Addis Ababa University

College of Natural and Computational Sciences

Center for Food Science and Nutrition

Use of Spent Coffee Ground (SCG) as ingredient in Bread Formulation

By: Teshome Daniel

Advisor: Dr. Paulos Getachew (PhD)

A thesis submitted to Addis Ababa University in partial fulfillment of the requirement for the Degree of Master of Science in Food Science and Nutrition

June, 2018

Addis Ababa, Ethiopia

DECLARATION

I, the undersigned certify that the thesis conducted as a partial fulfillment of MSc degree in Food Science and Nutrition is my own original work and has not previously been submitted by me in its entirety or in part to this or any other university for a degree. Also all the source materials used for writing the proposal have been duly acknowledged.

Name Teshome Daniel

Place: Addis Ababa University, Addis Ababa, Ethiopia

Date of submission _____

The thesis has been submitted as final approved thesis for partial fulfillment of MSc degree in Food Science and Nutrition as a university advisor.

Name Dr. Paulos Getachew

Signature_____

Date_____

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Approval by examining board:	Signature	Date
Dr. Paulos Getachew (Advisor)	_____	_____
Dr. Abera Belay (External examiner)	_____	_____
Dr. Kaleab Baye (Internal examiner)	_____	_____
Dr. Hasset Tamirat (Chairperson)	_____	_____

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ABBREVIATIONS

- CGA -Chlorogenic acids
- CSA – Central Statistical Agency
- DR-dark roast
- DF-dietary fiber
- DPPH-2,2-diphenyl-1-picrylhydrazyl
- EFSA- European Food Safety Authority
- GI-glycemic index
- HMF- Hydroxmethylfurfural
- IDF-insoluble dietary fractions
- ICP-AES- Inductively coupled plasma atomic emission spectroscopy
- MRP-Maillard reaction product
- MR-medium roast
- ROS-reactive oxygen species
- SDF-soluble dietary fractions
- SCG-Spent coffee ground
- TDF-total dietary fiber
- TFC-total flavonoids content
- TPC- total phenolics content
- UV-ultra violet

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ABSTRACT

Background: *Spent coffee ground (SCG) is the most abundant byproduct (45%) of instant coffee production. The increase in brewed coffee consumption worldwide parallels the amount of SCG generated. Around 6 million tons of SCG is generated per year. SCG is full of nutrients, yet it is not utilized properly except as compost in few areas. It is dumped as a waste material harnessing environmental degradation. But considering its biochemical composition, it can be used as ingredient in food industries such as bakery.*

Objective: *Therefore, the purpose of this study is to utilize SCG as a functional food ingredient in bread formulation. Through this valorization approach, the SCG incorporation proportion, glycemic index, overall acceptance and the physicochemical characteristics of the formulated bread were investigated.*

Materials and Methods: *Wheat flour blends for (2, 4, 6, 8 and 10% of SCG) for physicochemical and sensory properties also glycemic index and antioxidant activity of the formulated bread were investigated.*

Results: *There is considerable variation in antioxidant properties of bread baked from wheat flour and SCG has total phenolic content (TPC) 2768.53*μg GAE g^{-1} *and total flavonoids content (TFC) was 2409.24* μg QE g^{-1}. *Moisture content of SCG during collection reached to 48.98%, and dried to 8.47% to control microbial activity. The SCG based bread had improved proximate composition compared to that of wheat based bread. Specially, the SCG incorporation has shown enhancement in fiber content of the bread for instance the bread formulation with 10% SCG is 5.31 mg/100g which is much higher than the white bread which is 1.56 mg/100gm. Sensory evaluation of the new product had resulted similar overall acceptance for SCG based bread of 2% composition at $p<0.05$.*

With 2%of SCG, the bread has improved with proximate composition of protein 7.79g/100g, fiber 2.51g/100g and energy 264.48 Kcal. Compared to white bread mentioned proximate composition had significant increase for the amount with equivalent energy. In addition to proximate composition TPC and TFC of this incorporation was also improved which is 476.92μg GAE g^{-1} and 1154.43μg QE g^{-1} respectively.

In fact, the maximum concentration of SCG (10%) based bread had also showed glycemic index (GI) of 78.68 and minimum concentration 85.57.which is less than that of standard glucose and control white bread. Baking loss of the new product is also reduced due to the effect of the fiber complex water retention activity in SCG by 9.29% for maximum concentration in the formulation.

***Conclusion**: Spent coffee ground contains high antioxidant dietary fiber and rich with minerals. Mixing wheat flour with SCG affected physicochemical property and sensory value of the bread. Therefore, the new formulated bread can be optional source of food to people consumption.*

Key words: spent coffee ground, antioxidant capacity, fiber, glycemic index

CHAPTER ONE

1. Introduction

1.1. Background

Spent coffee ground (SCG) is the solid residue obtained during coffee brewing process (Campos-Vega et al., 2015). Coffee is one of the most popular beverages which are widely consumed (Mussatto et al., 2011; Kondamudi et al., 2008). According to International Coffee Organization (ICO, 2016), the global coffee production has increased by 6% since 2010, and the total coffee production in 2015 was 8.6 million tons (Getachew and Chun, 2016). Therefore, in parallel with the coffee production, the amount of spent coffee ground increases. Coffee producing countries generate residues from the coffee fruit amounting to >50% of the fruit mass (Tsai et al., 2012). About 2Kg of wet SCG are obtained from each Kg of instant coffee produced, with an annual generation of around 6 million tons worldwide (Mussatto et al., 2017). Yet, despite such large biomass residues being generated each year, they are underutilized, disposed of in landfills, only small amounts being utilized for composting worldwide (Liu and Price, 2011; Valipour, 2015). Apparently, as one of the leading coffee producing and consuming nation, in our country also spent coffee is one of the visible huge contributors of solid waste accumulation specially in urban areas. The piling of solid waste materials including SCG not only damages the environment, but also the potential to threat human life. The recent disaster that happened in Addis Ababa city in 2016 (i. e Koshe, solid waste disposal area,) due to solid garbage slide (death toll reached more than 120) can be a proof.

Spent coffee residue contains large amounts of organic compounds including fatty acids, amino acids, polyphenols, minerals, polysaccharides, melanoidins and dietary fiber (Getachew and Chun, 2016). These complex organic substances in the residue were found to have adverse impacts on land and the environment (Mussatto et al., 2011; Valipour et al., 2015). In contrast, if given the proper attention, the biochemical composition of SCG justifies its valorization (Getachew and Chun, 2016). Several researchers reported the extraction and potential applications of bioactive compounds from SCG. These compounds act as a source of antioxidants (Mussatto et al., 2011a), antimicrobial agents, protect against cell mutagens (Monente et al., 2015), good sources of dietary fiber (Martinez et al., 2017). Based on this hypothesis, Getachew and Chun (2016) reported that

applying subcritical water liquefaction of SCG (green waste valorization approach), SCG was found to be a useful source of bioactive compounds (i.e. polyphenols, flavonoids, proteins, maillard reaction products) with tremendous antioxidant and antimicrobial activities. Accordingly, SCG can also be considered as potential functional ingredients for the food industry. In fact, the increasing demand for foodstuffs free of artificial additives with added nutritional value induces the food industry to find new sources of antioxidants and bioactive compounds.

Therefore, SCG is utilized as landfill and composting, needed to utilize SCG industrially as food ingredient. Agreed upon SCG's utilization as food ingredient, the research question narrows down to which food industry? Recently, consumers are concerned about caloric content and glycemic index (GI) of foods under consumption. The benefits of low GI diets extend beyond weight loss and have favorable effects on obesity-related diseases such as type 2 diabetes. (Martinez et al., 2017). Related with this fact, commonly utilized foods including bakery products (especially white bread) and rice in our society are known to have high GI values. Hence, the search for healthier and tasty food like bakery products is a necessity in our population.

Martinez et al. (2017) reported the successful application of SCG in biscuit industry to process low GI bakery products. However, they were focused more on digestibility, safety and sensory properties for the innovated biscuits without a clear complete data concerning the chemical composition, physical property of the composite and glycemic index of the final product. Additionally, during both baking and coffee roasting maillard reaction is the main chemical event, which further supports SCG as ingredient in bakery industry in addition to its high dietary fiber content (i.e. low GI). In Ethiopia, as to our knowledge there is no study conducted on the biochemical composition of SCG and its utilization as a food ingredient. Therefore, the main objective of the present study was to evaluate the biochemical composition of SCG collected from major cafeterias in Addis Ababa and to utilize SCG for bakery industry as ingredient for antioxidant fiber rich bread production. Thus, different composition of SCG were blended with wheat flour to formulate new bakery product (bread) and proximate, sensory analysis, glycemic index and over all antioxidant capacity of the bread was analyzed.

1.2. Statement of the problem

Spent coffee ground (SCG) is the main residue of the coffee industry with a worldwide annual generation of 6 million tons. SCG contains large amounts of organic compounds (i.e., polyphenols, lignin, cellulose, hemicellulose, and other polysaccharides). As mentioned in the background coffee producing countries generate residues from the coffee fruit amounting to >50% of the fruit mass (Tsai et al., 2012). Despite such large biomass residues being generated each year, they are underutilized, disposed of in landfills (Valipour. 2015) worldwide. Ethiopia is also one of leading coffee producing and consuming coffee processing byproducts mostly spent coffee can be contributors of solid waste accumulation; specially in urban areas. The piling of solid waste materials including SCG not only damages the environment, but also it is a potential threat to human life.

In recent days, consumers are concerned about caloric content and glycemic index (GI) of the food as well as balanced nutrition. The benefits of low GI diets extend beyond weight loss and have favorable effects on obesity-related diseases such as type 2 diabetes. Food industries need to fulfill the increasing consumer's demand of healthier and tastier foods. Bakery products like white bread have the highest GI which should be improved through blending, considering its wide consumption worldwide. Accordingly, as reported by previous studies, SCG has high fiber and antioxidant compounds which can be blended in wheat flour to formulate healthier and nutritious bakery products (Rathinavelu and Graziosi, 2005).

So far, there is limited number of publications on SCG blended as ingredient in food processing. Martinez et al., 2017 produced biscuits supplemented with many functional ingredients e.g., maltitol, oligofructose, stevia and SCGs. To explore researchers around SCG this study can be considered as significant as a baseline for further investigation, world concern of recycling waste as well as reducing global warming determinants.

1.3. Objectives

13.1. General Objective

♦To utilize Spent Coffee Ground (SCG) in bakery industry as ingredient in bread processing and analyze physicochemical composition and overall acceptability of the product.

1.3.2. Specific Objectives

- To evaluate the proximate composition of SCG collected from cafeterias in Addis Ababa.
- To utilize the SCG as bakery ingredient to develop a low caloric, high fiber and antioxidant improved bread.
- To evaluate the biochemical composition of the new bread.
- To evaluate overall acceptance (sensory quality) of the new SCG based bread.
- To investigate the glycemic index of the SCG based bread.

CHAPTER TWO

2. Literature review

2.1. Overview on coffee plant

Coffee is a genus of flowering plants whose seeds, called coffee beans, are used to make various coffee beverages and products. It is a member of the family Rubiaceae. They are shrubs or small trees native to tropical and southern Africa and tropical Asia. Coffee ranks as one of the world's most valuable and widely traded commodity crops and is an important export product of several countries, including those in Central and South America, the Caribbean and Africa. Several species of coffee may be grown for the seeds. *Coffea Arabica* accounts for 75-80 percent of the world's coffee production, while *Coffea canephora* accounts for about 20 percent (Fujioka and Shibamoto 2008).

The trees produce edible red or purple fruits called "cherries" that are described either as epigenous berries or as indehiscent drupes. The cherries contain two seeds, the so-called "coffee beans", which despite their name are not true beans. In about 5-10% of any crop of coffee cherries, only a single bean, rather than the usual two, is found. This is called a pea berry, which is smaller and rounder than a normal coffee bean. It is often removed from the yield and either sold separately or discarded (Rathinavelu & Graziosi 2005).

When grown in the tropics, coffee is a vigorous bush or small tree that usually grows to a height of 3–3.5 m (9.8–11.5 ft). Most commonly cultivated coffee species grow best at high elevations, but do not tolerate freezing temperatures. The tree of Coffea *Arabica* will grow fruits after three to five years, and will produce for about 50 to 60 years (although up to 100 years is possible). The white flowers are highly scented. The fruit takes about 9 months to ripen *(Campos-Vega et al., 2012).* The coffee fruit (also called berry or cherry) consists of a smooth, tough outer skin or pericarp, usually green in unripe fruits but that turns red-violet or deep red when ripe (even yellow or orange in particular genotypes). The pericarp covers the soft yellowish, fibrous and sweet pulp or outer mesocarp. This is followed by a translucent, colorless, thin, viscous and highly hydrated layer of mucilage (also called the pectin layer) figure 1. Then, there is a thin endocarp yellowish in color, also called parchment. Finally, the silverskin covers each hemisphere of the coffee bean (endosperm) (Fujioka and Shibamoto 2008).

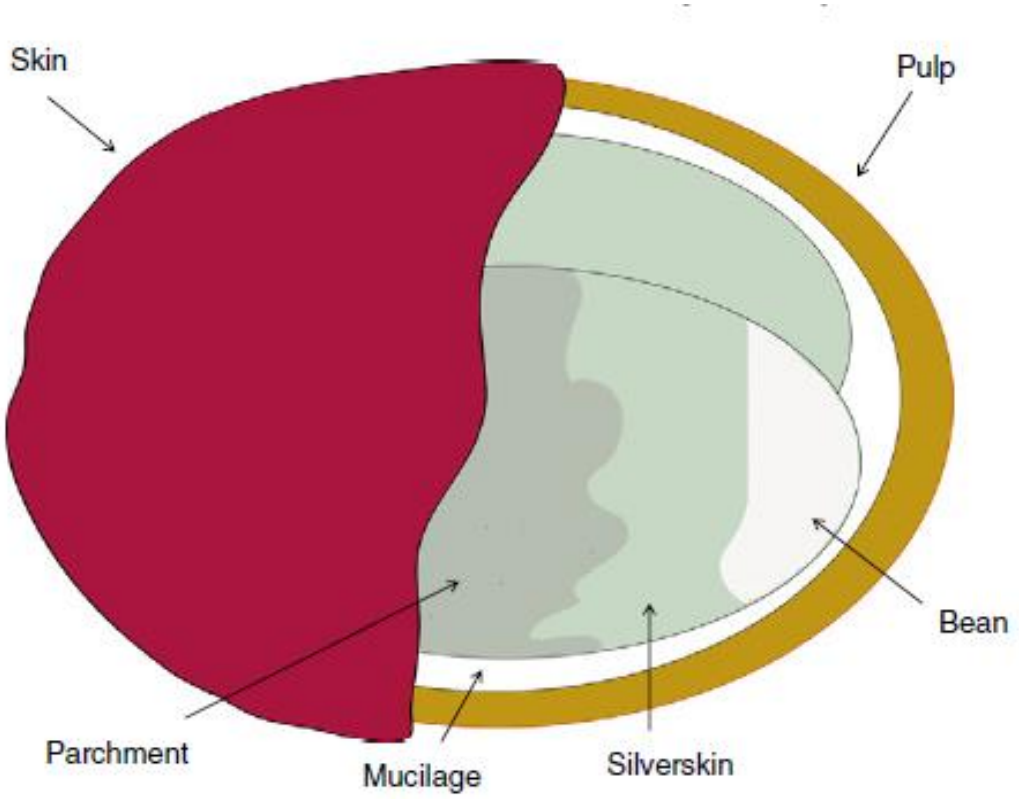

Figure 1: Anatomy of the coffee bean
Source: (Campos et al., 2012).

World coffee production has grown more than 100% from 1950 to 1960, and there was a prediction to grow more 0.5–1.9% by 2020 (Fujioka and Shibamoto 2008). Coffee is nowadays produced in a large number of countries worldwide. Nevertheless, the ten largest coffee producing countries are responsible for approximately 80% of the world production. Of this percentage, South America participates with around 43%, Asia with 24%, Central America 18%, and Africa with 16%. Brazil, Vietnam, Colombia, and Indonesia are respectively the first, second, and third largest world producers, responsible for more than half of the world supply of coffee. According to the International Coffee Organization (ICO 2010), in 2009 Brazil produced approximately 40 million bags of coffee.

The world consumption of coffee in 2007, estimated by the International Coffee Organization, has been around 124,636 million bags of 60 kg, representing an increase of 2.88% regarding the 121,150 million sacks consumed in 2006 (ICO, 2010). Despite the financial crisis, the world consumption of coffee in 2008 was around 128 million bags. According to ICO, the consumption of coffee was not affected by the crisis. The consumers will not stop drinking coffee, but instead of drinking high quality coffee, people will start to take coffee of middle quality.

Ethiopia is the origin of Coffee Arabica and the largest producer of coffee in Africa and the largest fifth coffee producer in the world (GAIN, 2014). Coffee production is vital to Ethiopian economy with about 15 million people directly or indirectly deriving their livelihoods from it. Coffee accounted for 19% of total Ethiopian export (Trading Economics, 2016). The area allocated for coffee production in 2015/16 Meher Season was 653,909.76 ha which was About 4.69% of the area under all crops in the country and 4,145,964.55 quintal was produced with average yield of 6.34 quintal/ha (CSA, 2016). In 2014/15 Meher Season, the area allocated for coffee production was estimated to be 561,761.82 ha from which about 4,199,801.56 quintal was obtained with average yield of 7.48 quintal/ha. Even though the area allocated has increased by about 92147 ha or 16.40%, yield obtained was decreased by 53837.01quintals or 1.28% and average yield of quintal per hectare also was decreased by 15.20% due to inadequate amount of rainfall in the country in 2015/16 production year. Coffee production in Ethiopia is constrained by lack of competitiveness, poor access to market, lack of infrastructure, in adequate access to services, low value addition, and in adequate technology transfer and research (Jose, 2012). Another constraint of coffee production in Ethiopia is limited extension and research facilities (World Bank, 2015).

2.2. Coffee brewing and its byproducts

Industrial processing of coffee fruit is done to isolate coffee powder by removing shell and mucilaginous part from cherries. Coffee is subjected to two methods of processing (washed and unwashed) such as pulping, washing, drying, curing, roasting, and brewing, and during the process, various by-products such as coffee pulp (CP), cherry husk (CH), parchment husk (PH), silver skin (SS), and spent coffee grounds (SCG) are obtained (Pushpa et al., 2008).

