

**PROXIMATE COMPOSITION, METAL, POLYPHENOL AND FLAVONOID  
CONTENTS OF GREEN AND BLACK TEA IN KAMPALA**

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**DECLARATION**

I, Denis Odur declare that this work is original and has never been submitted to any university or other higher institution of learning for a similar or other academic award.

Signature.....

Date.....

## APPROVAL

This is to certify that the work presented here in this dissertation is the student's original work, done by him under our supervision, and is now ready for submission for the award of the Degree of Master of Science in Food Technology of Kyambogo University.

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## **DEDICATION**

To my beloved wife Mirriam, and children; Abel, Abigail, Audrey and Alma Agenrwot.

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## LIST OF ACRONYMS AND ABBREVIATIONS

CTC	Crush, tear, and curl
ETC	European Tea Committee
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	Food and Agriculture Organization of the United Nations Statistical Databases
HM	Heavy Metal
mg GAE/g	Milligram Gallic Acid Equivalent per gram
mg RE/g	Milligram Rutin equivalent per gram
IGG	Intergovernmental Group on Tea
PAH	Polycyclic aromatic hydrocarbons
PCBs	Polychlorinated biphenyls
NAADS	National Agricultural Advisory Services
UNCTAD	United Nations Conference on Trade and Development

## ABSTRACT

Uganda is the second leading producer of tea in Africa. However, there are quality and safety concerns of Uganda's tea in the international market. This study determined the proximate composition, the essential and toxic elements, and total polyphenols and flavonoids in green and black tea from selected supermarkets in Kampala. Proximate analysis (moisture, ash content, protein, fat, and carbohydrate) was done as per the procedures of the Association of Official Analytical Chemists (AOAC). Essential elements; sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), manganese (Mn) and iron (Fe), and nonessential elements; mercury (Hg), lead (Pb), cadmium (Cd), arsenic (As), were determined using atomic absorption spectrophotometry (AAS). Total polyphenol and flavonoid contents were determined following the Folin–Ciocalteu method and aluminum chloride using colorimetric assay, respectively.

Moisture, fat, protein, ash, crude fiber and carbohydrate levels in the tea ranged from 2.64 to 8.43, 0.87 to 3.74, 12.41 to 29.28, 4.34 to 9.08, 12.12 to 31.46 and 28.65 to 61.13 %, respectively. Lead, As, Cd and Hg were not detected in all samples. Metal concentrations were in the range of 694.95 to 2681.83, 4.53 to 11.77, 17.51 to 32.59, 47.60 to 141.17, 39.77 to 280.94, 1.18 to 2.28 and 8.30 to 37.69 mg/kg correspondingly for K, Na, Mg, Mn, Ca, Cu and Fe. The total polyphenol and flavonoid amounts ranged from 82.17 to 159.31 mg GAE/g DW and 122.12 to 183.24 mg RE/g DW, respectively. Moisture and ash contents of the green and black tea were within recommended limits. Tea in Kampala is generally safe for human consumption because of the low levels of toxic elements. The high levels of polyphenols and minerals observed in green tea imply that green tea can be an additional source of these components in the diet. Therefore, drinking green tea may be more beneficial than drinking black tea for consumer.

## CHAPTER 1: INTRODUCTION

### 1.1 Background

Tea is the product of the dried leaf of the tea shrub *Camellia sinensis* (L.) O. Kuntze of the Theaceae family. It is one of the world's most popular beverages with an estimated 18 billion cups consumed daily (Rashid *et al.*, 2016; Brahma, Singh, Rawata, Bhagat, & Singh, 2017). World tea production reached 6.5 million metric tons in 2017 (FAOSTAT, 2019). Uganda is the second leading producer of tea in Africa with a production estimate of 73,486 MT after Kenya (458,850 MT) (FAOSTAT, 2019). Tea consumption is linked to low incidence of chronic diseases like cancer, cardiovascular disease (CVD) and diabetes (Zhang *et al.*, 2019). The composition of polyphenols, amino acids, carbohydrates, proteins, chlorophyll, volatile organic compounds and minerals is responsible for the positive health effects hence the tea quality (Yadav, Parajuli, Khatri, & Shiwakoti, 2018). Besides the many biologically active substances, tea bioaccumulates heavy metals (HM) such arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb). Heavy metals are important indicators of tea quality and safety (Zhang *et al.*, 2018).

The major teas consumed globally are black and green tea, which account for 75% and 15% of tea consumption, respectively (Zhang *et al.*, 2019). Black tea is most consumed because of its attractive aroma, taste and positive physiological properties. Tea is Uganda's third largest agricultural export commodity after coffee and fish, and earns the country *ca.* US\$ 104 million in foreign exchange (Kawooya, 2021). Uganda's tea is reported to fetch low premium on the export market where it is blended with Kenyan tea at the Mombasa auction because of its perceived low quality (Lakuma, Ezra, & Guloba, 2014). There is inadequate research on tea in Uganda because of limited tea expertise, finances, and infrastructure (Kawooya, 2021). Consequently, there is lack of sufficient data on the tea's quality (Lakuma, Ezra, & Guloba,

2014). Studies conducted on teas around the world have largely been on the proximate, minerals and HM composition (Zhang *et al.*, 2018), polyphenolic composition (Bobková *et al.*, 2021) and the antioxidant activity of the polyphenols (Yadav, Parajuli, Khatri, & Shiwakoti, 2018; Tong, Liu, Kang, Zhang, & Kang, 2019). The chemical composition of tea varies with the climate of the geographical source, season, horticultural practices and leaf age (Tong, Liu, Kang, Zhang, & Kang, 2019; Mozumder *et al.*, 2020).

## **1.2 Problem statement**

Heavy metals particularly As, Cd, Hg and Pb, have no known physiological function in humans moreover their actions can cause adverse developmental, reproductive, neurological, immunological, teratogenicity and genotoxic effects (Zhang *et al.*, 2018). Limited research has been done on tea in Uganda (Owuor *et al.*, 2011; Kawooya, 2021). The lack of data on Uganda's tea limits access to international markets and leads to reduced foreign exchange earnings, which in turn leads to reduced incomes for the farmers. The limited information also makes planning for the tea value chain rather difficult.

## **1.3 Justification**

Brewed tea contains many compounds that reduce the risk of disease (Khan & Mukhtar, 2007). Tea also contains soluble ingredients whose composition is dependent on the cultivar, climate, geographical location, plucking season, and position of the leaf on the plant (Tong, Liu, Kang, Zhang, & Kang, 2019). High levels of HM have been reported in teas around the world, but data on the HM safety of tea in Uganda is inadequate (Bamuwanye *et al.*, 2017). Therefore, this study assessed the proximate, metal and polyphenol contents of green and black tea consumed in Kampala. It was anticipated that the data obtained from the study would serve as baseline information for the quality and safety of tea in Uganda. Tea trade would be boosted

both at the local and international market. In addition, it would contribute towards bridging the gaps in literature, and the development of a national food composition database.

## **1.4 Objectives**

### **1.4.1 General objectives**

To assess the proximate composition, essential and toxic elements, polyphenol, and flavonoid content of green and black tea from selected supermarkets in Kampala.

### **1.4.2 Specific objectives**

1. To determine the proximate composition (moisture, lipid, total ash, crude protein, crude fiber and carbohydrate) of green and black tea.
2. To determine the amounts of Pb, Cd, As, Hg, K, Mg, Na, Zn, Cu, Mn and Fe in the green and black tea.
3. To determine the amounts of total polyphenol and total flavonoid of green and black tea.

## **1.5 Hypotheses**

1. There is no significant ( $p > 0.05$ ) difference in the proximate composition of green and black tea sold in Kampala.
2. There is no significant ( $p > 0.05$ ) difference in the amounts of essential and non-essential element content in green and black tea sold in Kampala.
3. There is no significant ( $p > 0.05$ ) difference in the total polyphenol and total flavonoid contents in green and black tea sold in Kampala.

## CHAPTER 2: LITERATURE REVIEW

### 2.1 History and distribution of tea

*Camellia* is a generic name from the name Kamel after Rev. Georg Kamel a Moravian-missionary to the Philippines (Short, 2006). While there are about 120 species in the genus *Camellia*, beverage tea is produced from the species *C.sinensis*. The species name *sinensis* means “from China” (Meegahakumbura *et al.*, 2018). Several varieties, cultivars and landraces of tea are known (Ahmed & Stepp, 2013). However, *C.sinensis* var. *sinensis* and *C.sinensis* var. *assamica* are the varieties commonly used for making tea (Kosińska & Andlauer, 2014; Yang *et al.*, 2016). Large leaves characterize Assamica types, while *sinensis* (China) types have very small leaves (Mukhopadhyay, 2017). *C.sinensis* var. *assamica* is a fast growing plant suitable for warm climates, whereas *C.sinensis* var. *sinensis* is more suitable for cold climates (Ahmed & Stepp, 2013). In general, green tea is obtained from the *sinensis* variety while black tea is obtained from the *assamica* variety (Senanayake, 2013; Kosińska & Andlauer, 2014). Because of its enormous yields, Assamica tea is the variety grown in Sri Lanka, India and African countries including Uganda (Senanayake, 2013).

*Camellia sinensis* grows well at optimum temperatures between 18°C and 20°C, and that do not fall below 12°C or go above 30°C (Ahmed & Stepp, 2013). Tea is nowadays cultivated in many countries with tropical or subtropical climate (Ahmed & Stepp, 2013; Hajiboland, 2017). In Africa, tea is largely grown in the East and Central regions of the continent. Tea was introduced into East Africa by European and Asian private investors from India at the beginning of the twentieth century (Owuor *et al.*, 2011).

## **2.2 Description of the tea plant**

. The tea plant can grow to up to 15 m in height over 30 to 50 years but tea bushes are maintained at a height of 2 m for ease of plucking. Tea has a sturdy taproot, and yellow-white flowers that have six to eight petals (Ahmed & Stepp, 2013). The plant has oval and tip pointed leaves, 4 to 15 cm long and 2 to 5 cm wide (Senanayake, 2013). Because of differences in chemical composition, leaves of different age produce tea of differing tea quality characteristics (Senanayake, 2013; Zhang *et al.*, 2020). Typically, the leaf buds or the first 2 to 3 leaves are hand-picked or harvested using a mechanical tea plucker (Lee, Kim, Kim, & Kim, 2014). The younger the tea leaf processed, the higher the quality and the more expensive the final product (Kosińska & Andlauer, 2014). A tea bush is mature enough to harvest a fair amount of leaves after about 2 years, then after 5 years, regular harvest can begin. The tea seeds that are contained in the 3-angled tea fruit, produce tea oil that can be used as seasoning and in cooking (Senanayake, 2013).

## **2.3 Cultivation and production of tea**

### **2.3.1 Cultivation**

Tea grows best in soils of pH 4.5 to 5.6 and elevation 1,500 to 2,250 ft above sea level (Owuor *et al.*, 2011; Hajiboland, 2017). Tea yield is influenced by the weather and uneven rainfall and temperature distributions, and air humidity (Owuor *et al.*, 2011). A positive correlation has been reported between the air temperature and the growth rate of the tea shoot (Muoki, Maritim, Oluoch, Kamunya, & Bore, 2020). The tea shoot requires a minimum air temperature of 13°C and an optimum of 18°C to 30°C to support its growth. Monthly temperatures above 26.6°C have adverse effects on tea yield. An additional 1°C at an average of 28°C a month reduces the yield by 3.8% (Jayasinghe & Kumar, 2021). Freezing night temperature and daytime

temperatures above 30°C result in leaf scorching and shoot growth restriction, respectively (Muoki, Maritim, Oluoch, Kamunya, & Bore, 2020).

The ideal relative humidity for tea growth is between 70% and 90%, and this is essential throughout the vegetative period (Mukhopadhyay, 2017). Humidity in tea physiology influences moisture loss by evapotranspiration (Jayasinghe & Kumar, 2021). High humidity reduces water loss, the tea plant grows more slowly, and the leaves acquire more flavour (Hajiboland, 2017). Low humidity (< 40%) increases water loss and besides, it increases water stress during drought. Drought accounts for 14 to 20% reduction in tea yield and 6 to 19% death of the tea plant in tea fields (Hajiboland, 2017). Heat waves can cause damage of up to 50% (Jayasinghe & Kumar, 2021). Because of this, while the minimum amount of rainfall required for tea growth is 1200 mm, the optimum amount required is between 2500 and 3000 mm (Hajiboland, 2017).

In Uganda, tea is majorly produced in the southwestern districts of Bushenyi, Buhweju, Kanungu and Kabale; the western districts of Kyenjojo, Kibale, Hoima and Kabarole; the central districts of Mukono, Buikwe, Mubende, Mityana and Masaka (Table 1). More than 67% of the tea is grown by smallholder farmers. The major tea varieties popularly grown by the farmers are: 303/577, 6/8, 31/8, 108/82 and 100/5 tea clones (Kawooya, 2021). Tea clones are selected depending on productivity and the levels of the pharmacologically active components (Syahbudin, Widyastuti, Masruri, & Meinata, 2019).

**Table 2.1:** Topographical and environmental features of the tea growing districts of Uganda

District	Geographical coordinates	Altitude (m)	Temp. (°C)	Rainfall (mm)
Kanungu	00°57'S, 29°47'E	2503	18.8	1200
Kabale	01°15'00"S, 29°59'24"E	2000	17.7	1000
Buhweju	00°20'40.0"S, 30°25'00.0"E	1800	20.3	104.14
Kabarole	00°36'N, 30°18'E	1527	20.2	1623
Bushenyi	0°32'30.0"S 30°11'16.0"E	1480	20.2	1449
Mubende	0°33'27.0"N, 31°23'42.0"E	1408	21.2	1085
Kyenjojo	0°36'36.0"N, 30°38'39.0"E	1400	25	1316
Masaka	00°30'S, 31°45'E	1288	21.1	1231
Buikwe	00°21'N, 33°02'E	1234	22	1100
Mityana	0°27'0"N, 32°3'0" E	1209	21	1588
Mukono	00°28'50.0"N, 32°46'14.0"E	1200	21.1	2015
Kibaale	00°47'00.0"N, 31°05'00.0"E	1130	20.2	1270
Hoima	01°24'N, 31°18'E	1120	22.6	1435

Temperature is the annual average temperature experience in the district; Rainfall is the annual average rainfall received in the district.

### 2.3.2 Production

The tea industry has grown rapidly with an increasing number of consumers and markets worldwide (Gunathilaka & Tularam, 2016). Tea production grew at an annual average rate of 4.4% in the decade ending 2018 (Voora, Bermúdez, & Larrea, 2019). China, India, Kenya, Sri-Lanka, Viet Nam and Turkey correspondingly contribute 43, 22, 8, 5, 5 and 5% towards the world total production of the tea leaf (Owuor *et al.*, 2011). Of the six countries, China and Turkey registered the highest production growth rates (FAO, 2018). Black tea and green tea

accounts for 78% and 20% respectively, of world production whereas the other tea types account for only 2% (Kosińska & Andlauer, 2014).

The world tea area has been increasing with some shifts within and between countries. The leading countries in acreage increases in the order Viet Nam, Sri Lanka, Kenya, India and China, which together contribute more than 80% of the world tea acreage. Similarly, the five countries account for 82% of the global production of tea. During the same period, China with the largest area of tea, had the highest production. However, the highest yields were in Iran followed by Turkey, India Vietnam and Argentina.

