FATTY ACID COMPOSITION OF OILS FROM GREEN COFFEE BEANS FROM DIFFERENT AGRO-ECOLOGICAL ZONES OF UGANDA

AMOS MUGABE, BFPT; KYU

16/U/13522/GMFT/PE

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DECLARATION

I, Amos Mugabe (Reg. No. 16/U/13522/GMFT/PE), declare that the dissertation which I hereby submit for the degree of Master of Science in Food Technology of Kyambogo University is entirely mine and has not been presented for any award at another University or higher institution of learning.

Signed .....Date..12/11/15.....

APPROVAL

This is to certify that the work presented herein this dissertation is the student's own and was done by him under our guidance and is now ready for submission to the board of examiners of Kyambogo University.

1. George William Byarugaba-Bazirake, PhD

Associate Professor-Food Biotechnology

Department of Food Technology,

Faculty of Science, Kyambogo University

Signature.....Date...12th Nov. 2018.....

2. Michael Bamuwamye, M. Sc (FST)

Natural Chemotherapeutics Research Institute

P. O. Box 4864, KAMPALA.

Signature.....Date...14/11/2018.....

DEDICATION

Dedicated to my dear parents (Rev. Solomon Ottawa and Mrs. Margaret Ottawa), my fiancée, Winifred Ssanyu Nakate and finally to my lovely daughter- Kolinda Audrey Mugabe.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA Analysis Of Variance

FA Fatty Acid(s)

FAME Fatty Acid Methyl Ester(s)

FAO Food and Agriculture Organization

FID Flame Ionization Detector

GC Gas Chromatography

GCBO Green Coffee Bean Oil

GDP Gross Domestic Product

ICO International Coffee Organization

IPCC Intergovernmental Panel on Climate Change

KYU Kyambogo University

MUFA Mono Unsaturated Fatty acid(s)

PUFA Poly Unsaturated Fatty Acid(s)

SaFA Saturated Fatty Acid(s)

UCDA Uganda Coffee Development Authority

UIA Uganda Investment Authority

USA United States of America

ABSTRACT

Coffee is an important agricultural commodity and beverage widely consumed throughout the world. Two-thirds of Ugandans depend on coffee as an important source of income. The quality of coffee determines the relative price and its end use. The lipid composition has been used to characterize Arabica and Robusta coffee. Oil yield, fatty acid (FA) composition and oil nutritional quality of green coffee beans; arabica coffee (*Coffea arabica*) from Bugisu, Rwenzori, Kisoro and Maracha, and robusta coffee (*Coffea canephora* var. *robusta*) from Iganga, Ibanda, Rukungiri, Mbarara, Ntungamo, Masaka, Kayunga and Luwero coffee regions of Uganda were determined. Green coffee beans were obtained from Uganda Coffee Development Authority (UCDA) and coffee dealers in Iganga, Luwero, Kisoro and Maracha Districts. Oil was extracted in a Soxhlet apparatus using n-hexane. Fatty acids were determined as the FA methyl esters (FAME) using flame ionization detection gas chromatography. Differences in oil yield were analysed by ANOVA. The PUFA/SaFA, MUFA/SaFA, (PUFA+MUFA)/SaFA and PUFA/MUFA ratios were used to evaluate the nutritional quality of the oil. Oil yield ranged between 1.75 and 15.45%. Arabica coffee from Bugisu gave a significantly ($p < 0.05$) higher oil yield. Robusta coffee from Ntungamo gave the lowest mean oil yield. Unsaturated fatty acids (UFA) predominated over saturated fatty acids (SaFA). Linoleic and oleic acids were the main unsaturated fatty acids accounting for 53% of the total. Palmitic acid was the major SaFA. Oil yield and FA composition were dependent on agro-ecological coffee sample source and coffee variety. Based on the obtained nutritional quality indices, green coffee oil can be used in foods on blending with other oils. Nevertheless, there is need to investigate the existence of the trans-fatty acids in green coffee oil.

KEY WORDS: *Coffea arabica*, *Coffea canephora*, fatty acid, green coffee beans, oil yield

CHAPTER 1: INTRODUCTION

1.1 Background

Coffee is one of the most important drinks all over the world. According to the International Coffee Organization (ICO), total coffee production in 2015 was about 143 million bags (ICO, 2017). The major producers include Brazil, Vietnam, Colombia, Indonesia and Ethiopia. Uganda is second largest producer of coffee in Africa, after Ethiopia (ICO, 2018). The annual coffee production in Uganda is 288,000 tons of which 95% is exported (UCDA, 2017). Coffee is consumed by around 40% of the world's population (Mussatto, Machado, Martins, & Teixeira, 2011; Murthy & Naidu, 2012) because of its pharmacological importance and primarily excellent taste and aroma (Grembecka, Malinowska, & Szefer, 2007; Butt & Sultan, 2011; Pohl, Stelmach, Welna, & Szymczycha-Madeja, 2013). It is a source of income for many developing countries (Jaramillo, *et al.*, 2011). About 100 million people derive their livelihoods from coffee with an annual retail value of US $ 90 billion (Bunn, Läderach, Rivera, & Kirschke, 2015). Africa's biggest robusta coffee producer is Uganda, and, with higher international prices, coffee generated more exports income (US$ 449 million) in 2001 than all other agricultural export goods together. Coffee is an income source for roughly 1.76 million rural households who have few opportunities to earn alternative income. A total of 282,284 hectares is under coffee cultivation, distributed across the major regions of Uganda. Coffee production in Uganda is dominated by smallholder farmers who are not adequately mobilized into viable economic units. They also largely produce on highly fragmented pieces of land, in most cases estimated at 0.33 hectares per household. The smallholder coffee production system embodies low input use, lack of improved technologies, insufficient business advisory and extension services, and unsustainable agronomic practices (UIA, 2016).

In Uganda, approximately 500,000 households, distributed over two-thirds of the country depend on coffee as an important source of income. Coffee beverage is produced by processing the green coffee beans (Valentin & Watling, 2013). Although more than 100 different species of the genus *Coffea* are known, the most popular species are *Coffea arabica* (arabica coffee), *Coffea canephora* var. *robusta* (robusta coffee) and *Coffea liberica* (liberica coffee, or excelsa coffee). Of these, only *C. arabica* (arabica coffee) and *C. canephora* var. *robusta* (robusta coffee) are used for the majority of coffee production (Cossignani, Montesano, Simonetti, & Blasi, 2016). Annual production in Uganda is on average made up of 20% arabica and 80% robusta (MAAIF, 2010). In addition to serving as a main source of income, coffee has many other uses and thus provides many opportunities for value addition investment. Coffee is a functional food with antioxidant properties and reduces the incidence of cancer, diabetes, asthma, headaches, Alzheimer's and liver diseases, protects against Parkinson's disease and reduces mortality risk (Jeszka-Skowron, Sentkowska, Pyrzyn´ska, & Peña, 2016). Green coffee beans contain high proportions of polysaccharides, monosaccharides, lipids, sterols, fatty acids, phenolic acids, polyphenols, alkaloids, proteins, free amino acids, vitamins, and minerals which are responsible for its health attributes (Parras, Martínez-Tomé, Jiménez, & Murcia, 2007).

The growing recognition of the importance of green coffee beans has made its unique composition and properties receive great attention in the recent times (Dziki *et al.*, 2015). Green coffee bean extract is currently among the world's most popular weight loss supplements. It contains a substance called chlorogenic acid, which is believed to be responsible for the weight loss effects. Other studies (in mice and rats) have shown that chlorogenic acid can reduce body weight, reduce fat absorbed from the diet, reduce fat stored in the liver and improve the function of the fat burning hormone adiponectin. Green coffee

bean extract may improve glucose metabolism and reduce blood pressure. This may have benefits for people who are at high risk of diabetes and heart disease (Messina *et al.*, 2015). The lipid fraction particularly contributes to the formation of aromas and flavours of coffee beverages hence playing a big role in determining the coffee cup quality. Lipids are responsible for flavour retention and influence the stability of foam in the coffee beverage. The lipid composition has also been suggested as a tool to characterize arabica and robusta coffee (Speer & Kölling-Speer, 2006). Coffee oils are popular for their applications in pharmacology and cosmetology. However, oils from arabica and robusta coffees in Uganda have been given little research attention and yet demands are high. Fatty acid profiles of oils from arabica and robusta coffee were characterized to provide basis for its commercial exploration.

1.2 Problem statement and justification of the study

Currently, one of the greatest challenges is the production of food, not only in sufficient amounts, but also with adequate nutritional quality (Tesfay, Tarekegn, & Kefarge, 2015). Coffee is the second most traded product by value after petroleum. It is the most popular beverage in the world with approximately 400 billion cups consumed per annum (Mussatto *et al.*, 2011). Consumption of coffee and, hence, its market is concentrated in the developed countries, with per capita consumption being highest in the Republic of Finland (UIA, 2016). Production is mainly from developing countries led by the Federative Republic of Brazil. In Africa, Uganda is second to Ethiopia in the contribution towards world coffee production (ICO, 2014). Africa's share of world coffee production has been declining whereas the output from Uganda has shown a steady increase since 1986 with annual production of about 288,000 tons (UCDA, 2017). Coffee remains a traditional cash crop in Uganda with notable contribution to foreign exchange earnings. Coffee is the most important agricultural export

with a contribution of 17.7% to the total national export value (UIA, 2016). The major challenge facing coffee production in Uganda is little value addition. The bulk of coffee exports are in the form of raw unprocessed beans. This may be partly explained by the limited capacity to undertake investment for value addition. In terms of market for processed coffee, 3% of coffee produced is consumed locally, with the remainder exported as green coffee beans. Most of the studies have focused on negative health effects rather than the positive effects of coffee (Messina *et al.*, 2015). This is attributed to the limited information on the chemical composition of Uganda's coffee yet fatty acid composition of coffee oil has been a subject of investigation in a number of coffee producing countries (Karl & Isabelle, 2006). Nutritionists recommend increased intake of polyunsaturated fatty acids (PUFA) and reduced intake of saturated fatty acids and *trans* fatty acids (TFA) because the latter are associated with an increased risk of cancer and heart disease (Ogwok, Muyinda, Nakisozi, & Bamuwamye, 2017).

Green coffee beans have unique chemical composition that has been widely recognized in promoting health (Dziki *et al.*, 2015). The lipid fraction particularly contributes to the formation of aromas and flavours of coffee beverages. These sensory attributes are desirable in determining the coffee cup quality. Lipids are responsible for flavour retention and influence the stability of foam in the coffee beverage. The lipid composition of coffee has been suggested as a tool to characterize arabica and robusta (Speer & Kölling-Speer, 2006). Oils from coffee are popular for their applications in pharmacology and cosmetology (Patay, Bencsik, & Papp, 2016). This study focused on the oil yield, fatty acids profile of the extracted oils and their nutritional quality to provide a basis for its commercial exploration.

1.3 Objectives of the study

1.3.1 Overall objective

To evaluate the oil content and fatty acid composition of the two major coffee varieties (*Coffea arabica* and *Coffea canephora* var. robusta) from different areas of Uganda.

1.3.2 Specific objectives

To determine the:

1. Oil content of arabica and robusta coffee varieties obtained from different regions of Uganda.
2. Fatty acid composition of the oil obtained from the two coffee varieties.
3. Nutritional quality of green coffee oil from the two varieties based on PUFA/SFA, MUFA/SFA, PUFA/MUFA and (PUFA+MUFA)/SFA ratios.

1.4 Hypothesis

Green coffee oil contains more unsaturated fatty acids and its yield and composition vary with geographical location and coffee variety.

CHAPTER 2: LITERATURE REVIEW

2.1 Coffee production trends

Coffea is an evergreen shrub of the *Rubiaceae* family and is native to tropical Africa, specifically Ethiopia, Sudan, Madagascar, the Comoros, Mauritius and Reunion in the Indian Ocean. The plant was exported from Africa to countries around the world and green coffee beans are now produced in more than 70 countries (Iwasa *et al.*, 2015). Coffee is one of the most consumed beverages in the world (Saura-Calixto & Goñi, 2006; Monente, Ludwig, Irigoyen, de Peña, & Cid, 2015). It represents an important financial source for developing countries, involving a large number of stakeholders in the coffee value chain (Coung, Ling, Jin, Jie, & Tiep, 2014). In 2015, total global coffee production was about 143 million bags, whereas the world consumption in 2014 was 149 million bags, implying an enormous demand for coffee (ICO, 2017). Coffee production is mainly by developing countries led by Brazil, Viet Nam, Indonesia, Colombia, Ethiopia, Peru, India, Honduras, Mexico and Uganda as the top 10 producers (UIA, 2016).

