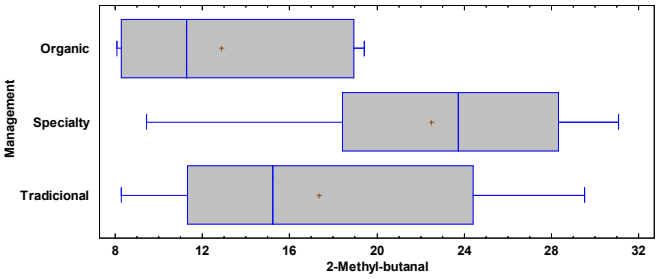
Management	Mean	Homogeneous groups
Traditional	1.6718	X
Organic	2.05775	X
Specialty	2.14582	X

Figure 10. Box-plot and representation of LSD of the Methacrolein for the different management practices



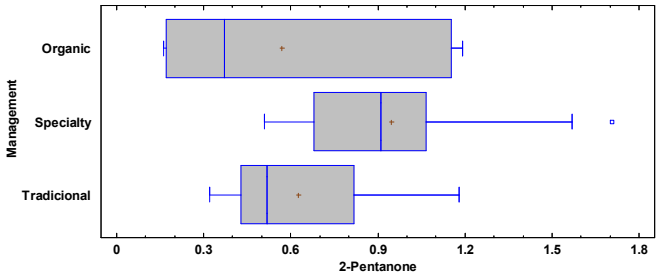
Management	Mean	Homogeneous groups
Organic	8.00967	X
Traditional	9.22452	X
Specialty	13.4809	X

Figure 11. Box-plot and representation of LSD of the 3-Methyl-butanal for the different management practices



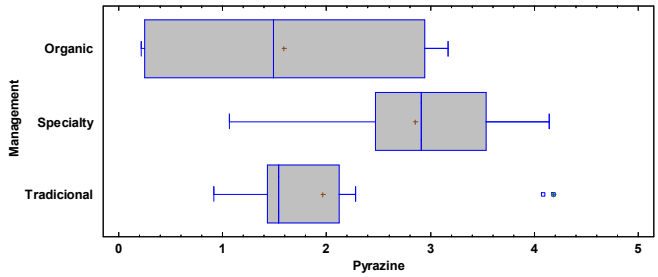
Management	Mean	Homogeneous groups
Organic	12.8894	X
Traditional	17.3274	XX
Specialty	22.4838	X

Figure 12. Box-plot and representation of LSD of the 2-Methyl-butanal for the different management practices



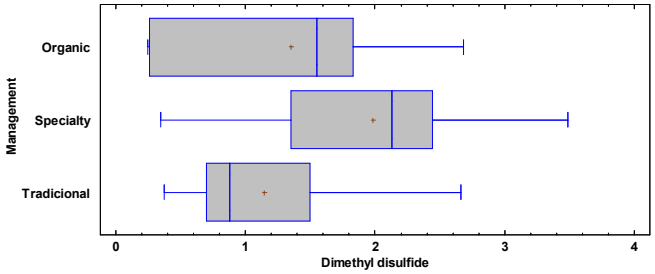
Management	Mean	Homogeneous groups
Organic	0.570117	X
Traditional	0.627208	X
Specialty	0.947042	X

Figure 13. Box-plot and representation of LSD of the 2-Pentanone for the different management practices



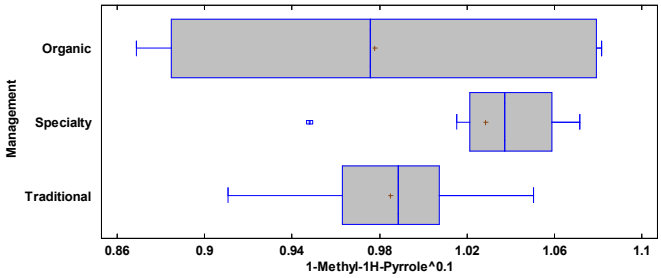
Management	Mean	Homogeneous groups
Organic	1.5933	X
Traditional	1.96447	XX
Specialty	2.85278	X

Figure 14. Box-plot and representation of LSD of the Pyrazine for the different management practices



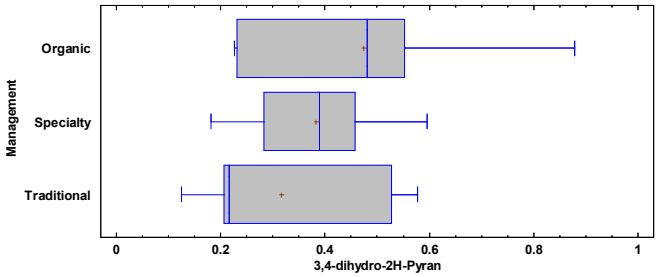
Management	Mean	Homogeneous groups
Traditional	1.14457	X
Organic	1.35432	XX
Specialty	1.98179	X

Figure 15. Box-plot and representation of LSD of the Dimethyl disulfide for the different management practices



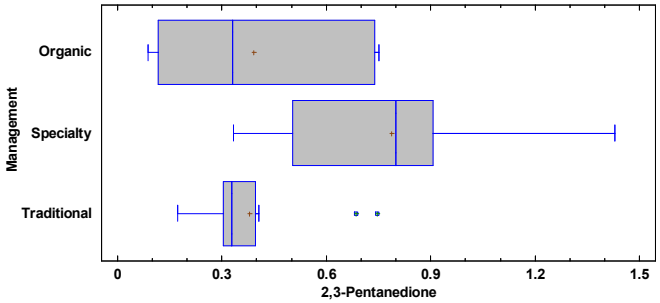
Management	Mean	Homogeneous groups
Organic	0.977748	X
Traditional	0.985029	X
Specialty	1.02862	X