**Table 2.2:** Area under tea cultivation, tealeaf yield and total production by major producing countries and regions for the period 2015 to 2019

Country/Region	Cultivated acreage (1000 ha)					Leaf yield (hg/ha)					Production (1000 metric tonnes)				
	2015	2016	2017	2018	2019	2015	2016	2017	2018	2019	2015	2016	2017	2018	2019
China	2127.4	2378.6	2860.1	2997.5	31855.3	10771	9779	8649	8758	8765	2291.4	2326.0	2473.8	2625.1	2791.8
India	566.7	577.5	621.6	623.0	628.2	21762	21654	21316	21489	22128	1233.1	1250.5	1325.1	1338.6	1390.1
Kenya	209.4	218.5	232.7	236.2	269.4	19059	21648	18902	20872	17032	399.1	473.0	439.9	493.0	458.9
Sri Lanka	215.0	216.5	202.5	202.5	200.3	14788	13513	15193	15001	14984	318.0	292.6	307.7	303.8	300.1
Viet Nam	117.8	118.8	123.2	116.6	115.9	20030	20198	21106	23150	23225	236.0	240.0	260.0	270.0	269.3
Turkey	76.2	76.4	82.1	78.1	84.9	31366	31823	28499	34556	30749	239.0	243.0	234.0	270.0	261.0
Indonesia	114.9	117.3	113.3	109.9	108.8	11543	12281	12907	12756	12672	132.6	144.0	146.2	140.2	137.8
Argentina	39.6	39.6	39.6	39.6	39.8	20831	21468	20575	20702	21543	82.5	85.0	81.5	82.0	85.7
Japan	44.	43.1	42.4	41.5	40.6	18068	18068	19340	20975	20123	79.5	80.2	82.0	86.3	81.7
Iran	18.6	20.7	15.9	17.2	16.2	105777	60257	63465	57801	55969	79.5	80.2	82.0	86.3	81.7
Uganda	28.4	19.2	24.4	34.8	35.6	20613	20458	20517	20576	20635	58.6	39.3	50.1	71.6	73.5
Asia	3461.7	3735.8	4251.7	4390.1	4571.6	14403	13311	12284	12442	12361	4986.0	4972.6	5223.0	5462.2	5650.8
Africa	367.5	369.5	388.1	416.1	459.1	18470	19781	17451	18531	16317	678.7	730.9	677.2	771.0	749.0
East Africa	353.3	354.9	373.0	400.8	445.5	18902	20290	17865	18970	16584	667.8	720.0	666.4	760.2	738.8
Americas	44.5	44.3	43.8	43.8	44.1	20433	20903	20069	19908	20712	90.9	92.7	87.9	87.2	91.4
Europe	0.661	0.893	0.732	0.718	0.623	6581	7088	10000	8412	6388	0.435	0.633	0.732	0.604	0.398
World	3878.3	41544.5	4688.3	4854.7	5079.4	14857	13967	12786	13033	12792	5762.0	580.3	5994.7	6326.9	6497.4

Source: FAOSTAT (2021)

### **2.3.3 Tea export**

Tea represents an important cash crop for many countries (UNCTAD, 2019). Domestic consumption comprises 50% of black tea and 78% green tea produced globally. About 1.1 million tones (93%) black tea is traded on the international tea market hence for export (Voora, Bermúdez, & Larrea, 2019). Ninety percent of tea exports from Africa comprise black tea (UNCTAD, 2019). Seventy percent of the tea in international trade is sold through public auctions (e.g. Calcutta, Colombo, Jakarta, Mombasa). During the period 2011 to 2017, Kenya with a market share of 17%, was the world's top exporter of tea (FAO 2016). However, Asian countries; China, India, Sri Lanka and Viet Nam remain dominant in the global tea market (UNCTAD, 2019). Uganda was eighth leading exporter of tea in the world, only second after Kenya in Africa (Table 2.3). According to FAO, Uganda's tea exports are growing at an average rate of 2.5% compared to the world rate of 0.8% per annum (FAO, 2018). Ninety percent of Uganda's tea is sold to exporters through the Mombasa Tea Auction where the tea is classified and priced according to quality attributes such as strength, aroma, liquor and appearance (Lakuma, Ezra, & Guloba, 2014).

**Table 2.3:** Tea exports (tons per year) for the top 10 exporting countries during 2011-2017

Country	2011	2012	2013	2014	2015	2016	2017
Kenya	388344	380357	448809	458728	410039	436924	400000
China	32258	313484	325206	301484	324949	332000	350000
India	322548	225082	254841	212606	235132	232920	240680
Sri Lanka	321074	318397	35525	325141	304835	-	281840
Viet Nam	134528	146899	141021	132252	125186	-	61850
Argentina	86650	78056	77335	76892	76089	-	72693
Indonesia	75450	70071	70842	66399	61915	-	43338
Uganda	55256	55210	62018	59687	53319	-	55589
UAE	-	48551	61788	71342	-	-	-
Malawi	46007	42494	43245	48227	38785	-	29290

UAE: United Arab Emirates; Source: FAO (IGG: TE ISM 17/3)

### 2.3.4 Tea consumption

World tea consumption grew at a rate of 4.5% per annum between 2007 and 2016. The increase in consumption is attributable to population growth, urbanization and rising incomes particularly in emerging and developing economies of the world (FAO, 2018). Major producing countries have played a role in the expansion of the demand for local consumption (FAO, 2018). Increased awareness about the health benefits of tea drinking has also been a factor (FAO, 2021). The consumption of tea ranges from 0.1 kg to 3.1 kg per person per year. The major consumers include Turkey, Kuwait, Ireland, Argentina, Paraguay, and the UK. Green and oolong teas are consumed mainly in China, Japan, India, and Thailand while black tea is majorly consumed in Africa and Western countries (Sharangi, 2009).

Accurate data on the consumption patterns of tea in Uganda is not readily available (Bamuwanye *et al.*, 2017). However, like in other African countries, domestic consumption represents a very low volume of the total tea production (FAO, 2018).

#### **2.4 Tea processing**

Tea processing comprises the steps involved in the transformation of the harvested tealeaf into made tea for brewing (Pou, Paul, & Malakar, 2019). Depending on the degree of oxidation, tea is classified into black, green, white and oolong tea. Black tea is a fully oxidized tea, oolong tea is a semi-oxidized, while green and white teas are un-oxidized forms of tea (Pou, 2016). The attainment of the characteristics of each of the different tea types may require specific unit operations (Table 2.4).

**Table 2.4:** Unit operations involved in tea processing

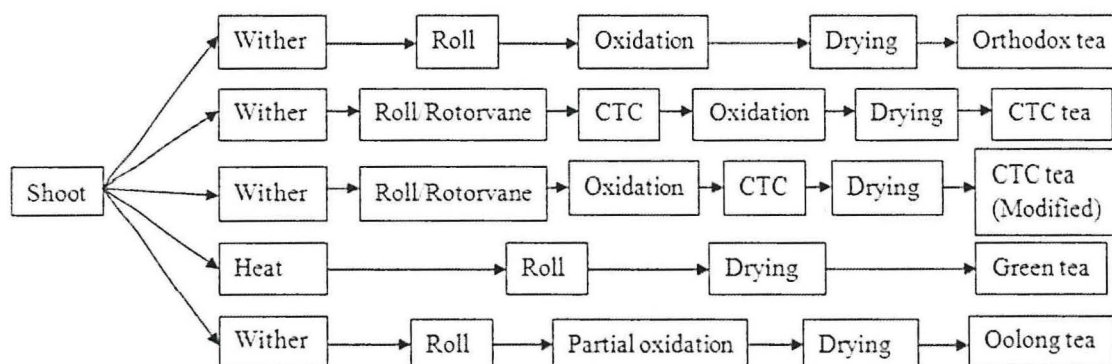
Process	Description
Fixation	Carried out by steaming, pan-firing, and sunning. The aim is to deactivate the endogenous enzymes present in the fresh tea leaves.
Withering	Natural and spontaneous process that allows the reduction of the moisture content of tea leaves. The aim of the withering is to improve the flavor in green, oolong, and black tea.
Rolling	Performed to obtain the different kinds of shape of tea leaves
Fermentation	It is so-called the enzymatic oxidation catalyzed by polyphenol oxidase (PPO) in order to oxidize polyphenols into tannins and complex quinones.
Drying	Unitary operation performed in order to stop the oxidation progress and to reach a moisture level of 3-4% (wet basis) to facilitate handling and transportation.

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Source: Cisneros-Yupanqui & Lante (2020)

There are two techniques of processing tea viz. (i) orthodox and (ii) crush, tear and curl (CTC). “Orthodox” refers to a method of producing black tea whereby the leaves undergo partial drying followed by oxidation (Kalauni, Joshi, & Joshi, 2020). Only the young shoot comprising the first 2 to 3 leaves harvested at the exact time of budding are processed into orthodox tea (Kalauni, Joshi, & Joshi, 2020). The leaves are withered, rolled on specially designed orthodox rollers and crushed to rupture the cells (Pou, Paul, & Malakar, 2019). The final step in the technique involves stopping the oxidation process by drying the crushed leaves quickly to a moisture content < 3%. Orthodox processing results in tea of fine quality due to its unique colour, aroma and flavor. Because of its quality, orthodox tea is in high demand among US and

EU consumers (Kalauni, Joshi, & Joshi, 2020). This implies that orthodox tea has a high potential to boost the foreign earnings of any given country because it also fetches a higher premium than CTC tea on the international market. Figure 2.1 shows the unit operations involved in the production of different types of tea.



**Figure 2.1:** Unit operations in the production of different types of tea.

Source: Pou (2016)

The most extensively consumed tea is the Crush, Tear, and Curl (CTC) black tea because of its convenience, low price, strong liquor, mild bitterness, unique flavor and color characteristics (Li *et al.*, 2021). This tea is also the preferred tea in the export market, particularly in the Middle East, United Kingdom and Ireland. In the CTC technique of producing tea, the leaves are withered and then passed through cylindrical rollers that macerate them (Pou, Paul, & Malakar, 2019; Li *et al.*, 2021). The final step involves firing or drying the tea to a moisture content of 2 to 3 percent for better shelf-life. CTC tea is the main tea product of China, Kenya, India, Vietnam, and Sri Lanka (Li *et al.*, 2021). As it is in Kenya and other tea producing countries in Africa, CTC technique is the main form of processing tea in Uganda (Munyambonera, Lakuma, & Guloba, 2014).

In the preparation of green tea, some thermal treatment that inactivates the enzymes is applied to prevent oxidation of many of the components. As a result, the green type of tea is rich in varieties of phytochemicals with beneficial effects on human health (Chacko, Thambi, Kuttan, & Nishigaki, 2010). The process by which oolong tea is made is similar to the one for green tea except that oolong tea is allowed to undergo a partial oxidation (Pou, 2016). The process involves withering fresh leaves under sunlight for a short period. Further withering combined with gentle rolling is done indoors for several hours, followed by brief oxidation, heat process and fast drying to stop oxidation (Cisneros-Yupanqui & Lante, 2020).

## **2.5 Health benefits of drinking tea**

Tea is an extraordinary remedy for the maintenance of human health (Afaq, Adhami, Ahmad, & Mukhtar, 2004). The Shang Dynasty used tea as medicine long before it was used as a beverage by the Zhou Dynasty (Saikia, 2020). According to Serafini, Rio, Yao, Bettuzzi, & Peluso (2011), consumption of green tea is correlated with low incidence of diseases in which oxidative stress is involved such as cancer and cardiovascular diseases; CVD (Chacko, Thambi, Kuttan, & Nishigaki, 2010). Similarly, green tea consumption reduces the risk of kidney stone formation (Wang *et al.*, 2021). Besides, green tea is an effective treatment for diarrhea and typhoid moreover it inhibits the growth of *Helicobacter pylori* (Chacko, Thambi, Kuttan, & Nishigaki, 2010). The health benefits associated with tea drinking include increased immunity, reduced cholesterol, increased metabolic rate, cancer prevention, blood pressure regulation and reduced risk of diabetes (Prathibhani, Kumarihami, & Song, 2018). The health benefits of tea are thought to derive from the phenolic antioxidants that constitute the main bioactive compounds present (Kopjar, Tadić, & Piližota, 2015).

## **2.6 Chemical composition of tealeaf**

Fresh tealeaf contains polyphenols, alkaloids, protein and amino acids, nucleotides, vitamins, trace amounts of lipids, carbohydrates, xanthic bases, pigments (chlorophyll, carotenoids), organic acids, carotenoids, volatile compounds and minerals (Street, Száková, Drábek, & Mládková, 2006; Tong, Liu, Kang, Zhang, & Kang, 2019). The composition of the tealeaf is dependent on the cultivar, climate, genetic strain, geographical origin, time of harvest, position of the leaf and the processing method (Tong, Liu, Kang, Zhang, & Kang, 2019). The major components in the tealeaf are described in Table 2.5.

**Table 2.5:** Major chemical components in tea

Serial No.	Compound	Main representatives
1.	Catechins	Flavanols: 12 catechins the main ones being; (+)-catechin, (-)-epicatechin, (-)-gallocatechin, (-)-epigallocatechin, (-)-catechin gallate, (-)-epicatechin gallate, (-)-gallocatechin gallate, (-)-epigallocatechin gallate
2.	Oxyaromatic acids	Gallic, caffeic, quinine, chlorogenic, n-coumaric acids
3.	Flavonols	Quercetin, kaempferol, myricetin
4.	Theaflavins	Theaflavin, theaflavin-3-O-gallate, theaflavin-3'-O-gallate, theaflavin-3-3'-O-gallate
5.	Teagallins	Teagallin
6.	Thearubigins	High-molecular weight polymers of catechin gallates with molecular weight from 1000 to 40000 Da
7.	Pigments	Carotenoids and chlorophyll
8.	Alkaloids	Caffeine, theophylline, theobromine
9.	Sugars	Glucose, fructose, saccharose
10.	Amino acids	Isoleucine, leucine, methionine, threonine, phenylalanine, glutamine, asparagine, alanine, serine, proline, histidine, glutamic acid, aspartic acid, theanine
11.	Vitamins	C, $\alpha$ -, $\beta$ -, $\gamma$ -, $\delta$ -tocopherols, riboflavin
12.	Dibasic acids	Succinic, malic, tartaric, citric, quinic, oxalic acids
13.	Minerals	$K^+$ , $Na^+$ , $Ca^{2+}$ and $Mg^{2+}$
14.	Trace elements	Fe, Zn, Cu, and Ni
15.	Lignans and saponins	Mixture of many compounds

Source: Reto, Figueira, Filipe, & Almeida (2007); Yashin, Nemzer, Combet, & Yashin (2015)

The chemical composition of processed tea may be different from that of the green tealeaf because many of the compounds may break down forming new products during processing (Maharramov, Jahangirov, & Maharramova, 2020).

### **2.6.1 Proximate composition of tea leaves**

The amount of moisture in the tealeaf determines the quality of the final product (You *et al.*, 2018; Sharma & Dutta, 2018). A high water content drastically reduces the long-term quality of green tea thereby limiting the shelf life to no more than six months. Excessive moisture initiates oxidation in the finished product, and potentially leads to the formation of off-flavors due to mold growth. The Uganda Standard US EAS 28:2019 specifies a moisture content of 7% (m/m) in black tea on a dry weight basis.