Coffee plays a vital part in the livelihood of many Ugandans, and contributes 17% of Uganda's foreign exchange earnings (UIA, 2016). The annual coffee production in Uganda is 200,000 tons with 3% of the total coffee produced consumed within the country (UCDA, 2017). According to the International Coffee Organization, Uganda produced 3744, 3650, 4962 and 5100 metric tons of green coffee in the 2014, 2015, 2016 and 2017, respectively. This trend reflects a 2.8% percentage increase between 2016 and 2017 against 3.2% for Africa as a whole (Figure 1). The productivity increased significantly due to new technologies, mechanization, use of chemicals and Government policies. 25% of the total surface area of Uganda (241,550.7 km^2) consists of rivers and lakes and much of it is roughly 1,000 metres above sea level making it suitable for both arabica and robusta coffee growth

(UIA, 2016). Much of Uganda's coffee exports are destined to the European Union, Sudan, United States of American (USA), Switzerland, Japan and Australia.

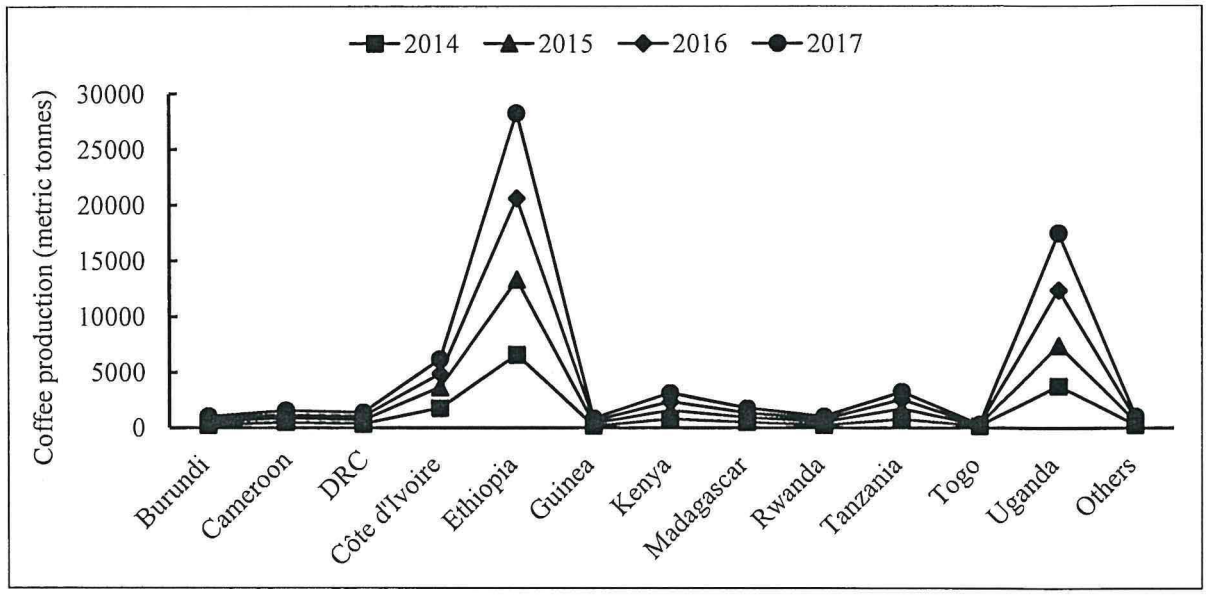

Figure 1: Trends of coffee production in African exporting countries
(Source: Data of ICO (2018))

Two types of coffee: Arabica and Robusta are majorly grown in Uganda in the ratio of 1: 4 repectively. Robusta coffee is grown in the low altitude areas of Central, Eastern, Western and South Eastern Uganda up to 1,200 meters above sea level while Arabica coffee is grown in the highland areas on the slopes of Mount Elgon in the East and Mount Rwenzori and Mount Muhabura in the South Western Region (1500-2,300 m above sea level)). Coffee is mostly grown in mixed stand where it is intercropped with food crops such as bananas and beans which ensure households' food security. It is also grown among shade trees that result into sustainable coffee production, with minimal use of agro-chemicals (fertilizers, pesticides and fungicides). Coffee farmers in Uganda use a low input system and producer households strongly rely on family labor.

2.2 Health benefits of coffee

Coffee is consumed because of its desirable bitter taste and medicinal benefits. The effect of coffee on human physiology varies from person to person and also with the quality and quantity of coffee consumed. Coffee is a complex chemical mixture. It is composed of over 1000 different chemical components. Although coffee has a long history of human food, until recently most of the studies on its health effects have focused on potential adverse and toxic effects (Messina *et al.*, 2015). Despite a vast amount of research, evidence to support the direct link of coffee with disease has been limited and inconsistent. However, although not yet proven, recent scientific literature suggests the potential beneficial health effects of coffee and several of its constituents. For example, coffee's positive effects on performance and protection against some types of cancers, liver disease, and radiation–induced tissue damage have been documented (Rajeev, Kaushal, Pavan, Rajesh, & Nitesh, 2016). In addition to caffeine, Trigonelline and chlorogenic acids, coffee contains substantial amounts of antioxidants, which may explain some of its potential beneficial effects. Caffeine is the major pharmacologically active compound in coffee is caffeine (methylxanthine), which is known to have effects on a number of body functions including the stimulation of the central nervous system and cardiac muscles, relaxation of smooth muscles especially bronchial muscle and to act on the kidney to produce diuresis. A number of other compounds present, such as chlorogenic acid are also pharmacologically active (Rajeev, Kaushal, Pavan, Rajesh, & Nitesh, 2016). There is a wide range of effects that are also attributed to caffeine such as increase of the pain killers' effect, reduction of tiredness and of migraines, increase of the stomach acid secretion, stimulation of the heart function (with hypertensive effects), stimulation of kidney function (with diuretic effect), stimulation of respiration and decrease of the vitamin B concentration. In addition, it is supposed that the occurrence of Parkinson's

disease may be reduced by a regular consumption of coffee and cola (Patay, Bencsik, & Papp, 2016).

Different parts of the coffee plant have for long been used as a medicine throughout the world healing a wide range of diseases or symptoms, such as diarrhea, intestinal pain, HIV/AIDS, flu, anemia, edema, asthenia, liver diseases, migraine, stomach pain, fever; against bleeding that accompanied abortion; as astringent, aphrodisiac, cough suppressant; for cardio-tonic and neuro-tonic effects, for tiredness, asthma, scorpion bites, and for the production of prolactin (Patay, Bencsik, & Papp, 2016). Coffee coal is used for the treatment of inflammatory diseases of the mouth and pharynx and as a treatment of festering wounds (Neuwinger, 2000). Regular consumption of coffee reduces the occurrence of kidney and liver cancers (Neuwinger, 2000). These effects are attributed to the content in caffeine, diterpenoids, caffeic acid, polyphenols, essential oils, and heterocyclic molecules (Patay, Bencsik, & Papp, 2016).

Coffee seed extracts present several health benefits when used in cosmetics and pharmaceuticals. The pharmacological benefits include a wide range of effects such as antioxidant, detoxifying, lipid reducing, cardio-protective, anti-inflammatory, analgesic, antineoplastic, diuretic, antibacterial, antiviral, antifungal, anti-osteoporotic, anti-cellulitic, and anti-age activity, the effect on central nervous and gastrointestinal systems, and on blood vessels (Boros *et al.*, 2010). According to Rodriguez *et al.* (2015), the hydro-alcoholic extract of coffee silver skin can be used for topical application because it has no irritant effects.

2.3 Applications of coffee

Coffee is one of the most popular beverages in the world, with an estimated more than 400 billion cups consumed per annum (ICO, 2014). Consumption of coffee and, hence, its market is concentrated in the developed countries, with per capita consumption being highest in Finland, followed by Denmark, Sweden, the Netherlands, Belgium, Norway, Switzerland, Germany, Austria and France in the top 10 consumers at levels of 12, 9.9, 8.7, 8.4, 8.2, 7.9, 6.8, 5.9, 5.8 & 5.5 Kg per capita respectively in the European Union (EU) region (ICO, 2014). Other than its traditional use as a beverage, coffee has been used in a number of products including flavor syrups, jet teas, fruit smoothies, bubble tea, concentrated coffee milk, fresh baked goods and assorted paper supplies. Current value adding applications of coffee wastes include biofuel, mushroom and fertilizer production, along with enzyme, dietary fibre and bioactive compound extraction (Murthy, & Naidu, 2012).

2.3.1 Coffee cup quality

Cup quality, often referred to as drinking quality or liquor quality, is an important attribute of coffee that acts as a yardstick for price determination (Muschler, 2001; Agwanda, Baradat, Eskes, Cilas, & Charrier, 2003). It also determines the usefulness of a given quantity of coffee (Agwanda, Baradat, & Eskes, 2003). Cup quality in coffee is affected by a great number of factors; agronomic, genetic and production related. The quality of coffee is extensive in its definition. Leroy *et al*, (2006) defines coffee quality on a number of levels. At the exporter or importer level coffee quality is linked to bean size, number of defects, regularity of provisioning, tonnage available and physical characteristics. At the roaster level coffee quality depends on moisture content, characteristic stability, origin, organoleptic (taste and smell) qualities and biochemical compounds. At the consumer level coffee quality is about taste and flavor, effects on health and alertness, geographical origin, and environmental

and sociological considerations. In 2004 the International Organization for Standardization (ISO) defined a standard for green coffee quality which entails defects, moisture content, size and some chemical compounds of beans as well as standardization of preparation of a sample from which to perform cup tasting. The quality of coffee also depends in part on the chemical composition of the green coffee beans. Uganda produces what is generally considered the world's best quality of robusta coffee, and, with its quality and volumes, it continues to find willing buyers (UIA, 2016). In contrast to 'off-notes', which also occur during the course of storage, and are mainly caused by undesired changes within the lipid fraction due to oxidation processes (Speer & Kolling-Speer, 2006), the causes of the progressive weakening of cup quality are still unknown. Therefore, green coffee bean quality is of critical importance to the coffee industry in terms of revenue.

2.4 Chemical composition of green coffee beans

2.4.1 Non-volatile components

The non-volatile fraction of green coffee is composed primarily of moisture (water), carbohydrates, fiber, proteins, free amino acids, lipids, minerals, organic acids, chlorogenic acids, trigonelline and caffeine (Table 1).

Table 1: Chemical composition of green *coffea arabica* and *coffea canephora* var. robusta beans (g/100g)

Component	*Coffea arabica*[1]	*Coffea canephora*[1]	*Coffea arabica*[2]	*Coffea canephora*[2]
Sucrose	6.0-9.0	0.9-4.0	9.0- 12.5	6.0- 11.5
Reducing sugars	0.1	0.4	0.2-0.5	0.2-0.5
Polysaccharides	34-44	48-55	46- 53	34- 44
Lignin	3	3	1.0-3.0	1.0-3.0
Pectin	2	2	-	-
Proteins	10.0-11.0	11.0-15.0	8.5- 12	8.5- 12
Free amino acids	0.5	0.8-1.0	-	-
Caffeine	0.9-1.3	1.5-2.5	0.8- 1.4	1.7- 4.0
Trigonelline	0.6-2.0	0.6-0.7	-	-
Coffee oil	15-17.0	7.0-10.0	15- 18	8.0- 12
Diterpenes	0.5-1.2	0.2-0.8	-	-
Minerals	3.0-4.2	4.4-4.5	3.0- 5.4	3.0- 5.4
Chlorogenic acid	4.1-7.9	6.1-11.3	6.7- 9.2	7.1- 12.1
Water	8.5-12.0	8.5-12.0	-	-
Aliphatic acid	1	1	-	-
Quinic acid	0.4	0.4	-	-

Source: 1. Farah (2012), 2. Berlitz *et al.* (2009)

2.4.1.1 Water

The moisture content of green coffee beans influences the way coffee roasts and the loss of weight during roasting, and is therefore an important quality parameter. According to Pitia *et al.* (2007), the optimum moisture content of *C. arabica* and *C. canephora* green beans varies between 8.5% and 12%. If coffee beans are too wet (> 12.5% moisture), moulds can easily

grow during storage thereby making it a health risk factor. Green coffee beans with low moisture content (< 8%) roast faster than those with high moisture content (Leroy *et al.*, 2006; ITC, 2002). Hydrolysis of coffee components especially triacylglycerols which are the major constituents of the coffee lipid causes staleness thus affecting the coffee integrity (Farah, 2012). Good levels of moisture content (10-12%) allow for high cupping scores, balanced acidities and great aroma. Over-drying also affects the quality and taste of coffee. At 9% moisture, a loss in aroma, freshness and clarity is expected. If the moisture content drops below 8%, then the roasted bean would contain hardly any flavour at all (Pittia, Nicoli, & Sacchetti, 2007). Various authors have this linked to the loss of viability (Sivetz & Desrosier, 1979), but scientific proof for this statement is lacking.