Since more than 50% of the coffee fruit is not used for production of the commercialized green coffee and, therefore, is discarded during processing, it should be interesting to find applications

for these by-products. Up to now, most progress has been achieved in their use for industrial purposes other than food industry, such has energy production (Kondamudi et al., 2008), adsorption of compounds and manufacturing of industrial products, such as particleboards, ethanol, gibberellic acid and α-amylase. Commercialized extracts from the coffee fruits, which contain CGA, condensed proanthocyanidins, quinic and ferulic acid, have shown interesting results for facial skin care. However, in spite of the known high phenolic antioxidant and phytonutrient levels of the coffee fruit, only limited progress has been achieved on its use as a functional ingredient (Heimbach, et al., 2010).

The consequence of this big market, the coffee industry is responsible for generating large quantities of residues; among which, spent coffee grounds (SCG) and coffee silverskin (CS) are the most significantly generated. SCG is the residual material obtained during the treatment of coffee powder with hot water or steam for the instant coffee preparation. Almost 50 % of the worldwide coffee production is processed for soluble coffee preparation, which generates around 6 million tons of SCG per year (Mussatto et al., 2011).

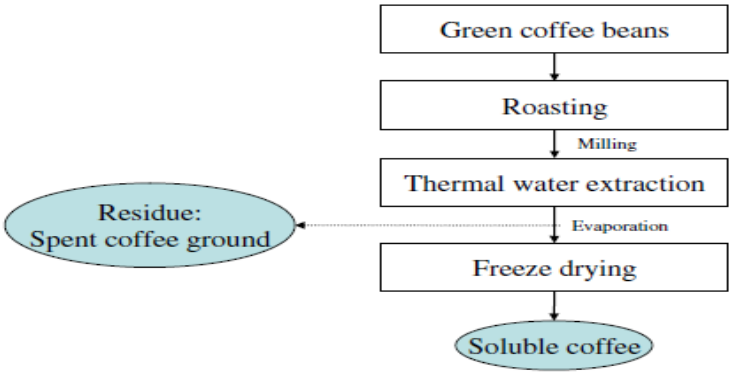

Figure 2: Coffee brewing flow chart

Source: (Mussatto et al., 2011)

Nowadays, there is a great political and social pressure to reduce the pollution arising from industrial activities. For that reason, it is necessary to focus on the exploitation of SCG and CS, and their profitable utilization, adding value to these unused materials and decreasing their impact to the environment. Despite that some characteristics of SCG and CS have been recently

reported in the literature, to the best of our knowledge, there is not any study that shows a complete characterization of both materials. Such information is of great importance to identify the possible areas for application of these residues.

2.2.1. Coffee husks, skin and pulp

Coffee husks, skin and pulp can be a source of phytochemicals for the food and pharmaceutical industries (Ramírez et al., 2004) found four major classes of polyphenols (viz., flavan-3-ols, hydroxycinnamic acids, flavonols and anthocyanidins) in Arabica coffee pulp. For instance, the phenolic compounds tentatively identified by HPLC in fresh coffee pulp are: chlorogenic acid (5-caffeoylquinic acid) (42.2% of the total of identified phenolic compounds), epicatechin (21.6%), 3,4-dicaffeoylquinic acid, (5.7%), 3,5-dicaffeoylquinic acid (19.3%), 4,5-dicaffeoylquinic acid (4.4%), catechin (2.2%), rutin (2.1%), protocatechuic acid (1.6%) and ferulic acid (1.0%). (Later on, Clifford and Ramírez-Martínez 1991) additionally identified 5-feruloylquinic acid in coffee pulp. More recently, described the use of fresh coffee husks as a potential source of the anthocyanin cyanidin-3-rutinoside. In a similar study, but using peels and pulp derived from wet-processed fruits, (Esquivel et al., 2010) identified cyanidin-3-rutinoside, cyanidin-3-glucoside and its aglycone as the major anthocyanins present before and after tissue browning. Moreover, they also found important levels of caffeine in these coffee by-products.

2.2.2. Coffee mucilage

The coffee mucilage fraction remains adhered to the coffee bean in the wet processing after depulping without enzymatic degradation. This method allows separation and concentration of this fraction. The mucilage is composed of water (84.2%), protein (8.9%), sugar (4.1%), pectic substances (0.91%) and ash (0.7%). (Belitz et al., 2009) The composition analysis of the alcohol-insoluble residues showed the presence of pectic substances (30%), cellulose (8.1%) and neutral noncellulosic polysaccharides (18%). Pectins contained uronic acids (60%) with high degree of methyl esterification and moderate degree of acetylation (Avallone, et al., 2001).

2.2.3. Coffee parchment

The strong fibrous endocarp that covers both hemispheres of the coffee seed and separates them from each other is called the parchment. In the dry processing, the parchment is separated from the green coffee beans together with the peel and pulp, in a single step. However, in the wet processing the parchment is removed after drying and hulling separate steps (Belitz et al., 2009).
The latter process permits collection and use of parchment separately from other byproducts. Coffee parchment is composed by (α-) cellulose (40-49%), hemicellulose (25-32%), lignin (33-35%) and ash (0.5-1%) Similar to the mucilage, authors do not know any study on the functional characteristics of coffee parchment.

2.2.4. Coffee silverskin

As mentioned above silverskin remnants still attached to the green coffee beans are removed during roasting (Belitz et al., 2009). They can be easily found as a coffee processing by-product in coffee roasting plants and are presently used as fuel or for composting and recommended the use of silverskin as functional ingredient, based on the low amount of fats and reducing carbohydrates, high contents of soluble dietary fiber (60%) and marked antioxidant activity. The latter is probably consequence of the high contents of melanoidins generated during roasting, because silverskin has low contents of free phenol compounds. Additionally, silverskin supports growth of bifidobacteria in vitro, which might have some beneficial effects (Borrelli et al., 2004).

2.2.5. Low-grade green coffee

Coffee with imperfections, such as black or dark brown color, insect damage, spots, bits, from immature fruits, etc., is graded during processing and termed as low-grade coffee beans. These beans comprise about 15–20% of coffee production (Ramalakshmi et al., 2009).

2.2.6. Spent Coffee Ground

Spent coffee ground (SCG) is the main residue of the coffee industry with a worldwide annual generation of 6 million tons. SCG have no commercial value and are currently disposed of as a solid waste or, in some cases, used as fertilizers or burned. Due to their high organic material content and the presence of compounds which can have negative effects on the environment, the disposal of SCG needs to be properly managed (Saenger et al., 2001). Similarly, burning of SCG can result in the release of greenhouse gases into the atmosphere. This has stimulated efforts to find ways of reducing their environmental impact and/or transforming them into value-added products. SCG contains large amounts of organic compounds (i.e. polyphenols, lignin, cellulose, hemicellulose, and other polysaccharides) that can be exploited as a source of value-added products (Belitz et al., 2009).

In the late 19th century, used coffee grounds were used to adulterate pure coffee. In gardens, coffee grounds may be used for composting or as much as they are known to slowly release nitrogen into the soil .The coffee grounds are rich in potassium, magnesium and phosphorus. They are especially appreciated by worms and acid-loving plants such as blueberries. Used coffee grounds have other homemade uses in wood staining, air fresheners, and body soap scrubs. They may also be used industrially in biogas production or to treat wastewater (Menéndez & Pis, 2007).

Bioactive compounds can be used as antioxidants, antimicrobial agents in the food, cosmetics and pharmaceutical industries. Thus, adding value to SCG is not only important for the production of bioactive materials from waste but is also helpful in combating the environmental damage caused from disposing SCG to landfills or burning as fuel (Avallone et al,. 2001). As it is known, coffee beans contain several classes of health related chemicals such as phenolic compounds, melanoidins, diterpenes, xanthines, and vitamin precursors. Caffeine is the most studied coffee component because of its well-established psychoactive effects and promotion of energy metabolism. Coffee phenolics have attracted much interest in recent years due to their strong antioxidant and metal-chelating properties. These properties are believed to provide in vivo protection against free radical damage and reduce the risk of degenerative diseases associated with oxidative stress (Acevedo et al,. 2013).

Chlorogenic acids (CGA) are the main components of the phenolic fraction of green coffee seeds. Several studies demonstrate that the consumption of CGA-rich beverages may result in remarkable health benefits including reduced incidence of atherosclerosis, diabetes, and various types of cancer. In addition, the main CGA present in coffee are highly bioavailable, being easily absorbed and/or metabolized throughout the gastrointestinal tract (Ownby et al., 2006). During coffee processing, CGA may undergo chemical transformations such as isomerization, hydrolysis, or degradation into lower molecular weight compounds; the high temperature of roasting also leads to a reduction of the amount of CGA by transformation into quinolactones and melanoidins. Other biologically active coffee components with potential beneficial health effects are nicotinic acid, trigonelline, quinolinicacid, tannic acid, and pyrogallicacid. Antioxidant properties such as reactive oxygen species (ROS) scavenging have also been recently proposed for caffeine, the most abundant alkaloid present in coffee beans (Loewenstein et al., 2006).

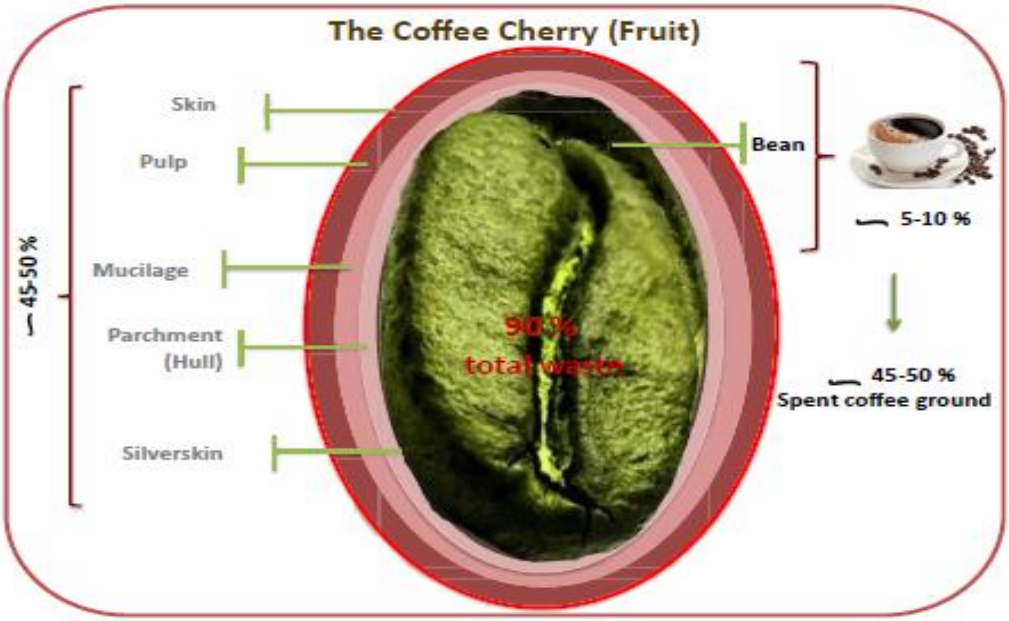

Figure 3**:** The coffee cherry fruit and its by product.
Source: (Campos et al., 2012)

2.3. Biochemical composition of coffee and its health benefits

Coffee traditionally recommended as a beverage to reduce or omit because of a risky global profile, coffee has progressively moved to a less negative position due to its better known phytochemistry. Coffee includes a complex mixture of compounds, where caffeine has been perhaps the most widely known; however, coffee is also rich in other bioactive substances with a wide array of physiological effects. The list comprises upto 1000 described phytochemicals. Among them, are polyphenols, including chlorogenic and caffeic acid, lactones, diterpenes, including cafestol and kahweol, niacin, and the vitamin B3 precursor trigonelline. Moreover, coffee is rich in vitamin B3, magnesium and potassium (Mussatto et al., 2011). There is now some significant evidence that coffee have distinct benefits. This is not to say that all of coffee's effects are healthy. It certainly is a cardiac stimulant and if one is prone to arrhythmias, it may exacerbate them; it can also increase reflux as well as tremor and agitation.

Peterson 2007 studied the results of recent prospective studies which assess the relative risk of developing Type II Diabetes according to coffee consumption. Most studies confirm a protective effect against Type II Diabetes, with some dose-response related to the degree of daily coffee consumption. The study found that, after adjustment for confounders, the relative risk of Type II Diabetes in coffee drinkers compared to non-drinkers was 0.87 for up to 1 cup per day, 0.58 for 2 or 3 cups per day, and 0.53 for 4 or more cups per day (P for trend <0.0001). Associations were fairly similar for caffeinated and decaffeinated as well as for brewed (filtered) and instant coffee. When tea consumption of even 4 or more cups per day was compared with no tea consumption, there was no significant effect on the risk of type II diabetes.

A prospective study reported by Rheum 2007 looked at a large cohort of 45,869 men with no history of gout at baseline. Long-term coffee consumption was associated with a lower risk of incident gout: the relative risks according to coffee consumption of 0, <1, 1-3, 4-5 and 6 or more cups per day were respectively 1.00, 0.97, 0.92, 0.60 (95% confidence interval 0.41-0.87), and 0.41 (95% CI 0.19-0.88), (P for trend = 0.009). Decaffeinated coffee seemed to work almost as well.

Coffee use also seems to have a positive effect on chronic liver disease. In a study reported in the British Journal of Cancer 2007, August 6;97(3):426-8, the multivariate adjusted odds ratio (95% CI) for mortality from hepatocellular carcinoma was 0.49 overall for daily coffee drinkers versus non-coffee drinkers. When analyzed separately in HCV-positive and HCV-negative individuals, it was 0.31, and 0.75 respectively. Benefits reported in several other interesting studies include a lower prevalence of non-melanoma skin cancer in Caucasian women, and a lower risk of Parkinson's disease. On the other hand, this is not to say that coffee is without negative effects. Some article suggested that it might be prudent for pregnant women to limit coffee consumption to three cups per day, or no more than 300 mg/day of caffeine, to exclude any increased probability of spontaneous abortion or impaired fetal growth (Cano-Marquina et al., 2013).

The effect of coffee on cardiovascular health is an ongoing controversy; coffee consumption was shown to have adverse effects on serum cholesterol, blood pressureand plasma homocysteine. However, the effects of coffee on epinephrine concentrations, hyperglycemia, and blood pressure all appear to be weaker than the effects of the same amount of caffeine used in isolation. The harmful cardiovascular effects of caffeine may be offset by the beneficial effects of other compounds in coffee on the biological pathways involved in the development of coronary heart disease (Cano-Marquina et al., 2013)

Osteoporosis, the association of coffee intake with bone metabolism, bone or bone fracture has been a matter of debate for years. The background supporting association resides in initial findings that the intake of coffee increased urinary calcium output, most probably as a result of the acidic load favored by coffee (Bhatti, S.K. et al. 2013).

Moreover, the traditional description of coffee as a risk factor for hypertension or cardiovascular disease seems to vanish. Much of the contrast between the former prevention and the present view may be influenced by the past association of coffee effects to caffeine in the presence of an insufficient number of clinical studies. The subsequent arrival of more and better quality clinical data, together with the improvement in the knowledge of such coffee components as phenolic acids, has contributed to the change. It may be concluded, therefore, that the labeling of coffee as a

mostly harmful beverage lacks support in the light of present knowledge reference (Hatem et al., 2018).

2.4. Spent coffee and its bioactive compounds

Bioactive compounds are value-added products, justifying their isolation from the industrial wastes. These residues, in fact, could be an alternative source for obtaining natural antioxidants, which are considered completely safe in comparison with synthetic antioxidants (Moure et al. 2001). An understanding of the physiological effects of coffee is drastically limited by the complexities deriving from two factors, the vast array of components included in the brewed product, and the varied effects of each compound. The most abundant bioactive compounds in SCG are caffeine and chlorogenic acid. The free fatty acid profile of the lipid fraction (mainly linolenic and palmiticacids) from SCG is similar of that of coffee beans indicating that the processing of instant coffee production does not affect it. Kahweol and cafestol were found to be available in relatively high concentrations in SCG. SCG and defatted SCG were found to be rich in several polyphenol compounds with high antioxidant activity (Acevedo et al., 2013).

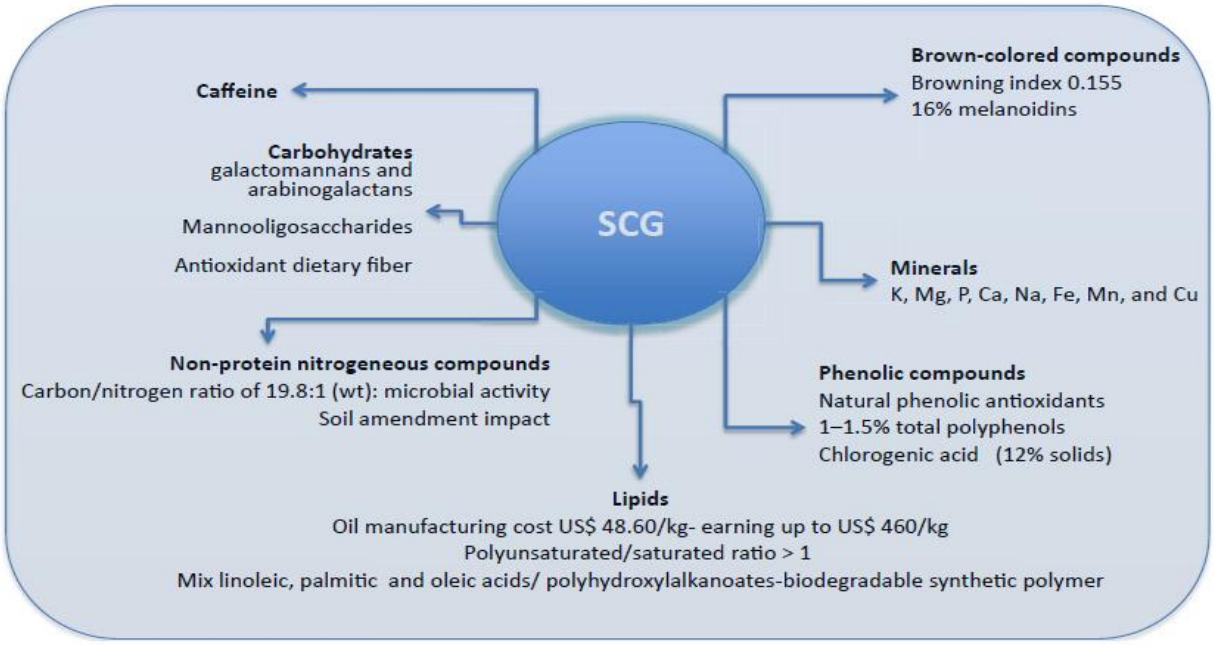

Figure 4**:** Major bioactive compounds in SCG
Source: (Campos-Vega et al, 2012.)