Lipids, amino acids, glycosides and carotenoids are the precursor to tea aroma compounds (Ho, Zheng, & Li, 2015). Tea lipids may have no direct influence on the quality of made tea (Rahman, Kalam, Salam, & Rana, 2013). The lipid composition of tea depends on genetics and differences in geographical origin (Rahman, Kalam, Salam, & Rana, 2013). According to Rahman, Kalam, Salam, & Rana (2013), green tea leaves contain between 15% and 20% protein. Amino acids of the tea protein are an important contributor to the tea infusion taste, and are used as descriptor for tea quality (Jia *et al.*, 2018). The amino acid L-theanine responsible for calm and alertness in the human body is characteristically found in the tealeaf. The differentiation of amino acids has been used as a chemometric descriptor for classifying different tea types. Glutamine, arginine and L-theanine have been reported as the main constituent amino acids in high-quality Japanese teas (Jia *et al.*, 2018). Carbohydrates in tea leaves are present largely as flavorless compounds known as glycosides (Jeganathan *et al.*, 2016). Quercetin, kaempferol and myricetin are the common glycosides found in tea

(Jeganathan *et al.*, 2016). Several kaempferol and quercetin glycosides of glucose, galactose and rhamnose have been reported in tea. During processing, these compounds are hydrolysed by enzymes to liberate volatile aromatic compounds such as monoterpenoid alcohols and aromatic alcohols (Ho, Zheng, & Li, 2015). Quercetin and kaempferol showed antioxidant and anti-inflammatory effects, and reduced risk of CVD in *in vitro* and in animal studies, respectively (Dabeek & Marra, 2019). Green tea is rich in kaempferol glycosides, while oolong and black teas are richer in the quercetin glycosides. Oolong tea also contains moderate quantities of myricetin glycosides (Jiang, Engelhardt, Thräne, Maiwald, & Stark, 2015).

Total ash relates to the mineral amounts in the tea (Jayawardhane *et al.*, 2016). Tea ash content and composition varies with the age of the tealeaf to a limited extent. The total ash of tea has been listed as a quality parameter and the US EAS 28:2019 specifies a total ash content between 4 and 8% (Jayawardhane *et al.*, 2016).

The major part of the insoluble matter of tea is the crude fibre, which is largely made up of cellulose and hemicelluloses (Aroyeun, 2013). The organoleptic properties (taste, flavor and appearance) that determine tea acceptability by consumers are influenced by the crude fibre content hence an important quality parameter (Chaturvedula and Prakash 2011). Young tealeaf contains a much lower fibre content than the old tealeaf. The fibre content in tea products ranges between 7 and 20% (Aroyeun, 2013). However, US EAS 28:2019 standard specifies the maximum amount of fibre in black tea as 16.5%. Higher amounts of fibre translate into a decline in the majority of other quality characteristic (Aroyeun, 2013). Table 2.6 shows the proximate composition of teas from different tea producing countries.

**Table 2.6:** Proximate composition of tea from different countries around the world

Country	Tea species and type	N	Moisture	Lipids	Ash	Proteins	Fibre	Carbohydrates	Source
India	<i>C.sinensis</i> <sup>b</sup>	-	12.13	16.16	5.94	22.86	16.05	39.86	(Pradhan & Dubey, 2020)
India	<i>C.assamica</i> <sup>b</sup>	-	14.17	1.97	5.04	20.35	18.01	40.46	(Pradhan & Dubey, 2020)
Bangladesh	<i>C.sinensis</i>	-	9.46	2.72	7.3	13.65	7.69	59.2	Haque <i>et al.</i> (2019)
South Korea	<i>C.sinensis</i> <sup>b</sup>	3	6.41	1.79	5.17	19.45	-	67.45	Tong <i>et al.</i> (2019)
South Korea	<i>C.sinensis</i> <sup>g</sup>	3	2.79	2.12	4.91	33.55	-	56.14	Tong <i>et al.</i> (2019)
Pakistan	<i>C.sinensis</i> <sup>g</sup>	-	4.88	2.49	5.6	18.06	15.35	53.68	Ahmad <i>et al.</i> (2014)
Pakistan	<i>C.sinensis</i> <sup>b</sup>	10	5.53	1.32	4.94	0.96	14.46	72.79	Adnan <i>et al.</i> (2013)
Pakistan	<i>C.sinensis</i> <sup>g</sup>	5	6.46	1.68	4.57	1.23	16.16	76.36	Adnan <i>et al.</i> (2013)
China	<i>C.sinensis</i>	-	7.2	10.1	7.1	9.75	14.0	51.850	Mohammed & Sulaiman (2009)
Kenya	<i>C.sinensis</i>	-	7.2	9.30	7.2	5.5	14.02	56.780	Mohammed & Sulaiman (2009)
India	<i>C.sinensis</i>	-	7.5	10.30	5.1	7.688	14.10	55.313	Mohammed & Sulaiman (2009)
Sri Lanka	<i>C.sinensis</i>	-	6.5	10.50	4.9	6.938	18.82	52.342	Mohammed & Sulaiman (2009)
Mambila	<i>C.sinensis</i>	-	5.6	10.6	7.2	6.938	18.04	51.622	Mohammed & Sulaiman (2009)

<sup>b</sup>: black tea; <sup>g</sup>: green tea; N: number of samples analyzed

### 2.6.2 Minerals

The main minerals in tea are Na, K, Mg, Mn, calcium (Ca), phosphorus (P), fluorine (F) and aluminium (Al) (Alam, Huda, & Chowdhury, 2020). Tea also contains small amounts of the essential elements Fe, Zn, Cu, chromium (Cr), molybdenum (Mo), selenium (Se), cobalt (Co) and nickel (Ni). Therefore, if consumed regularly, tea may supplement the dietary intake of essential elements in the diet in addition to the large amount of K relative to Na that make it beneficial in the management of hypertension.

According to Bamuwamye, Ogwok, Tumuhairwe, Eragu, & Nakisozi (2017), most studies conducted on tea have largely centered on Cu, Zn, Fe, Cd, Pb and Mn. Potassium, Na, Al, Ni and Cr have been moderately researched. Few studies reported on the levels of Mg, As and Hg in tea, in the period between 1998 and 2017 (Jalbani, Kazi, Arain, Jamali, & Afridi, 2007; Bamuwamye, Ogwok, Tumuhairwe, Eragu, & Henriettah Nakisozi, 2017). The genetics, tea agrotype, geographical origin, environmental conditions and agronomic conditions determine the quantity of minerals in tea (Brzezicha-Cirocka, Grembecka, & Szefer, 2016; Zhang *et al.*, 2018; Alam, Huda, & Chowdhury, 2020). According to Fernández-Cáceres, Martín, Pablos, & González (2001), the mineral content of green and black teas is very similar. Table 2.7 shows the essential and toxic metal contents of green and black teas from selected studies documented in literature.

**Table 2.7:** Essential and toxic metal contents in green and black tea from selected studies in literature

Element	B <sup>1</sup>	B <sup>2</sup>	B <sup>3</sup>	G <sup>3</sup>	G <sup>4</sup>	D <sup>5</sup>
K	8.54-28.38	1.38-2.39	-	-	9290-19858	15145.4
Na	0.08-0.89	20-100	-	-	14.3-137.6	-
Mg	-	0.15-0.28	58.03	38.13	1224-2426	1978.2
Al	74.75-2085	200-630	-	-	-	1074
Cu	0.5-30.9	-	5.64	8.4	5.70-17.9	-
Mn	2.37-1820	159-2290	-	-	193-1547	824.8
Fe	ND-489	57-275	33.29	65.66	40.7-290	-
As	ND-2.0	0.02-0.221	-	-	-	-
Cd	ND-1.5	-	< 0.001	7.75	2.13-19.2	-
Pb	ND-8.39	0.093-0.937	26	38.9	ND-311	-
Hg	ND-0.47	-	-	-	-	-

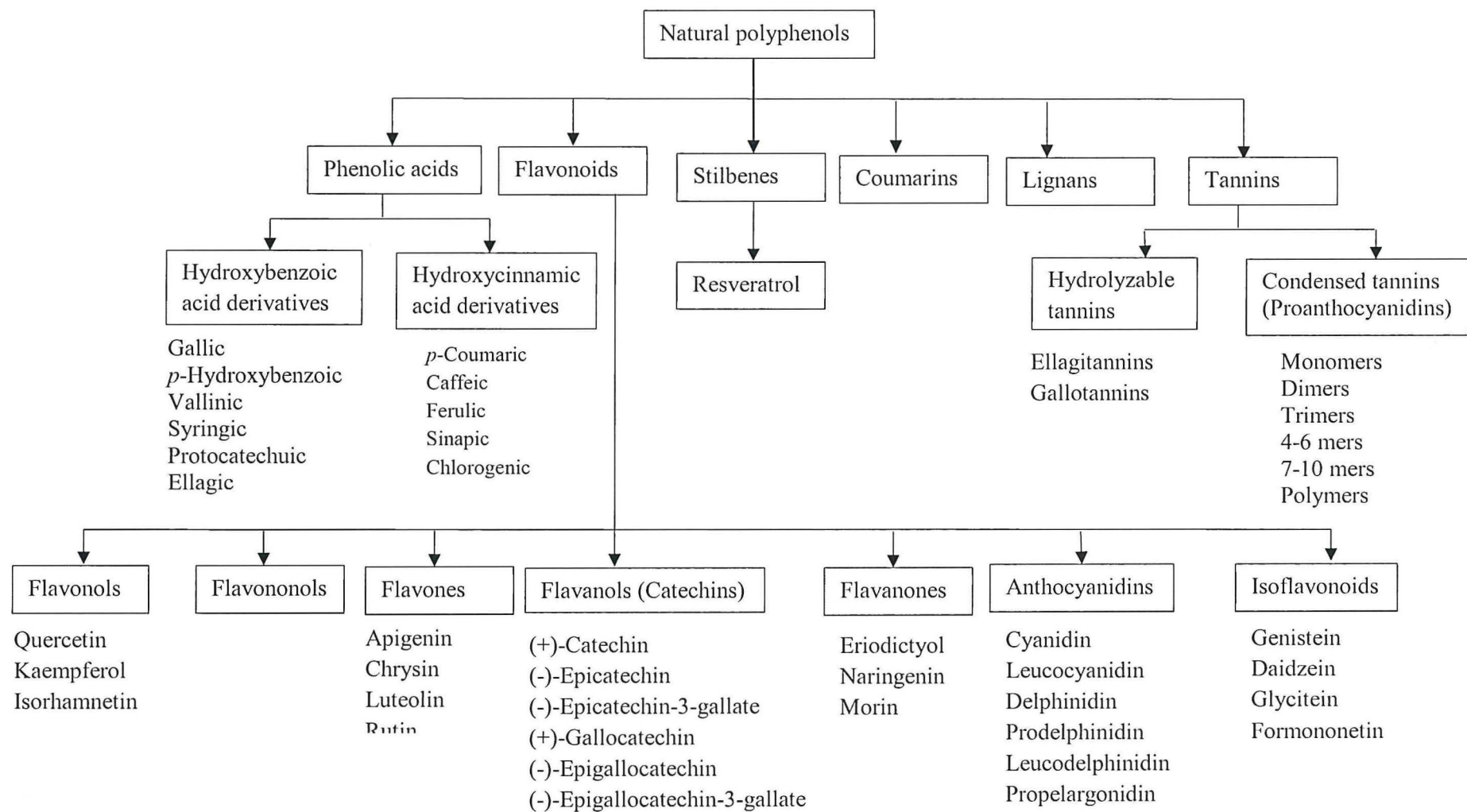
B: black tea; G: green tea; D: different types of tea; <sup>1</sup>Bamuwanye, Ogwok, Tumuhairwe, Eragu, & Henriettah Nakisozi (2017); <sup>2</sup>Zhang *et al.* (2018); <sup>3</sup>Gonzalez-Weller *et al.* (2015); <sup>4</sup>Koch *et al.* (2018); <sup>5</sup>Fernández-Cáceres, Martín, Pablos, & González (2001).

### 2.6.3 Tea polyphenols

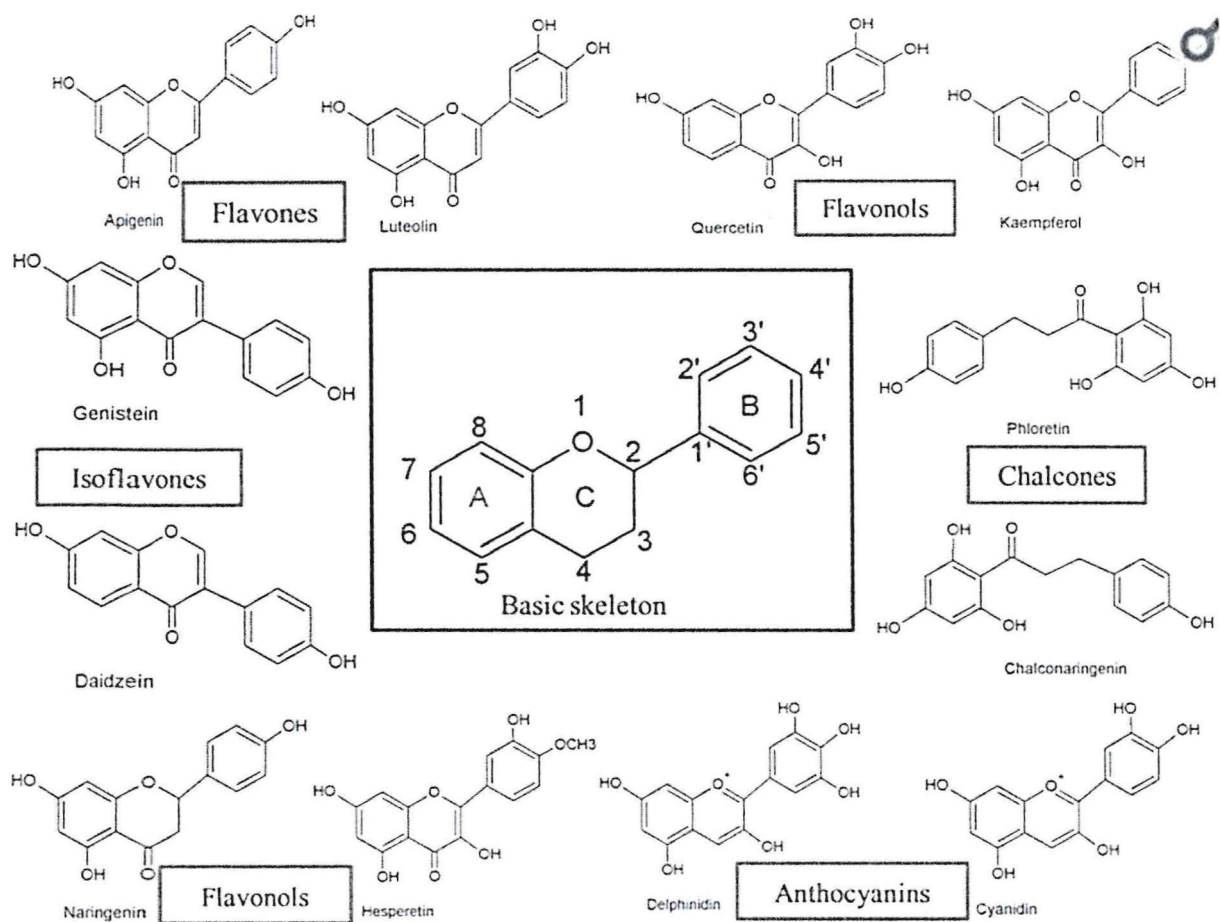
Polyphenols are pharmacologically active compounds responsible for a diversity of the health benefits of tea (Khan & Mukhtar, 2019). Tea polyphenols contain a characteristic benzene (C<sub>6</sub>H<sub>6</sub>) ring with a hydroxyl group; -OH (Cano- Avendaño, Carmona-Hernandez, Rodriguez, Taborda-Ocampo, & González-Correa, 2021). The location and number of -OH groups, ring substitution and orientations determine polyphenol functionality. The presence of multiple -OH groups and the delocalized pi ( $\pi$ ) bond system make polyphenols efficient antioxidants (Cano-Avendaño, Carmona-Hernandez, Rodriguez, Taborda-Ocampo, & González-Correa,

2021). Polyphenols also have good anti-inflammatory and anticancer effects, and regulate lipid metabolism (Yan, Zhong, Duan, Chen, & Li, 2020).