2.4.1.2 Carbohydrates

Carbohydrates are major constituents of coffee and account for more than 50% of the dry weight. They are precursors for the Maillard and caramelization reactions, which are important for color and aroma development during coffee roasting. Soluble and insoluble polysaccharides account for approximately 44% and 47% of dry matter weight in *C. arabica* and *C. canephora,* respectively (Berlitz *et al*, 2009). Coffee polysaccharides are considered bioactive compounds due to their potential role as substrates for probiotic microorganisms in the human intestine. Of the main polysaccharides in coffee, galactomannan and arabinogalactan are water soluble (Farah, 2012). High-molecular-weight polysaccharides in addition give body in the coffee brew. Sucrose is important for coffee flavor and quality; it accounts for up to 9% of dry weight of *C. arabica* and 4.5% in *C. canephora*. A higher sucrose content is one of the reasons for the superior aroma and overall flavor of arabica coffee (Farah, 2012). Small amounts of simple carbohydrates such as fructose, glucose, mannose, arabinose, and rhamnose and oligosaccharides such as raffinose and stachyose have

also been identified in green coffee and reported to contribute to the acidity of the coffee brew after roasting.

2.4.1.3 Soluble dietary fiber

Soluble dietary fiber in coffee consists of high-molecular-weight polysaccharides that increase the brew's viscosity (Nunes & Coimbra, 2001). The hot water-soluble green coffee type II arabinogalactans (Figure 2) are highly branched and extremely complex polysaccharides, and are covalently linked to proteins in which 10% of the amino acid chains are 4-hydroxyproline residues. These compounds cannot be digested by humans; therefore, they reach the colon intact, potentially serving as substrates for beneficial colonic microbiota fermentation (Farah, 2012). A high intake of dietary fiber was also positively associated with several beneficial physiologic and metabolic effects such as lowering blood cholesterol and modulating the blood glucose and insulin responses (Gntechwitz, Reichardt, Blaut, Steinhart, & Bunzel, 2007).

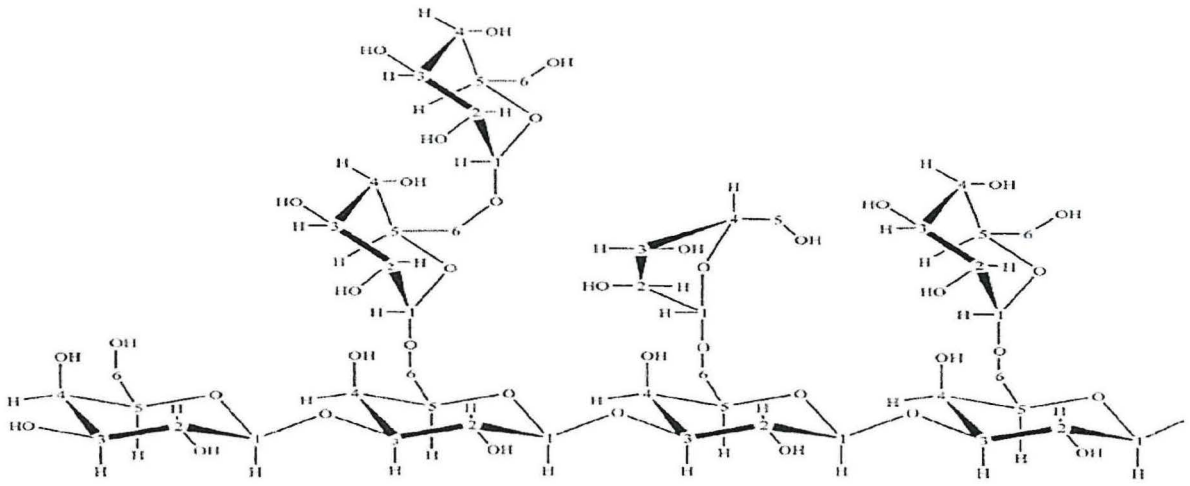

Figure 2: The fragment of molecular structure of arabinogalactan with numbered C atoms

2.4.1.4 Protein, peptides, and free amino acids

Protein, peptides, and free amino acids are vital for coffee flavor because like carbohydrates, they are a key component of the Maillard reaction (Farah, 2012). They are precursors for the formation of volatile compounds such as furans, pyridines, pyrazines, pyrroles, aldehydes, and melanoidins. Melanoidins are responsible for coffee's color and to some extent, its antioxidant activity. The total nitrogenous compounds excluding caffeine and trigonelline account for 9 to 16% of the green coffee bean chemical composition, with a slightly higher content in *C. canephora* than *C. arabica (*Farah, 2012). However, coffee is not a good nutritional source of protein because it is deficient in essential amino acids. Alanine was reported as the amino acid with the highest content (1.2 g/kg) in robusta coffee beans, followed by asparagine (0.68 g/kg) and 0.8g/kg and 0.36 g/kg in arabica respectively (Murkovic & Derler, 2006). Asparagine is actually an essential component of proteins involved in signaling, neuronal development and transmission across nerve endings. In addition, asparagine is known for its key role in the biosynthesis of glycoproteins, as well as in the synthesis of many other proteins. However, thermal degradation of free asparagine in the presence of sugars in the Maillard reaction could lead to the formation of acrylamide which is a neurotoxin and probably carcinogenic (Lea, Sodek, Parry, Shewry, & Halford, 2007).

2.4.1.5 Lipids

The lipid fraction of green coffee beans comprises mainly triacylglycerols, sterols, tocopherols, and diterpenes of the kaurene family (Speer & Kölling-Speer, 2006; Ferrari, Angelis, & Navarini, 2010). *Coffea arabica* and *Coffea canephora var. Robusta*, contain between 7 and 17 % fat. Arabica coffee has been observed to have higher lipid content than robusta coffee. Coffee oil is composed mainly of triacylglycerols with fatty acid composition

similar to those of some common edible vegetable oils (Farah, 2012). Coffee oil has been widely used as flavoring in ice cream, beverages and instant coffee industries or as natural ingredient for cosmetic companies (Hurtado-Benavides, Dorado, & Sánchez-Camargo, 2016). In terms of quality, arabica contains almost 60% more lipids than robusta which may play an important role in aroma retention (Oestreich-Janzen, 2010). Coffee lipids contribute to the texture and mouthfeel of the beverage as they carry flavours and fat-soluble vitamins (Oestreich-Janzen, 2010). Yang *et al* (2013) reported linoleic acid (C18:2), palmitic acid (C16:0), oleic acid (C18:1) and arachidic acid (C20:0) to be the major fatty acids in green coffee oils. However, small amounts of other fatty acids such as linolenic acid (C18:3), docosanoic acid (C22:0), tetracosanoic acid (C24:0), eicosenoic acid (C20:1), myristic acid (C14:0), and tricosanoic acid (C23:0) we also reported to be present (Yang *et al.,* 2013). Karl and Kooling (2006) and Farah (2012) (Table 2), reported C18:2 and C16:0 as the major fatty acids. Fatty acids are known to be important components of the flavor and aroma of coffee (Jham *et al*., 2008).

Table 2: Fatty acid composition of green coffee beans

Fatty acid	Kooling			Oliviera	Farah
	Dewaxed beans	Robusta	Arabica	Beans	Beans
C14:0	0.2	trace	trace	trace	-
C15:0	-	trace	trace	-	-
C16:0	33.3	27.2-32.1	26.6 -27.8	34	-
C17:0	-	trace	trace	-	-
C18:0	7.3	5.8-7.2	5.6-6.3	7	-
C20:0	2.5	2.7-4.3	2.6-2.8	3	-
C21:0	-	trace	trace	-	-
C22:0	0.5	0.3-0.8	0.5-0.6	0.7	-
C23:0	-	trace	trace	-	-
C24:0	Trace	0.3-0.4	0.2-0.4	-	-
C16:1ω7	-	trace	trace	trace	-
C20:1	-	0.2-0.3	trace - 0.3	0.3	-
C18:1ω9	6.6	9.7-14.2	6.7-8.2	9	14-7
C18:2ω6	47.7	43.9-49.3	52.2-54.3	44	43-54
C18:3ω6	1.7	0.9-1.4	2.2-2.6	-	-
C18:3ω3	-	-	-	1.5	1-2.6
C20:3ω3	-	-	-	0.3	-

2.4.1.6 Minerals

Potassium accounts for approximately 40% of the mineral content of green coffee (1-2g/100g) (Farah, 2012). Phosphorus is another important mineral in coffee, accounting for 4%. The remaining mineral content consists of approximately 30 different elements (Antonio *et al.*, 2011). Among these are micro or trace elements including zinc, strontium, silicon, manganese, iron, cupper, barium, and boron. Trace elements are essential bio-elements that play important roles as part of enzymes, vitamins, hormones and pigments in plants and animals. They accelerate biochemical processes in the body, and stimulate the synthesis of

starch, sugar, pectin and nucleic acids in plants (Grembecka, Malinowska, & Szefer, 2007; Ashu & Singh, 2011; Pohl, Stelmach, Welna, & Szymczycha-Madeja, 2013).

2.4.1.7 Diterpenoids

The pentacyclic diterpene alcohols cafestol and kahweol (Figure 3) constitute the major diterpenes in coffee.

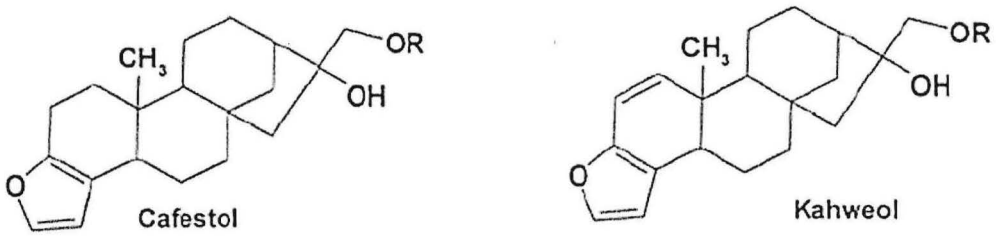

Figure 3: Structure of cafestol and kahweol

These compounds and their derivative salts or esters of saturated and unsaturated fatty acids, represent approximately 20% of the lipid fraction of coffee (Wattenberg, 1983). Cafestol is the primary constituent of the unsaponifiable fraction of coffee oil, accounting for approximately 0.2%–0.6% of coffee weight. Kahweol is less abundant because it is sensitive to heat, oxygen, light and acids (Flament, Gautschi, Winter, Willhalm & Stoll, 1968). Higher levels of diterpenes are found in *C. arabica* than in *C. canephora*. Coffee diterpenes exhibit anticarcinogenic and hepatoprotective properties *in vitro* (Wattenberg, 1983). However, high consumption of these compounds is associated with elevated homocysteine and low-density lipoprotein levels in human plasma, which could increase the risk of cardio vascular disease (Olthof, Hollman, Zock, & Katan, 2001). Considerable amounts of these compounds are present primarily in unfiltered coffee since they are poorly soluble in water and are therefore trapped by paper filters.