Analysis of proximate composition varies from articles to articles. However the most reputable used to determine moisture (method 925.10), lipid (method 920.39), ash (method 923.03), and nitrogen (method 920.87) contents of the ground bean samples (coffee beans and SCG). Moisture was assessed based on weight loss after oven drying at 105 °C until constant weight was reached. Nitrogen content was determined using the micro-Kjeldahl method with sodium sulfate as catalyst. Protein content was calculated as nitrogen × 6.25. Lipid content was obtained from Soxhlet extraction (6 h) with petroleum ether. Ash content was calculated from the weight of the sample after incineration in a muffle furnace at 550 °C for 2 h. Carbohydrate values were obtained by difference (Association of Official Analytical Chemists, 2002).

2.4.1. Carbohydrate

Carbohydrates, the most abundant coffee bean (CB) constituent increased (68%) on roasting from medium to dark due to polysaccharide depolymerizationand/or increase in galactomannans, the main polysaccharides in roasted coffee with increasing degree of roast. Also, it has been suggested that, during roasting, oligomers and especially monomers are converted very rapidly into Maillard and pyrolysis products (Oosterveld et al., 2003).

Carbohydrate content of SCG (60% w/w, dry weight) higher (13.4%) from dark roast (DR) than from medium roast (MR) reflecting the higher amount of insoluble polysaccharides (9.4% vs 3.1% for DR and MR, respectively) bound to the SCG matrix, and the possible supports of low molecular weight brown compounds by the high molecular weight galactomannans. Carbohydrates accounted for 60% of the dry weight of SCG from medium roasted coffee. Total dietary fiber (mostly insoluble fiber $\geqslant$ 95%). Despite the fact that DF was unaffected by roasting intensity, galactomannans degraded only moderately during roasting, and those remaining in the bean showed no evidence of molecular weight changes even after a dark roast. (Nunes et al., 2006).

Spent coffee ground is rich in sugars polymerized into cellulose and hemicellulose structures, which correspond to almost half (45.3%, w/w, dry weight) of the material. SCG contains 46.8% mannose, 30.4% galactose, 19% glucose, and 3.8% arabinose, with mannans as the major polysaccharides. (Mussatto & Teixeira 2012).

2.4.2. Crude fat

SCG total lipids range from 9.3 to 16.2%, 10-15% and 14-15.4% from espresso coffee residues, filter and industrial soluble coffee, respectively. Also, the yield of SCG oil extracted using Soxhlet, is a function of extraction conditions, particularly, the choice of solvent and the duration of extraction (Cruz et al., 2012). Commercial SCG contains higher oil (16.7 & 17.2%) compared to raw (9-12.6%), roasted (12-15%), or laboratory extracted SCG (7.9-14%); free fatty acids and lower unsaponifiable matter (5.9-9.4% vs 9-13.2%) relative to those produced in the laboratory. Coffee brews prepared by different methods showed that lipids (90.2%) mainly remained in SCG with the following lipid composition (% total lipids), 84.4% triglycerols, 12.3% diterpene alcohol esters, 1.9% sterols, 1.3% polar material, and 0.1% sterol esters. The lipid composition is similar to those of boiled or filtered coffee with 87-93% triglycerides, 7-13% diterpene alcohol esters, 0.2-0.9% sterols, and up to 0.8% polar material (Ratnayake et al., 1993). The lipid composition of SCG may vary analogous to those of green coffee oil depending on the source, although generally up to 80 – 90% of the oil will be glycerides, including free fatty acids, with the rest of the lipids containing terpenes, sterols and tocopherols (Jenkins et al., 2014).

2.4.3. Crude protein

SCG contain significant amount of proteins (13.6%, w/w). Total coffee nitrogen compounds are relatively stable between species or even during roasting, ranging from 8.5 to 13.6%. Crude protein reported by (Cruz et al., 2012) in espresso coffee residues vary between 12.8 and 16.9%. The mean protein content of SCG is 13.6% after soluble coffee preparation (Belitz et al., 2004). Roasted coffee contains on average 3.1% (w/w) protein. The protein content in SCG is higher than in the coffee bean due to concentration of the non-extracted components during instant coffee preparation. The protein content in SCG may be overestimated due to the presence of other nitrogen-containing substances (caffeine, trigonelline, free amines and amino acids). However, many authors report similar protein contents, varying between 6.7% and 9.9% (Arya &Rao 2007). The protein profile of coffee changes during roasting, the proteins are both fragmented and polymerized, and integrated into melanoidins. Other protein components such as peptides and free amino acids constitute up to 1.5% of green coffee, whereas alkaloids (3-4%), of which trigonelline represents about 1%, are transformed during roasting (Elbl et al., 2014)

2.4.4. Crude fiber

Dietary fiber including cellulose, hemicellulose, lignin, pectic substances, gums, and mucilages is known as the edible part of plants that is resistant to digestion and absorption in the human small intestine, with complete or partial fermentation in the large intestine (Betancur et al., 2004). Dietary fiber fractions, containing soluble dietary fractions (SDF), and insoluble dietary fractions (IDF) were determined following the enzymatic-gravimetric method. Resistant starch was quantified following the gravimetric method of (Saura et al., 1993) described briefly in the study (Campos et al., 2009). Resistant starch, often considered as dietary fiber increased, (without attaining statistical significance) with roasting intensity (18.8%) from MR to DR, thereby inducing similar increase (10.7%) in their corresponding SCG. The thermal treatment (roasting) potentially induced interactions resulting in modified starch formation resisting enzymatic action and increasing TDF and their fractions (Lintas & Cappeloni, 1988).

The content of total dietary fiber (TDF) in SCG is (60.46 % w/w). Additionally, insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) are also present in higher amounts in SCG. However, SCG contains similar proportion of IDF and SDF with respect to the total fiber composition, being IDF correspondent to 84% of the TDF in SCG and SDF correspondent to 16%. The higher content of IDF than SDF in the samples is justifiable since cellulose, hemicellulose, and lignin are part of the insoluble fibers and significant amounts of these fractions are present in the composition of SCG (Borrelli et al., 2004). The SDF values in SCG reveal larger soluble fiber potential of these coffee residues when compared to other materials such as Jack bean (Carnavalia ensiformis) (6.04 %), lima bean (Phaseoluslunatus) (2.61 %), rice husk (2.23 %), wheat straw (6.48 %), and okara (10.17 %) (Kuan and Liong 2008).

It is important emphasizing that each type of fiber (insoluble and soluble) has specific properties. For instance, SDF possess large water retention, promotes the creation of bacterial flora, and decreases the absorption of fat and sugars. On the other hand, IDF has low water retention, accelerates the movement of food through the digestive system, and promotes stool regularity. SCG is byproduct with high levels of SDF and IDF; and therefore, they have great potential to be used as raw material in the development of functional foods (Borrelli et al., 2004).

Table 1**:** Chemical composition, fiber and resistant starch content of medium/dark roasted coffee beans and spent coffee grounds.

Proximate	Medium roasted		Dark roasted	
	Coffee bean	*Spent coffee grounds*	*Coffee bean*	*Spent coffee grounds*
Protein	16.5 ± 0.5	15.8 ± 0.1	15.1 ± 0.1	11.5 ± 0.4
Lipid	15.9 ± 0.0	15.1 ± 0.0	15.7 ± 0.1	15.3 ± 0.1
Carbohydrates	58.5 ± 0.5	60.3 ± 0.1	62.5 ± 0.2	68.4 ± 0.4
Ash	6.2 ± 0.1	1.8 ± 0.0	4.3 ± 0.0	1.1 ± 0.0
Total fiber	48.6 ± 0.5	57.1 ± 0.9	48.2 ± 1.6	58.6 ± 0.6
Soluble fiber	2.1 ± 0.1	1.6 ± 0.1	2.2 ± 0.2	1.5 ± 0.2
Insoluble fiber	46.5 ± 0.5	55.5 ± 0.9	45.9 ± 1.7	57.1 ± 0.7
Resistant starch	4.8 ± 0.8	5.6 ± 0.2	5.7 ± 0.0	6.2 ± 0.0

Source: (*Hernández et al., 2017)*

2.4.5. Crude ash

SCG also contains ash (1.6 %), which, according to the ICP-AES analysis, consists of several minerals. Potassium is the most abundant element, followed by phosphorus and magnesium Potassium is also the predominant mineral in coffee beans, corresponding to 40% of the oxide ash. Most minerals are easily extracted with hot water during instant coffee preparation. Total mineral (K, Mg, P, Ca, Na, Fe, Mn, and Cu) content of espresso SCG varies from 0.82 to 3.52%, confirming mineral leaching during espresso coffee preparation, although not as exhaustive as with soluble coffee. Potassium, the major mineral of espresso SCG, ranges from 3.12 to 21.88 mg/g (Mussatto et al., 2011). The industrial SCG contains lower absolute (3.55 mg/g) and relative amounts (22%) of this element. Coffee is regarded as an important source of Mg, comprising 11% of the SCG minerals, again higher than those of industrial SC (Ballesteros et al., 2011).

2.4.6. Caffeine and Total polyphenolic compounds

Phenolic compounds have received considerable attention due to their beneficial effects on human health, such as a protective action against chronic degenerative diseases (cataracts, macular degeneration, neurodegenerative diseases, and diabetes mellitus), cancer and cardiovascular diseases, and others which have been ascribed to their antioxidant activity (Acevedo et al., 2013). Three polyphenols were identified in SCG, chlorogenic acid, gallic acid and rutin. The predominance of chlorogenic and gallic acids and trace amounts of rutin in SCG has been reported. The chlorogenic acid content of SCG is equivalent to those present in 35 mL of coffee assuming that a cup of coffee (200 mL) contains 95.8 mg of chlorogenic acid. Ascorbic acid was higher than individual phenolic acid content with SCG retaining 78% of ascorbic acid present in coffee bean. (Nardini & Scaccini, 2002).

In a recent study, extracts produced from SCG exhibited anti-tumor and anti-allergic activities, which were related to the presence of phenolic compounds such as chlorogenic acid in their composition. In fact, chlorogenic acid, which is one of the most abundant phenolic compounds in SCG, has been reported to have a number of beneficial health properties related to their potent antioxidant activity as well as hepatoprotective, hypoglycemic, anti-bacterial, antiviral, anti-inflammatory and anti-carcinogenic activities. Due to these important biofunctionalities, phenolic compounds have found numerous applications in food and pharmaceutical areas (Mussatto et al., 2011)

2.4.7. Caffeine and chlorogenic acid (CGA)

Caffeine has raised many health concerns over the past decade. Caffeine 1, 3, 7-trimethyl-xanthine, a purine alkaloid, is the quintessential single most popular compound recognized in coffee and coffee products/ingredients. This alkaloid is removed from coffee beans by the decaffeinating process commonly used in the industrial scale. Although the caffeine content in coffee waste is lower than that in coffee beans, a large amount of caffeine still remains. Higher caffeine can be extracted from coffee husks than from SCG. Caffeine concentrations range from 0.734 to 41.3 μg/mg of spent coffee ground extracts, obtained by low-pressure extraction (ultrasound and

Soxhlet) and supercritical fluid CO2 extraction (SFE) varying in yield from 9 to 15% (Andrade et al., 2012).

Caffeine and CGA are the major bioactive compounds of coffee. Freeze-dried SCG showed values of 200 mg caffeine/100 g and 10 mg CGA/100 g SCG, respectively. CGA content values vary for those described by different authors. These differences might be attributed to solvent extraction, set conditions, method of quantification, brewing method and origin of SCG. However, caffeine content perfectly fit in approximate fractions (Bravo et al., 2012; Mussatto et al., 2011).

2.4.8. Maillard reaction Products

Thermal processes are frequently used in food manufacturing to obtain safe products with a prolonged shelf-life and have a strong impact on the final quality of foods. Baking, toasting, frying, roasting, sterilization result in desired and undesired effects due to various chemical reactions being Maillard reaction (MR), caramelisation and lipid oxidation the most prominent. Melanoidins are high molecular weight brown-colored compounds originating from the Maillard reaction between amino groups and reducing sugars. They may account for up to about 25% of the dry weight of roasted coffee beans, but their chemical structure remains largely unknown (Van Boekel et al., 2010).

Heating also destroys enzymes and micro-organisms and lowers the water activity of the food thereby preserving the foods. On the other hand, it is well known that some substances arising from the heating processes can play a positive role on human health. Many neo-formed compounds showing antioxidant, antimicrobial and antiallergenic effects as well as modulating activity in vitro have been detected in heated foods (Borrelli and Fogliano, 2005). Recently, two neo-formed contaminants have gained much interest because of their high toxicological potential and their wide occurrence in foods: acrylamide and 5-hydroxymethylfurfural. In particular acrylamide and HMF can be regarded as the most important heat-induced contaminants occurring in bread and bakery products (Knize et al., 1999).

Acrylamide has been added to the list of food-borne toxicants since in 2002 Swedish National Food Administration found out relevant amount of acrylamide in several heat treated, carbohydrate-rich foods such as potato chips and crisps, coffee and bread (Swedish National Food

Administration, 2002). Shortly after its discovery in foods, it has been clearly established that the major pathway for acrylamide formation in foods is Maillard reaction with free asparagine as main precursor. An Acrylamide level in roasted coffee is 253µg/kg (EFSA, 2009).

Coffee melanoidins have high antioxidant activity, which is due, at least in part, to their ability to incorporate or bind none covalently CGA. Evidence to date also suggests that they can modulate bacterial growth in the colon, exert anti-inflammatory, anti-glycative effects, and more importantly, inhibit matrix metallo-proteinases, a family of endo-peptidases that are thought to play a key role in tumor growth and metastasis. Furthermore, molecular weight of the brown material linked to galactomannans is higher in the dark-roasted than in the light-roasted coffee (Nunes et al., 2006).

2.5. Mineral composition

SCG contains (1.30-2% w/w) of ash. A variety of mineral elements including potassium, calcium, magnesium, sulfur, phosphorus, iron, manganese, boron, copper, and others is present in the composition. Potassium is the most abundant mineral element in both SCG, followed by magnesium and phosphorus. The most important minerals present in SCG are considered as essential micronutrients for human health (Kuan et al., 2011).

Minerals of SCG regulate multiple metabolic and physiological functions of the human body including hormonal and enzymatic activities, electrolyte balance, and normal growth .These minerals also support vital processes such as respiration, digestion, and circulation. Thus, the micronutrients found in SCG can be used for the production of nutrient added foods (Kuan et al., 2011).

2.6. Useful biological activities

2.6.1. Overall antioxidant capacity

Chlorogenic acid (CGA) has antioxidant activity due to its cation chelation properties (Robertson and Eastwood 1981). However, other phenolics could also contribute to antioxidant activity marginally. The SCG has been reported for radical scavenging activity, oxygen radical absorbance capacity, antitumor, and anti-allergic activity (Ramirez-Coronel et al., 2004). Free radical scavenging potential of coffee by-product conserves extracted with aqueous isopropanol was tested by the DPPH method. Among the different coffee by-products, the SCG showed 61%, 66%, and 70% antioxidant activity at 100, 200, and 500 ppm. Antioxidants are believed to intercept the free radical chain of oxidation by donating hydrogen from the phenolic hydroxyl groups, thereby forming a stable end product, which does not initiate or propagate oxidation of the lipids (Chung et al., 1998).

The presence of phenolic, CGAs, and brown pigments in appreciable quantity makes these by-products a source of natural antioxidants. By-products like grape skins, citrus peel, apple pomace, onion by-products, and potato peel waste are also found rich in compounds with antioxidant activity Likewise, the recovered antioxidants from coffee by-products have shown good free radical scavenging activities and could be used as a pharmaceutical and food supplement (Vasso Oreopoulou and Russ, 2007).

Table 2**:** Overall antioxidant activities of SCG and Defatted SCG obtained by Soxhlet method.

Parameter	SCG	Defatted SCG
Polyphenol content (mg GAE/g dm)	273.34 ± 34.17	255.61 ± 32.20
DPPH method (μmol Trolox/g dm)	82.65 ± 8.81	101.63 ± 13.48
IC_{50} DPPH (mg/L)	148.40 ± 30.43	165.89 ± 6.77
Inhibition of linoleic acid oxidation (μmol Trolox/g dm)	2.12 ± 0.08	1.6 ± 0.06
Capability to inhibit β-carotene blanching (μmol Trolox/g dm)	0.0564 ± 0.0007	0.0569 ± 0.0031

Sourse : (Acevedo et al., 2013)

2.7. Utilization of spent coffee ground for bakery industries

Consumption of natural bioactive compounds such as polyphenols, carotenoids and dietary fiber offers health benefits including protection against cardiovascular diseases, cancer and other degenerative diseases. Nutritionists worldwide recommend consumption of bakery product rich in antioxidant and dietary fiber. Therefore, an increased interest of western consumers about the relationship between food processing and health benefits has risen. Ammonia caramels are the most common antioxidant colour agent used in bakery formulations, although their high sugars content. An alternative could be coffee melanoidins, which are brown colored compounds with antioxidant properties, readily available from instant coffee (Adams and Engstrom 2000).

Supplementation of biscuits with antioxidants has been a common approach to potentially extend their shelf life. Biscuits supplemented with antioxidants from green and roasted coffee were shown to slow down fat oxidation during 12 weeks (Budryn & Nebesny 2013). Supplementation with aqueous spent coffee grounds extracts did not contribute to acrylamide formation (Martinez et al., 2017). It is confirmed in literatures that SCG natural source of antioxidant insoluble dietary fiber, proteins, essential amino acids and low glycemic sugars. SCG can be blended as food ingredient in bakery industry without affecting the conventional food preparation and the final quality of the product. These bakery food blended with SCG can be favored to people with reduced energetic intake and particular requirements.

2.8. Glycemic Index

The glycemic index (GI) concept was introduced by (Jenkins et al., 1998). GI is defined as the incremental blood glucose area (0-2h) following ingestion of 50 g of available carbohydrates as a percentage of the corresponding area following an equivalent amount of carbohydrate from a standard reference product.

The GI was originally meant to be an index of the blood glucose raising potential of the available carbohydrate in foods. Monro (2002) has shown that GI does not indicate the glycemic impact of a food. He rightly points out that GI is a property of the carbohydrates in foods, not a property of foods, and also that GI is a value which is independent of the portion size of the food or the amount of carbohydrate consumed (Monro, 2003).