Polyphenols are categorized in five major classes of which flavonoids are the most prominent (Figure 2.3). The flavonoids are made up of a 15-carbon base structure, which consists of two phenyl rings (A and B), and the pyran ring C (Figure 2.3). Depending on the chemical structure, flavonoids may belong to one of the subgroups; flavanols or catechins, flavones, flavonols, isoflavones, flavanones, anthocyanins and chalcones (Panche, Diwan, & Chandra, 2016). Isoflavones are the compounds in which the B ring is linked in position 3 of the pyran ring while neoflavonoids are those in which the link is in position 4. In the remaining subgroups, the B ring is linked in position 2 of the pyran ring.

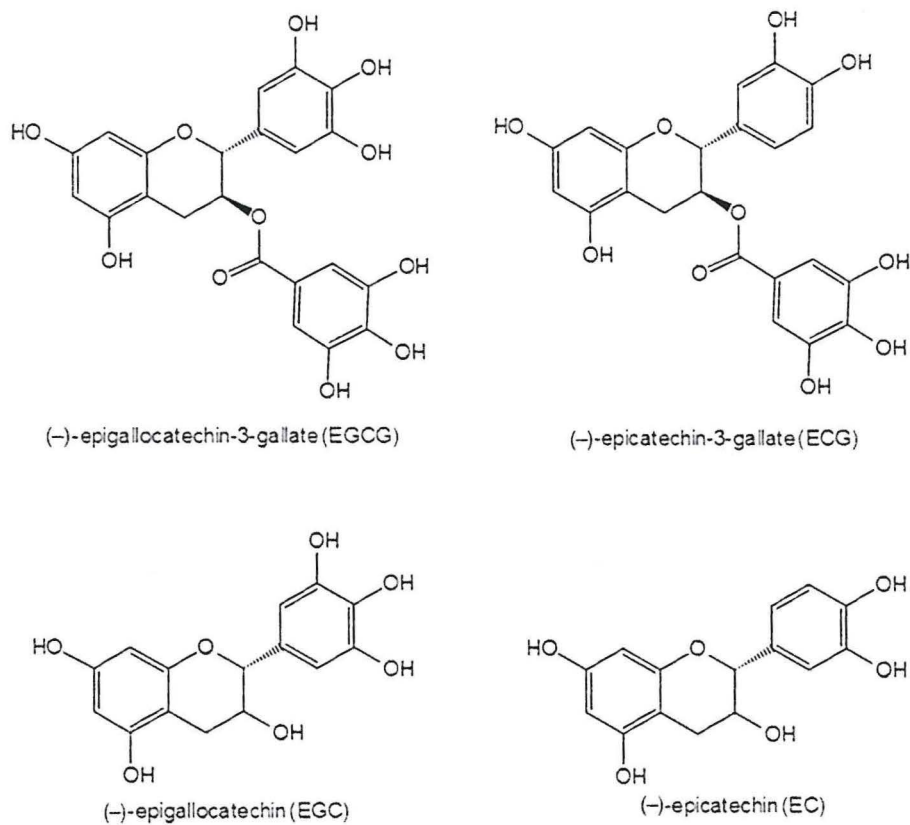


**Figure 2.2:** Classification of phenolic antioxidants Source: Shahidi & Ambigaipalan (2015).



**Figure 2.3:** Chemical structure of flavonoids; Source: Panche, Diwan, & Chandra (2016).

The major and most studied tea flavonoid is the catechin epigallocatechin-3-gallate (EGCG) (Nagle, Ferreira, & Zhou, 2007; Singh, Shankar, & Srivastava, 2011; Khan & Mukhtar, 2019; Bae, Kim, Shin, Kim, & Kim, 2020). Epicatechin-3-gallate (ECG), epigallocatechin (EGC), epicatechin (EC) and catechin are other catechins present in low quantities (Figure 2.4).



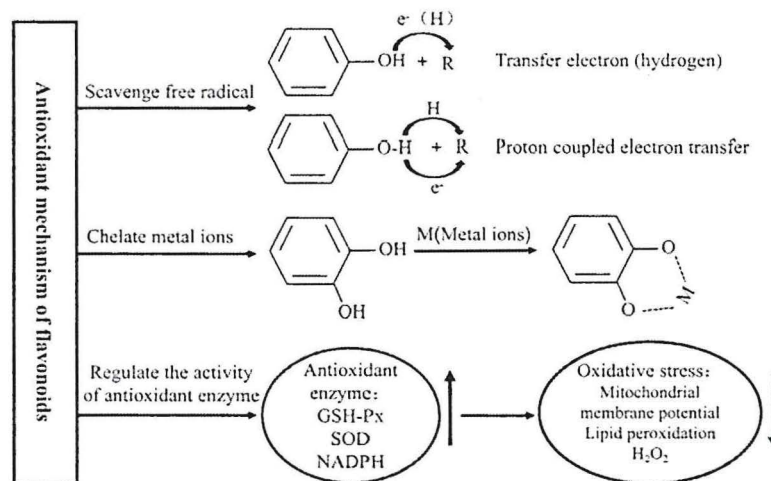
**Figure 2.4:** Major tea catechins

**Source:** Yan, Zhong, Duan, Chen, & Li (2020)

Epigallocatechin-3-gallate is reported to have anti-viral properties (Steinmann, Buer, Pietschmann, & Steinmann, 2013). Different modes of the antiviral action of EGCG have been demonstrated against viruses such as Retroviridae, Orthomyxoviridae and Flaviviridae, hepatitis B, hepatitis C and *Bacillus anthracis* (Steinmann, Buer, Pietschmann, & Steinmann, 2013; Falcinelli, Shi, Friedlander, & Chua, 2017). Further, by activating the synthesis of collagen and inhibiting the production of matrix metalloproteinase enzymes, catechins directly affect the human skin (Bae, Kim, Shin, Kim, & Kim, 2020). The distribution of the catechins within a shoot varies with the age of the leaves (Turkmen, Sarı, & Velioglu, 2009).

## 2.7 Mechanism of the antioxidant effect of tea polyphenols on human health

Tea polyphenols scavenge free radicals and regulate many oxidase enzymes thus reducing the extent of cellular and DNA damage (Yan, Zhong, Duan, Chen, & Li, 2020; Lv *et al.*, 2021). They also reduce the risk of many chronic diseases by preventing inflammation triggered by oxidative stress. Tea polyphenols inhibit oxidation due to catalysis by metal ions for example  $\text{Ca}^{2+}$  and  $\text{Fe}^{2+}$  by chelating the cations thereby reducing on the amount of free radical formed in the body (Yan, Zhong, Duan, Chen, & Li, 2020). Besides, polyphenols retain their key structural feature after ingestion and metabolism in the body. These mechanisms enable polyphenols to protect and prevent humans against cardiovascular diseases (CVDs), cancer and diabetes. Figure 2.5 shows the general mechanism for the antioxidant activity of tea flavonoids (Lv *et al.*, 2021).



**Figure 2.5:** Antioxidant mechanism of tea flavonoids.

## 2.8 Factors that influence tea quality

Tea quality hinges on the content of characteristic organoleptic components viz. amino acids, organic acids, sugars, lipids, as well as some bioactive compounds such as polyphenols. The amino acid theanine is very important in determining tea quality (Ruan *et al.*, 2019).

Polyphenols are important in determining the colour, flavour and brightness of tea (Turkmen, Sari, & Velioglu, 2009). Most tea in Uganda is classified as “Plainer” and fewer than six tea estates are categorized as “Lower medium” teas (Lakuma, Ezra, & Guloba, 2014). There is a 30% price difference between lower medium and good medium tea from Uganda and Kenyan, respectively. There is need to enforce internationally accepted standards along the tea value chain in Uganda so as to improve on the tea quality considering that tea price is dependent on the quality (Lakuma, Ezra, & Guloba, 2014).

## **2.9 Chemical food safety of tea**

Tea quality and safety are major aspects of consumer demand in the international market. According to the European Tea Committee (ETC), natural constituents of tea do not present any safety risks requiring control measures (ETC, 2014). Nonetheless, tea may be contaminated with environmental contaminants such as pesticide residues, dioxins, polycyclic aromatic hydrocarbons (PAH) and HM, adulterants such as the ant-slimming drug sibutramine and residues from fumigation (Zachara, Gałkowska, & Juszczak, 2018; Rivera-Pérez, Romero-González, & Frenich, 2021). Contamination can also occur during transportation and handling, and from the tea processing machinery. Environmental pollution results in increased levels of HM from various sources such as industrial wastes and emissions from nearby road traffic. Lead, Cd and Fe are primary metal contaminants in tea, and originate from agricultural practices and pressing procedures (Rivera-Pérez, Romero-González, & Frenich, 2021). Excessive Pb in tea has been the focus of tea safety worldwide, with varying permissible limits (PL) among different countries. China, EU and UK set the PL at 5, Australia, Canada and India at 10, and Japan at 20 mg/kg (Yu, Sun, & He, 2020).

Improper roasting can also lead to the contamination of the final product with polycyclic aromatic hydrocarbons (PAH) (Zachara, Gałkowska, & Juszcak, 2018). The PAH are a diverse group of compounds comprising more than two rings fused together in various configurations. Sixteen PAH are regularly found in environmental samples and are the candidates for monitoring for safety (Zelinkova & Wenzl, 2015). They include naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, chrysene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene (B[*a*]P), indeno[1,2,3-*cd*]pyrene, benzo[*g,h,i*]perylene, and dibenz[*a,h*]anthracene. The PAH are cytotoxic, immunotoxic, genotoxic, teratogenic and carcinogenic yet they are well absorbed in the gastrointestinal tract (GIT) especially in high fat diets (Abdel-Shafy & Mansour, 2016). B[*a*]P is carcinogenic to humans and is used as the indicator for PAH exposure. Teas made from *C.sinensis* tend to be contaminated with PAH but black tea is reported to contain higher levels of B[*a*]P (Ciemniak, Kuz'micz, Rajkowska-Mys'liwiec, & Cadena, 2019).

## CHAPTER 3: MATERIALS AND METHODS

### 3.1 Materials

Tea leaves of different brands were purchased from six major supermarkets in Kampala city. Chemicals used were of analytical grade. Sulphuric acid ( $\text{H}_2\text{SO}_4$ ; 98%), sodium hydroxide ( $\text{NaOH}$ ;  $\geq 98\%$ ) anhydrous pellets, Boric acid ( $\text{H}_3\text{BO}_3$ ; 99.95%), hydrochloric acid ( $\text{HCl}$ ; 37%), copper(II) sulphate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ;  $> 98\%$ ), petroleum ether (PE; boiling range 60-80°C) and phosphate buffer pH 4 and 7 were obtained from Sigma-Aldrich (The Federal Republic of Germany). The Reagecon ICP, ICP-MS, ICP-OES Multi Element Standard (1000  $\mu\text{g/l}$ ) in 2-5% Nitric Acid ( $\text{HNO}_3$ ) were obtained from Reagecon Diagnostics Limited (Ireland). The Multi Element Standards were used in the quantification of Pb, Cd, As, Hg, K, Mg, Al, Cu, Mn and Fe in the tea. Phosphate buffered saline (PBS; tablet) was to prepare the tea extract, while methanol ( $\text{CH}_3\text{OH}$ ), 10% Folin–Ciocalteu reagent, sodium hydrogen carbonate ( $\text{NaHCO}_3$ ; 7.5%), aluminium chloride hexahydrate ( $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ ; 99%), sodium nitrite ( $\text{NaNO}_2$ ; 98%) and gallic acid (GA) used to determine total polyphenolic content (TPC) were also purchased from Sigma-Aldrich.

### 3.2 Equipment

The K, Na, Fe, Pb, Cd, As, Hg, Mg, Al, Cu and Mn concentration in the tea leaves was determined by atomic absorption spectrophotometry (AAS, AA6800, Shimadzu (Japan)). The machine has a deuterium lamp for background correction, an autosampler, and is equipped with a data processing software. The hot air oven (Memmert UFE-600; Germany) was used to dry the samples. Weights were taken on a AA-200DS Mettler-Toledo (Germany) analytical balance. Polyphenol and flavonoid content was determined on a Shimadzu UV-1800 UV-Spectrophotometer (Japan).

### **3.3 Methods**

#### **3.3.1 Sampling and sample treatment**

A total of thirty (n=30; Green=10 and Black=20) local and imported tea samples were purchased as granular powder of the particular brand from supermarkets; MS in Kampala Central, CS in Ntinda, PB in Nakawa Division, JR in Makindye Division, KS in Kawempe Division and KJ in Lubaga Division (Table 3.1). For each tea type and brand, 3 samples were obtained from each of the supermarkets. Podwika, Kleszcz, & Krośniak (2018), Pradhan & Dubey (2020) and Zhou *et al.* (2022) analyzed a total of 27 samples each. Tea leaves were packaged in labeled bags and transported to the Uganda National Bureau of Standards (UNBS) chemistry laboratory for analysis. At the laboratory, the samples were composited to make 30 laboratory samples and the composites oven dried at 105°C for 6 h. The dry samples were stored under cool dry conditions till analysis.