2.4.1.8 Caffeine

Caffeine is a methyl xanthine (Figure 4), responsible for 10% of the perceived bitterness of the coffee beverage (Flament, Gautschi, Winter, Willhalm, & Stoll, 1968). It is a heat stable alkaloid whose concentration in *C. canephora* is approximately twice that found in *C. arabica.*

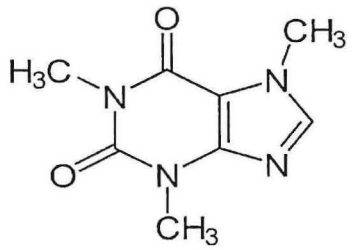

Figure 4: Structure of caffeine

The effects of caffeine on health are controversial (Shlonsky, Klatsky, & Armstrong, 2003). Low to moderate caffeine intake was reported to be generally associated with increased alertness, learning capacity, exercise performance, and better mood, while high doses produce negative effects such as anxiety, tachycardia and insomnia in sensitive individuals (Toci, Farah, & Trugo, 2006). Some studies associated caffeine with high blood cholesterol, coronary heart disease, and cancer, while others suggested its consumption could lower the incidence of suicide and hepatic cirrhosis (Farah, de Paulis, Trugo, & Martin, 2006). Acute caffeine consumption has negative effects on glucose tolerance, glucose disposal, and insulin sensitivity in lean, obese, and persons with type II diabetes, and increases the urinary excretion of minerals such as calcium (Ribeiro-Alves, Trugo, & Donangelo, 2003; Shearer, Sellars, Farah, Graham, & Wasserman, 2007).

2.4.1.9 Trigonelline

Trigonelline (Figure 5), is an alkaloid biologically derived from enzymatic methylation of nicotinic acid. Like caffeine, trigonelline contributes to the bitterness of the coffee brew and in addition, it is a precursor for the formation of different classes of volatile compounds such as pyrroles and pyridines. The amount of trigonelline in *C. canephora* is approximately two-thirds of that found in *C. arabica*.

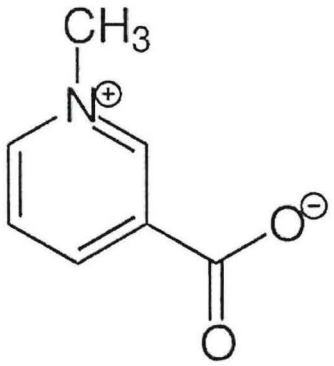

Figure 5: Chemical structure of Trigonelline

Trigonelline inhibits the invasiveness of cancer cells *in vitro* (Hirakawa, Okauchi, Miura, & Yagasaki, 2005). It also regenerates dendrites and axons in animal models, suggesting that it improves memory (Tohda, Kuboyama, & Komatsu, 2005). It is considered a novel phytoestrogen (Allred, Yackley, Vanamala, & Allred, 2009). Phytoestrogens are plant-based compounds that mimic estrogen in the body. They have been found to be beneficial in combatting symptoms and conditions caused by estrogen deficiency. This may be of particular benefit to premenopausal and post-menopausal women. Trigonelline demethylation during coffee roasting produces nicotinic acid, a B-complex vitamin also known as niacin and this vitamin lowers cholesterol levels in the body (Trugo, 2003).

2.4.1.10 Chlorogenic acids

Chlorogenic acids (Figure 6) comprise a major class of phenolic compounds, which are derived primarily from esterification of *trans*-cinnamic acids such as caffeic, ferulic and *p*-coumaric acid with (−)-quinic acid. The main subclasses of chlorogenic acids in green coffee are caffeoylquinic acids, dicaffeoylquinic acids, feruloylquinic acids, *p*-coumaroylquinic acids and caffeoyl-feruloylquinic acids. Among these classes, caffeoylquinic acids account for approximately 80% of the total chlorogenic acids content in green coffee beans. Chlorogenic acids confer astringency, bitterness, and acidity to the coffee brew.

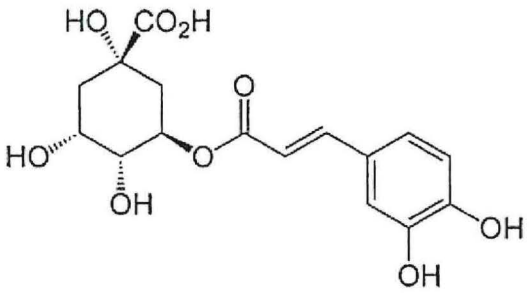

Figure 6: Structure of chlorogenic acid

High amounts in green coffee, particularly caffeoylquinic and feruloylquinic acids may produce undesirable flavour due to oxidation and degradation products formed before roasting (Farah, Monteiro, Calado, & Trugo, 2006). Chlorogenic acids are precursors of phenols and catechol that may confer unpleasant sensory off notes that are formed during roasting (Trugo, 2003). The content of chlorogenic acid in *C. canephora* is generally one and a half to two times higher than in *C. arabica,* but this concentration varies considerably in both species.

2.4.2 Volatile compounds in green coffee beans

The volatile fraction of unroasted green coffee beans gives them a weak but characteristic aroma. Approximately 100 different volatile compounds have been identified in green coffee beans (Farah, 2012). The most abundant classes of volatile compounds are alcohols, esters, hydrocarbons and aldehydes. Ketones, pyrazines, furans, and sulphur compounds have also been identified (Toci & Farah, 2008). The production of microbial volatile compounds during fermentation results in coffee with richer aroma quality (Gonzalez-Rios *et al.*, 2007). The roasting of coffee beans is another very important step in coffee processing, since specific organoleptic properties (flavors, aromas, and color) develop and affect the quality of the coffee and the excellence of the coffee beverage, as a consequence (Hernández, Heyd, & Trystram, 2008). This process is time–temperature dependent and leads to several changes in the chemical composition and biological activities of coffee as a result of the transformation of naturally occurring polyphenolic constituents into a complex mixture of Maillard reaction products (Sacchetti, Mattia, Pittia, & Mastrocola, 2009), as well as the formation of organic compounds resulting from pyrolysis. Sulfur compounds also change by oxidation, thermal degradation, and/or hydrolysis (Kumazawa & Masuda, 2003), and the vanillin content increase considerably during the roasting process.

2.5 Processing of coffee cherries into green coffee beans

2.5.1 Primary processing

Primary coffee processing is the major post-harvest process that involves wet processing or dry processing of red ripe coffee cherries to improve their appearance (Figure 7). The resulting clean coffee can then be roasted and ground to obtain the coffee powder for human consumption.

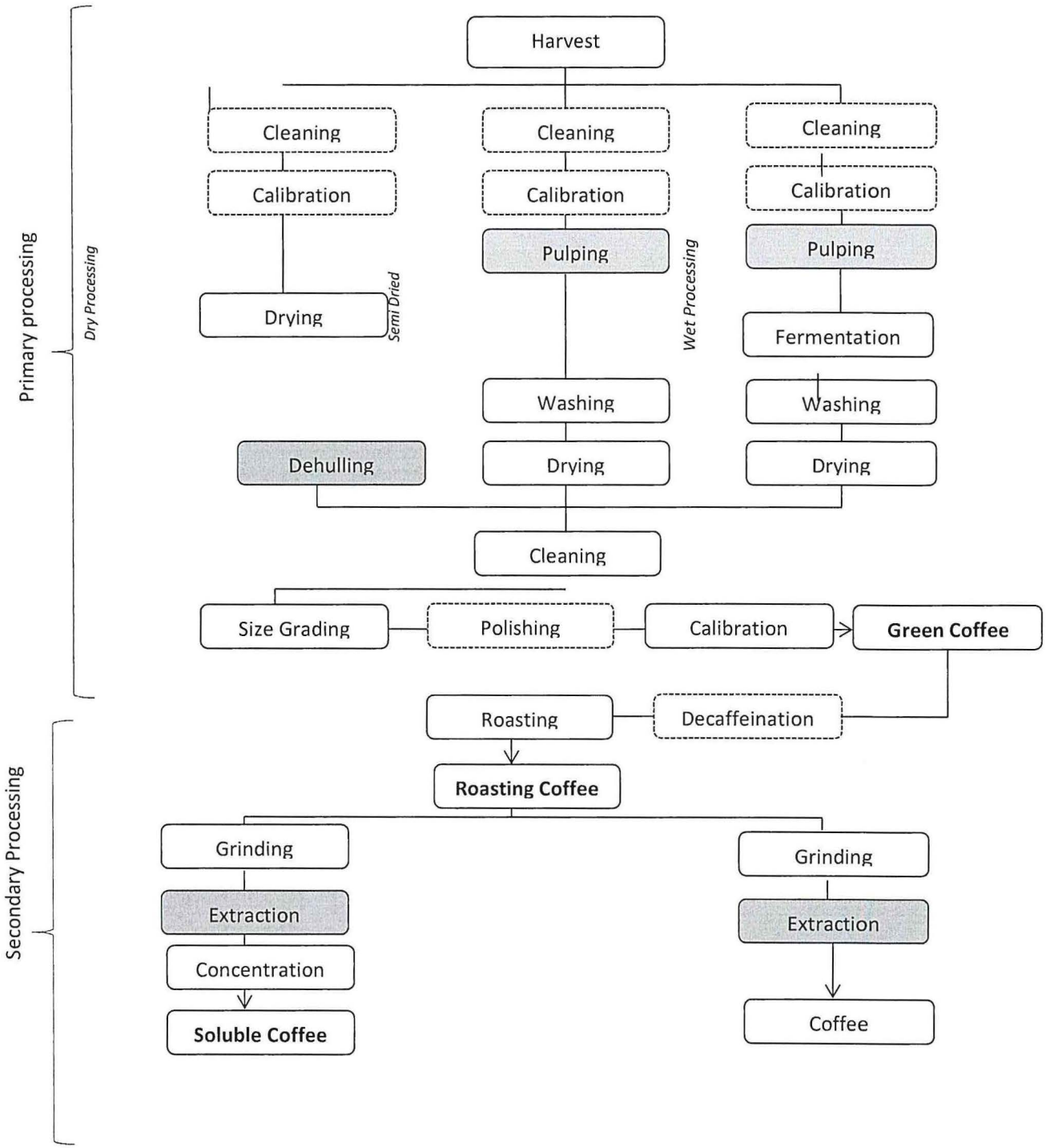

Figure 7: Coffee processing and residues produced

(Source: Cruz, 2014)

2.5.1.1 Dry Processing

Dry processing is used mainly for robusta coffee although it can also be used for arabica coffee. Dry processing involves the freeing of the wet parchment of mucilage at moisture contents of 50 – 60% to the required 12% to ensure their preservation. Dry processing involves drying of coffee cherries either by the sun on raised stands or on mats or in solar driers immediately after harvest (Cruz, 2014). Care must however be taken to prevent dust and dirt blown onto the produce as well as damage from unexpected rainstorms that can soak the produce very quickly without warning. After coffee drying, hulling commences. This is the removal of the pericarp either by a pestle and mortar or in a mechanical huller (Clarke, 2003). The mechanical hullers usually consist of a steel crew, the pitch of which increases as it approaches the outlet so removing the pericarp.

2.5.1.2 Wet processing

Wet processing is mainly used for arabica coffee although it can also be done for robusta coffee especially clonal coffee leading to wash robusta coffee (Clarke, 2003). Wet processing involves three stages: removal of pulp and mucilage, followed by washing to obtain clean wet parchment. Pulping involves the removal of the exocarp and the white fleshy pulp and the separation of the pulp and the beans. The amorphous gel of mucilage around the bean consists of hemicelluloses, pectic substances and sugars and is water soluble. It can therefore be removed by use of chemicals, warm water or by a pulper (Cruz, 2014). However, for small scale units, fermentation is the most feasible. Fermentation involves the beans being placed in a plastic bucket or tank and left until the mucilage has been broken down. Natural enzymes in the mucilage and bacteria in the environment work together to break down the mucilage (Clarke, 2003). The beans should be stirred occasionally and a few beans tested by washing them in water. The beans are ready when the mucilage can be washed off and the beans feel

gritty. After this, the wet processed beans are dried to prevent cracking and this should be done slowly to 10% moisture content and similar drying methods can be used for this as for the dry processed coffee. After drying, the coffee should be rested for 8 hours in a well-ventilated place and the thin parchment around the coffee removed by hand, pestle and mortar or in a small huller.

2.5.2 Secondary processing

Secondary processing is the final post-harvest process before coffee is exported. This stage involves: pre-cleaning and de-stoning, size grading, gravimetric sorting and finally for export of green coffee beans, bag-off which entails bagging coffee in jute bags of 60 Kg which are then loaded into a container for transportation to the port (UIA, 2016). Apart from exporting green coffee beans, the coffee beans can also be processed to make higher value-added coffee beverage products. This level of coffee processing involves roasting, grinding, making of instant coffee, extraction of soluble coffee solids and other products using appropriate technologies (Cruz, 2014).