The terms ‘glycemic index’ and ‘glycemic response’ should also not be confused because these entities have different mathematical and statistical properties (Wolever, 1992). Theoretically, the GI adjusts glycemic response areas to each individual’s response to a reference food, thus correcting for between-subject variation. In order to test this hypothesis, we determined the glycemic responses of bread, rice and spaghetti in 12 subjects with diabetes with each subject repeating each food four times (Willett et al., 2002). The results showed that the glycemic responses (i.e. incremental AUC values) differed significantly for the different foods and also differed significantly in the different subjects. Indeed, 62% of the total variance was accounted for by variation between subjects. The exact magnitude of between-subject variation of glycemic responses depends on the homogeneity of the subjects chosen, and in this case was large because subjects were chosen to be dissimilar by including subjects with both type 1 and type 2 diabetes on a variety of different treatments (Narushima et al., 2005).

The GI of dietary carbohydrates directly or indirectly influences many physiological processes which are relevant to health and performance, including physical activity, cognitive function, appetite regulation, energy balance, body composition, maternal and fetal weight gain during pregnancy, gastrointestinal function and tooth decay (Wolever et al., 2006).

A large body of evidence suggests that low-GI foods may be beneficial for the prevention or treatment of a number of chronic diseases including diabetes, cardiovascular disease (CHD and stroke) and cancer. Five prospective studies have examined the effect of diet GI on the risk of type 2 diabetes (Salmero´n et al., 1997; Stevens et al., 2002; Hodge et al., 2004; Schulze et al., 2004). In four of these studies, a high-GI diet was associated with statistically significantly greater risk of developing type 2 diabetes, after adjusting for confounding variables.

2.9. Glycemic load

The GL was originally defined as the sum, for all foods in the diet, of the products of the carbohydrate content per serving of each food in the diet times the average number of servings of that food per day times its GI. The resulting GL variable was adjusted for total energy using the residuals method (Salmero´n et al., 1997). On the other hand, when being applied to diets in

intervention studies, the resulting GL variable is sometimes adjusted for energy using the residuals method (Wolever and Mehling, 2002) and sometimes by dividing by 1000 kcal (Ebbeling et al., 2003). When applied to individual foods, the GL is defined as GI times grams of carbohydrate divided by 100 (Brand-Miller et al., 2003). If, for a single meal, GL is GI times grams of carbohydrate (not adjusted for energy), then the GL for the day's diet would be the sum of the GL for each meal not adjusted for energy (Bell and Sears, 2003).

2.10. Sensory analysis

Sensory evaluation methods may be divided into two broad classes: affective and analytical methods of Institute of Food Technologists Hill, (2008). Affective methods use consumer panels or trained panelists to answer questions such as the following: Which product do you prefer? Which product do you like? How well do you like this product? How often would you buy/use this product? Affective methods require a much larger panel size than do analytical methods in order to have greater confidence about the interpretation of the results. The most common analytical methods of sensory evaluation used in the wine industry are discrimination (or difference) and descriptive methods. Discrimination tests can be used to determine if products are different, if a given wine characteristic is different among samples, or if one product has more of a selected characteristic than another Bruce and Zoecklein (2016).

The hedonic rating scales are used to quantify affective dimension of the consumer perception of foods (Tuorila et al., 2009). Among the hedonic rating scales, the 9-point degree of liking scale, also called the 9-point hedonic scale, is probably the most commonly used (Lawless and Heymann, 2010). The scale was invented in the 1940s and has been carefully developed, tested and evaluated during the years (Lawless and Heymann, 2010). In the test participants/consumers are asked to give their hedonic opinion to a product sample by choosing and marking one of nine alternatives, (ranging from 1 = like extremely to 9 = dislike extremely). The 9-point hedonic scale is nowadays present in several different appearances (Lawless and Heymann, 2010). The verbally anchored scale is probably one of the most used forms (Tuorila, 2009).

CHAPTER THREE

3. Materials and methods

3.1. Location of the study area

The study was conducted at Addis Ababa University College of Natural and Computational science center of Food Science and Nutrition, Addis Ababa Ethiopia in 2018.

3.2. Sample collection

3.2.1. Wheat flour and Spent Coffee Ground

Raw coffee by-product: Spent coffee was collected from industrial soluble coffee production of the Arabica species provided by Aroma coffee (Ethiopia) Mamocacha coffee (Ethiopia) and Tomoca coffee (Ethiopia) instant coffee beverage producers and dried using oven drying at 40 ^{o}C for 48hrs until constant moisture obtained. Finally the grounded and sealed sample was stored at room temperature for preparation of the bread and intended analysis as a method mentioned by Martinez et al. (2016).

Wheat flour: Soft wheat flour (100%) labeled in the factory was purchased randomly from three different known local supermarkets in Addis Ababa and stored at room temperature until the preparation of the bread and physicochemical analysis. Additional ingredients were purchased from local supermarket.

3.1.2. Frame Work of the Experiment

The SCG with soft wheat flour blended and the newly formulated breads were cooled to room temperature and then assessed for their proximate composition, bread characteristics (loaf weight, volume, baking loss as well as sensory attributes like colour, taste, aroma, texture and general acceptability.

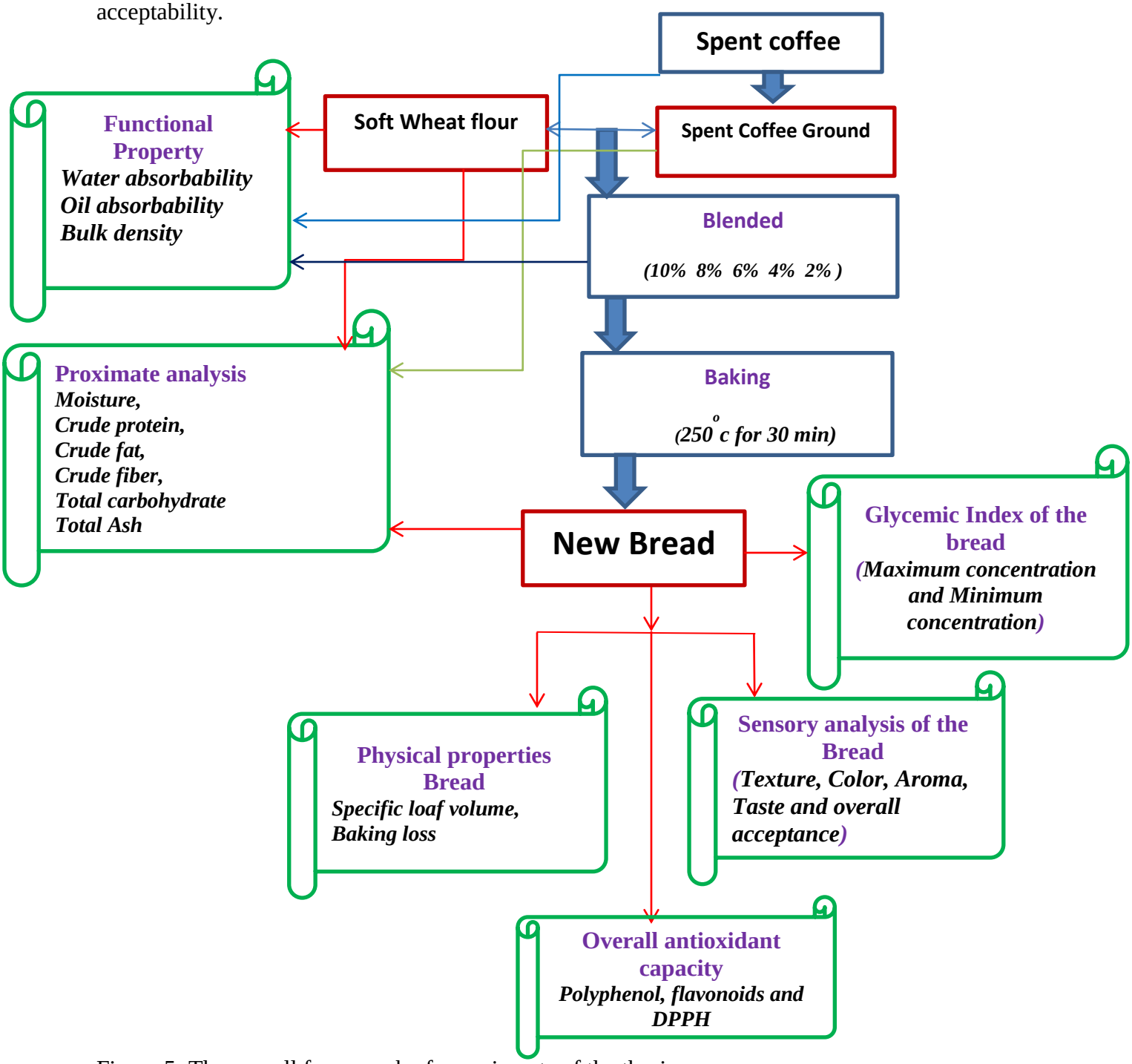

Figure 5: The overall framework of experiments of the thesis

3.2. Methods

3.2.1. Experimental Design

Completely randomized design (CRD) was used in the study and the principal factor was bread types (White Bread, SCGB 1, SCGB 2, SCGB 3, SCGB 4 and SCGB 5). The samples were analyzed for proximate composition, baking characteristics, physical property of the composite, glycemic index and sensory attributes. The effects of the principal factor on these parameters were determined.

3.2.2. Preparation of Bread

Straight dough method: a single-mix process of making bread. The wheat flour (White Bread) and composite breads Spent Coffee Ground Breads (SCGBs) were made by mixing the flour with following ingredients; 2% of salt, 4% of fat, 2% shortening, 2% of yeast and 4% of sugar in 60% of water followed by stirring using a Kenwood mixer (Model A 907 D) for 5 min to obtain a dough. The dough was allowed to ferment in a bowl covered with wet clean muslin cloth for 55 min at room temperature (~25°C). Later, the dough was punched and scaled to 140gm dough pieces. The dough pieces were proofed in a proofing cabinet for 90 min at 30°C in 85% relative humidity and baked at 250°C for 30 min. (Giami et al., 2004). The breads were cooled to room temperature and then assessed for their proximate composition, bread characteristics (loaf volume and baking loss), sensory attributes like colour, taste, aroma, texture and general acceptability, overall antioxidant activities as well as glycemic index. The dough was baked in an electrical oven at 235^{o}C for 35 mins in Furno Bakery one of known standard bakery in Addis Ababa Ethiopia.

Table 3**:** Baker's percentage of bread making (Miñarro et al., 2012)

Ingredients	Percentage	Amount by gm.
Wheat flour & SCG	100%	56.17
Water	60%	33.70
Sugar	4%	2.24
Fat	4%	2.24
Yeast	2%	1.12
Salt	2%	1.12
Bread improver	2%	1.12
Total		180gm

3.2.1. Recipe formulation

A total of five high fiber SCG bread (SCGB1, SCGB2, SCGB3, SCGB4 and SCGB5) were formulated as indicated in Table 4. SCG bread was prepared using as basic ingredients mentioned above in table 3. SCG were included as antioxidant insoluble dietary fiber (Martinez et al., 2017). The amount of SCG added to the wheat flour ranged from 2% – 10%, in order to achieve amount of fiber which accounts to quarter of the daily intake recommendation of WHO in 100g of SCG based bread, WHO (2003).

Table 4**:** Formulation of SCG and wheat flour in SCG bread

Baker's percentage converted to SCG blend						
Ingredients	SCGB1	SCGB2	SCGB3	SCGB4	SCGB5	White bread
Randomized ingredients						
Wheat flour	90% (50.55gm)	92% (51.56gm)	94% (52.59gm)	96% (53.64gm)	98% (54.71gm)	100% (56.17gm)
SCG	10% (5.61gm)	8% (4.61gm)	6% (3.58gm)	4% (2.53gm)	2% (1.46gm)	0% (0gm)
Constant ingredients						
Water	60% (33.70gm)					
Sugar	4% (2.24gm)					
Fat	4% (2.24gm)					
Yeast	4% (2.24gm)					
Shortening	2% (1.12gm)					
Salt	2% (1.12gm)					

3.2.2. Process flow chart

Bread was prepared by straight dough bread production process (mixing and kneading, bulk fermentation, molding, rounding, intermediate proofing, molding, final proofing, baking, cooling and packaging). The baking formula included wheat flour, SCG, yeast, shortening, salt, sugar and water. All ingredients were mixed in a dough mixer (model: A 907 D mixer). The dough was fermented in a bowl covered with polyethylene plastic for 30 minutes at room temperature. It was then knocked back and molded. The dough pieces were then allowed to ferment for 90 minutes in a proofing room of temperature 30^0C and relative humidity of 80 %. The fermented dough was baked in baking oven (Model: KL-2) at time-temperature combination of 30 min at 250^0C. (Miñarro et al., 2012). The experiment was conducted in Furno bakery local bread producer in Addis Ababa Ethiopia.

Figure 6: SCG based bread baking Flow chart

3.2.3. Laboratory analysis

3.2.3.1.Proximate composition analysis

Moisture content

Moisture content of the samples were determined according to Association of Official Analytical Chemistry AOAC (2000) using the official method 925.09 by oven drying method. A crucible was cleaned and dried in an oven (CHINCAN, GHG-9055A, china) at 105^{o}C for 1 hour and placed in desiccators to cool. The weight of the crucible (W1) was determined. 5gm samples (in triplicate) was weighed in the dry crucible (W2) dried at 105^{o}C for 3 hours and after cooling in desiccators to room temperature it is again weighed (W3). The moisture content was determined using Equation.

$$\textbf{Moisture content} = \frac{\mathbf{W2-W3}}{\mathbf{W2-W1}} * \mathbf{100} \quad \text{..... Equation 1}$$

Where:

W1= the weight of the crucible

W2= the weight of the crucible plus weight of the sample before dried

W3= the weight of the crucible plus weight of the sample after dried

Ash content

The ash content was determined by AOAC (2000) using the official method 923.03. Porcelain dishes were placed in a muffle furnace for 30min at 550^{o}C. The dishes were cooled in desiccators (with granular silica gel) for about 30 minutes at room temperature and weighed to the nearest milligram (W1). About 2.5g of fresh sample (in triplicate) were placed in dish and weighed (W2). Dishes were placed on a hot plate furnace under a fume-hood and the temperature was slowly increased until smoking ceases and the samples become thoroughly charred. The dishes with sample were placed inside the muffle furnace at 550 ^{o}C for 5 hours and cooled in desiccators for 1 hour. The ash in each dish was clean and white in appearance. When cooled to room temperature, each dish with ash was reweighed to the nearest milligram (W3).

$$\textbf{Total ash}\ (\%) = \frac{\textbf{W3}-\textbf{W1}}{\textbf{W2}-\textbf{W1}} * \textbf{100} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Equation 2}$$

Where:

W1= the weight of the crucible

W2= the weight of the crucible plus weight of the sample before dried

W3= the weight of the crucible plus weight of the sample after dried

Crude fiber content

Crude fiber was determined by the method of AOAC (2000), as the combustible and insoluble organic residue was obtained after the samples were subjected to acid digestion and then alkaline distillation. Clean crucible was dried in oven maintained at 105^0C for one hour and placed in desiccators to cool. One gram of each sample was measured in the dried crucible using analytical balance (W_1). Two hundred ml of 1.25%H_2SO_4 (R1) solution was added to each beaker and allowed to boil for 37 minutes. The acid later drained using vacuum pump; sample was cooled for five minutes and then washed three times using distilled water. The same step (as H_2SO_4) was followed using 1.25% NaOH solutions NaOH solution (R2) except that column was used instead of beaker. Crucibles containing residue was dried at 130^0C for two hours by drying oven cooled in desiccators and weighted (W_2).The crucibles were transferred to muffle furnace and kept for three hours at 525^0C.Crucible containing ash was later cooled in desiccators and weighted (W_3).The crude fiber content was measured using the following formula.

$$\textbf{Crude Fiber in}\ \frac{\textbf{g}}{\textbf{100g}} = \frac{\textbf{W2}-\textbf{W3}}{\textbf{W1}} * \textbf{100} \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Equation 3}$$

Where,

W2=mass of the crucible

W3=mass of the crucible and the sand

W1= weight of sample

Crude fat content

Crude fat was determined according to AOAC (2000) using the official method 4.5.01. About 2g of flour was extracted with 50 ml petroleum ether or diethyl ether for a minimum period of 4 hours in the soxhlet extractor. The solvent was then evaporated and the extracted fat was dried in the oven and cooled in a desiccator. The crude fat was determined according to the following equation.

$$\textbf{Crude fat, percent by weight} = \frac{\mathbf{W2-W1}}{\mathbf{W}} * \mathbf{100} \quad \text{..... Equation 4}$$

Where:

W1= weight of the extraction flask (g)

W2 = weight of the extraction flask plus the dried crude fat (g)

W = weight of sample (g)

Where: (W2-W1) is sample mass in g on dry base and (W3-W1) mass of ash in g.

Crude protein content

Crude protein in bread sample was determined by Kjeldahl method. Three basic steps was required in such determination, i.e., digestion, distillation and titration according to the method of AOAC 950.36 (2000).

Digestion: 0.5 g of each sample was placed in the digestion flask. Mixed catalysts such as 5 g of potassium sulphate and 1 g of Copper sulfate added into the flask. 6 ml of concentrated sulfuric acid and 3.5ml of 30% hydrogen peroxide was also placed in the flask. The contents digested in the digestion rack for 3 hours until frothing cease. The samples then kept cool before distillation.

Distillation: 50 ml of distilled water and 25 ml 40% sodium hydroxide was added to the digested samples and the flasks was connected to the distillation apparatus. The addition of sodium hydroxide converts the ammonium sulfate into ammonia gas. 25ml of distilled water and 25ml of boric acid and 5-7 drops of methyl red added to receiving flask 250ml capacity which is connected to the distiller by tube. The distillation process was terminated when the volume of the receiving flask reach between 200 to 250ml.

$(NH_4)2SO_4 + 2NaOH \longrightarrow 2NH_3 + 2H_2O + NaSO_4$

The ammonia gas formed liberates from the solution and move out of the digestion flask into the receiving flask which contain excess of boric acid.

$NH3 + H_3BO_3$ (boric acid) $\longrightarrow NH_4^+ + H_2BO_2^-$ (borate ion)

Titration: The nitrogen content was estimated by titration of the ammonium borate formed with 0.1NHcl.

$H_2BO_3^- + H^+ \longrightarrow H_3BO_3$

Moles of Hcl = Moles of NH_3 = moles of N in the sample

$$\textbf{Nitrogen}\ (\%) = \frac{\textbf{VHcl for sample} - \textbf{VHcl for blank} * \textbf{NHcl}}{\textbf{Wo}} * \mathbf{14} * \mathbf{100} \text{.....Equation 5}$$

$$\text{Protein}(\%) = 6.25 * \%\ \text{Nitrogen}$$

Determination of Carbohydrates

The available carbohydrate content of SCG flour samples were determined by difference that is by subtracting the sum percentage of crude protein, crude fat, crude ash and crude fiber from 100.