Figure 16. Box-plot and representation of LSD of the 1-Methyl-1H-pyrrole for the different management practices



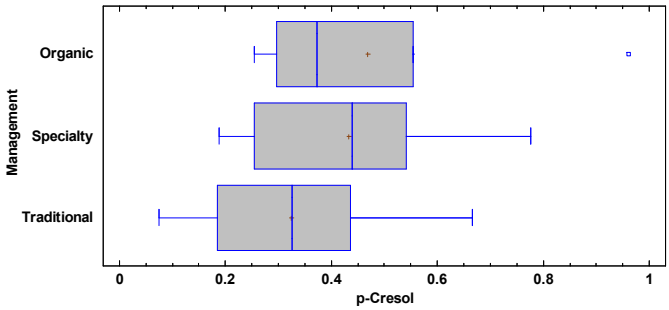
Management	Mean	Homogeneous groups
Traditional	0.317067	X
Specialty	0.383317	X
Organic	0.474933	X

Figure 17. Box-plot and representation of LSD of the 3,4-dihydro-2H-pyran for the different management practices



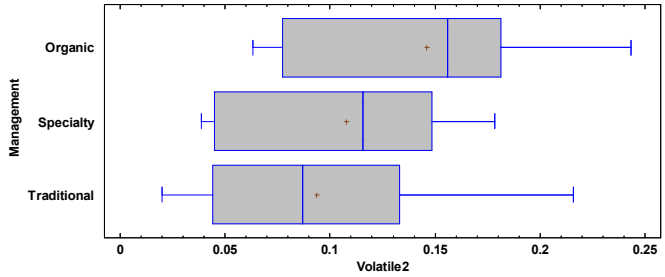
Management	Mean	Homogeneous groups
Traditional	0.378208	X
Organic	0.392633	X
Specialty	0.786633	X

Figure 18. Box-plot and representation of LSD of the 2,3-Pentanedione for the different management practices



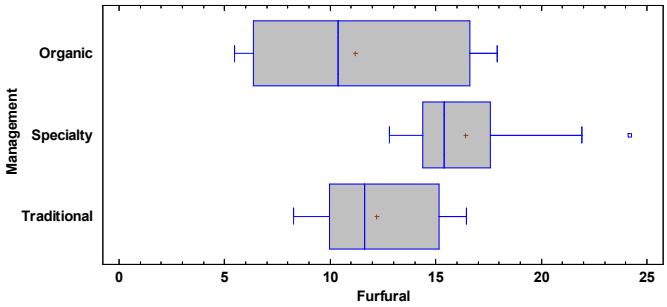
Management	Mean	Homogeneous groups
Traditional	0.323408	X
Specialty	0.432733	X
Organic	0.468567	X

Figure 19. Box-plot and representation of LSD of the p-Cresol for the different management practices



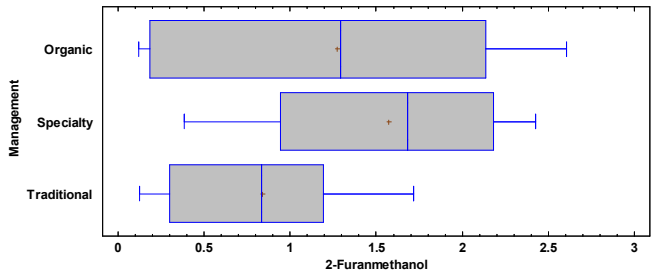
Management	Mean	Homogeneous groups
Traditional	0.0934417	X
Specialty	0.107858	X
Organic	0.146183	X

Figure 20. Box-plot and representation of LSD of the Volatile2 for the different management practices



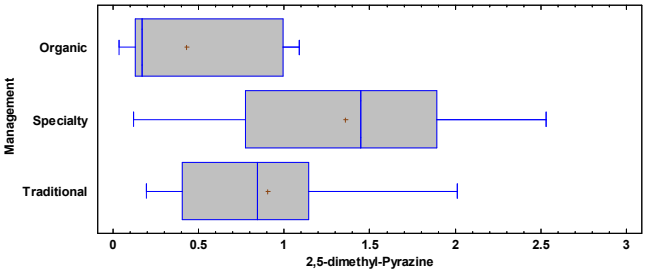
Management	Mean	Homogeneous groups
Organic	11.1826	X
Traditional	12.1978	X
Specialty	16.426	X

Figure 21. Box-plot and representation of LSD of the Furfural for the different management practices



Management	Mean	Homogeneous groups
Traditional	0.840725	X
Organic	1.27167	XX
Specialty	1.57017	X

Figure 22. Box-plot and representation of LSD of the 2-Furanmethanol for the different management practices



Management	Mean	Homogeneous groups
Organic	0.432283	X
Traditional	0.905925	XX
Specialty	1.35742	X

Figure 23. Box-plot and representation of LSD of the 2,5-Dimethyl-pyrazine for the different management practices

Table 5. Classification table from the Discriminant Analysis according to Variety and management

Management & Variety	Group Size	Predicted Management & Variety			
		Organic Bourbon	Specialty Castillo	Specialty Tabi	Traditional Castillo
Organic Bourbon	6	3	1	0	2
		(50,00%)	(16,67%)	(0,00%)	(33,33%)
Specialty Castillo	6	0	5	1	0
		(0,00%)	(83,33%)	(16,67%)	(0,00%)
Specialty Tabi	6	0	2	1	3
		(0,00%)	(33,33%)	(16,67%)	(50,00%)
Traditional Castillo	12	2	2	2	6
		(16,67%)	(16,67%)	(16,67%)	(50,00%)

Percent of cases correctly classified: 50,00%