CHAPTER 3: MATERIALS AND METHODS

3.1 Description of Study area

Study area composed of twelve (12) different coffee districts of Uganda. These were: Maracha in the North West, Kisoro in South West, Masaka, Luwero and Kayunga in the Central region, Iganga and Bugisu in the East and Mbarara, Ibanda, Rukungiri, Ntungamo and Rwenzori in the West (Figure 8).

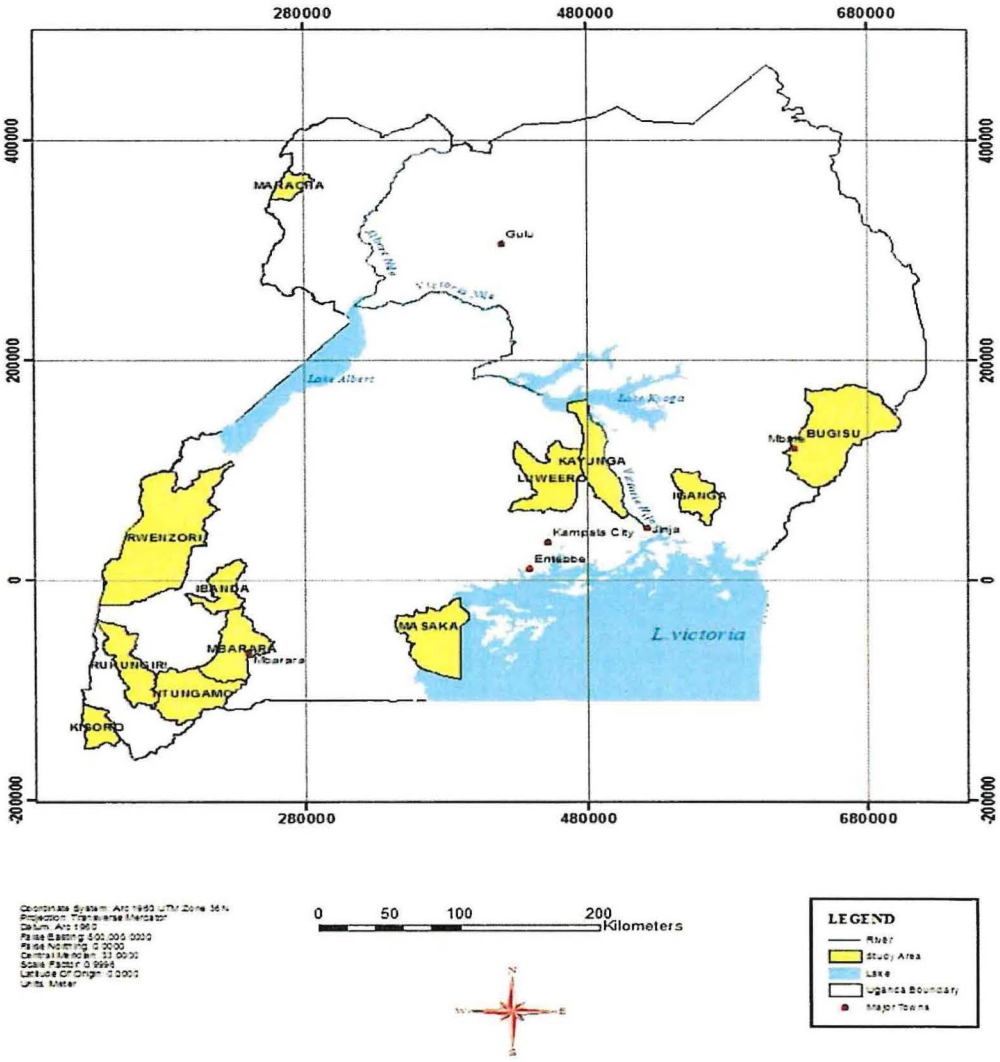

Figure 8: Map of Uganda showing sampled areas

The geographical coordinates of these areas are as shown in the table below.

Table 3: Geographical coordinates of the study areas

Study area	Geographical coordinates
Maracha	03 17N, 30 56E
Kisoro	1°17'06.0"S, 29°41'06.0"E
Bugisu (Mbale)	00 57N, 34 20E
Rwenzori	0.1699° N, 30.0781° E
Mbarara	0036 48S, 30 39 30 E
Masaka	0°20'28.0"S, 31°44'10.0"E
Ntungamo	0°52'55.0"S, 30°15'55.0"E
Rukungiri	0.7518° S, 29.9278° E
Ibanda	$0^0$08'05.0"S, $30^0$29'42.0"E
Kayunga	01 00N, 32 52E
Luwero	00 50N, 32 30E
Iganga	0°36'54.0"N, 33°29'06.0"E

Uganda lies astride the Equator, between latitudes 4° 12′ N and 1° 29′ S and longitudes 29° 34′ W, and 35° 0′ E. Temperatures are in the range of 15°–30°C. More than two-thirds of the country is a plateau, lying between 1 000–2 500 metres above sea level. Precipitation is fairly reliable, varying from 750 mm in Karamoja in the Northeast to 1 500 mm in the high rainfall areas on the shores of Lake Victoria, in the highlands around Mt. Elgon in the east, the Ruwenzori mountains in the southwest and some parts of Masindi and Gulu (FAO, 2006).

3.2 Equipment

The green coffee beans were dried in a hot air oven (DSO-D/DSO DF series, Taiwan). The dry beans were ground to fine powder using an electric grinder. Fatty acids were determined as fatty acid methyl esters (FAME) on a Varian 3800 GC chromatograph with flame ionization detection (GC-FID) manufactured by Varian Chromatography Systems, USA; using a Chrompack CP-Sil 88 TM fused silica capillary column (100 m × 0.25 mm i.d., 0.2 μm film thickness; Varian Inc., Walnut Creek, CA, USA).

3.3 Chemicals and reagents

All chemicals used were of analytical grade. Green coffee oil was extracted with n-hexane (boiling range 62-68°C, 96%) manufactured by Sharlab S. L. Spain. Pure standards of fatty acid methyl esters (FAME); FAME standard mixtures (Supelco 37 component FAME MIX) obtained from Supelco/Sigma-Aldrich (USA) were used to identify the fatty acids. High-purity deionized water was used for making solutions and cleaning of glassware.

3.4 Methods

3.4.1 Sampling

Twelve areas were considered for composite sampling and quartering and these included; Kisoro, Maracha, Bugisu and Rwenzori for arabica coffee and Mbarara, Kayunga, Ntungamo, Luwero, Masaka, Ibanda, Rukungiri and Iganga for robusta coffee. The samples (Bugisu, Rwenzori, Mbarara, Kayunga, Ntungamo, Masaka, Ibanda and Rukungiri) were obtained from Uganda Coffee Development Authority (UCDA) in Kampala. Luwero, Iganga, Maracha and Kisoro samples were obtained from coffee dealers in the respective areas. Green coffee beans were sealed in polyethylene bags and transported to the Natural Chemotherapeutics Research Institute chemistry laboratory for oil extraction.

3.4.2 Sample preparation

Oil was extracted using the Soxhlet procedure. The coffee beans were dried for 4.5 hours in the hot air oven at 105°C. The dry beans were ground to fine powder in a grinder, and the powder (10 g), was extracted using pro-analysis grade n-hexane. The solvent was evaporated on a rotary evaporator and the residual oil dried in a hot air oven at 80°C for 1.5hr. The oil yield was estimated based on green coffee bean weight used for extraction. The extracted oil was packaged in amber colored bottles and kept in a deep freezer until analysis. Fatty acid analysis was performed at Chemiphar (Uganda) Limited, an internationally accredited analytical laboratory (accreditation no: 167-TEST/INSP).

3.4.3 Determination of fatty acid profile of the oil

Fatty acids were determined as Fatty acid methyl esters (FAME) on a Varian 3800 GC chromatograph with flame ionization detection (GC-FID). A Chrompack CP-Sil 88 TM fused silica capillary column (100 m × 0.25 mm i.d., 0.2 μm film thickness; Varian Inc., Walnut Creek, CA, USA) was used. Fatty acid profile was determined according to the method described by Ogwok, Muyinda, Nakisozi, & Bamuwamye (2017). By this method, the FA profile of green coffee bean oil was determined using gas chromatography with flame ionization detection (GLC-FID) based on the ISO 5509:2000 transesterification method. The FA profile was analyzed with a Chrompack CP 9001 chromatograph equipped with a split-splitless injector, an FID and a Chrompack CP-9050 autosampler. The temperature of the injector and detector was 250°C. Nitrogen was used as a carrier gas at an internal pressure of 120 kPa. The column temperature was 140°C for a 5 min hold. It was then programmed to increase to 220°C at a rate of 4°C/min and then held for 10 min. The split ratio was 1:20, and the injected volume was 1.2 mL. The results were expressed in relative percentage of each FA, calculated by internal normalization of the chromatographic peak area. Fatty acid

identification was made by comparing the relative retention times of FAME peaks from the samples with standards.

3.5 Data analysis

All experiments were performed in triplicate and results expressed as mean ± standard deviation. Analysis of variance (ANOVA) using R statistical software was used to determine differences in oil yield among the green coffee bean varieties and also differences in oil yield based on sample source. Mean differences were considered significant at $p < 0.05$.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Oil yield of green coffee beans

Oil content of green coffee beans ranged from 2.81 to 12.78% (Table 4). The highest content was obtained from arabica coffee from Bugisu and the lowest from robusta coffee from Ntungamo. Within varieties, Bugisu had a significantly high ($p < 0.05$) oil yield in arabica coffees while the lowest yield was arabica from Maracha. For robusta coffees, Rukungiri had a significantly higher ($p < 0.05$) oil yield while Ntungamo had the lowest yield.

Table 4: Oil yield (%w/w) of the Coffea arabica and Coffea canephora var. Robusta green beans

Area	Coffee type	Min	Max	Mean	SD
Kisoro	Arabica	3.21	5.2	4.42[a]	1.06
Maracha	Arabica	3.92	4.7	4.31[a]	0.39
Bugisu	Arabica	10.4	15.45	12.78[b]	2.56
Rwenzori	Arabica	4.83	6.36	5.51[a]	0.78
Mbarara	Robusta	3.96	4.44	4.21[a]	0.24
Kayunga	Robusta	1.75	4.02	3.18[a]	1.24
Ntungamo	Robusta	2.53	3.33	2.81[a]	0.45
Luwero	Robusta	2.87	3.92	3.38[a]	0.53
Masaka	Robusta	2.74	3.27	3.05[a]	0.27
Ibanda	Robusta	3.97	5.56	4.57[a]	0.87
Rukungiri	Robusta	4.68	6.98	5.71[ac]	1.17
Iganga	Robusta	4.38	6.25	5.09[a]	1.01

Means with different superscripts within the same column are significantly different ($p<0.05$)

Studies have reported lipid content in the range of 13.0 to 17.0 g/100 g DW in green coffee beans (De Castro, Marraccini, 2006; Joët *et al.*, 2010). Other studies reported lipids in green coffee beans in the range between 7 and 17% (Kurzrock & Speer, 2001; Oestreich-Janzen, 2010), which is also generally higher than that of this study. A higher content of lipid is normally found in arabica relative to robusta coffee (Oestreich-Janzen, 2010), which is in agreement with the findings of this study. This is because arabica starts to store oil earlier and in higher concentrations than robusta (Joet *et al.*, 2009). Lipid content is also subject to environmental factors, such as shade, which results in higher levels (Odeny *et al.*, 2014; Vaast *et al.*, 2006). High altitude is another factor in favor of fat accumulation (Avelino *et al.*, 2005).The famine early warning systems network (FEWSNET) 2012, showed in a study on climate change in Uganda that rains had decreased during the past 25 years. Increased temperatures have the general effect of reducing the amount of accumulated lipids (Fábio, Adriana, Bruna, & Marcos, 2010). Annual global temperatures have increased by *ca.* 0.4°C, with even larger changes observed in several regions (IPCC, 2001). Lipid content is influenced by the coffee cultivars (Wenjiang, Lehe, Jianping, Rongsuo, & Minquan, 2015) as was observed in this study.