% Available carbohydrates = 100 - [%Moisture +%Fat +%Protein +%Ash +%Dietary fiber].....Equation 6

Gross energy determination in kilo calories

The gross energy of each samples were estimated (in Kcal/g) by multiplying the percentage of crude protein, crude fat and total carbohydrate with recommended factors.

Total energy (Kcal/100g) = (4*crude protein+9*crude fat+4*carbohydrate).....Equation 7

Mineral determination

Acid digestion procedure (Wet ashing method)

The dried selected SCG based samples of air dried in the laboratory were removed and mixed uniformly by coning, pulverized and quartering. The samples were sieved through a 2 mm sieve to remove coarse particles. 1g of sieved of the SCG samples were weighed out into 100ml conical flask. The samples were digested by the addition of 20ml of aquaregia (mixture of HCl and HNO_3, ratio 3:1) and 10ml of 30% H_2O_2. The H_2O_2 was added in small portions to avoid any possible overflow leading to loss of material from the conical flask. The analyt was digesting for 2hr in 100ml conical flask covered with a watch glass, and reflex over a hot plate at 90°C for 2 hours. The conical flask wall and watch glass was washed with distilled water and the sample was filtered out to separate the insoluble solid from the supernatant liquid. The volume was adjusted to 100ml with distilled water. Blank solution was handled as detailed for the samples.

The absorbance of the samples was measured using flame atomic absorption spectrophotometer by aspirating de-ionized water. Sample blank solution was run with the sample solution. The concentrations of the samples were calculated from the absorbance values of each samples using Beer-Lambert Law plot.

$$\textbf{Metal content}\left(\frac{\textbf{mg}}{\textbf{100g}}\right) = \frac{(\textbf{MC})*(\textbf{V})}{(\textbf{W})} * \textbf{DF} * \textbf{100} \quad \text{.....Equation 8}$$

Where,

DF= Dilution factor.

MC=Mineral concentration found in mg/L

V=volume of volumetric flask in L

W=Sample weight in g

3.2.3.2.Functional property of the raw materials

A. Bulk density (g.cm-3)

Bulk density was measured using an approximately 10ml volume cylinder. The container was filled with raw/sample material, and then the material will slightly compacted to ensure absence of large void space. The bulk density then calculated by dividing the weight of the material by the volume of material in the cylinder.

$$\boldsymbol{d = \frac{m}{v}}$$ ……..Equation 9

Where: d is the bulk density

m is mass of the sample

v is volume of the sample compacted and measured

B. Water absorption Index (WAI)

Water absorption Index (WAI) was determined with the method reported by (Sosulski and Wu, 1988). 10 ml of distilled water is added to a sample of 1g flour (W1) in a weighed centrifuge tube (W2) and stirred six times for 1 min at 10 min intervals. The mixtures were centrifuged at 3000 rpm for 25 min and the clear supernatant was decanted and discarded. Pellets were dried at 105^{o}C for 60min. The adhering drops of water were removed and then reweighed (W3). The amount of water retained in the sample was recorded as weight gained and was taken as water absorbed. Water absorption index was expressed as the weight of water bound by 100 g dried flour.

The calculation of water absorption capacity is calculated as follows:

$$\mathbf{WAI} = \boldsymbol{W3} - \frac{\boldsymbol{W1+W2}}{\boldsymbol{W1}}\boldsymbol{X}\ \mathbf{100}$$…….. Equation 10

C. Oil absorption Index (OAI)

The method used by (Sathe and Salunkhe, 1982) was used for oil absorption index. 10 ml (V_1) of refined sunflower oil with density of 0.99 mg/ml was added to one gram of flour in a 25 ml centrifuge tube. The content of the centrifuge tube was stirred for 2 min and then centrifuged at 4000 rpm for 20 min. The amount of oil separated as supernatant will be decanted and measured

using 10 ml cylinder (V_2). The difference in volume was taken as the oil absorbed by the sample. Oil absorption index was expressed as ml of oil bound by 100 g dried flour.

$$\mathbf{OAI} = \frac{\boldsymbol{V1\ X\ density\ of\ oil}}{\boldsymbol{Weight\ of\ sample}}\ \boldsymbol{X}\ \mathbf{100}$$.....Equation 11

Where,

V1= volume of the oil

3.2.3.3.Antioxidant property

a. Analysis Total phenol content

The total phenol content (TPC) was determined by spectrophotometer, by methanol extraction preferred by (Angkawijaya et al., 2014) using Gallic acid as a standard, according the method described by (Singleton and Rossi 1965). Briefly, 0.2 mL of the diluted sample extract transferred in tubes containing 1.0 mL of a 1/10 dilution of Folin-Ciocalteu's reagent in water. After waiting for 10 minutes, 0.8 mL of a sodium carbonate solution (7.5% w/v) was added to the sample. The tubes are then allowed to stand at room temperature for 30 min before absorbance at 765nm. The TPC was expressed as Gallic acid equivalents (GAE) in mg/100 mL of fruit juice. The concentration of polyphenols in samples was derived from a standard curve of Gallic acid ranging from 0.2 to 4 mg/L.

b. Total Flavonoids

Flavonoids in SCG extracts were estimated using the colorimetric assay previously described by (Chang et al., 2012) with some modification. A volume of 30 µl of each filtered extract was added in a 96-well microplate and subsequently, a sequential addition of 90 µl methanol, 6 µl aluminum chloride at 10% (w/v), 6 µl potassium acetate (1 mol/l), and 170 µl distilled water to each extract sample was performed. Samples were maintained during 30 min in the dark at room temperature. The absorbance of the mixture was then measured at 415 nm against a blank of distilled water. A calibration curve was prepared with a standard solution of Quercetin (50, 100, 150, 200,250 mg/l).

The content of total flavonoids was expressed as milligram Quercetin equivalent per dry weight material (mg QE/g).

c. Free radicals scavenging activities

DPPH assay

The radical scavenging ability of SCG and the bread was tested on the basis of the radical scavenging effect on the DPPH free radical. The DPPH molecule is a stable free radical which has a deep-violet color, characterized by absorption at 515–520 nm.

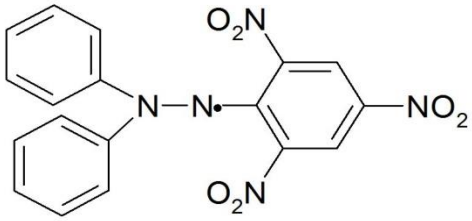

Figure 7**:** Chemical structure of 2, 2-diphenyl-1-picrylhydrazyl
Source: (Kiers et al., 1976).

The DPPH radical-scavenging assay is based on the reduction of DPPH when mixed with an antioxidant such as polyphenol, which leads to loss of its violet color and a reduction in its absorption at 520 nm (Molyneux, 2004). The effect of methanolic extracts on DPPH radical was estimated according to (Kirby and Schmidt, 1997). A 0.004% solution was mixed with 1ml of various concentrations (0.25-7.5mg/mL) of extracts in methanol. Finally, the samples were incubated for 30min in the dark at room temperature. Scavenging capacity was read spectrophotometrically by monitoring the decrease in the absorbance at 517nm.This absorption maximum was first verified by scanning freshly prepared DPPH from 200-800nm using the scan mode of spectrophotometer. Ascorbic Acid was used as standard and mixture without extract was used as the control. The extract concentration providing 50% of radicals scavenging activities (IC_{50}) was calculated RSA percentage against extract concentration (Barros & Baptita 2007).

Inhibition of free radical DPPH in percent (IC_{50}%) was then calculated as:

$$\boldsymbol{Radical\ scavenging\ activity = Ao - \frac{A1}{Ao} X100\%} \quad \text{.....Equation 12}$$

Where,

A_o = Absorbance of control

A_1 = Absorbance of test sample

3.2.3.4.Glycemic Index Testing

Glycemic index (GI) study was conducted using internationally recognized GI methodology and method used by Arvidsson-Lenner, et al., (2004) which has been validated by small experimental studies and large multicenter research trials. The experimental procedures used in this study were according with international standards for conducting ethical research with humans and was approved by Ethics Review Committee of Addis Ababa University. The study was carried out after getting permission from the institutional review board (IRB) of Addis Ababa University College of Natural and Computational Sciences, (See Annex VI).

I. Study Participants (Subjects)

A group of 10 healthy, non-smoking, people aged between 18-45 years was recruited from the staff and student population of the Addis Ababa University Center for Food Science and Nutrition. People who were volunteer to participate in the study was excluded if they were overweight, dieting, had a family history of diabetes, were suffering from any illness or food allergy, or were regularly taking any medication. During data collection the participants were well inform about the purpose of the study, procedure of collection and assurance on confidentiality and the participation was fully decided by the participants. Any unwilling individuals were excluded. All participants were given a written consent before taking part in the study, (See ANNEX II).

II. Test Foods

Control Bread (White bread) with pure water used as the standard reference food and was consumed by each study participant on three different occasions. Each of the types of SCG blended bread was consumed by each study participant on one occasion only. The reference food and the two breads were all served in amounts containing 50 grams of available (digestible) carbohydrate.

For each study participant, the reference food prepared by the day before and consumed. The study participants were consumed the entire portion of the food, with pure water and within 5 minuts in there confortable pace.

III. Blood sample collection

Subjects were asked to be on fasting 8 hours prior consuming the samples. Once consuming the bread, capillary blood from fingertip was collected at each fifteen minutes and analyzed by glucometer for rest of two hours.

IV. Calculation of glycemic index (GI)

The GI was calculated by the method of (Jenkins et al., 1981).The values of blood glucose were plotted against time. For each person, the area under his 2hrs blood glucose response (glucose AUC) and for the food eaten (50gm of available carbohydrate) was then being measured. The glycemic index value for the test food was then being calculated for each person by dividing their glucose AUC for the test food by their glucose AUC for the reference food. The final glycemic index value for the test food was the mean glycemic index value for the 10 people. However, this is because the reduction in between-subject variation reduces total variation when expressed as coefficient of variation $CV = 100\,X\frac{SD}{mean}$ expressing results as GI reduces between-subject variation without changing variation between foods or variation within subjects. GI was calculated by the formula:

$$\boldsymbol{GI = \frac{AUC\ of\ the\ Test\ Food}{AUC\ of\ referenceGlucose}\ X\ 100} \quad \text{……..Equation 13}$$

V. **Calculation of glycemic load (GL)**

GL refines the concept of GI to quantify the impact that a carbohydrate containing meal or a single food eaten in a normal portion has on blood sugar. The GL was calculated as the GI (%) multiplied by the grams of carbohydrate in the serving of food eaten. The GL for a meal would be the sum total of the GL of each food that is part of the mean GL was calculated using the formula stated by Farukh Tabassum1 et al., (2013).

$$\boldsymbol{GL = Net\ carbohydrate\ in\ a\ typical\ serving\ X\ \frac{GI}{100}} \quad \text{……Equation 14}$$

3.2.3.5.Sensory analysis

Overall acceptance of breads sensory acceptability for texture, color, taste, aroma and overall acceptance were done as of (Lim, 2011). The bread samples were homogenously sliced and served with water in identical containers for twenty semi trained panelists in normal physiologic condition and non-smoking were selected from students and staff of the Center of Food Science and Nutrition, Addis Ababa University Ethiopia, to perform the evaluation using a hedonic scale of 9 points (Like extremely, Like very much, Like moderately, like slightly, Neither like nor dislike, Dislike slightly, Dislike moderately, Dislike very much or Dislike extremely), (See ANNEX I).

3.2.3.6.Physical Properties of Bread

a. Specific Volume

Specific volume was calculated as volume to mass ratio (cm^3/g) (Okafor et al., 2012). The loaf weight was measured using a laboratory scale and Loaf volume was measured 50 minutes after loaves were removed from the oven by using the rapeseed displacement method as modified by Hegazy and Faheid (1990). A box of fixed dimensions (24.00 x 15.70 x 18.95 cm) of internal volume 7140 cm^3 was put in a tray, half filled with rapeseed, shaken vigorously 4 times, then filled till slightly overfilled, so that overspill fell into the tray. The box was shaken again twice, and then a straight edge (or rule) was used to press across the top of the box once to give a level surface.

The rapeseed were decanted from the box into a receptacle and measured by using measuring cylinder. The procedure was repeated three times and the mean value for rapeseed weight was noted (B g). A weighed loaf was placed in the box and weighed (850 g) were used to fill the box and leveled off as before. The overspill was weighed and from the weight obtained the weight of dough around the loaf and volume of seed displaced by the loaf were calculated by the following equations.

Dough displaced by loaf (L) = B g + overspill weight – 4500 g

$$\textbf{Loaf Volume}(\textbf{LV}) = \frac{\textbf{LX7140}}{\textbf{B}}\ \textbf{cm3} \quad \text{…….…} \text{ Equation 15}$$

Specific Loaf Volume = Loaf Volume / Loaf Weight (cm^3/g)

b. Baking Loss

The baking loss is done according to (Kotoki & Deka 2010) and is obtained by subtracting loaf weight (g) from initial dough weight (140.00g).

Baking Loss = Dough Weight (g) - Loaf Weight (g)……..Equation 16

3.3. Data Analysis

Statistical analyses were performed using SPSS (version 20.0) software. Data were expressed as the mean value ± standard deviation. Analysis of variance (ANOVA) and the Fisher post hoc test were applied to determine differences between means. Differences were considered significant at $p < 0.05$.

CHAPTER FOUR

4. Result and Discussion

4.1. Physico-chemical Characteristics and Functional Properties of SCG and Wheat Flour

Spent coffee ground (SCG) is the main residue of the coffee industry that contains large amounts of organic compounds. SCG obtained from instant coffee producers in Addis Ababa, Ethiopia was analyzed for physicochemical property.

The laboratory analysis balanced towards now day's consumers concern for caloric content and glycemic index (GI) of the food as well as balanced nutrition (Mussato et al., 2011). Widely consumed bakery products like white bread have lowest fiber and nutritional content except clear calorie in it which derives the need for research in improving the nutritional quality. Therefore, the present study investigated the formulation of SCG with the traditional bread ingredients and evaluated physico-chemical compositions, antioxidant property, sensory attributes of the new formulations. The results are discussed below.

Spent Coffee (SC) was collected from Aroma, Mamocacha and Tomoca cafeterias in Addis Ababa. The moisture content of the fresh SC ranged between (46-48)%. Slightly higher moisture was observed for Tomoca coffee which depends on the amount of water used in brewing, while the lowest value was for SCG obtained from Aroma Coffee. The SC was oven dried at 60 ^{o}C for 48 hours until constant weight of 20% moisture obtained. The moisture content of the bread was analyzed 30 minutes after baking. There is no significant difference in moisture content between the blends and the control bread (Table 5). After drying the sample was grinded and sieved through a 2 mm sieve to remove coarse particles as well as to obtain the size equivalent to wheat flour.

Table 5: Proximate composition of SCG, wheat flour and bread formulations

Bread formulations	Moisture (g/100g)	Protein (g/100g)	Ash (g/100g)	Fat (g/100g)	Fiber (g/100g)	Total CHO (g/100g)	Energy (Kcal)
WB	37.97 ± 3.60^{b}	7.39 ± 0.20^{e}	2.75 ± 0.14^{a}	11.18 ± 0.32^{a}	1.56± 0.15^{b}	76.92 ± 0.51^{b}	426.68 ± 1.69^{a}
SCGB1	32.74 ± 0.69^{bc}	10.68 ± 0.02^{b}	3.00 ± 1.15^{a}	10.04 ± 0.00^{b}	5.31 ± 0.14^{e}	70.47 ± 0.94^{c}	404.92 ± 1.73^{b}
SCGB2	29.59 ± 2.58^{c}	10.05± 0.04^{bc}	2.25 ± 0.14^{b}	11.19 ± 0.36^{a}	4.97 ± 0.28^{d}	71.54± 0.23^{c}	415.88 ± 3.07^{b}
SCGB3	36.47 ± 2.98^{b}	9.54 ± 0.16^{c}	2.00 ± 0.29^{b}	11.47 ± 0.01^{a}	4.13 ± 0.09^{cd}	72.86 ± 0.13^{c}	421.36 ± 2.04^{a}
SCGB4	37.46 ± 0.15^{b}	8.23 ± 0.09^{d}	2.25 ± 0.14^{b}	11.61 ± 0.03^{a}	3.28 ± 1.04^{c}	74.63 ± 1.08^{b}	424.32 ± 2.55^{a}
SCGB5	34.20 ± 0.54^{bc}	7.79 ± 0.05^{de}	2.50 ± 0.00^{b}	10.74 ± 0.50^{ab}	2.51 ± 0.97^{b}	76.46 ± 0.62^{b}	422.92 ± 2.87^{a}
SCG	20.27 ± 1.25^{a}	11.38 ± 0.50^{b}	2.00 ± 0.29^{b}	10.30 ± 0.12^{b}	42.18 ± 1.20^{a}	34.14 ± 0.35 ^{d}	264.48 ± 1.84^{d}
Wheat Flour	11.70 ± 2.16^{d}	13.80 ± 0.24^{a}	0.89 ± 0.48^{c}	1.80 ± 0.21^{c}	1.60 ± 0.35^{b}	81.91± 0.45^{a}	397.24 ± 3.31^{c}

Results were expressed as Mean ± standard deviation, n=3. Means in the same column with the same letter are not significantly different at $p < 0.05$ as assessed by Post Hoc multiple comparison tests. WB=bread without spent coffee ground SCGB1, 2, 3, 4, 5= SCG based bread with 10%, 8%, 6%, 4%, 2% Spent Coffee Ground, SCGB= SCG Spent Coffeeground

a) Proximate composition

Protein content was variable among composites and ranged from 7.79 ± 0.05 up to 10.68 ± 0.02g/100g. The bread with 10% SCG (maximum amount) has the highest protein content, which might be as a result of higher protein content in SCG (Table 5).White bread from 100% wheat has the lowest the protein content. As the amount of SCG blending increased the protein content increased also. Thus, with 10 and 8% blending of SCG, significant protein increment was found compared with the wheat bread at $P < 0.05$ (Table 6).

The fat content for the various bread formulations was ranged between 10.74 ± 0.50 and 10.04 ± 0.00 g/100g, which is similar with the values reported by (Cruz et al., 2012). However, the fat content of SCG was affected by coffee brewing and the amount of oil used as a beker's percentage (Jenkins et al., 2014). Due to the reason, the mentioned value of fat on table 6 might not be dependent on SCG.