**Table 3.1:** Nature and origin of tea samples used in the study

Tea type	Batch No.	Origin	Sampling date	Manufacture date	Expiry date	Supermarket
G1	10/2021	Uganda	14/9/2021	10/2021	10/2024	JR
G2	3217	Uganda	14/9/2021	5/2021	5/2023	JR
G3	APA	Uganda	15/9/2021	7/2021	12/2022	MS
G4	24/7/ 2021	Rwanda	15/9/2021	7/2021	7/2023	MS
G5	12/6/2020	Rwanda	15/9/2021	6/2020	6/2023	MS
G6	A1055	Kenya	28/11/2021	8/2021	8/2024	KJ
G7	10/11/2020	China	28/10/2021	11/2020	11/2022	KJ
G8	L0049	U.K	14/9/2021	2/2021	2/2023	CS
G9	52	Kenya	14/9/2021	5/2021	5/2023	CS
G10	RCL0721	Uganda	14/9/2021	7/2021	7/2023	CS
B1	C21	Uganda	15/9/2021	9/2021	9/2023	KS
B2	RCL 0221	Uganda	15/9/2021	2/2021	2/2023	KS
B3	3/2021	Uganda	15/9/2021	3/2021	3/2024	KS
B4	30/3/2021	Uganda	21/9/2021	3/2021	3/2023	PB
B5	2021204	Uganda	21/9/2021	9/2021	9/2023	PB
B6	02/11/21	Uganda	14/9/2021	11/2021	10/2023	CS
B7	23	Kenya	14/9/2021	2/2021	2/2024	CS
B8	EUCHU	Uganda	15/9/2021	11/2020	11/2022	KS
B9	16/9/2021	Uganda	15/9/2021	9/2021	9/2022	KS
B10	RCL052021	Uganda	15/9/2021	4/2021	4/2023	KS
B11	02/1/2020	Uganda	15/9/2021	1/2020	11/2022	MS
B12	03. 2021	Uganda	15/9/2021	3/2021	4/2023	MS
B13	8/2021	Uganda	28/10/2021	8/2021	8/2023	JS
B14	1/2021	Uganda	28/10/2021	1/2021	1/2023	JS
B15	04:13:52	Kenya	28/10/2021	5/2021	5/2024	JS
B16	1/2021	Uganda	28/10/2021	1/2021	12/2022	PS
B17	9/2021	Uganda	28/10/2021	9/2021	9/2023	PS
B18	5/5/2021	Uganda	15/9/2021	5/2021	5/2023	KS
B19	7/2021	Uganda	15/9/2021	7/2021	7/2024	KS
B20	2/2020	Uganda	15/9/2021	2/2020	2/2022	KS

B: black tea; G: green tea.

### **3.3.2 Proximate analysis**

Standard methods described by the Association of Official Analytical Chemists (AOAC) were used for proximate analysis (AOAC, 2019). The oven drying method (AOAC Official Method No. 930.15) was used to determine the moisture content. Kjeldahl's method (AOAC Official Method No. 920.103) was used to determine the crude protein. Total fat was determined in a Soxhlet apparatus (AOAC Official Method No. 930.09) using PE as extraction solvent. Total ash was determined by incinerating the sample in a muffle furnace at 550°C (AOAC Official Method No. 920.100). Crude fiber content was determined as per the procedure described in AOAC Official Method No. 985.29 (AOAC, 2019). The carbohydrate content was determined by difference, i.e., the weight after subtracting the amounts of moisture, protein, fat, crude fibre and ash found by analysis.

### **3.3.3 Determination of metal amounts in tea leaves**

Samples were digested following the method described by Usman, Al-Ghouti, & Abu-Dieyeh (2019). The equipment was calibrated using standards prepared from multi-element standard stock solutions (1000 ppm) supplied by Spex<sup>®</sup>. Standard solutions of the metals were prepared at 0.05, 0.5, 1.0, 2.0 and 4.0 ppm. The calibration curves summarized in Table 3.2 were generated.

**Table 3.2:** Calibration data used in the quantification of metal elements by Atomic Absorption Spectrophotometry

Element	Wavelength (nm)	Concentration range (mg/L)	Equation of the line	R <sup>2</sup>
K	766.5	0.0035-0.0698	$y = 0.0074x - 0.0023$	0.9939
Na	589.0	0.2332-1.990	$y = 0.1918x - 0.0059$	0.9879
Mg	285.2	0.1620-9.9840	$y = 0.9821x + 0.1804$	0.9997
Ca	422.7	0.1729-0.8910	$y = 0.0363x - 0.0087$	0.9992
Cu	324.8	0.0011-0.3810	$y = 0.0956x - 0.0068$	0.9991
Fe	248.3	0.0103-0.3893	$y = 0.0971x + 0.0021$	0.9999
Mn	279.5	0.0221-0.8001	$y = 0.1989x + 0.0023$	0.9998
Pb	283.3	0.00176-0.0678	$y = 0.017x + 0.0003$	0.9994
Cd	228.8	0.0035-0.1559	$y = 0.1544x + 0.0017$	0.9999
As	193.7	0.055-0.30	$y = 0.0709x + 0.0131$	0.9970
Hg	253.7	0.003-0.210	$y = 0.0266x - 0.0008$	0.9981

The tea (5 g) was carbonized by heating on a hotplate then ashed at 450°C for 4 h. To the resulting white ash was added HNO<sub>3</sub> (10%; 10 mL) and the mixture warmed to dissolve completely. The digest was filtered in a 50 mL volumetric flask and topped up to the mark with deionized water. The absorbances of the elements; Pb, Cd, As, Hg, K, Na, Mg, Al, Cu, Mn and Fe were determined following direct aspiration into the AAS. The absorbances were used to determine the metal concentrations (mg/kg) of the different tea samples from the calibration curves.

### **3.3.4 Determination of polyphenols in the tea leaves**

#### **3.3.4.1 Preparation of tea extract**

Tea extracts were prepared using the method of Hossain, Nibir, Zerin, & Ahsan, (2014) and Nibir, Sumit, Akhand, Ahsan, & Hossain (2017). The tea powder (50 g) was boiled in 300 mL distilled water for 45 min followed by filtering on a Whatman No.1 filter paper. The filtrate was in each case freeze dried and the dry powder obtained stored at 4°C. A working solution of 400 mg/mL was prepared by dissolving the powder in PBS whenever required (Nibir, Sumit, Akhand, Ahsan, & Hossain, 2017).

#### **3.3.4.2 Determination of total polyphenol**

The total polyphenol content was determined following the method described by Nibir, Sumit, Akhand, Ahsan, & Hossain (2017). In this method, total polyphenol content is determined against Gallic acid (GA) as standard using spectrophotometry. The CH<sub>3</sub>OH extract (1 mg/mL; 0.5 mL) was mixed with Folin-Ciocalteu (10%; 2.5 mL) reagent dissolved in distilled water and NaHCO<sub>3</sub> (7.5%; 2.5 mL) in a 10 mL test-tube and the mixture vortexed. The resultant mixture was allowed to stand at room temperature for 60 min. The absorbance (A) of the tea extract was determined against water at  $\lambda_{\text{max}} = 765 \text{ nm}$  and from this absorbance, the total polyphenol content was determined. A blank was prepared with methanol instead of extract solution. The procedure was repeated for the gallic acid and a standard curve generated (Appendix XII). The total polyphenol content was expressed as Gallic acid equivalents (GAE) in mg of GA/g of tea extract.

### **3.3.4.3 Determination of the total flavonoid content**

Total flavonoid content of the aqueous tea extract was spectrophotometrically determined against rutin as standard using aluminum chloride colorimetric assay. The tea extract (30 mg/mL; 1 mL) was dissolved in AlCl<sub>3</sub> (10%; 0.3 mL) and NaNO<sub>2</sub> (5%; 0.3 mL) followed by NaOH (1M; 200 mL). The mixture was incubated at room temperature for 1 h. The  $\lambda$  of the solution was measured against water at  $\lambda_{\text{max}}=510$  nm. A standard curve (Appendix XIII) was generated following the same procedure. The total flavonoid content was determined from the standard curve and the results expressed as rutin equivalents in mg/g of tea extract.

### **3.4 Statistical analysis**

All experiments were done in triplicate ( $n = 3$ ) and results expressed as the mean  $\pm$  SD of the replicates. Results of proximate composition and trace metals in the samples were subjected to multiple comparisons using one-way analysis of variance (one-way ANOVA) followed by Tukey post hoc test. Data on total polyphenol and total flavonoid content were subjected to paired  $t$  test to compare the means, and the relationship between the two examined using Pearson's bivariate correlation analysis. All statistical analyses were executed using Minitab Statistical Software (v21.0, Minitab Inc., USA). Means were considered significantly different at  $p < 0.05$ .

## CHAPTER 4: RESULTS AND DISCUSSION

### 4.1 Proximate composition of tea

#### 4.1.1 Moisture

Moisture content of selected brands of green and black teas from supermarkets in Kampala city, Uganda ranged from 2.64 to 8.43% (Table 4.1). Overall, green tea had higher ( $p < 0.05$ ) moisture contents than the black tea. Majority (86.7%) of the tea had moisture content greater than 4%. The remaining (13.3%) samples were black teas with moisture content falling between 2.64 and 3.98%. The moisture content values obtained for teas in this study are comparable to the values of 2.46 to 7.47% reported by Adnan *et al.* (2013). According to Dmowski and Ruszkowska (2018), the moisture content of black tea should not be less than 2% to avoid loss of its essential oils through volatilization. However, it should not go beyond 9% to avoid the growth of mold. Moisture content of tea leaves from Kampala were within the recommended limits (US EAS 28:2019).

#### 4.1.2 Lipid

Lipid content of the green and black teas were in the range of 1.31 to 2.82% and 0.87 to 3.74%, respectively. Studies on the proximate composition of teas reported lipid contents between 1.3 and 16.2% (Adnan *et al.*, 2013; Abbasi *et al.*, 2022). However, according to Ifemeje *et al.* (2020), the lipid content of tea should range between 0.95 and 1.62% to avoid deterioration of its flavor when the lipids degrade (Chen *et al.*, 2022). The results of this study showed that 14 out of 30 samples (46.7%) had a total fat content outside the recommended limits (Table 4.1).

**Table 4.1:** Proximate composition of green and black tea in Kampala

Sample	Moisture	Total fat	Crude protein	Ash	Crude fibre	Carbohydrate
G1	6.97±0.11 <sup>efg</sup>	1.39 ±0.05 <sup>kl</sup>	17.99±0.33 <sup>kl</sup>	5.76±0.29 <sup>b</sup>	12.12±0.03 <sup>m</sup>	55.77±0.71 <sup>c</sup>
G2	5.16±0.06 <sup>kl</sup>	1.31±0.00 <sup>kl</sup>	13.23±0.01 <sup>j</sup>	5.82±0.20 <sup>b</sup>	13.36±0.14 <sup>kl</sup>	61.13±0.17 <sup>a</sup>
G3	8.23±0.10 <sup>ab</sup>	2.82±0.11 <sup>b</sup>	19.77±0.07 <sup>b</sup>	9.08±2.74 <sup>a</sup>	31.46±0.34 <sup>a</sup>	28.65±2.23 <sup>m</sup>
G4	4.31±0.04 <sup>mn</sup>	1.56±0.00 <sup>ijk</sup>	17.80±0.75 <sup>ijk</sup>	6.45±3.64 <sup>ab</sup>	15.34±0.13 <sup>ghijk</sup>	54.54±4.03 <sup>dc</sup>
G5	7.05±0.09 <sup>defg</sup>	1.42±0.13 <sup>kl</sup>	20.07±0.11 <sup>fe</sup>	4.34±0.19 <sup>b</sup>	14.55±0.09 <sup>ijkl</sup>	52.58±0.19 <sup>efg</sup>
G6	5.18±0.01 <sup>ijkl</sup>	1.38±0.09 <sup>ijkl</sup>	15.25±0.06 <sup>ghi</sup>	5.41±0.80 <sup>b</sup>	13.80±0.20 <sup>ijkl</sup>	58.98±0.63 <sup>b</sup>
G7	8.26±0.07 <sup>ab</sup>	2.21±0.04 <sup>cd</sup>	16.72±0.14 <sup>ghi</sup>	5.39±0.40 <sup>b</sup>	22.50±0.35 <sup>b</sup>	44.92±0.80 <sup>ijkl</sup>
G8	7.22±0.05 <sup>cdef</sup>	1.63±0.05 <sup>ghij</sup>	17.39±0.05 <sup>ghi</sup>	5.01±0.07 <sup>b</sup>	23.54±0.03 <sup>b</sup>	45.23±0.06 <sup>ijkl</sup>
G9	5.73±0.11 <sup>hij</sup>	1.49±0.03 <sup>ijk</sup>	18.14±0.36 <sup>ghi</sup>	5.64±0.59 <sup>b</sup>	15.32±0.21 <sup>ghijk</sup>	53.69±0.95 <sup>def</sup>
G10	6.50±0.07 <sup>gh</sup>	1.58±0.01 <sup>ghijk</sup>	17.23±0.22 <sup>ghi</sup>	5.32±0.02 <sup>b</sup>	14.76±0.14 <sup>ijkl</sup>	54.32±0.72 <sup>def</sup>
B1	7.69±0.04 <sup>bc</sup>	1.57±0.07 <sup>ghijk</sup>	26.49±1.32 <sup>b</sup>	5.66±0.03 <sup>b</sup>	15.57±0.14 <sup>ghij</sup>	43.03±1.23 <sup>kl</sup>
B2	5.94±0.04 <sup>hi</sup>	1.42±0.04 <sup>ijkl</sup>	27.96±1.13 <sup>b</sup>	5.91±0.03 <sup>b</sup>	16.51±0.58 <sup>ghij</sup>	42.26±0.80 <sup>kl</sup>
B3	6.12±0.08 <sup>ab</sup>	1.18±0.07 <sup>l</sup>	12.41±0.70 <sup>k</sup>	5.31±0.02 <sup>b</sup>	15.22±0.56 <sup>ijk</sup>	59.76±0.91 <sup>b</sup>
B4	2.64±0.08 <sup>o</sup>	2.41±0.07 <sup>c</sup>	25.71±0.87 <sup>c</sup>	5.36±0.00 <sup>b</sup>	17.55±0.19 <sup>def</sup>	46.33±1.08 <sup>ijk</sup>
B5	7.52±0.11 <sup>cde</sup>	1.46±0.11 <sup>ijkl</sup>	29.28±0.45 <sup>a</sup>	5.32±0.02 <sup>b</sup>	14.67±0.15 <sup>ijkl</sup>	41.75±0.32 <sup>l</sup>
B6	5.14±0.12 <sup>kl</sup>	1.97±0.01 <sup>def</sup>	22.16±0.45 <sup>dc</sup>	6.47±0.00 <sup>ab</sup>	18.92±0.15 <sup>dc</sup>	45.35±0.43 <sup>ijk</sup>
B7	7.16±0.05 <sup>cdef</sup>	1.61±0.03 <sup>ghij</sup>	17.83±0.35 <sup>ghi</sup>	5.19±0.05 <sup>b</sup>	17.88±0.07 <sup>def</sup>	50.34±0.25 <sup>ghij</sup>
B8	2.87±0.06 <sup>o</sup>	0.87±0.04 <sup>m</sup>	17.88±0.07 <sup>ghi</sup>	5.68±0.12 <sup>b</sup>	12.87±0.32 <sup>l</sup>	59.85±0.34 <sup>b</sup>
B9	4.89±0.11 <sup>lm</sup>	1.58±0.08 <sup>ghijk</sup>	19.91±0.06 <sup>g</sup>	5.14±0.07 <sup>b</sup>	17.12±0.34 <sup>efgh</sup>	51.36±0.35 <sup>fghi</sup>
B10	7.00±0.03 <sup>efg</sup>	1.96±0.03 <sup>def</sup>	20.23±0.50 <sup>fe</sup>	5.35±0.06 <sup>b</sup>	20.24±0.11 <sup>cd</sup>	45.23±0.46 <sup>ijkl</sup>
B11	8.36±0.09 <sup>a</sup>	2.03±0.00 <sup>dc</sup>	21.18±0.81 <sup>ed</sup>	6.37±0.03 <sup>ab</sup>	16.44±0.12 <sup>ghij</sup>	45.61±0.89 <sup>ijk</sup>
B12	6.80±0.11 <sup>fg</sup>	1.84±0.00 <sup>efg</sup>	19.14±0.07 <sup>ghi</sup>	6.05±0.02 <sup>b</sup>	17.67±1.48 <sup>def</sup>	48.50±1.58 <sup>ghij</sup>
B13	8.43±0.05 <sup>a</sup>	1.66±0.16 <sup>ghij</sup>	21.57±0.16 <sup>def</sup>	5.80±0.35 <sup>b</sup>	15.30±0.54 <sup>ghijk</sup>	47.24±0.86 <sup>ghijk</sup>
B14	5.18±0.03 <sup>kl</sup>	2.73±0.00 <sup>b</sup>	19.68±0.26 <sup>g</sup>	5.56±0.00 <sup>b</sup>	17.53±0.29 <sup>efg</sup>	49.31±0.55 <sup>ghij</sup>
B15	3.97±0.09 <sup>n</sup>	1.50±0.33 <sup>ijk</sup>	21.36±0.14 <sup>cd</sup>	6.49±0.01 <sup>ab</sup>	14.94±0.33 <sup>ijkl</sup>	51.75±0.66 <sup>efgh</sup>
B16	7.38±0.18 <sup>cdef</sup>	1.81±0.04 <sup>efgh</sup>	17.23±0.80 <sup>ghi</sup>	5.64±0.05 <sup>b</sup>	21.59±0.08 <sup>c</sup>	46.34±0.91 <sup>ghijk</sup>
B17	5.39±0.90 <sup>ijkl</sup>	3.74±0.02 <sup>a</sup>	19.67±0.35 <sup>g</sup>	6.03±0.36 <sup>b</sup>	17.12±0.15 <sup>fghi</sup>	48.06±1.25 <sup>ghijk</sup>
B18	7.62±0.16 <sup>cd</sup>	1.71±0.14 <sup>fghi</sup>	20.26±0.05 <sup>ed</sup>	6.49±0.19 <sup>ab</sup>	21.64±0.16 <sup>b</sup>	42.28±0.29 <sup>ijkl</sup>
B19	2.72±0.09 <sup>o</sup>	1.97±0.02 <sup>def</sup>	19.02±0.35 <sup>ghi</sup>	5.85±0.76 <sup>b</sup>	14.98±0.23 <sup>ijk</sup>	55.46±0.63 <sup>cd</sup>
B20	6.06±0.11 <sup>hi</sup>	1.46±0.02 <sup>ijkl</sup>	17.48±0.36 <sup>ghi</sup>	5.84±0.39 <sup>b</sup>	15.12±0.54 <sup>ijk</sup>	54.04±0.70 <sup>def</sup>