4.2 Fatty acid composition green coffee beans

A total of nineteen fatty acids were detected in the oils from *Coffea arabica* and *Coffea canephora* var. Robusta. In both varieties and in all samples, palmitic acid (C16:0), linoleic acid (LA, C18:2ω6), oleic acid (OA, C18:1ω9), α- linolenic acid (ALA, C18:3ω3) and arachidic acid (C20:0) were quantifiable in the oil. These fatty acids accounted in total for more than 95% of the total fatty acids in both varieties (Tables 5 and 6). Palmitic acid was the major saturated fatty acid and it ranged from 30.48 to 47.4%. Linoleic acid was the major unsaturated fatty acid and it ranged from 33.23 to 42.11%. Elaidic acid (C18:1ω9*t*) was relatively low. Arachidic (C20:0) and behenic acids (C22:0) were also detected in the *Coffea* oils. Erucic acid (C22:1ω9) and eicosatrienoic acid (C20:3ω3) were detected in small amounts in the *Coffea* oils from Kisoro and Maracha districts. Arachidonic acid (AA, C20:4ω6), C22:1ω9 and (C18:1ω9) were present in Robusta coffee oils from Mbarara, Kayunga, Ntungamo, Luwero and Masaka. Stearic acid (C18:0) was notably absent in *Coffea* oils from Masaka and Rukungiri *Coffea* oils. Fatty acid composition in this study is affected more by geographical sample source as opposed to green coffee variety. This is evidenced by the occurrence of some and absence of some fatty acids especially the minor ones like heneicosanoic, trocosanoic and myristoleic acids among others in oil from different areas. Leroy *et al.* (2006), states that coffee quality depends on biochemical compounds and organoleptic quality. These are affected by geographical origin, environmental and sociological aspects.

Table 5: Fatty acid composition (g/100g) of *Coffea arabica*

Fatty acid	Kisoro	Maracha	Bugisu	Rwenzori	Minimum	Maximum	Mean	SD
C14:0	ND	ND	0.12	0.11	ND	0.12	_	_
C15:0	ND	ND	0.08	ND	ND	0.08	_	_
C16:0	35.47	30.48	47.4	46.19	30.48	47.4	39.89	8.25
C18:0	0.11	0.14	0.11	0.11	0.11	0.14	0.12	0.02
C20:0	2.82	1.82	1.21	ND	ND	2.82	_	_
C21:0	0.46	1.57	ND	ND	ND	1.57	_	_
C22:0	1.15	0.88	0.07	0.08	0.07	1.15	0.55	0.55
C18:1ω9*c*	14.44	16.53	12.61	13.13	12.61	16.53	14.18	1.75
C18:1ω9*t*	1.39	1.57	0.07	0.98	0.07	1.57	1.00	0.67
C22:1ω9	0.74	1.09	ND	ND	ND	1.09	_	_
C18:2ω6	41.51	41.71	37.1	34.61	34.61	41.71	38.73	3.48
C18:3ω6	0	0.04	0.08	ND	ND	0.08	_	_
C20:4ω6	0.35	0.39	ND	ND	ND	0.39	_	_
C18:3ω3	1.33	3.09	1.09	2	1.09	3.09	1.88	0.9
C20:3ω3	0.18	0.47	ND	ND	ND	0.47	_	_

Notes: ND: not detected. Means and standard deviations on the measure of FA composition

Table 6: Fatty acid profile (g/100g) of *Coffea canephora*

Fatty acid	Mbarara	Kayunga	Ntungamo	Luwero	Masaka	Ibanda	Rukungiri	Iganga	Minimum	Maximum	Mean	SD
C14:0	ND	ND	ND	ND	ND	0.16	0.16	0.15	ND	0.16	_	_
C16:0	37.04	33.89	36.49	33.85	33.92	43.39	47.72	40.67	33.85	47.72	38.37	5.12
C17:0	ND	0.01	ND	ND	ND	ND	ND	ND	ND	0.01	_	_
C18:0	0.09	0.09	0.09	0.12	ND	16.18	16.47	0.1	ND	16.47	_	_
C20:0	0.84	0.74	0.49	1.28	0.99	1.4	1.26	1	0.49	1.4	1.00	0.31
C21:0	0.2	ND	ND	0.2	ND	ND	ND	ND	0.2	0.2	_	_
C22:0	0.35	0.31	0.22	0.52	0.48	0.04	0.06	0.04	0.04	0.52	0.25	0.19
C14:1	ND	ND	ND	ND	ND	0.09	ND	0.05	ND	0.09	_	_
C16:1ω7	ND	ND	ND	ND	ND	0.04	0.04	ND	ND	0.04	_	_
C20:1	ND	ND	ND	ND	ND	0.12	ND	0.16	ND	0.16	_	_
C18:1ω9*c*	19.35	20.88	20.03	20.13	21.45	3.65	16.47	20.21	3.65	21.45	17.77	5.90
C18:1ω9*t*	0.69	0.73	0.72	0.7	0.75	ND	ND	0.65	ND	0.75	_	_
C22:1ω9	0.15	0.11	0.05	0.21	0.15	ND	ND	ND	ND	0.21	_	_
C18:2ω6	39.43	41.2	39.9	40.86	42.1	34.24	33.23	35.03	33.23	42.1	38.25	3.51
C18:3ω6	ND	0.02	0.04	0.02	ND	0.08	0.5	ND	ND	0.5	_	_
C20:4ω6	0.26	0.2	0.12	0.35	0.16	ND	ND	ND	ND	0.35	_	_
C18:3ω3	1.6	1.82	1.83	1.74	ND	0.62	0.55	1.94	ND	1.94	_	_

Notes: ND: not detected. Means and standard deviations on the measure of FA composition

Previous studies have presented C18:2 and C16:0 as predominant fatty acids in green coffee bean oil. Levels of fatty acids in the oil of this work were in agreement with earlier studies (Karl & Kooling, 2006; Yang *et al.,* 2013). Coffee oils are poor in short chain and medium chain fatty acids. Linoleic acid is an essential fatty acid in coffee oil (Romijin, Wiseman, Scheek, de Fouw & van Tol, 1998). Palmitic acid raises total, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol and is a risk factor for thrombosis (Godswill *et al.*, 2010). It raises the serum total cholesterol concentration, compared with unsaturated fatty acids. A high blood cholesterol level and high level of low-density lipoprotein cholesterol (LDL-C) are associated with increased risk of heart disease. Stearic acid has a neutral effect on the concentration of total serum cholesterol. Linoleic and α- linolenic acids are required for body tissue development and must be obtained from the diet (Ribeiro *et al.*, 2009). Differences in the oil composition is attributable to climate, altitude, and shade that play an important role through temperature, availability of light and water during the ripening period (Decasy *et al.*, 2003). Rainfall and sunshine distributions have a strong influence on flowering, bean expansion, and ripening. Temperature is the most important element, which affects coffee bean quality.

4.3 Nutritional quality indices of green coffee oil

Trans fatty acids (TFA) were highest in Maracha coffee oils and were not detected in Ibanda and Rukungiri oils (Table 7). Averagely, arabica coffee had more TFA compared to robusta coffee. Dietary TFA are associated with increased risk of cardiovascular disease (Mozaffarian, Aro, & Willett, 2009). Recommended daily allowance for TFA by WHO is < 1% (*ca.* 2g/day) for all foods (Ogwok *et al.*, 2017).

Table 7: Nutritional quality of green coffee oil

	Arabica coffee				Robusta coffee							
Fatty acids	Kisoro	Maracha	Bugisu	Rwenzori	Mbarara	Kayunga	Ntungamo	Luwero	Masaka	Ibanda	Rukungiri	Iganga
SaFA	40.06	35.11	48.99	46.49	38.52	35.04	37.33	35.99	35.39	61.16	49.2	41.95
MUFA	16.56	19.19	12.75	16.91	20.19	21.72	20.8	21.04	22.34	3.89	16.51	21.07
PUFA	43.38	45.7	38.26	36.61	41.29	43.24	41.87	42.97	42.27	34.94	34.29	36.98
TFA	1.39	1.57	0.07	0.98	0.69	0.73	0.72	0.7	0.75	ND	ND	0.65
ω-3 PUFA	1.51	3.56	1.09	2	1.6	1.82	1.83	1.74	ND	0.62	0.55	1.94
ω-6 PUFA	41.87	42.15	37.17	34.61	39.69	41.42	40.05	41.23	42.27	34.32	33.73	35.03
ω-6/ω-3	27.73	11.84	34.1	17.31	24.81	22.76	21.89	23.7	_	55.35	61.33	18.06
P/S	1.08	1.3	0.78	0.79	1.07	1.23	1.12	1.19	1.19	0.57	0.7	0.88
M/S	0.41	0.55	0.26	0.36	0.52	0.62	0.56	0.58	0.63	0.06	0.34	0.5
(P + M)/S	1.5	1.85	1.04	1.15	1.6	1.85	1.68	1.78	1.83	0.63	1.03	1.38
P/M	2.62	2.38	3	2.16	2.05	1.99	2.01	2.04	1.89	8.98	2.08	1.76

Notes: P: PUFA, S: SaFA, M: MUFA.

Levels of TFA in this study are rather low. However, given their health implications, it is necessary to minimize their consumption. TFA have unique adverse effects on serum lipids (Dhaka *et al.*, 2011). They increase LDL-C, lower high-density lipoprotein cholesterol (HDL-C) and increase the ratio of total cholesterol to high-density lipoprotein (HDL) (Dhaka *et al.*, 2011). Consumption of oils containing TFA also increases serum triglyceride levels and may reduce LDL particle size, which increases the risk of coronary heart disease (Ogwok, Muyinda, Nakisozi, & Bamuwamye, 2017).

Saturated FA varied between 35.04 to 61.16% in the green coffee. Ibanda coffee oil had higher SFA value than other coffee oils. Results are in accordance with previous reports which highlighted that coffee bean oil contains much more unsaturated fatty acids than saturated fatty acids (Romano *et al.*, 2014). Total MUFA were in the range of 3.89 and 22.34%. Masaka coffee oil had the highest MUFA value and Ibanda coffee oil had the lowest. Total PUFA ranged from 34.29 to 45.70%. Maracha coffee oil had the highest concentration of PUFA, whereas Rukungiri coffee oil had the lowest amounts. The coffee oils had a PUFA/SaFA ratio in the range of 0.57 to 1.23, whereas that of PUFA/MUFA was 1.76 to 8.98. The ratio of MUFA/SaFA was in the range of 0.06 to 0.63. The ratios of ΣUFA/ΣSaFA were higher than 1.0 for all samples, which indicates that green coffee oil can serve as a food supplement in the diet to decrease the level of fats and cholesterol, potentially preventing some cardiovascular diseases (Yang *et al.*, 2013).

The PUFA/SaFA ratio is a good indicator of nutritional value and health attributes of food (Parunović *et al.*, 2012; Kaić, Mioč, Kasap, & Potočnik, 2016). It is also considered to be a risk factor for cardiovascular diseases in humans. A balanced PUFA/SaFA ratio greater than 0.45 in

food has been recommended for a healthy diet (Takahashi, & Carvalho, 2010; Okrouhlá *et al.*, 2013). In this study, the ratios in all coffee oils were higher than 0.45. However, according to Ogwok *et al.* (2017), large amounts of dietary MUFA can lead to elevated plasma and liver lipids. Therefore, the PUFA/SaFA ratio alone cannot be sufficient to predict changes in plasma cholesterol levels. A MUFA/SaFA ratio < 1.5, PUFA/MUFA ratio > 0.67, and (PUFA + MUFA)/SaFA ratio < 3.0 have in addition been suggested (Chang, & Huang, 1999). The coffee oil in this study had desired PUFA/SFA, MUFA/SaFA, PUFA/MUFA and (PUFA + MUFA)/ SaFA ratios. Excessive amounts of omega-6 polyunsaturated fatty acids (PUFA) and a very high omega-6/omega-3 ratio promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increased levels of omega-3 PUFA (a low omega-6/omega-3 ratio) exert protective effects. Ratios between 5:1 and 10:1 are recommended by the WHO and FAO (Simopoulos, 2002). This is below the obtained ratios of this study. The high ω -6 FA/ω-3 FA ratio could be attributable to a substantially high amount of LA observed in coffee oils in this study.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Oil content of green coffee beans was generally low (2.81-12.7%) with variations noted in samples from different regions and between varieties. Palmitic, linoleic, oleic and α-linolenic were predominant. Green coffee beans under investigation can be a source of dietary fatty acids especially linoleic acid. The coffee oil in this study had desired PUFA/SaFA, MUFA/SaFA, PUFA/MUFA and (PUFA + MUFA)/ SaFA ratios and therefore healthy for consumption. The ω-6/ω-3 ratio was far beyond recommendations by WHO and FAO.