Ash gives an indication of inorganic elements that are present in food as minerals. The ash content in the SCGB1 was significantly higher than the other formulations ($p < 0.05$) (Table 5). This might suggests that SCG can provide better content of inorganic elements than the conventional wheat flour. The result had similar as amount of ass reported by Oliveira et al., (2006).

The benefits of high dietary fiber diets extend beyond weight loss and have favorable effects on obesity related diseases. (Rathinavelu and Graziosi, 2005). Dietary fiber was the main constituent of SCGs (42.18%). Similar values were reported by Vardon et al., (2013). Thus, among the formulations SCGB1 (maximum of 10% SCG) had the highest fiber amount (5.31%). Meanwhile, the lowest value was found with WB (1.56%). In fact, in this study the maximum incorporation of 10% SCG in the formulations was set based on WHO's dietary fiber guideline Williams et al., (1995) to achieve quarter of recommended daily intake of IDF which is up to 30mg day. Accordingly, in this study, the fiber content of all SCG based breads significantly increased compared with the white bread. Martínez et al., (2017) also reported a similar finding on SCG based biscuit formulations.

The total carbohydrate contents of SCGB4 and the SCGB5 were significantly higher than all the other formulations except that of white bread at $p < 0.05$ (Table 6). Hence, the newly formulated breads can be a good source of energy.

Minerals contents

Magnesium, Potassium, Iron and Calcium

SCG had potassium content between 40.21 ± 1.27 to 47.12 ± 1.00 mg/100g. With 2-10 % SCG formulated breads the magnesium content ranged between17.52 ± 1.02 to 17.52 ± 1.02mg/100 g, also significantly different from the control ($p < 0.05$) table 6. The iron content was between7.74 ± 0.21 to 32.79 ± 1.00 mg/100 g which is significantly different from the control (Table 7). This is similar with the result reported by (Mussatto et al., 2011).

Potassium is the most abundant mineral element in both SCG and new bread followed by magnesium and Iron. The most important minerals present in SCG are considered as essential micronutrients for human health (Kuan et al., 2011). So the minerals identified in the new bread can be a good source of health ingredient.

Table 6**:** Iron, Calcium, Magnesium and Potassium content (mg/l00g) of the SCG based bread

Samples	Ca (mg/100g)	Mg (mg/100g)	Fe (mg/100g)	K (mg/100g)
WB	16.06 ± 0.01^{a}	9.50 ± 0.51^{c}	7.38 ± 0.27^{c}	36.19 ± 1.30^{c}
SCGB1	11.24 ± 0.13^{c}	17.52 ± 1.02^{b}	32.79 ± 1.00^{a}	47.12 ± 1.00^{b}
SCGB2	12.64 ± 0.34^{c}	15.95 ± 0.67^{b}	26.71 ± 1.03^{b}	46.29 ± 1.08^{b}
SCGB3	13.03 ± 1.01^{c}	14.89 ± 0.30^{b}	9.24 ± 0.13^{c}	45.95 ± 1.04^{b}
SCGB4	14.05 ± 0.12^{b}	10.73 ± 0.12^{c}	9.19 ± 0.31^{c}	42.65 ± 1.17^{c}
SCGB5	14.75 ± 0.42^{b}	9.58 ± 0.63^{c}	7.74 ± 0.21^{c}	40.21 ± 1.27^{c}
SCG	12.27 ± 1.30^{c}	20.53 ± 0.41^{a}	36.05 ± 1.07^{a}	51.19 ± 0.96^{a}

Results were expressed as Mean ± standard deviation, n=3. Means in the same column with the same letter are not significantly different at $p < 0.05$ as assessed by Post Hoc multiple comparison tests. WB=bread without spent coffee ground SCGB1, 2, 3, 4, 5= SCG based bread with 10%, 8%, 6%, 4%, 2% Spent Coffee Ground, SCGB= SCG Spent Coffee Ground Bread.

4.2. Flour characteristics and Functional properties of the composite

Bulk density

The bulk density of wheat flour (WF) and SCG is 0.83g/cm^3 and the composite flour is significantly higher which is 0.91g/cm^3 $p<0.05$ (table 7). As reported by Ryu & Walker 1995, a high bulk density is very important in packaging and transportation, and is desirable as it can significantly reduce costs. The bulk density is influenced by a range of factors. These include the amount of air entrapped in the powder particles (occluded air), the overall density of the particle (determined by the composition),the air between the individual powder particles (interstitial air), the particle size distribution and the particle shape. Hence, the effect bulk density of SCG, wheat flour and composite for the new product was analyzed as composite flour with 10% SCG and 90% wheat flour as well as individual samples (i.e. wheat flour and SCG).

The bulk density of the composite flours with SCG and wheat flour was significantly different ($p < 0.05$) than only Wheat flour. The composite flour has the highest bulk density (Table 7). Higher bulk density is desirable for greater ease of dispersibility of flours (Appiah et al., 2011). Therefore, blending wheat with SCG will improve the bulk density of the flour, which is very important for the bakery industry.

Table 7**:** Bulk density, Water absorption index (WAI) and Oil Absorption index (OAI) of SCG based composite flours

Composite sample	BD (g/cm3)	WAI (g/100g)	OAI (g/100g)
WF	$0.83 + 0.00^{b}$	7.23 ± 0.03^{a}	3.28 ± 0.28^{c}
SCG	$0.83 + 0.00^{b}$	3.04 ± 0.25^{b}	4.85 ± 0.41^{b}
Composite flour(CF)	$0.91 + 0.01^{a}$	3.13 ± 0.52^{b}	6.33 ± 0.03^{a}

Results were expressed as Mean ± standard deviation, n=3. Means in the same column with the same letter are not significantly different at $p < 0.05$ as assessed by Post Hoc multiple comparison tests. BD=bulk density WAI= water absorption Index OAI= Oil absorption Index WF=Wheat flour without spent coffee ground CF= composite flour (SCG 10% wheat flour 90% composite), SCG= 100% spent coffee ground.

Water absorption index (WAI)

Composite flour of SCG and wheat flour (CF) had the minimum WAI 3.13 ± 0.52 g/100g, while wheat flour has the maximum 7.23 ± 0.03 g/100g (Table 7).Which is similar to Codex standard for wheat flour. (Codex stan,1995). The increase in the water absorption index has always been associated with increase in the amylose leaching and solubility, and loss of starch crystalline structure (Butt and Batool, 2010).

Oil absorbability index (OAI)

The maximum oil absorption obtained for composite flour (CF) was 6.33 ± 0.03 g/100g, while the minimum oil absorption index was obtained for wheat flour (control) 3.28 ± 0.28 g/100g (Table 7).

As it is reported by (Suresh and Samsher 2013)**,** the oil binding capacity of food protein depends upon the intrinsic factors like amino acid composition, protein conformation and surface polarity or hydrophobicity. Composite flour having highest OAI could have the availability of the proteins to bind oil. Which make the new bread useful in food system where optimum oil absorption is desired (Doxasta et al., 2002). The OAI also makes the flour suitable in facilitating enhancement in flavor and mouth feel when used in food preparation.

4.3. Physical property of the formulated bread

Loaf Volume of Bread

The lowest SCG incorporation (SCGB5) the highest specific loaf volume was found in Figure 7. In contrast, with the highest SCG incorporation the reverse was obtained. This might be attributed from SCG's high WAC which resulted from fiber matrix (Tokimoto et al., 2005).

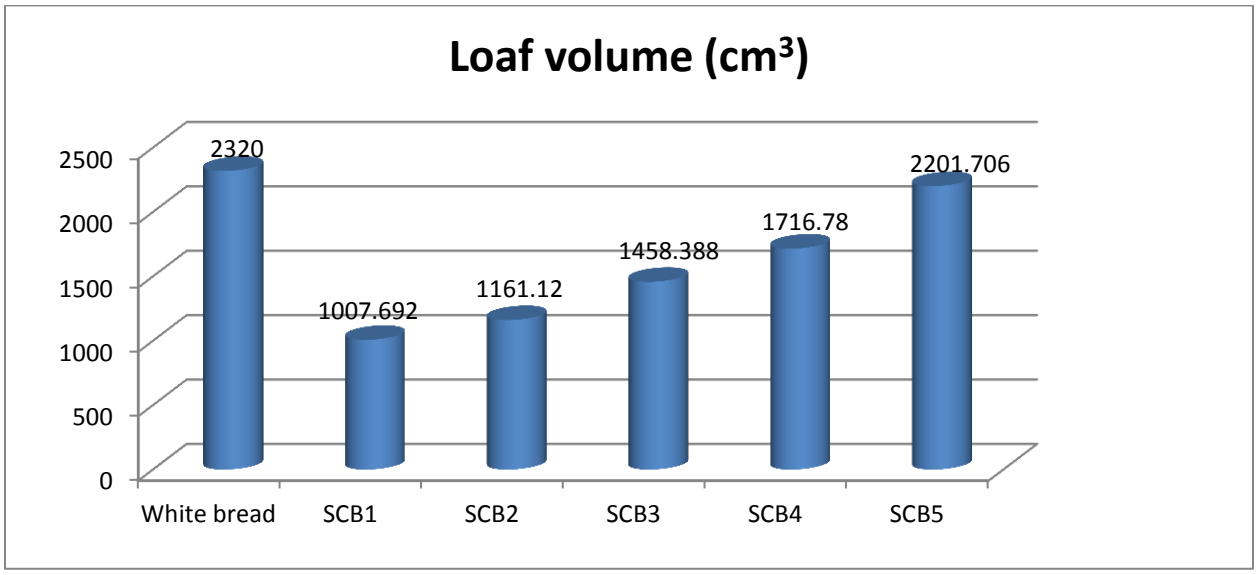

Figure 8**:** The effect of SCG on specific loaf volume of bread

Baking loss

Bread baked at highest SCG combinations lost less water (9.29 %), as a result the bread become heavier in weight. This might be due to high water retention capacity of fiber, which is the major composition of SCG (Borrelli et al., 2004). The bread baked with rest of SCG combinations loss more water (10.71-10.72%) figure 9.

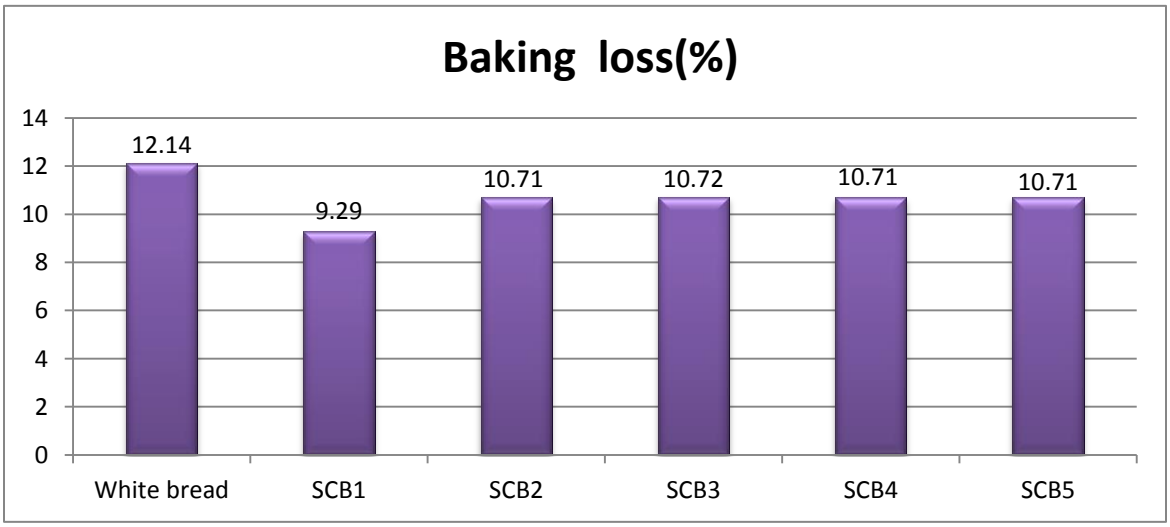

Figure 9**:** Baking loss of SCG based bread formulation

4.4. Overall Antioxidant Property

3.2.4. Total phenolic and Flavonoid content

The level of phenolic compounds in methanol extracts of the SCG, control bread and SCG based bread formulations are shown in Table 8. SCG has the highest total phenolic content. The methanolic extract of the SCG had high amounts of TPC and TFC All the bread formulations with SCG had higher TPC and TFC as compared with the 100% wheat based bread ($p < 0.05$) (Table 8). Apparently, this is resulted from the high TPC and TFC contents of SCG. Martinez et al. (2017) also reported similar result from SCG. The high TPC and TFC contents in the formulated bread products supported one of the basic hypotheses of this study. Phenolic compounds play vital roles in human health (Rathinavelu and Graziosi, 2005).

Table 8**:** Values for total phenolic content, total flavonoid content, and antioxidant activities of SCG and its composite breads (n=3)

Bread formulation	**Total Phenolic content (TPC)** **(µg GAE g^{-1})**	**Total Flavonoid content (TFC)** **(µg QE g^{-1})**	**DPPH scavenging activity** **(IC_{50}µ/mL^{-1})**
SCG	2768.53 ± 5.28^{a}	2409.24 ± 30.76^{a}	3.30
WB	383.69 ± 21.39^{g}	1021.51 ± 23.33^{f}	5.36
SCGB1	1391.20 ± 6.31^{b}	2338.09 ± 43.15^{b}	4.01
SCGB2	1015.16 ± 13.59^{c}	2253.54 ± 20.86^{c}	4.45
SCGB3	871.21 ± 21.92^{d}	1727.82 ± 36.46^{d}	4.69
SCGB4	585.12 ± 44.94^{e}	1227.78 ± 12.83^{e}	4.87
SCGB5	476.92 ± 4.66^{f}	1154.43 ± 9.68^{e}	6.17

Means in the same column followed by different letters are significantly different ($P < 0.05$). WB=bread without spent coffee ground SCGB1, 2, 3, 4and 5= SCG based bread with 10, 8, 6, 4 and 2% Spent Coffee, SCG=Spent coffee ground. TPC = Total Phenolic Content, TFC: T Flavonoid Content

3.2.5. DPPH scavenging activity

The IC_{50} of SCG, White bread (WB), SCGB1, SCGB2, SCGB3, SCGB4 and SCBG5 was 3.30, 5.36, 4.01, 4.45, 8.99, 4.87 and 6.17 mg/mL respectively. As compared with positive control ascorbic acid (IC_{50}= 1.99), SCG1 was the most potent of all, that could scavenge most free radical as shown in figure 9. In contrast, SCG 5 had the highest IC_{50}, (i.e. the least potent).

DPPH scavenging method has been widely used in antioxidant activity studies (Pinelo et al., 2004). In fact, this free radical scavenging method is based on the concept that the reduction of alcoholic DPPH solutions in the presence of hydrogen donating antioxidant (Koleva et al., 2002). IC50 was calculated formulas per the method by Tomoko et al. (2014); with two points enclosing 50% inhibition was selected and the regression line Y=AX + B was drawn and X value was calculated when the value of Y is substituted by 50.

Both SCG and SCG based bread formulations had the highest potential of scavenging free radical DPPH as compared to control white bread. This was because of the high amount of antioxidants and polyphenolic compounds in SCG (Table 9). SCG passes through high heat treatment with the formation of millard reaction products (MRPs). Controlled MRPs have health benefits including anti-oxidation property. Martinez et al. (2016) also reported high antioxidant capacity of SCG enriched biscuit products. As shown in Figure 10, the DPPH scavenging activity of the SCG incorporated bread is higher than the white bread increasing with amount of SCG. Therefore, the incorporation of SCG in the bread formulations is important to improve the antioxidation property of bakery products. This result was obtained after baking with considerably high temperature. Antioxidant compounds are bulky and heat sensitive; thus in the future it would be logical to optimize the baking conditions for the maximum retention of anti-oxidant compounds. It can be concluded that SCG based bread had higher radical scavengers than those of clear calorie breads.

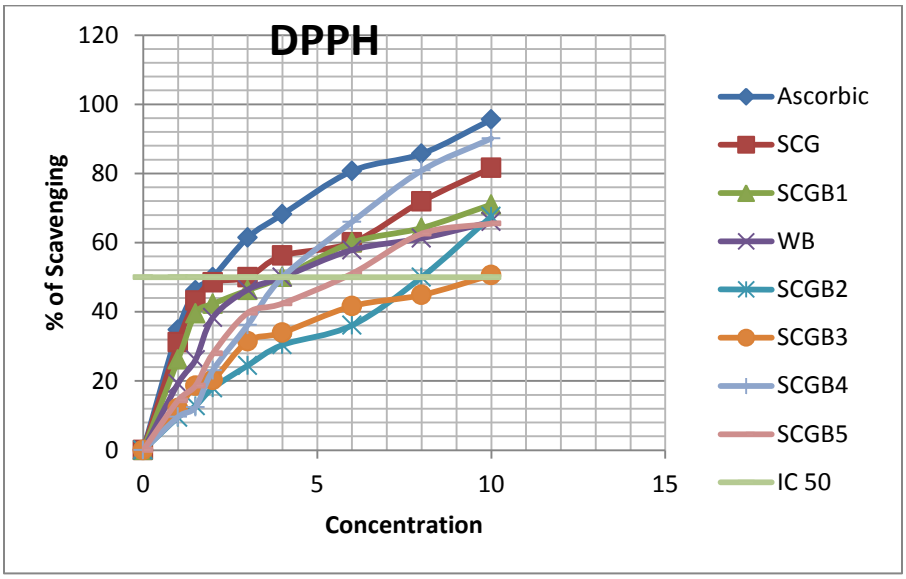

Figure 10**:** DPPH scavenging activity

4.5 Glycemic index

Mean values of the GI for standard glucose, maximum concentration of SCG1 (10%) and minimum concentration of SCG5 (2%) was determined in a total of 210 tests in the whole group of 10 volunteers. There was a significant difference when comparing samples vs. standard glucose ($p = 0.05$) and SCG1 vs. SCG5 ($p < 0.05$) the glycemic index chart could be seen (Figure 11). In addition the GI of white bread is higher than both spent coffee ground based bread. Table 9 The AUC of the sample with highest amount of SCG had less this is due to the complex chemical composition and high dietary fiber content of SCG. Dietary fiber acts on intermediate metabolism by slowing the absorption rate of glucose and fat from the small intestine; moreover, it ferments in the gut and produces short-chain fatty acids than can contribute to the modulation of glucose and lipid metabolism in the liver. (Jenkins et al., 1983)

Figure 11**:** Capillary blood sample collection using lancet.