Results are presented as mean ± standard deviation of 3 analyses. G: Green tea; B: Black tea. Values with different superscript letters in a column are significantly different (p<0.05).

#### **4.1.3 Crude protein**

Green and black tea had protein content varying from 13.23 to 20.07% and 12.41 to 29.28%, respectively. The differences between the crude protein content of the different tea brands were not significant ( $p > 0.05$ ). Ramdani, Chaudhry, & Seal (2013) and Pradhan & Dubey (2020) reported protein contents of teas at 24.2% and 22.86%, respectively. Similarly, protein contents of 37.015% and 20.215% have been reported in Chinese green and black teas, respectively (Czernicka, Zagula, Bajcar, Saletnik, & Puchalski, 2017). Most of the foregoing reports found proteins in teas at levels that are comparable to the values recorded in this study. The proteinaceous content of tea include enzymes (polyphenol oxidase and peroxidase) and amino acids (majorly L-theanine, L-arginine and L-aspartic acid). The enzymes are required for catechin oxidation and black tea processing while the amino acids are believed to ease anxiety, stress and reduce insomnia (Wong, Sirisena, & Ng, 2022).

#### **4.1.4 Ash content**

The ash content of the tea leaves ranged from 4.34 to 9.08% and 5.14 to 6.49% for green and black tea respectively. Generally, the ash content of black teas ranges between 5 to 6% while that of green tea may be higher (Adnan *et al.*, 2013; Abbasi *et al.*, 2022). High ash content of tea is an indication of its richness in minerals that are essential for human health (Malongane, McGaw, & Mudau, 2020). One sample (G3) had a total ash content above the ISO recommendation.

#### **4.1.5 Crude fiber**

Crude fiber content ranged from 12.12 to 23.54% and 12.87 to 21.64% for green and black teas, respectively. These values are comparable to Adnan *et al.* (2013) who reported crude fiber content of green and black teas to be in the range of 12.75 to 17.21% and 11.23 to 16.33%,

respectively. Fiber is part of plants in form of lignin, cellulose, hemicellulose and pectin that cannot be digested and assimilated by the human gastrointestinal tract. According to ISO 3720:2011, crude fibers in black teas should not be more than 16.5% (ISO, 2021). Ten out of 20 of the black tea samples had crude fiber contents above the recommended content of 16.5%.

Carbohydrates ranged from 28.65 to 61.13% in green tea and 41.75 to 59.85% in black tea. The amount of carbohydrate observed in this study is in line with that reported in black teas from Kenya (Shevchuk, Megías-Pérez, Zemedie, & Kuhnert, 2020). According to Wong, Sirisena, & Ng (2022), the carbohydrate content of green tea leaves comprises cellulose (13.3%), some free sugars (including glucose, fructose, sucrose, raffinose and stachyose) and the rest is lignin. Free sugars are responsible for the synthesis of catechins, formation of heterocyclic flavour compounds during processing of black tea and contributing towards water-soluble solids in tea liquor (Lv *et al.*, 2022). Differences in the composition of tea is attributable to variety, climatic conditions, soil properties, plucking season, position of the leaf, and processing and storage conditions (Lee *et al.*, 2014; Yan *et al.*, 2020; Bobková *et al.*, 2021). Young tea leaves should be used if useful constituents are required in the final processed tea (Rahman *et al.*, 2013).

#### **4.2 Concentration of metals in green and black teas**

Macroelements, also called major elements, are needed in amounts greater than 100 mg per day moreover each of these elements make up more than 0.01% of the human body weight. Sodium, K, Mg, and Ca are macro minerals. Microelements also called minor or trace elements, on the other hand are needed in quantities of less than 100 mg per day. Copper, Fe, and Mn are microelements.

#### 4.2.1 Macro-elements

The highest content of essential metals occurred in black teas as compared to the green teas. Among the macro-elements, K was present at the highest concentrations ranging from 694.95 to 2,207.83 mg/kg in green tea and 911.91 to 2,681.83 mg/kg in black teas (Table 4.2). In general, *C.sinensis* accumulates high concentrations of K evidenced in different reports from around the world. Bamuwanye *et al.* (2017) reported mean concentrations of K of 14,740.41 mg/kg, 10,003.69 mg/kg and 9,969.74 mg/kg in locally branded, unbranded and imported teas in Kampala city. Fernández-Cáceres *et al.* (2001) reported K mean concentration of 15,145.4 mg/kg in green, black and instant teas from China, Japan, India, Kenya and Sri Lanka. Potassium is required for translocation of water, nutrients and carbohydrates in plant tissues, and in photosynthesis (Sardans & Peñuelas, 2021). Potassium and Mg fertilizer application was observed to increase the contents of free amino acids and caffeine of various tea types with the maximum increase found in the treatment including both nutrients (Ruan, Wu, & Hårdter, 1999). The content of polyphenols in tea leaves is also increased by K application. Based on the ISO recommendations, black and green tea leaves were within the limit

Sodium ranged from 6.20 to 11.47 mg/kg in green tea and 4.53 to 11.77 mg/kg in black tea. The observed Na concentration is lower than that reported by Bamuwanye *et al.* (2017) for imported, locally branded and unbranded teas in Kampala. According to Islam & Ebihara (2017), Na, Fe and Zn are present in tea leaves in the range of 3 to 58 mg/kg. Although Na is a major mineral in animals it is found in low amounts in many plants (Adeyeye & Oyarekua, 2015).

Magnesium was present at concentrations ranging from 17.51 to 29.20 mg/kg and 25.62 to 32.59 mg/kg in green and black tea, respectively. Magnesium concentration was lower than

that reported in black tea in Japan, and green and black tea from China, Japan, India, Kenya and Sri Lanka (Fernández-Cáceres *et al.*, 2001). Magnesium forms the core of the chlorophyll that is required for photosynthesis in plants and is an important cofactor of many enzymes (Sardans, & Peñuelas, 2021). Similarly in humans, Mg acts as a cofactor for more than 300 enzymes, regulating a number of fundamental functions such as muscle contraction, neuromuscular conduction, glycemic control, myocardial contraction, and blood pressure (Al-Alawi, Majoni, & Falhammar, 2018).

Calcium concentration ranged from 39.77 to 191.75 mg/kg and 74.48 to 280.94 mg/kg correspondingly in green and black tea. A lower concentration of Ca was observed in the teas of this study than in previous studies (Fernández-Cáceres *et al.*, 2001). Calcium is an essential macronutrient in plants, with concentrations in the shoot ranging from 0.1 to over 5% of dry weight (Thor, 2019). It serves both as a structural component of cell walls and membranes and as intracellular second messenger. In humans, Ca is essential for muscle contraction, oocyte activation, building strong bones and teeth, blood clotting, nerve impulse transmission, regulation of heartbeat and fluid balance within cells (Pravina, Sayaji, & Avinash, 2013).

**Table 4.2:** Concentration of bio-elements and toxic metals (mg/kg) in green and black tea obtained from supermarkets in Kampala city