5.2 Recommendations

1. Green coffee oils can serve as a food ingredient to decrease the level of fats and cholesterol since the ratios of ΣUFA/ΣSaFA were larger than 1.0 thus reducing the risk of developing non-communicable diseases such as diabetes and cardiovascular diseases.
2. Although levels of TFA were low in coffee oil in this study, there is a need to fully study their existence as they are associated with increased risk of cardiovascular disease.
3. Since the quality of the green coffee beans affects both yield and fatty acids composition, the regulatory authorities should keep monitoring the quality of the coffee to keep it at the acceptable standards required.
4. Also the composition of other coffee species in Uganda should be explored to determine their viable applications in food technology and hence their commercialization.

REFERENCES

Agwanda, C., Baradat, P., Eskes, A., Cilas, C., & Charrier, A. (2003). Selection for bean and liquor qualities within related hybrids of Arabica coffee in multilocal field trials. *Euphytica, 131*(1), 1-14.

Allred, K. F., Yackley, K. M., Vanamala, J., & Allred, C. D. (2009). Trigonelline is a novel phytoestrogen in coffee seeds. *Journal of Nutrition*, 61, 1833–1838.

Antonio, A. G., Iorio, N. L., Pierro, V. S., Candreva, M. S., Farah, A., N., D. S, & ′ Maia, L. C. (2011). Inhibitory properties of Coffea canephora extract against oral bacteria and its effect on demineralisation of deciduous teeth. Archives of Oral Biology, 556–564.

AOAC. (2000) Official methods of analysis of AOAC. *International 17th edition; Gaithersburg, MD, USA Association of Analytical Communities.*

Ashu R. and Singh Chandravanshi B. (2011). Concentration levels of metals in commercially available Ethiopian roasted powder and their infusion, *Bulletin of the Chemical Society of Ethiopia.* 25(1).

Avelino, J., Barboza, B., Araya, J. C., Fonseca, C., Davrieux, F., Guyot, B., *et al.* (2005). Effects of slope exposure, altitude and yield on coffee quality in two altitude terroirs of Costa Rica, Orosi and Santa Maria de Dota. Journal of the Science of Food and Agriculture, 85(11), 1869-1876.

Berlitz, H.-D., Grosch, W., Schieberle, P., 2009. Food Chemistry. Springer Berlin Heidelberg, Berlin, Heidelberg. *131*(1), 1-14

Boros B, Jakabová S, Dörnyei A, Horváth GY, Pluhár ZS, Killár F, Felinger A. 2010. Determination of polyphenolic compounds by liquid chromatography-mass spectrometry in thymus species. *Journal of Chromatography* A. 1217:7972–7980. DOI: 10.5772/intechopen.68149

Bunn, C., Läderach, P., Rivera, O. O., & Kirschke, D. (2014). A bitter cup: climate change profile of global production of Arabica and Robusta coffee. *Climatic Change, 129*(1–2), 89–101. doi:10.1007/s10584-014-1306-x

Butt, M. S., & Sultan, M. T. (2011). Coffee and its consumption: benefits and risks. *Critical Reviews in Food Science and Nutrition, 51*(4), 363-73. doi:10.1080/10408390903586412

Chang, N. W., & Huang, P. C. (1999). Effects of the Ratio of Polyunsaturated and Monounsaturated Fatty Acid to Saturated Fatty Acid on Rat Plasma and Liver Lipid Concentrations. *Journal of Lipid, 33.*

Clarke, R. J. Coffee: green coffee/roast and ground. In: *Encyclopedia of Food Science and Nutrition*, 2nd edition, Caballero, B., Trugo, L. C., Finglas, P., eds. Oxford: Academic Press; 2003, Vol. 3.

Cossignani, L., Montesano, D., Simonetti, M. S., & Blasi, F. (2016). Authentication of Coffea arabica according to Triacylglycerol Stereospecific Composition. *Journal of Analytical Methods in Chemistry, 2016*(7482620), 7. DOI:10.1155/2016/7482620

Coung, T. V., Ling, L. H., Jin, S., Jie, S. S., & Tiep, T. D. (2014). Effect Of Roasting Conditions On Concentration In Elements Of Vietnam Robusta Coffee. *Acta Universitatis Cibiniensis Series E: Food technology, xviii*(2), 14. DOI 10.2478/aucft-2014-0011

Cruz, R. 2014. Coffee by-products: Sustainable Agro-Industrial Recovery and Impact on Vegetables Quality: dissertation thesis. Universidade de Porto. *131*(1), 1-14

De Castro, R.D.; Marraccini, P. Cytology, biochemistry and molecular changes during coffee fruit development. *Brazilian Journal of Plant Physiology*. 2006, *18*, 175–199.

Decasy, F., Avelino, J., Guyot, B., Perriot, JI., Pineda, C., Cilas, C. 2003. Quality of different Honduran coffees in relation to several environments. *Journal of Food Science.* 68

Dhaka, V., Gulia, N., Ahlawat, K.S. and Khatkar, B.S. (2011), "Trans fats sources, health risks and alternative approach– a review", *Journal of Food Science and Technology*, DOI: 10.1007/s13197-010-0225-8

Dziki, D., Gawlik-Dziki, U., Pecio, Ł., Różyło, R., Świeca, M., Krzykowski, A., & Rudy, S. (2015). Ground green coffee beans as a functional food supplement—Preliminary study. *LWT-Food Science and Technology, 63*(1), 691–699.

Fábio M. DaMatta, Adriana Grandis, Bruna C. Arenque, Marcos S. Buckeridge., 2010. Impacts of climate changes on crop physiology and food quality. Food Research International 43.

Farah, A. (2012). Coffee Constituents. In J. Wiley, & Y.-F. Chu (Ed.), *Coffee: Emerging Health Effects and Disease Prevention* (1st Edition ed., pp. 21-50). Washington: Blackwell Publishing Ltd.

Farah, A., & dos Santos, T. F. (2015). The coffee plant and beans: An introduction. In V. R. Preedy (Ed.), Coffee in health and disease prevention (pp. 5–10). Elsevier Inc: Academic Press.

Farah, A., de Paulis, T., Trugo, L. C., & Martin, P. R. (2006). Chlorogenic acids and lactones in regular and water-decaffeinated arabica coffee. *Journal of Agricultural and Food Chemistry, 54*(2), 374–381. DOI:10.1021/jf0518305

Farah, A., Monteiro, M., Calado, V., & Trugo, L. C. (2006). Correlation between the chemical attributes of coffee and cup quality. *Food Chemistry*, 373–380.

Ferrari, M., Angelis, E. D., & Navarini, L. (2010). Interfacial properties of coffee oils. *Colloids and Surfaces A Physicochemical and Engineering Aspects, 365*(1), 79-82. DOI: 10.1016/j.colsurfa.2010.02.002

FEWSNET.2012. http://pubs.usgs.gov/fs/2012/3062/FS2012-3062.pdf.

Fischer G, Shah M, Tubiello F N and van Velhuizen H 2005 Socio-economic and climate change impacts on agriculture: an integrated assessment, 1990–2080 *Philosophical Transactions of the Royal* Society. B 360

Flament, I., Gautschi, F., Winter, M., Willhalm, B., & Stoll, M. (1968). Les composants furanniques de l'arome ˆ cafe: quelques aspects chimiques et spectroscopiques. International conference on coffee science, 197–215.

Food and Agriculture Organization (FAO). Country pasture/Forage Resource profiles (2006). Standard Reference Release 27 .

Gntechwitz, D., Reichardt, N., Blaut, M., Steinhart, H., & Bunzel, M. (2007). Dietary fiber from coffee beverage: degradation by human fecal microbiota. *Journal of Agricultural and Food Chemistry, 55*(17), 6989–6996.

Godswill, N.-N., Constant, L.-L.-N. B., Martin, B. J., Kingsley, T.-M., Albert, D.-M. J., Thierry, K. S., Emmanuel, Y. (2016). Effects of Dietary Fatty Acids on Human Health: Focus on Palm oil from Elaeis guineensis Jacq. and Useful Recommendations. *Food and Public Health, 6*(3), 75-85. DOI: 10.5923/j.fph.20160603.03

Gonzalez-Rios, O., Suarez-Quiroza, M. L., Boulanger, R., Barel, M., Guyot, B., Guiraud, J.-P., *et al.* (2007). Impact of "ecological" post-harvest processing on coffee aroma: I. Green coffee. Journal of Food Composition and Analysis, 20.

Grembecka, M., Malinowska, E., & Szefer, P. (2007). Differentiation of market coffee and its infusions in view of their mineral composition. *Science of the Total Environment* DOI: 10.1016/j.scitotenv.2007.04.031

Hernandez, J. A., Heyd, B., & Trystram, G. (2008). On-line assessment of brightness and surface kinetics during coffee roasting. Journal of Food Engineering, 87.

Hirakawa, N., Okauchi, R., Miura, Y., & Yagasaki, K. (2005). Anti-invasive activity of niacin and trigonelline against cancer cells. *Bioscience, Biotechnology and Biochemistry, 69*(3), 653–658.

Hurtado-Benavides Andrés, Dorado Daniela A. and Sánchez-Camargo Andrea del Pilar. (2016). Study of the fatty acid profile and the aroma composition of oil obtained from roasted Colombian coffee beans by supercritical fluid extraction. Journal of Supercritical Fluids 113 (2016) 44–52. DOI.org/10.1016/j.supflu.2016.03.008

ICO. (2014). *Coffee Market Report.* Retrieved september, 2014, from international cooffee organization: http://www.ico.org/

ICO. (2017). *International Coffee Organisation.* Retrieved May 11, 2017, from International Coffee Organisation: http://www.ico.org/

ICO.(2018). Total coffee production by all exporting countries. Retrieved April 2018, from international Coffee Organization: http://www.ico.org/

IPCC (Intergovernmental Panel on Climate Change Working Group 2) 2001 *Climate Change 2001: Impacts, Adaptation and Vulnerability* IPCC Working Group 2

ITC. 2002. Coffee An exporter's guide. International Trade Centre UNCTAD/WTO. Geneva. pp. 243-289

Iwasa, K., Setoyama, D., Shimizu, H., Seta, H., Fujimura, Y., Miura, D., Wariishi. (2015). Identification of 3-methylbutanoyl glycosides in green Coffea arabica beans as causative determinants for the quality of coffee flavors. *Journal of Agricultural and Food Chemistry*, 3742–3751.

Jaramillo J, Muchugu E, Vega FE, Davis A, Borgemeister C, Chabi-Olaye A (2011) Some Like It Hot: The Influence and Implications of Climate Change on Coffee Berry Borer (*Hypothenemus hampei)* and Coffee Production in East Africa. PLoS ONE 6(9): e24528. https://doi.org/10.1371/journal.pone.0024528

Jeszka-Skowron, M., Sentkowska, A., Pyrzyn´ska, K., & Peña, M. P. (2016). Chlorogenic acids, caffeine content and antioxidant properties of green coffee extracts: influence of green coffee bean preparation. *European Food Research* and *Technology, 242*, 1403–1409. DOI 10.1007/s00217-016-2643-y

Jham GN, Berhow M., Manthey LK, Palmquist D., Vaughn SF (2008). The use of fatty acid profile as a potential marker for Brazilian coffee (*Coffea arabica* L.) for corn adulteration. Journal of the Brazilian Chemical Society 19:1462-1467;doi:10.1590/S0103 50532008000800004.

Joet, T., Laffargue, A., Salmona, J., Doulbeau, S., Descroix, F., Bertrand, B, & Dussert, S. (2009). Metabolic pathways in tropical dicotyledonous albuminous seeds: *Coffea arabica* as a case study. New Phytologist, 182(1), 146-162.

Joët, T.; Laffargue, A.; Descroix, F.; Doulbeau, S.; Bertrand, B.; de Kochko, A.; Dussert, S. Influence of environmental factors, wet processing and their interactions on the biochemical composition of green *Arabica coffee* beans. *Food Chemistry.* 2010, *118*, 693–701.

Kaić, A., Mioč, B., Kasap, A. and Potočnik, K. (2016), "Meta-analysis of intramuscular fatty acid composition of mediterranean lambs", *Archives Animal Breeding*,Vol. 59.