The glycemic response to the same food or meal may be influenced by the time, composition and GI of a previous meal. A prolonged glucose response after a breakfast meal has been demonstrated to improve glucose tolerance at lunch (Arvidsson et al., 2004).

Table 9**:** Glycemic load, Glycemic index GI and GI variation between individuals of SCG based bread and standard glucose in 10 volunteers (7 men, 3 women).

Bread type	AUC	GI	GL(g)	CV
Standard glucose	12.48	100	50	-
White bread	12.30	98.55 +13.56[a]	49.27	13.75%
SCGB5	10.63	85.57 ± 12.06[b]	42.78	13.13%
SCGB1	9.82	78.68 ± 8.84[c]	39.34	10.47%

Glycemic Index by mean ± SD [%] $p<0.05$ SCG1= Spent Coffee Ground of 10% in the flour SCG1 = Spent Coffee Ground of 10% in the flour AUC= Area under Curve CV= Coefficient of variation between Individual subjects.

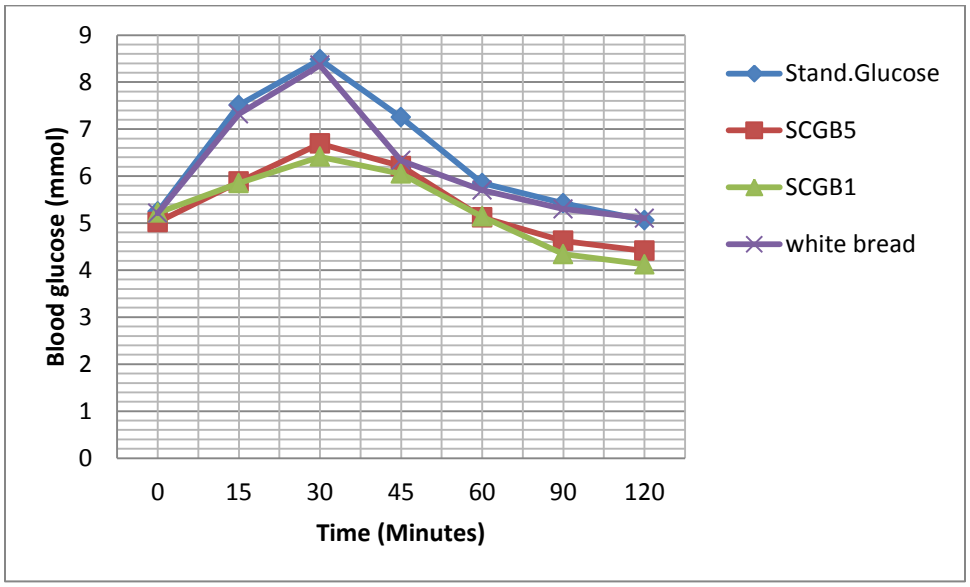

Figure 12: Glycemic index of maximum and minimum concentration of SCBG based bread and white bread compared with standard glucose

3.3. Sensory Evaluation

The mean sensory scores of control and mixed bread with SGCs are shown in Table 10 and illustrated in Figure 12. Significant differences ($p < 0.05$) in color were observed between control and SCG supplemented breads. With increased SCG incorporation the color acceptability of the bread decreased; apparently due to the dark coloration of SCG. There was no significant difference in aroma between white bread and bread with 10, 8 and 6 % SCG incorporations. During coffee brewing most of the volatile aromatic compounds will be lost or extracted with the aquas phase; reducing the amount of aromatics in the SCG. This might entail no significance difference in aroma. The result was similar with the findings of (Hatem et al., 2018).

Table 10: Sensory evaluation of SCG based bread

Bread	Sensory attributes			
Formulation	Colour	Taste	Aroma	Texture
White Bread	8.20 ± 0.20^{a}	7.80 ± 0.20^{a}	7.45 ± 0.37^{a}	7.90 ± 0.23^{a}
SCGB1	5.75 ± 0.61^{b}	5.30 ± 0.48^{c}	6.35 ± 0.38^{ab}	6.15 ± 0.53^{b}
SCGB2	5.60 ± 0.55^{b}	6.15 ± 0.43^{b}	6.50 ± 0.41^{ab}	6.05 ± 0.57^{b}
SCGB3	6.40 ± 0.44^{b}	6.20 ± 0.39^{b}	5.95 ± 0.47^{b}	6.90 ± 0.44^{ab}
SCGB4	6.60 ± 0.39^{b}	6.75 ± 0.38^{a}	5.90 ± 0.42^{b}	6.80 ± 0.43^{ab}
SCGB5	6.75 ± 0.39^{b}	6.70 ± 0.45^{a}	6.20 ± 0.42^{ab}	7.30 ± 0.32^{ab}

Values with the same letters in the same column are not significantly different. SCGB1 = 10% concentration of SCG, SCGB2 = 8% concentration of SCG, SCGB3 = 6% concentration of SCG, SCGB4 = 4% concentration of SCG, SCGB5 = 2% concentration of SCG and White bread is without SCG.

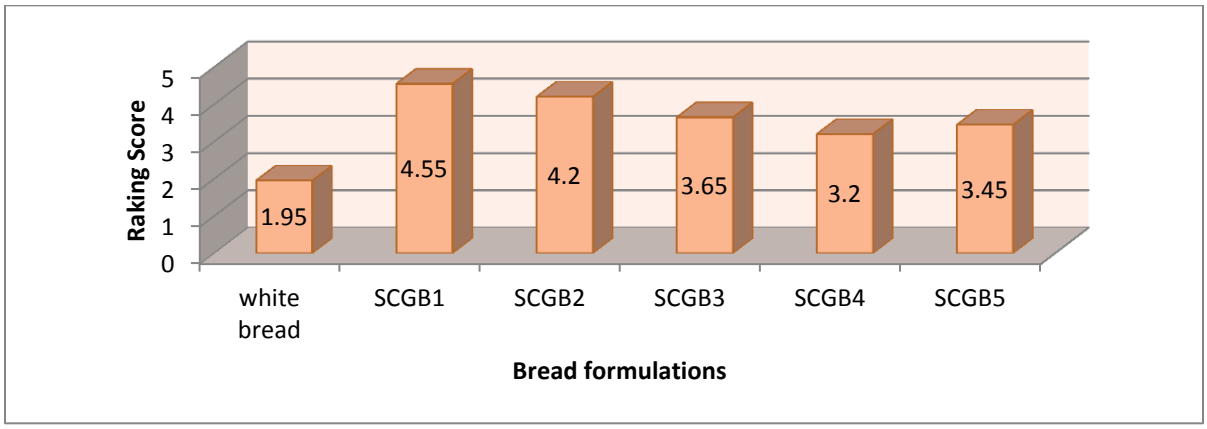

Figure 13**:** Over all acceptance of the bread by ranking

With SCGB 1 and 2, there was a significant difference in texture sensory quality compared with the control bread. Due to visible difference in color and aroma compared with white bread also, the low appearance attribute for the bread sample mixed with 10% SCGs may be due to the darkening of the product (Ballesteros et al., 2014).

The 100% wheat bread has the highest sensory acceptability in all attributes. With increased SCG incorporation, the sensory acceptability of the breads decreased. Among the formulations, 2% SCG based bread is preferable in all aspects than other bread formulation. Based on this 2-4% of SCG flour is selected as optimum blend ratio and used for further work (Figure 12).

5. Conclusion

Spent coffee is the main byproduct of instant coffee brewing with high contents of dietary insoluble fibers, protein, lipids and ashes. In this study, the physicochemical properties of SCG collected from three main cafeterias ware evaluated. The SCG composite had the highest dietary fiber amount. SCG (2, 4, 6, 8 and 10) % was mixed with wheat flour to prepare bread. With higher incorporation of SCG, the dietary fiber amount was improved. With its tremendous total phenolic content, it improved the anti-oxidation property of white bread.

2% SCG had sensory acceptability similar to that of white bread, even with this amount, the bread has improved with proximate composition of Protein 7.79 g/100g, Fiber 2.51 g/100g and K 40.21mg/100g, Mg 9.58 mg/100g. As well as its TFC and TPC was improved.

In addition to proximate composition TPC, TFC and IC_{50} SCG based bread at 2% incorporation was 476.92µg GAE g^{-1}, 1154.43µg QE g^{-1} and 6.17 $IC_{50}µ/mL^{-1}$. Furthermore, this formulation had lower GI than the control bread.

Therefore, the addition of SCG in all the tested flours improved bread quality by increasing the content of nutrients (proteins), dietary fiber, polyphenolic substances, flavonoids as well as minerals and increased antioxidants power. Considering this findings, SCG based bread can be a good source of antioxidant dietary fiber and nutritionally enriched type bread for people consumption.

6. Recommendation

The following are recommendations for future study:

- Further optimization using Surface Response Method for roasting coffee to obtain better composition SCG flour.
- Need to formulate SCG based other bakery products
- Evaluate the millard reaction products in the formulated bread
- Evaluate the effects of variable baking time-temperature combination on properties of SCG supplemented breads.
- Evaluate the role of SCG flour supplementation in the extension of the shelf life of bread
- Effect of incorporating gluten powder for production of value added bread from SCG composite flour as a means of getting high loaf volume breads

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Annexes

ANNEX I:

INFORMED CONSENT FORM FOR SENSORY EVALUATION PANELISTS TO PARTICIPATE IN:

Descriptive Analysis for Bakery product Study

Dear panelist, you are invited to participate in a study involving bread baked with Spent Coffee Ground blend evaluation. The overall objective of this study is to develop a descriptive list of terms for the bread to be tested. This product will be evaluated using a sensory evaluation method known as descriptive analysis. You will be oriented to identify, name and classify a range of **color**, **taste, aroma** and **texture** characteristics of these samples. You will be asked to taste and expectorate the samples, and to rate the samples for intensity of each characteristic. If you have prior experience of any allergic reactions to coffee products and bread (wheat), you should not participate in this study. If you experience allergic reactions any time during the study, you should discontinue the study. There is no direct benefit to you for participating in this study. You are free to withdraw from the study at any time and for any reason. We also reserve the rights to terminate your participation of the study at any time and for any reason.

Your performance and data in this research is confidential. Responses are coded to be confidential and any publications or presentation of the results of the research will only include information about group performance. Names or other identifiable information will not be disclosed or published.

You are encouraged to ask any questions that you might have about this study whether before, during, or after your participation. Questions can be addressed to Mr. Teshome Daniel (+251929006539, anna.dadae@gmail.com).

I understand the above information and voluntarily consent to participate in the study described above. I have been given a copy of this consent form.

Signature Date

ANNEX II

Informed Consent and Laboratory procedure for Glycemic index

INFORMED CONSENT FORM FOR GLYCEMIC IDEX TO PARTICIPATE IN:

Glycemic Index Analysis for Bakery product Study

Dear sir/madam, you are invited to participate in a study involving bread baked with Spent Coffee Ground blend evaluation. The overall objective of this study is to develop a descriptive list of terms for the bread to be tested. This product will be evaluated by level of Glycemic Index analysis from bread baked from Spent Coffee Ground and wheat flour. You will be asked to be on fasting 8 hours prior consuming the samples. Once consuming the bread, capillary blood from fingertip will be collected at each fifteen minutes and analyzed by glucometer for rest of two hours. If you have prior experience of any allergic reactions to coffee products and bread (wheat), you should not participate in this study. If you experience allergic reactions any time during the study, you should discontinue the study. There is no direct benefit to you for participating in this study. You are free to withdraw from the study at any time and for any reason. We also reserve the rights to terminate your participation of the study at any time and for any reason.

Your performance and data in this research is confidential. Responses are coded to be confidential and any publications or presentation of the results of the research will only include information about group performance. Names or other identifiable information will not be disclosed or published.

You are encouraged to ask any questions that you might have about this study whether before, during, or after your participation. Questions can be addressed to Mr. Teshome Daniel (+251929006539, anna.dadae@gmail.com).

I understand the above information and voluntarily consent to participate in the study described above. I have been given a copy of this consent form.

Signature Date

ANNEX III

Laboratory procedure and Selection criteria for Glycemic index and Factors Affecting level of Glycemic Index

Parameter	Comments
Subjects	> 10 test subjects
	Healthy subjects appropriate (lower within-subject variation than e.g. diabetic subjects using drugs)
Time of day	Mornings (more pronounced glycaemic response)
Background diet	Fasting subjects
	Standardised evening meal may reduce within-subject variation
Physical activity	Standardisation so far not successful in decreasing variation
Determination of glycaemic carbohydrates	"By difference" (total CHO minus dietary fibre): Acceptable estimation of digestible CHO in most normal foods, but may overestimate digestible CHO in products with undigestible CHO not determined as dietary fibre (e.g. oligosaccharides, resistant starch, and sugar alcohols).
	Specific assay of the CHO profile recommended for scientific purpose
Carbohydrate load	Typically 50 g glycaemic CHO (linear response for 25–50 g)
Reference product	White bread or glucose
	Other reference than glucose: GI characteristics of the reference maintained over time and recalculated/disseminated using glucose reference
Blood sampling/analyses	Capillary blood (preferable): higher postprandial glucose concentration, less variation
	Venous blood: lower glycaemic response, larger variation, higher GI than capillary blood
	Blood glucose analyses based on approved analytical methods, not enzymatic recognition in dry systems
Calculation	Typically 2 h incremental area (3 h area may be useful for products with extreme lente characteristics)

CHO = carbohydrates.

ANNEX IV

Amharic version of informed consent for Glycemic Index analysis subjects

<u>አዲስ አበባ ዩኒቨርሲቲ</u>

<u>የተፈጥሮ እና ኮምፒውቴሽናል ሳይንስ ኮሌጅ</u>

የምግብ ሳይንስ እና ኒውትሪሽን ማዕከል

በአዲስ መልክ የተዘጋጀ ዳቦ በደም ግሉኮስ መጠን እና የቅምሻ ምዘና ላይ ያላቸውን አጠቃላይ ለማጥናት የተዘጋጀ መተማመኛ ሰነድ

<u>ስምምነት</u>

እኔ ተሾመ ዳንኤል እባላለሁ::የመመረቂያ ጥናቴን በምግብ ሳይንስና ኒውትሪሽን በአዲስ አበባ ዩኒቨርሲቲ ውስጥ በመስራት ላይ ስገኝ ጥናቱም የሚያተኩረው ህብረተሰቡ በብዛት እየተጠቀማቸው ያሉ ዳቦ ላይ የቡና አተላ ዱቄት በመቀላቀል የደቦዉን ይዘት በማሻሻል በውስጣቸው ያለው ንጥር ነገር በበቂ ሁኔታ መገኘቱንና እንዲሁም የዚህን ዳቦ ይዘት ማነፃፀርና ለጤና ያለዉን ጠቀሜታ መወቅ ሲሆን ስንመገባቸው የደም ግሉኮስ መጠናችንን በምን ያህል እንደሚጨምሩ እና አጠቃላይ ጣዕም ያለዉን ለዉጥ ጥናት ማካሄድ ይሆናል::

ከላይ ለመግለጽ እንደተሞከረው የዳቦዉን በደም ግሉኮስ መጠን እና አጠቃላይ ጣዕም ምዘና ላይ ያላውን ለውጥ ለማወቅ ከ18-45 የዕድሜ ክልል ላይ የሚገኙ 10 ተሳታፊዎችን ለደም ጉሉኮሰ እንዲሁም 20 ተሳታፊዎችን ለቅምሻ ምዘና የሚፈልግ ጥናት ሲሆን እነዚህም ሰዎች ከማንኛውም ሱስ ነጻ የሆኑ፣በቤተሰባቸውም ሆነ በራሳቸው ከስኳር በሽታ ነጻ የሆኑ፣የማታጠባ ሴት፣ከእርግዝና ነጻ የሆነች ሴት፣ትክክለኛ ክብደት ያላቸው፣ከማንኛውም መድሀኒት መውሰድ እና ከተጠቀሰዉ ምግብ አለረጅይ ነጻ የሆኑ መሆን ይኖርባቸዋል::

በዚህ ጥናት ላይ ለመሳተፍ ሲወስኑ የደም ጉሉኮስ ጥናቱ የሚካሄደው በ5 ቀናት ልዩነት ለ3ቀን ሲሆን ጠዋት ቁርስ ከመብላትዎ በፊት አንድ ጠብታ ደም ይወሰዳል በመቀጠል ዳቦዉን ከወሰዱ በኋላ በ15 ደቂቃ ልዩነት ለ2 ሰዓት ከጣትዎ ላይ አንድ አንድ ጠብታ ደም በአውቶማቲክ ላንሴት በጤና ባለሙያ አማካኝነት የሚወሰድ ሲሆን ይህም የእርስዎን የደም ግሉኮስ መጠን ያሳውቀናል::ከላይ በተጠቀሰው በተጨማሪ ለቅምሻ ምዘና ደግሞ ዳቦዉን መቅመስና መትፋት ከዛም የተቀመጡ መጠይቆችን መሙላት ለ1 ቀን ይካሄዳል ማለት ነው::

በዚህ ጥናት ላይ የሚያደርጉት ታሳትፎ በፍቃደኝነት ላይ ብቻ የተመሰረተ ሲሆን ነገር ግን የጥናቱ ግኝት ለተጠቃሚ ህብረተሰብ፣ ለምግብ አምራች ድጅቶች፣ለተቆጣጣሪ አካላት እና ለተለዩ የምርምር ተቋማት ያለውን ጉልህ ሚና ታሳቢ በማድረግ የእርስዎ ተሳትፎ በእጅጉ አስፈላጊ መሆኑን ተገንዝበው ለመሳተፍ ይስማማሉ ብዬ ተስፋ አደርጋለሁ::

ደም በሚወሰድበት ጊዜ ላንሴቱ በነካዎት ጣት ላይ የተወሰነ ስሜት ሊኖረው ይችላል ነገር ግን ማንም ሰው ምንም አይነት ችግር አያጋጥመውም::

ለመሳተፍ ከመወሰንዎ በፊት ማንኛውም አይነት ጥያቄ ካለዎት ያሳውቁን::በተጨማሪም በጥናቱ ሂደት ውስጥም ማንኛውም አይነት ጥያቄ መጠየቅ ይችላሉ::ከዚህ በታች ስምዎን ፅፈው ከፈረሙ በዚህ ጥናት ላይ ተሳታፊ ለመሆን ተስማምተዋል ማለት ነው:: እናመሰግናለን!