Sample	K	Na	Mg	Ca	Cu	Fe	Mn	Pb	Cd	As	Hg
G1	2207.83 ± 2.97 <sup>b</sup>	11.47 ± 0.15 <sup>c</sup>	18.24 ± 0.14 <sup>l</sup>	46.19 ± 0.22 <sup>l</sup>	1.34 ± 0.01 <sup>kl</sup>	8.46 ± 0.31 <sup>l</sup>	141.17 ± 1.01 <sup>a</sup>	ND	ND	ND	ND
G2	1502.71 ± 0.54 <sup>cd</sup>	10.73 ± 0.12 <sup>cd</sup>	29.20 ± 0.2 <sup>ghij</sup>	67.84 ± 0.14 <sup>kl</sup>	2.28 ± 0.02 <sup>a</sup>	11.22 ± 0.18 <sup>ijkl</sup>	92.13 ± 0.31 <sup>def</sup>	ND	ND	ND	ND
G3	758.36 ± 2.90 <sup>kl</sup>	6.40 ± 0.10 <sup>ijkl</sup>	17.51 ± 0.40 <sup>m</sup>	39.77 ± 0.25 <sup>m</sup>	1.61 ± 0.02 <sup>ghijk</sup>	33.72 ± 0.17 <sup>c</sup>	62.57 ± 0.25 <sup>ijkl</sup>	ND	ND	ND	ND
G4	847.33 ± 2.75 <sup>ijkl</sup>	6.60 ± 0.20 <sup>ijkl</sup>	22.26 ± 0.22 <sup>ijkl</sup>	82.75 ± 0.23 <sup>ijkl</sup>	1.58 ± 0.03 <sup>ijk</sup>	10.62 ± 0.20 <sup>kl</sup>	67.67 ± 0.51 <sup>ijkl</sup>	ND	ND	ND	ND
G5	694.95 ± 2.85 <sup>m</sup>	6.20 ± 0.10 <sup>ijkl</sup>	25.40 ± 0.36 <sup>ijkl</sup>	191.75 ± 0.25 <sup>cd</sup>	1.26 ± 0.03 <sup>l</sup>	8.30 ± 0.20 <sup>m</sup>	47.60 ± 0.36 <sup>m</sup>	ND	ND	ND	ND
G6	1432.63 ± 0.48 <sup>de</sup>	8.80 ± 0.10 <sup>ghij</sup>	26.33 ± 0.32 <sup>ijk</sup>	113.84 ± 0.15 <sup>ijkl</sup>	1.51 ± 0.02 <sup>ijkl</sup>	23.76 ± 0.21 <sup>ghijk</sup>	78.47 ± 0.35 <sup>ghijk</sup>	ND	ND	ND	ND
G7	935.49 ± 0.29 <sup>ijkl</sup>	7.13 ± 0.15 <sup>ijkl</sup>	24.62 ± 0.20 <sup>ijkl</sup>	104.38 ± 0.22 <sup>ijkl</sup>	1.60 ± 0.02 <sup>hijk</sup>	11.61 ± 0.22 <sup>ijkl</sup>	61.80 ± 0.40 <sup>ijkl</sup>	ND	ND	ND	ND
G8	821.69 ± 1.42 <sup>ijkl</sup>	7.43 ± 0.15 <sup>ijk</sup>	19.32 ± 0.25 <sup>ijkl</sup>	118.65 ± 0.63 <sup>ijk</sup>	1.48 ± 0.03 <sup>ijkl</sup>	13.64 ± 0.16 <sup>ijkl</sup>	58.43 ± 0.35 <sup>ijkl</sup>	ND	ND	ND	ND
G9	711.64 ± 0.55 <sup>l</sup>	6.70 ± 0.10 <sup>ijkl</sup>	18.56 ± 0.19 <sup>kl</sup>	127.82 ± 0.50 <sup>ijk</sup>	1.18 ± 0.02 <sup>m</sup>	29.35 ± 0.22 <sup>i</sup>	55.40 ± 0.36 <sup>kl</sup>	ND	ND	ND	ND
G10	856.69 ± 1.47 <sup>ijkl</sup>	6.87 ± 0.06 <sup>ijkl</sup>	23.41 ± 0.36 <sup>ijkl</sup>	165.42 ± 0.47 <sup>def</sup>	1.56 ± 0.04 <sup>ghijk</sup>	15.42 ± 0.16 <sup>ijkl</sup>	60.47 ± 0.31 <sup>ijkl</sup>	ND	ND	ND	ND
B1	1114.70 ± 0.36 <sup>ghijk</sup>	10.40 ± 0.20 <sup>def</sup>	30.51 ± 0.30 <sup>de</sup>	158.52 ± 0.30 <sup>ghij</sup>	1.86 ± 0.02 <sup>ghijk</sup>	23.71 ± 0.10 <sup>ghijk</sup>	97.70 ± 0.46 <sup>de</sup>	ND	ND	ND	ND
B2	911.91 ± 0.30 <sup>ijkl</sup>	4.53 ± 0.21 <sup>m</sup>	30.39 ± 0.22 <sup>def</sup>	204.47 ± 0.32 <sup>c</sup>	1.84 ± 0.02 <sup>ijkl</sup>	29.61 ± 0.22 <sup>de</sup>	68.70 ± 0.20 <sup>ijkl</sup>	ND	ND	ND	ND
B3	966.70 ± 0.85 <sup>ijk</sup>	8.71 ± 0.22 <sup>ghijk</sup>	27.40 ± 0.20 <sup>hijk</sup>	107.53 ± 0.55 <sup>ijkl</sup>	1.87 ± 0.20 <sup>l</sup>	19.67 ± 0.15 <sup>ijk</sup>	70.53 ± 0.25 <sup>hijk</sup>	ND	ND	ND	ND
B4	1362.73 ± 0.41 <sup>def</sup>	11.71 ± 0.2 <sup>b</sup>	27.87 ± 0.14 <sup>ghijk</sup>	140.71 ± 0.20 <sup>hijk</sup>	1.64 ± 0.02 <sup>c</sup>	21.69 ± 0.20 <sup>hijk</sup>	101.67 ± 0.35 <sup>cd</sup>	ND	ND	ND	ND
B5	1033.87 ± 0.35 <sup>hijk</sup>	8.58 ± 0.24 <sup>ghijk</sup>	28.55 ± 0.15 <sup>ghijk</sup>	142.54 ± 0.39 <sup>ghijk</sup>	1.56 ± 0.03 <sup>ijk</sup>	11.42 ± 0.18 <sup>ijkl</sup>	88.43 ± 0.35 <sup>def</sup>	ND	ND	ND	ND
B6	1622.07 ± 2.00 <sup>c</sup>	9.60 ± 0.20 <sup>def</sup>	30.39 ± 0.21 <sup>efg</sup>	157.24 ± 0.39 <sup>ghij</sup>	1.75 ± 0.02 <sup>efgh</sup>	43.92 ± 0.25 <sup>a</sup>	105.80 ± 0.40 <sup>c</sup>	ND	ND	ND	ND
B7	1245.97 ± 0.21 <sup>efg</sup>	11.62 ± 0.16 <sup>b</sup>	25.62 ± 0.16 <sup>hijkl</sup>	74.48 ± 0.34 <sup>ijkl</sup>	1.66 ± 0.02 <sup>ghij</sup>	25.43 ± 0.36 <sup>ghfghi</sup>	79.43 ± 0.35 <sup>ghij</sup>	ND	ND	ND	ND
B8	1047.63 ± 0.71 <sup>ghijk</sup>	5.61 ± 0.22 <sup>l</sup>	32.42 ± 0.42 <sup>b</sup>	280.94 ± 0.44 <sup>a</sup>	1.64 ± 0.02 <sup>ghijk</sup>	24.76 ± 0.14 <sup>ghijk</sup>	69.37 ± 0.21 <sup>ijk</sup>	ND	ND	ND	ND
B9	2681.83 ± 0.42 <sup>a</sup>	11.77 ± 0.16 <sup>a</sup>	29.61 ± 0.21 <sup>ghij</sup>	174.55 ± 0.27 <sup>de</sup>	1.27 ± 0.05 <sup>kl</sup>	29.45 ± 0.15 <sup>def</sup>	114.53 ± 0.31 <sup>b</sup>	ND	ND	ND	ND
B10	1002.37 ± 0.74 <sup>ijk</sup>	8.87 ± 0.15 <sup>ghij</sup>	30.28 ± 0.11 <sup>fghi</sup>	206.76 ± 0.37 <sup>b</sup>	1.74 ± 0.02 <sup>fghi</sup>	35.52 ± 0.29 <sup>def</sup>	79.83 ± 0.21 <sup>ghij</sup>	ND	ND	ND	ND
B11	1062.94 ± 1.27 <sup>ghijk</sup>	9.23 ± 0.15 <sup>fgh</sup>	30.31 ± 0.30 <sup>efgh</sup>	169.44 ± 0.19 <sup>def</sup>	1.93 ± 0.02 <sup>b</sup>	24.20 ± 0.19 <sup>ghij</sup>	82.37 ± 0.31 <sup>efgh</sup>	ND	ND	ND	ND
B12	1137.57 ± 0.50 <sup>ghij</sup>	9.60 ± 0.20 <sup>def</sup>	32.20 ± 0.21 <sup>b</sup>	159.29 ± 0.92 <sup>fghi</sup>	1.79 ± 0.03 <sup>def</sup>	19.49 ± 0.30 <sup>ijkl</sup>	84.57 ± 0.25 <sup>efg</sup>	ND	ND	ND	ND
B13	1166.32 ± 0.50 <sup>ghij</sup>	8.30 ± 0.20 <sup>ijk</sup>	29.19 ± 0.30 <sup>ghijk</sup>	159.55 ± 0.74 <sup>efgh</sup>	1.86 ± 0.02 <sup>cd</sup>	28.47 ± 0.33 <sup>efg</sup>	80.47 ± 0.35 <sup>fghi</sup>	ND	ND	ND	ND
B14	1167.65 ± 0.45 <sup>fghi</sup>	9.53 ± 0.25 <sup>efg</sup>	30.59 ± 0.32 <sup>cd</sup>	148.83 ± 0.31 <sup>ghijk</sup>	1.67 ± 0.03 <sup>ghij</sup>	21.34 ± 0.29 <sup>ijk</sup>	78.83 ± 0.45 <sup>ghij</sup>	ND	ND	ND	ND
B15	1847.01 ± 1.57 <sup>b</sup>	10.50 ± 0.36 <sup>de</sup>	27.38 ± 0.44 <sup>ijk</sup>	113.51 ± 0.34 <sup>ijkl</sup>	1.49 ± 0.02 <sup>ijkl</sup>	26.37 ± 0.40 <sup>efgh</sup>	121.63 ± 0.35 <sup>b</sup>	ND	ND	ND	ND
B16	948.87 ± 0.76 <sup>ijkl</sup>	7.43 ± 0.35 <sup>ijk</sup>	30.43 ± 0.25 <sup>def</sup>	224.78 ± 0.33 <sup>b</sup>	1.84 ± 0.04 <sup>def</sup>	28.67 ± 0.32 <sup>def</sup>	48.57 ± 0.31 <sup>kl</sup>	ND	ND	ND	ND
B17	1151.64 ± 0.56 <sup>ghij</sup>	8.47 ± 0.31 <sup>hijk</sup>	30.40 ± 0.20 <sup>def</sup>	165.81 ± 0.27 <sup>def</sup>	1.55 ± 0.04 <sup>ijkl</sup>	37.69 ± 0.27 <sup>b</sup>	76.87 ± 0.15 <sup>ghijk</sup>	ND	ND	ND	ND
B18	1323.84 ± 0.29 <sup>def</sup>	9.53 ± 0.15 <sup>efgh</sup>	32.59 ± 0.22 <sup>a</sup>	152.60 ± 0.49 <sup>ghij</sup>	1.77 ± 0.02 <sup>efg</sup>	24.39 ± 0.37 <sup>ghij</sup>	69.47 ± 0.49 <sup>ijk</sup>	ND	ND	ND	ND
B19	1173.73 ± 1.19 <sup>def</sup>	8.73 ± 0.21 <sup>ghij</sup>	29.43 ± 0.16 <sup>ghij</sup>	146.20 ± 0.34 <sup>ghijk</sup>	1.86 ± 0.02 <sup>de</sup>	22.84 ± 0.21 <sup>ghijk</sup>	77.80 ± 0.36 <sup>ghijk</sup>	ND	ND	ND	ND
B20	1397.77 ± 0.34 <sup>def</sup>	9.23 ± 0.15 <sup>ghij</sup>	31.22 ± 0.18 <sup>c</sup>	163.19 ± 0.38 <sup>efg</sup>	1.68 ± 0.03 <sup>ghij</sup>	31.79 ± 0.16 <sup>cd</sup>	86.47 ± 0.35 <sup>def</sup>	ND	ND	ND	ND

Results are presented as mean ± standard deviation of 3 analyses. ND = Not detected. G: Green tea; B: Black tea; K: potassium; Na: sodium; Mg: magnesium; Ca: calcium; Cu: copper; Fe: iron; Mn: manganese; Pb: lead; Cd: cadmium; As: arsenic; Hg: mercury. Values with different superscript letters in a column are significantly different (p<0.05).

#### 4.2.2 Trace elements

The concentration of Cu ranged from 1.18 to 2.28 mg/kg in green tea and 1.27 to 1.93 mg/kg in black tea (Table 4.2). Green and black tea had comparative values. Copper concentration was lower than that reported by Bamuwanye *et al.* (2017). Differences in copper content of teas from different countries are attributable to differences in cultivars, grades and geographical area of production of the tea. Copper is an essential element in tea processing that is pivotal in the functioning of polyphenol oxidase enzyme (Taranto *et al.*, 2017). Polyphenol oxidase enzyme observed in a few cases is associated with the formation of flavour compounds that positively influence the food processing quality in the production of black tea (Taranto *et al.*, 2017). In humans, Cu plays an important role in Fe metabolism but excessive Cu intake may lead to nausea, kidney damage and hemolytic anemia (Bost *et al.*, 2016). The Tolerable Upper Intake Level (UL) of Cu for adults is 10 mg/day, a value based on protection from liver damage as the critical adverse effect (Bost *et al.*, 2016).

The concentration of Fe recorded in the tea ranged from 8.30 to 33.72 mg/kg for green tea and 11.42 to 37.69 mg/kg for black tea. Bamuwanye *et al.* (2017) reported the Fe content of black tea in Uganda to range from 1.1 to 39.89 mg/kg implying that the findings of this study are within range. Iron levels as high as 193.82 mg/kg have also been reported in black tea in Egypt, 56.7 mg/kg in green tea, and 115.6 mg/kg in black tea from China, Nepal, Japan and Korea (Bobková *et al.*, 2017). Iron is an essential micronutrient for almost all living organisms because it plays a critical role in production of haemoglobin in red blood cells and for energy production, metabolic processes such as DNA synthesis and repair, respiration, and photosynthesis (Rout & Sahoo, 2015). Further, many metabolic pathways are activated by iron, and it is a prosthetic group constituent of many enzymes.

Manganese concentration ranged from 47.60 to 141.17 mg/kg for green and black tea. Levels present are generally low compared to most studies. Because of its biochemical importance, Mn has been the most analyzed element in tea leaves (Karak & Bhagat, 2010; Bamuwanye *et al.*, 2017). Tea leaves from most countries have Mn content in the range of 300 to 900 mg kg<sup>-1</sup>, except those from Turkey and Japan where much higher Mn contents (1,100 to 2,678 mg kg<sup>-1</sup>) have been reported (Karak & Bhagat, 2010). Manganese content as high as 2,210 mg/kg has also been observed in tea leaves from Kenya (Podwika, Kleszcz, Krośniak, & Zagrodzki, 2018). The difference in manganese content is attributed to bioaccumulation by the tea leaves. According to Tseng & Lai (2022), Mn hyper accumulation is a characteristic of tea trees. The availability of the element mainly depends on soil pH. Manganese is essential for intracellular activities; it functions as a cofactor for a variety of enzymes, including arginase, glutamine synthetase (GS), pyruvate carboxylase, and Mn superoxide dismutase (Mn-SOD) (Chen, Bornhorst, & Aschne, 2018; Balachandran *et al.*, 2020). It plays important roles in development, digestion, reproduction, antioxidant defense, energy production, immune response, and regulation of neuronal activities. However, human over exposure to Mn can result in a neurological disorder referred to as manganism, presenting symptoms similar to those inherent to Parkinson's disease (Nyarko-Danquah *et al.*, 2020). Excess Mn tends to accumulate in the liver, pancreas, bone, kidney, and brain, with the latter being the major target of Mn intoxication (Chen, Bornhorst, & Aschne, 2018).

#### **4.2.3 Non-essential and toxic elements**

Lead, As, Cd, and Hg were not detected in tea (Table 4.2). Bamuwanye *et al.* (2017) did not detect As in black tea marketed in Kampala city. However, the authors reported Hg concentration in the range below the detection limit (BDL) to 0.35 mg/kg while Pb and Cd ranged from BDL to 8.39 mg/kg. According to the available literature, low levels of toxic

elements are detected in green tea (Karak & Bhagat, 2010). Green tea from India had the lowest Pb and Cd contents (0.010 mg/kg and 0.0003 mg/kg) (Brzezicha-Cirocka *et al.*, 2016). Although tea consumption is good for health, there are concerns associated with drinking tea laden with heavy metals (Nagara, Sarkar, Luo, Biswas, & Datta, 2022). Tea plants are known to be metal accumulators, and contamination could occur during the growth or the manufacturing process (Karak & Bhagat, 2010; Brzezicha-Cirocka, Grembecka, & Szefer, 2016). Low levels of heavy metals in both green and black tea from Kampala could be resulting from low amounts in the environment. Ugandan teas are grown in the southwestern districts of Bushenyi, Buhweju, Kanungu and Kabale; the western districts of Kyenjojo, Kibale, Hoima and Kabarole; the central districts of Mukono, Buikwe, Mubende, Mityana and Masaka are known to have low industrial waste disposal as well as vehicle emissions.

#### **4.3 Total polyphenol and total flavonoid content in green and black teas**

The total polyphenol ranged from 82.17 to 133.10 mg GAE/g DW in black tea and 88.93 to 159.31 mg GAE/g DW in green tea. The total flavonoid content ranged from 122.12 to 177.10 mg RE/g DW in black tea and 43.36 to 183.24 mg RE/g DW in green tea. In general, black tea had a lower total polyphenol content than green tea. A similar trend was observed for the total flavonoid content (Table 4.3). These findings are consistent with those of Bobková *et al.* (2021) who reported the total polyphenol content of green tea to be higher than that of black tea from the same area. Black tea fermentation involves oxidative processes that yield products such as thearubigins and theaflavins. Within the polyphenols, the tea catechins and theaflavins, have been identified as the bioactive phytochemicals of green tea and black tea respectively, and shown to be responsible for their antioxidant activity (Peluso & Serafini, 2017). Differences in total polyphenols and total flavonoids of teas is attributable to the genetic composition, and climatic and soil conditions notably soil nitrogen, light and temperature (Bibi *et al.*, 2022).

**Table 4.3:** Total polyphenol and total flavonoid content of green and black tea

Sample	Total polyphenol content (mg GAE/g)	Total flavonoid content (mg RE/g)
B1	103.43 ± 2.81 <sup>fg</sup>	143.08 ± 1.01 <sup>gh</sup>
B2	112.7 ± 0.51 <sup>de</sup>	167.73 ± 8.58 <sup>ed</sup>
B3	98.71 ± 1.63 <sup>fg</sup>	153.10 ± 0.50 <sup>fg</sup>
B4	84.76 ± 1.52 <sup>jk</sup>	177.10 ± 1.84 <sup>ab</sup>
B5	94.87 ± 0.53 <sup>ghi</sup>	122.12 ± 0.70 <sup>i</sup>
B6	95.69 ± 0.40 <sup>gh</sup>	153.89 ± 1.88 <sup>ef</sup>
B7	133.10 ± 1.15 <sup>b</sup>	160.26 ± 1.37 <sup>de</sup>
B8	121.15 ± 8.66 <sup>c</sup>	171.81 ± 2.85 <sup>bc</sup>
B9	82.17 ± 0.16 <sup>k</sup>	161.63 ± 1.73 <sup>de</sup>
B10	90.27 ± 0.91 <sup>hij</sup>	176.99 ± 0.24 <sup>ab</sup>
G1	159.31 ± 2.89 <sup>a</sup>	43.36 ± 0.47 <sup>l</sup>
G2	153.35 ± 0.47 <sup>ab</sup>	47.05 ± 0.99 <sup>l</sup>
G3	88.93 ± 4.16 <sup>ij</sup>	183.24 ± 0.36 <sup>a</sup>
G4	152.05 ± 3.13 <sup>ab</sup>	102.62 ± 0.58 <sup>j</sup>
G5	133.10 ± 1.15 <sup>b</sup>	160.26 ± 1.37 <sup>ef</sup>
G6	153.89 ± 0.49 <sup>ab</sup>	148.28 ± 6.25 <sup>fg</sup>
G7	116.99 ± 0.53 <sup>d</sup>	50.11 ± 1.71 <sup>l</sup>
G8	106.50 ± 0.77 <sup>ef</sup>	75.59 ± 1.49 <sup>k</sup>
G9	148.07 ± 0.98 <sup>ab</sup>	154.74 ± 2.91 <sup>ef</sup>
G10	151.22 ± 1.02 <sup>ab</sup>	136.35 ± 0.49 <sup>h</sup>

G: Green tea; B: Black tea; Results are presented as mean ± standard deviation of triplicate analyses; mg GAE/g = milligram Gallic Acid Equivalent per gram, mg RE/g = milligram Rutin Equivalent per gram. Values with different superscript letters in a column are significantly different (p<0.05).