Karl Speer and Isabelle Kölling-Speer (2006). The lipid fraction of the coffee bean. *Brazillian Journal of plant physiology*, 18(1) 201-216

Kolling-Speer, L., & Speer, K. (2005). The Raw Seed composition. In A. Illy, & R. Viani, *Espresso Coffee, the Science of Quality* (pp. 148–178). Italy: Elsevier Academic Press.

Köseoglu Yılmaz, P., Hacıbekiroglu, I., & Kolak, U. (2014). Effect of roasting on antioxidant and anticholinesterase capacities of coffee. *Journal of Food and Nutrition Research*, 53, 232–239

Kumazawa, K., & Masuda, H. (2003). Investigation of the change in the flavor of a coffee drink during heat processing. Journal of Agricultural and Food Chemistry, 51, 2674–2678.

Kurzrock, T., & Speer, K. (2001). Diterpenes and diterpene esters in coffee. Food reviews international, 17(4), 433- 450.

Lea, P. J., Sodek, L., Parry, M. A., Shewry, P. R., & Halford, N. (2007). Asparagine in plants. *Annals of Applied Biology, 150*, 1–26. https://doi.org/10.1111/j.1744-7348.2006.00104.x

Leroy T., R. F., Bertrand, B., Charmetant, P., Dufour, M., Montagnon, C., Marraccini, P., & Pot, D. (2006). Genetics of coffee quality. *Brazilian Journal of Plant Physiology, 18*(1), 229-242.

Messina G, Zannella C, Monda V, Dato A, Liccardo D, *et al.* (2015) The Beneficial Effects of Coffee in Human Nutrition. Biology and Medicine (Aligarh) 7:240. doi: 10.4172/0974-8369.1000240

Ministry of Agriculture, Animal Industry & Fisheries (2010). Agriculture Sector Development Strategy and Investment Plan: 2010/11-2014/15.

Monente, C., Ludwig, I., Irigoyen, A., de Peña, M., & Cid, C. (2015). Assessment of total (free and bound) phenolic compounds in spent coffee extracts. *Journal of Agricultural and Food Chemistry*, 63. DOI: 10.1021/acs.jafc.5b01619

Mozaffarian, D., Aro, A. and Willett, W. (2009), "Health effects of trans-fatty acids: experimental and observational evidence", *European Journal of Clinical Nutrition*, Vol. 63. DOI: 10.1007/s13197-010-0225-8

Murkovic, M., & Derler, K. (2006). Analysis of amino acids and carbohydrates in green coffee. *Journal of Biochemical and Biophysical Methods, 69*, 25–32.

Murthy, P. S., & Naidu, M. M. (2012). Recovery of phenolic antioxidants and functional compounds from coffee industry by-products. Food and Bioprocess Technology, 5(3), 897-903. http://dx.doi.org/10.1007/ s11947-010-0363-z.

Mussatto, S. I., Machado, E. M., Martins, S., & Teixeira, J. A. (2011). Production, Composition, and Application of Coffee and Its Industrial Residues. *Food Bioprocess Technology , 4*, 661–672. doi:10.1007/s11947-011-0565-z

Neuwinger, H. D. (2000) African traditional medicine: a dictionary of plant use and applications. *Medpharm Scientific, Stuttgart, Germany*, 589 pp.

Nunes, F. M., & Coimbra, M. A. (2001). Chemical characterization of the high molecular weight material extracted with hot water from green and roasted Arabica coffee. *Journal of Agricultural and Food Chemistry*, Vol.1773.

Odeny, D., Chemining'wa, G., & Shibairo, S. (2014). Beverage quality and biochemical components of shaded coffee. In Paper presented at the the 25th international conference on coffee science, Colombia.

Oestreich-Janzen, S. (2010). Chemistry of coffee. Comprehensive Natural Products, II, 1085-1117.

Ogwok, P., Muyinda, R., Nakisozi, H., & Bamuwamye, M. (2017). Fatty acid profile of wild edible and cultivated mushrooms (Pleurotus ostreatus, Amanita spp and Termitomyces microcarpus). *Nutrition & Food Science, 47*(3), 357-368. doi:10.1108/NFS-08-2016-0122

Okrouhlá, M., Stupka, R., Čítek, J., Šprysl, M. and Brzobohatý, L. (2013), "Effect of dietary linseed supplementation on the performance, meat quality, and fatty acid profile of pigs", 102, 582–592. *Czech Journal of Animal Science*. 58.

Olthof, M. R., Hollman, P. C., Zock, P. L., & Katan, M. B. (2001). Consumption of high disease of Chlorogenic acid present in coffee, or of black tea increases plasma total homocysteine concentrations in humans. *American Journal of Clinical Nutrition, 73*(3), 532–538. doi:10.1093/ajcn/73.3.532

Paola Pittia, Maria Cristina Nicoli and Giampiero Sacchetti (2007). Effect of moisture and water activity on textural properties of raw and roasted coffee beans. Journal of Texture Studies 38(1):116 – 134

Parras, P., Martínez-Tomé, M., Jiménez, A. M., & Murcia, M. A. (2007). Antioxidant capacity of coffees of several origins brewed following three different procedures. *Food Chemistry*. Vol. 102.

Parunović, N., Petrović, M., Matekalo-Sverak, V., Trbović, D., Mijatović, M. and Radović, Č. (2012), "Fatty acid profile and cholesterol content of m. longissimus of free-range and conventionally reared Mangalitsa pigs", South African Journal of Animal Science. DOI: 10.4314/sajas.v42i2.2

Patay, E. B., Bencsik, T., & Papp, N. (2016). Phytochemical overview and medicinal importance of Coffea species from the past until now. *Asian Pacific Journal of Tropical Medicine, 9*(12), 1127–1135. DOI:10.1016/j.apjtm.2016.11.008

Pittia, P., Sacchetti, G., Nicoli, M.C. (2007). Effect of moisture and water activity on textural properties of raw and roasted coffee beans. Journal of Texture Studies 38(1). DOI: 10.1111/j.1745-4603.2007.00089.x

Pohl Pawel, Stelmach Ewelina, Welna Maja, Szymczycha-Madeja Anna-2013-Determination of Elemental composition of coffee using instrumental methods, *Food Analytical Methods*, 6: p.598-613;

Rajeev, R., Kaushal, K., Pavan, K., Rajesh, R., & Nitesh, K. (2016). Green Coffee Extract Improves Human Health: A Systematic Review. World Journal of Pharmaceutical Research. Volume 5, Issue 12, 295-321. DOI: 10.20959/wjpr201612-7415.

Ribeiro, B., Pinho, P.G., Andrade, P.B., Baptista, P. and Valentão, P. (2009), "Fatty acid composition of wild edible mushrooms species: a comparative study", *Microchemical Journal*. DOI: 10.1016/ j. microc. 2009.04.005.

Ribeiro-Alves, M., Trugo, L. C., & Donangelo, C. (2003). Use of oral contraceptives blunts the calciuric effect of caffeine in young adult women. *Journal of Nutrition*, Vol. 3933

Rodrigues, N., de Jesus Garcia Salva, T., & Bragagnolo, N. (2015). Influence of coffee genotype on bioactive compounds and the in vitro capacity to scavenge reactive oxygen and nitrogen species. *Journal of Agricultural and Food Chemistry*, 4815–4826. DOI: 10.1021/acs.jafc.5b00530

Romano, R., Santini, A., Grottaglie, L. L., Manzo, N., Visconti, A., & Ritieni, A. (2014). Identification markers based on fatty acid composition to differentiate between roasted Arabica and Canephora (Robusta) coffee varieties in mixtures. *Journal of Food Composition and Analysis, 35*, 1–9. http://dx.doi.org/10.1016/j.jfca.2014.04.001

Romijin, D.; Wiseman, S.A.; Scheek, L.M.; de Fouw, N.J.; van Tol, A. A linoleic acid enriched diet increases serum cholesterol esterification by lecithin: Cholesterol acyltransferase in meat-fed rats. *Annals of Nutrition and Metabolism*. 1998. DOI: 10.1159/000012740.

Sacchetti, G., Di Mattia, C., Pittia, P., & Mastrocola, D. (2009). Effect of roasting degree, equivalent thermal effect and coffee type on the radical scavenging activity of coffee brews and their phenolic fraction. Journal of food engineering, 90, 74–80.

Saura-Calixto, F., & Goñi, I. (2006). Antioxidant capacity of the Spanish Mediterranean diet. *Food Chemistry*, 442–447. DOI:10.1016/j.foodchem.2004.11.033

Shearer, J., Sellars, E., Farah, A., Graham, T. E., & Wasserman, D. H. (2007). Effects of chronic coffee consumption on glucose kinetics in the conscious rat. *Canadian Journal of Physiology and Pharmacology, 85*(8), 823–830. DOI:10.1139/y07-070

Shlonsky, A. K., Klatsky, A., & Armstrong, A. (2003). Traits of persons who drink decaffeinated coffee. *Annals of Epidemiology, 13*(1), 273–279.

Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & Pharmacotherapy, 56*(8), 365–379. doi:10.1016/S0753-3322(02)00253-6

Sivetz M, Desrosier NW. 1979. Hulling, classifying, storing, and grading green coffee beans. In: Sivetz M, Desrosier NW, eds. Coffee technology. Westport, CT: The Avi Publishing Company, 117–169.

Speer, K., & Kölling-Speer, I. (2006). The lipid fraction of the coffee bean. *Brazilian Journal of Plant Physiology, 18*(1), 201-216.

Sunitha Elizabeth George, Kulathooran Ramalakshmi, and Lingamallu Jagan Mohan Rao (2008). A perception on the health benefits of coffee. *Critical reviews in Science and Nutrition*, 48:464-486

Takahashi, J. and Carvalho, S. (2010), "Nutritional potential of biomass and metabolites from filamentous fungi-current research, technology and education topics in applied microbiology and microbial biotechnology", in Méndez-Vilas, A. (Ed.), Microbiology, Vol. 2, FORMATEX, pp. 126-1135.

Tesfay Gebretsadik, Tarekegn Berhanu, Belete Kefarge. Levels of Selected Essential and Nonessential Metals in Roasted Coffee Beans of Yirgacheffe and Sidama, Ethiopia. American Journal of Environmental Protection. Vol. 4, DOI: 10.11648/j.ajep.20150404.13

Toci, A. T., Farah, A. Volatile compounds as potential defective coffee seeds' markers. (2008). *Food Chemistry.*108. DOI: 10.1016/j.foodchem.2007.11.064.

Tohda, C., Kuboyama, T., & Komatsu, K. (2005). Search for natural products related to regeneration of the neuronal network. *Neurosignals 2005*. DOI: 10.1159/000085384.

Trugo, L. C. (2003). Coffee Analysis . In B. Caballero, & L. C. Trugo, *In: Encyclopedia of Food Science and Nutrition* (p. 498). Oxford, UK: Oxford Academic Press.

UCDA. (2017, May 15). *Uganda Coffee Development Authority*. Retrieved May 15, 2017, from Uganda Coffee Development Authority: http://ugandacoffee.go.ug/fact-sheets/

UIA. (2016). *Coffee Sector Investment Profile Uganda.* Kampla: Uganda Investment Authority.

USDA. (2014). *coffee sector.* new york: usda.

Valentin, J. L., & Watling, R. J. (2013). Provenance establishment of coffee using solution ICP-MS and ICP-AES. *Food Chemistry, 141*(1), 98-104. doi:10.1016/j.foodchem.2013.02.101

Wattenberg, L. W. (1983). Inhibition of neoplasia by minor dietary constituents. *Cancer Research*, 2448s–2453s.

Wenjiang, D., Lehe, T., Jianping, Z., Rongsuo, H., & Minquan, L. (2015). Characterization of Fatty Acid, Amino Acid and Volatile Compound Compositions and Bioactive Components of Seven Coffee (Coffea robusta) Cultivars Grown in Hainan Province, China. Vol.16.

Yang, K.; Wu, D.; Ye, X.Q.; Liu, D.H.; Chen, J.C.; Sun, P.L. (2013). Characterization of chemical composition of bee pollen in China. *Journal of Agricultural and Food Chemistry.* Vol. *61*. DOI: 10.1021/jf304056b

APPENDIX 1: THE COFFEE PLANT

Source: Murphy and Naidu, 2012

APPENDIX 2: PHOTOGRAPHIC IMAGE GREEN COFFEE BEANS