የተሳታፊ ስም_____

የተሳታፊ ፊርማ(ከ18 ዕድሜ በላይ ለሆኑ)_____

ቀን_____

የጥናቱ ተመራማሪ ስምና አድረሻ

ተሾመ ዳንኤል

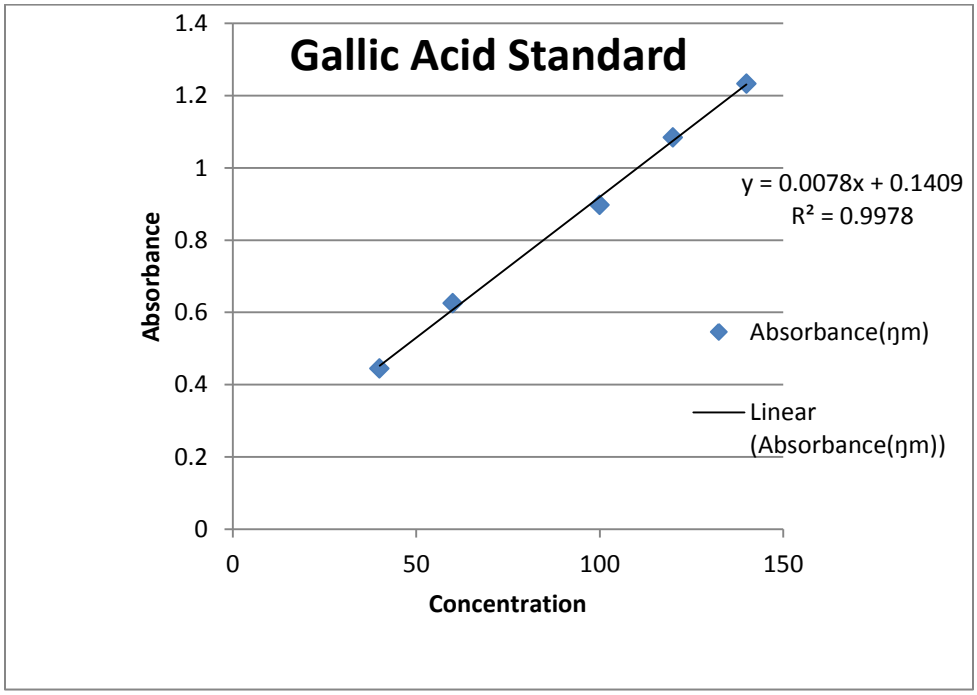
ስልክ ቁጥር፡09 29 00 65 39 (09 88 11 75 07)

ኢሜል አድራሻ፡anna.dadae@gmail.com

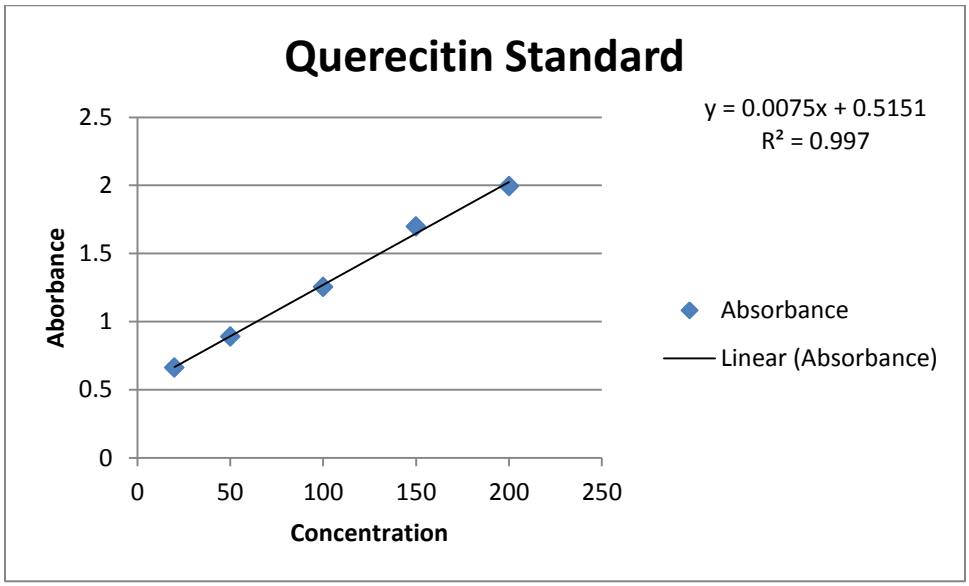
ANNEX VI

Different Standards calibration Curve

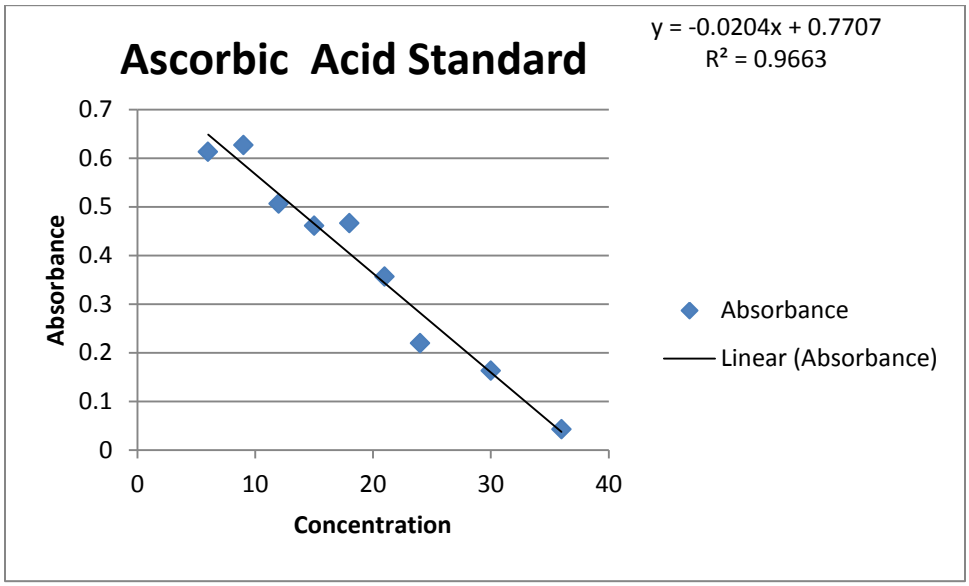
1. Gallic Acid Standard for polyphenol analysis



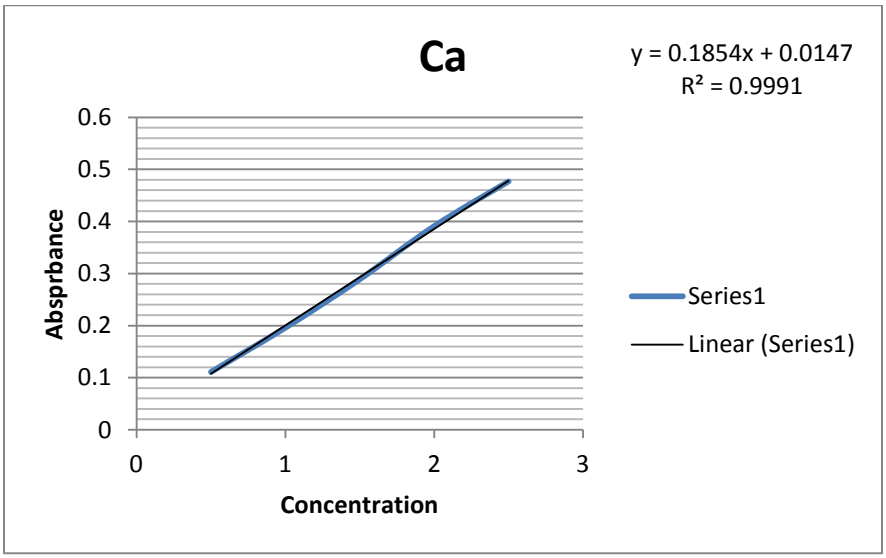
2. Querecetin standard for Flavonoids analysis

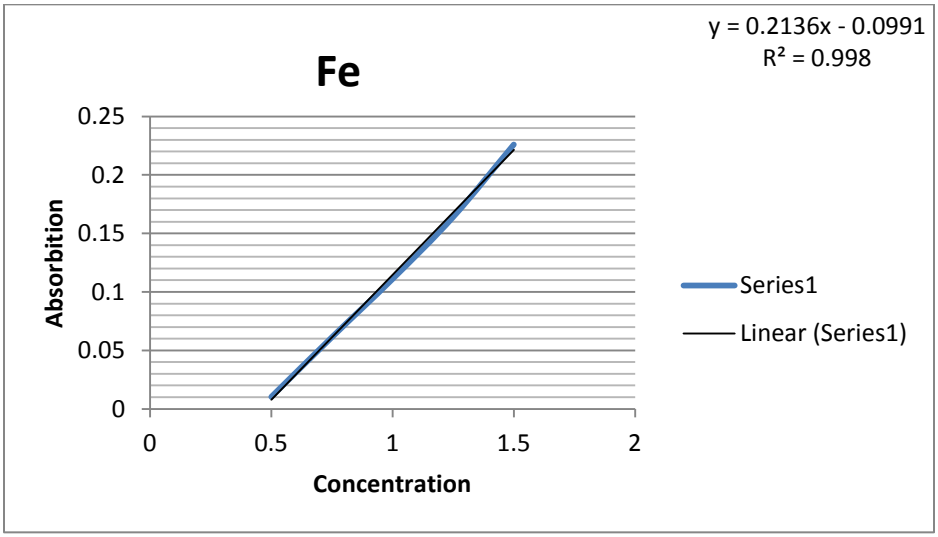


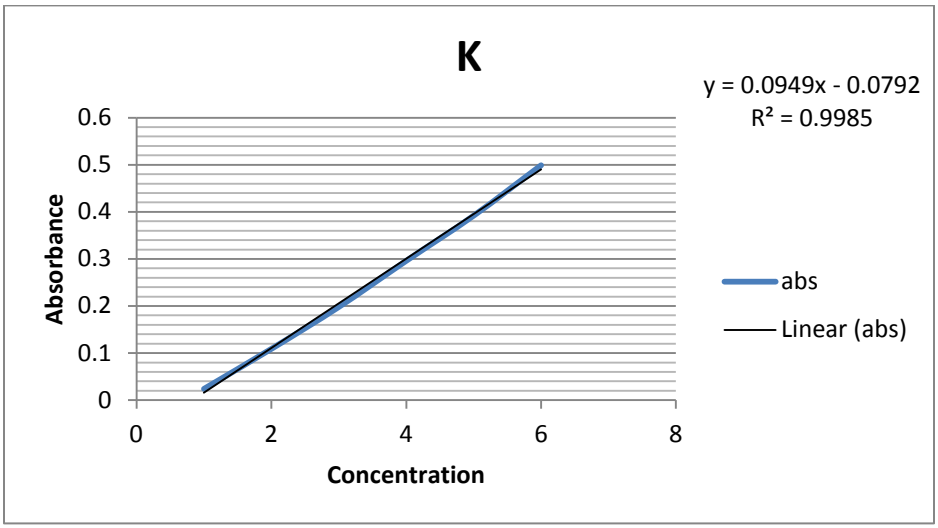
3. Ascorbic Acid Standard

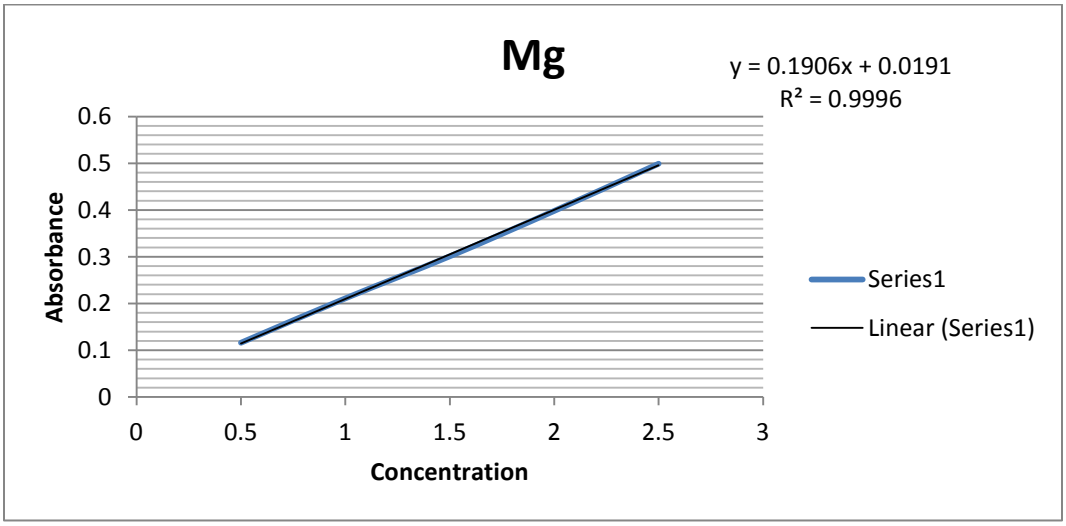


4. Minerals


Ca
y = 0.1854x + 0.0147
R² = 0.9991
Absprbance
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
Concentration
Series1
Linear (Series1)

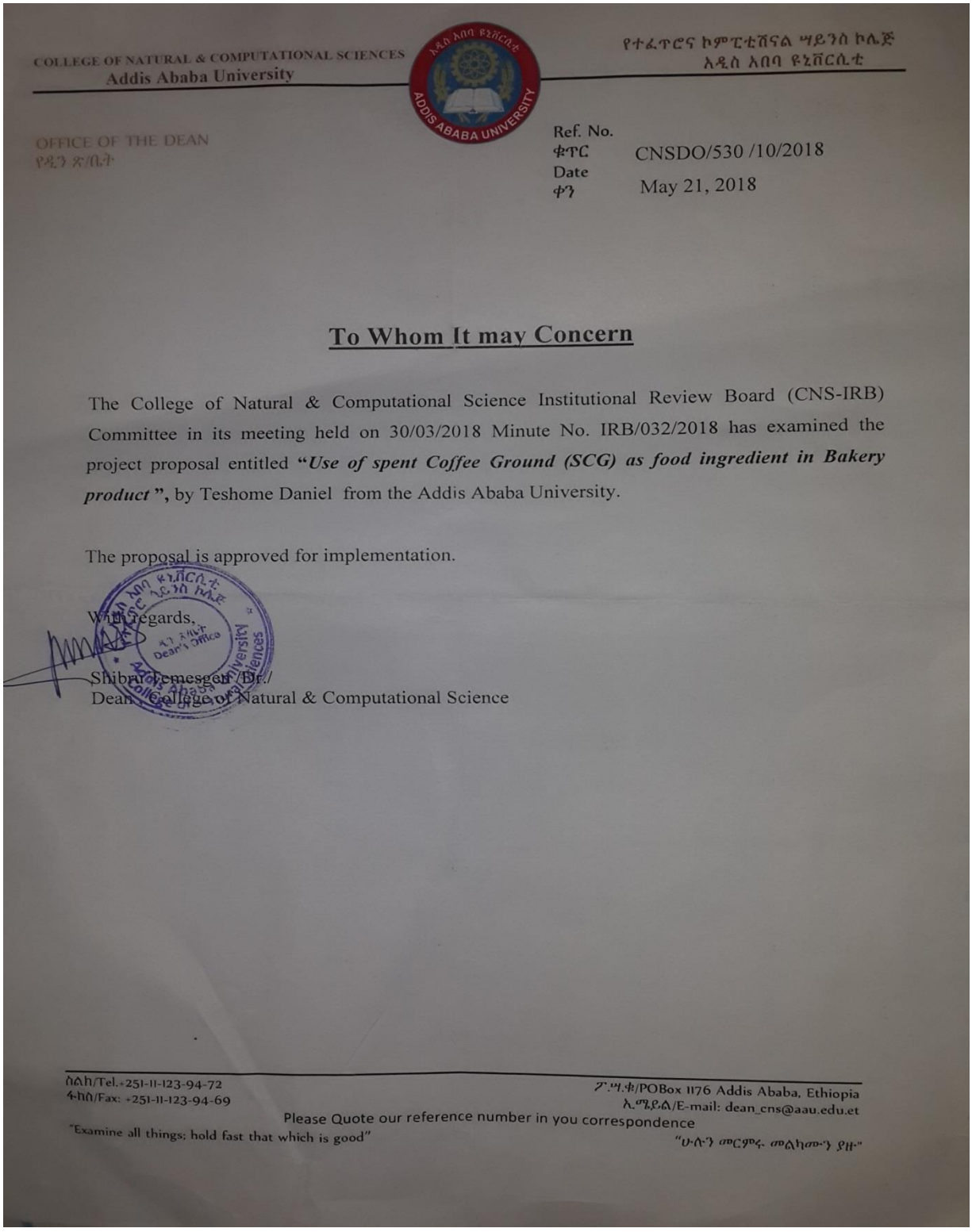

Fe
y = 0.2136x - 0.0991
R² = 0.998
Absorbition
0.25
0.2
0.15
0.1
0.05
0
0
0.5
1
1.5
2
Concentration
Series1
Linear (Series1)


K
y = 0.0949x - 0.0792
R² = 0.9985
Absorbance
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
Concentration
abs
Linear (abs)


Mg
y = 0.1906x + 0.0191
R² = 0.9996
Absorbance
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.5
1
1.5
2
2.5
3
Concentration
Series1
Linear (Series1)

ANEXX VI

Ethical clearance



COLLEGE OF NATURAL & COMPUTATIONAL SCIENCES
Addis Ababa University

የተፈጥሮና ኮምፒቲሽናል ሣይንስ ኮሌጅ
አዲስ አበባ ዩኒቨርሲቲ

OFFICE OF THE DEAN
የዲን ጽ/ቤት

Ref. No. ቁጥር CNSDO/530 /10/2018
Date ቀን May 21, 2018

To Whom It may Concern

The College of Natural & Computational Science Institutional Review Board (CNS-IRB) Committee in its meeting held on 30/03/2018 Minute No. IRB/032/2018 has examined the project proposal entitled **"*Use of spent Coffee Ground (SCG) as food ingredient in Bakery product*"**, by Teshome Daniel from the Addis Ababa University.

The proposal is approved for implementation.

With regards,

Shibru Temesgen (Dr./
Dean, College of Natural & Computational Science

ስልክ/Tel.+251-11-123-94-72
ፋክስ/Fax: +251-11-123-94-69

ፖ.ሣ.ቁ/POBox 1176 Addis Ababa, Ethiopia
ኢ.ሜይል/E-mail: dean_cns@aau.edu.et

Please Quote our reference number in you correspondence

"Examine all things; hold fast that which is good"

"ሁሉን መርምሩ. መልካሙን ያዙ"