Polyphenols are bioactive components that form the core source of dietary antioxidants of tea, and constitute up to 35% of the tea by dry weight (Tong *et al.*, 2019). In vitro and animal studies provide strong evidence that polyphenols derived from tea may possess the bioactivity to affect the pathogenesis of several chronic diseases (Khan & Mukhtar, 2007). As tea fermentation is usually steam-based, this may reduce the catalytic activity of polyphenol oxidase enzyme responsible for production of phenolic compounds in teas. Thus, lower total polyphenol and total flavonoid content in black tea may be inevitable because they are fully fermented teas (Tanaka & Matsuo, 2020).

## CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

### 5.1 Conclusions

The moisture and ash contents were within internationally recommended limits. Lipids, crude protein, crude fiber, and carbohydrates were within the acceptable limits. The presence of the toxic elements; lead, arsenic, cadmium and mercury in tea were generally low and found to be within the safe limits making them fit for human consumption. Green tea is a rich source of macro and trace metals than black tea. Green tea can provide a significant dietary source of polyphenols, and an additional source of minerals.

### 5.2 Recommendations

1. Black and green tea leaves are safe for consumption based on the low levels of lead, arsenic, cadmium and mercury.
2. Green tea leaves could be a better source of nutrients than black tea to regular consumers given their higher mineral content and superior polyphenol amounts. It is important to have characterization study conducted on the polyphenols to identify dominant phytochemicals.
3. In order to ensure a quality final product, young tea leaves should be harvested and processed.

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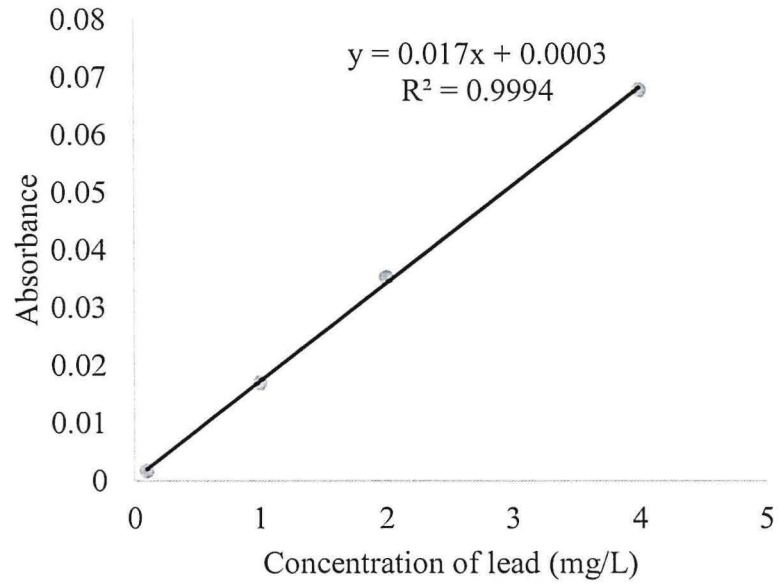
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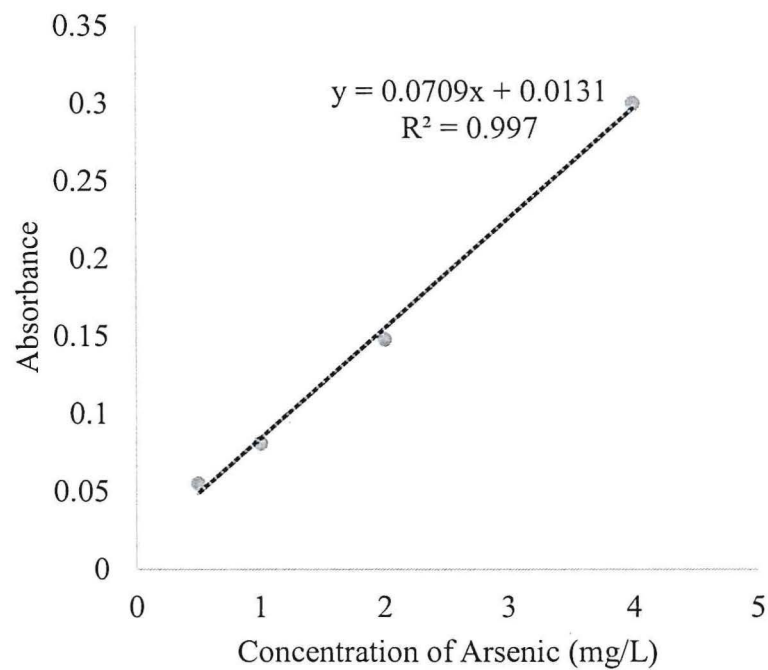
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## APPENDICES

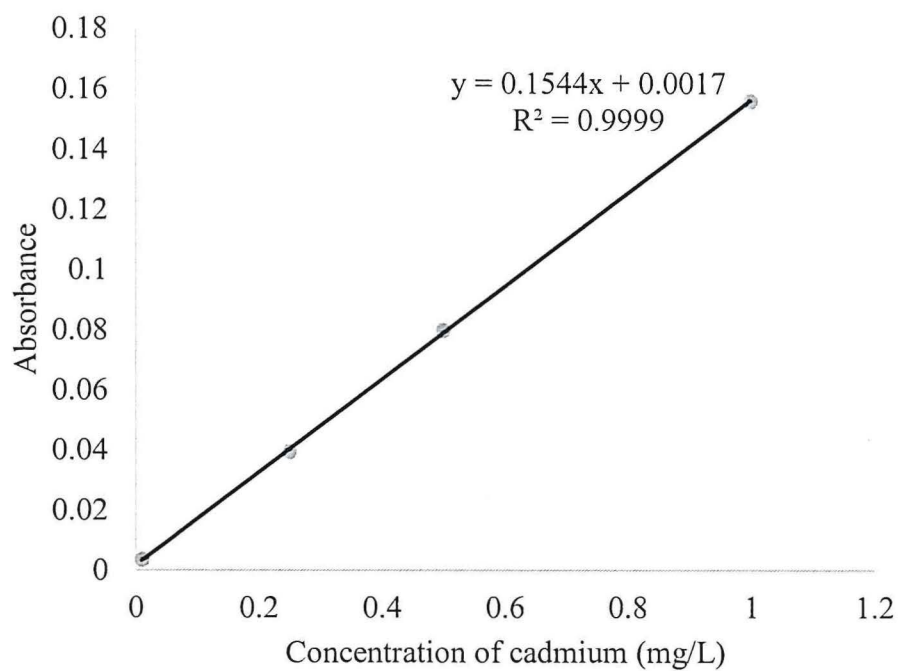
### APPENDIX I: Standard curve used to analyze lead



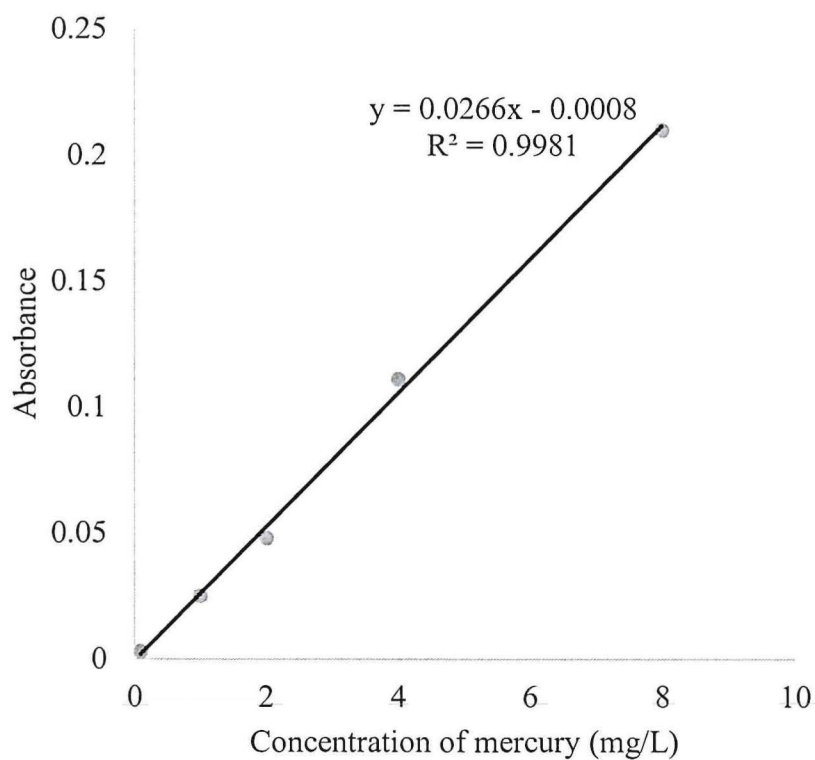
### APPENDIX II: Standard curve used to analyze arsenic



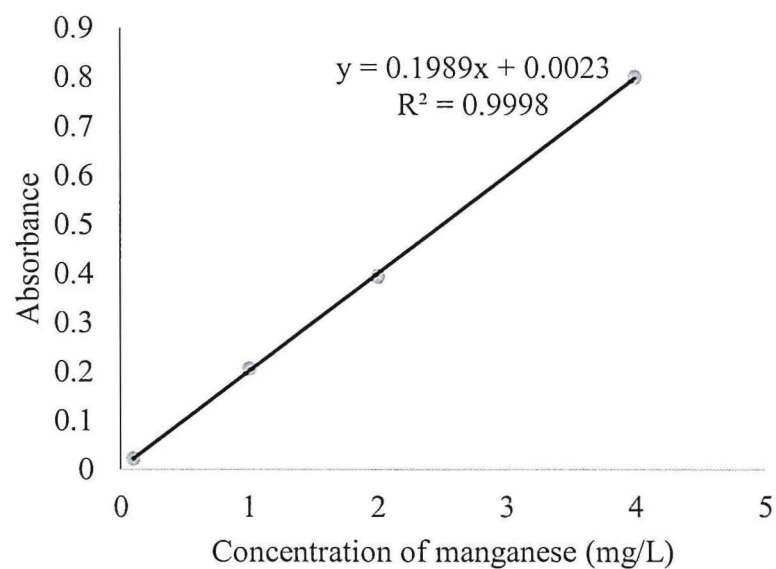
**APPENDIX III:** Standard curve used to analyze cadmium



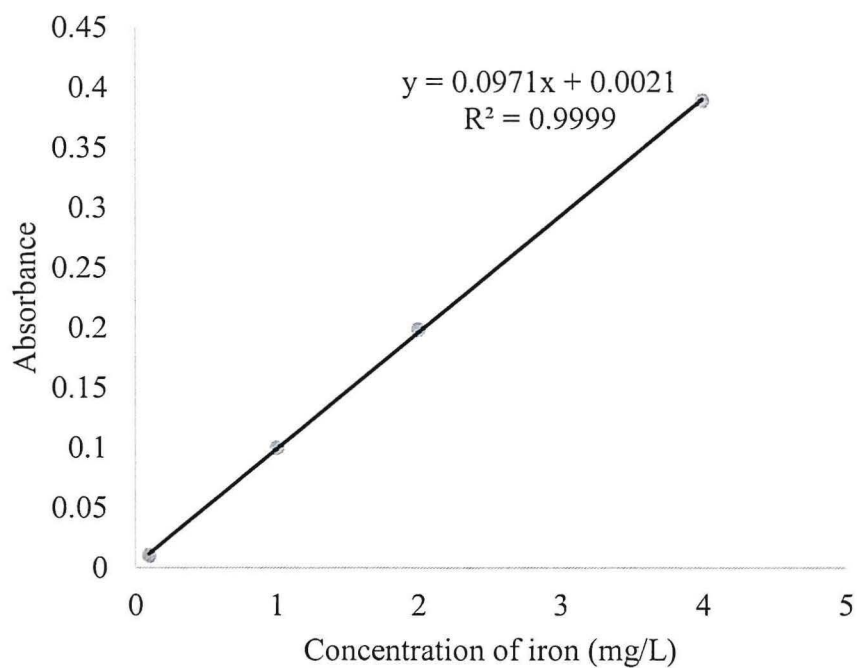
**APPENDIX IV:** Standard curve used to analyze mercury



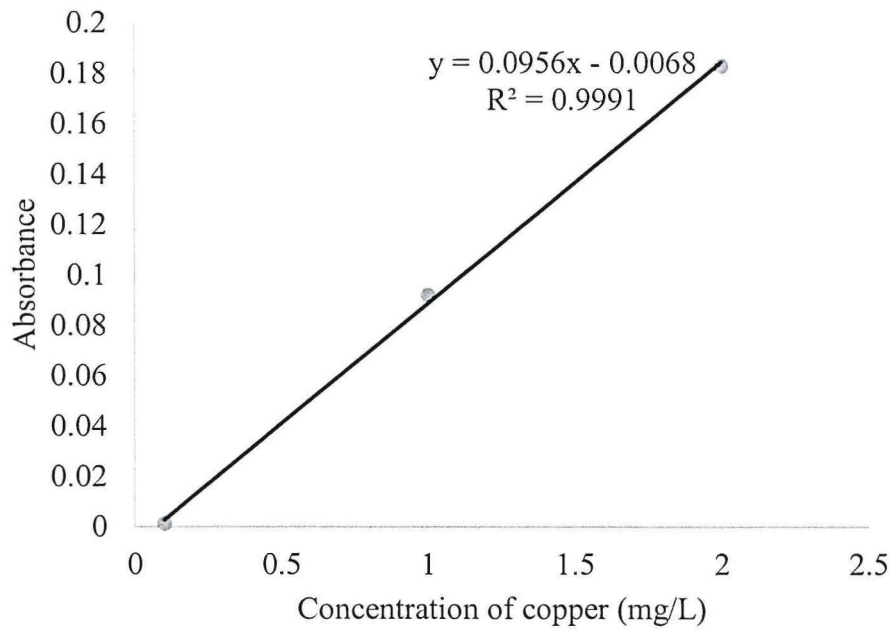
**APPENDIX V: Standard curve used to analyze manganese**



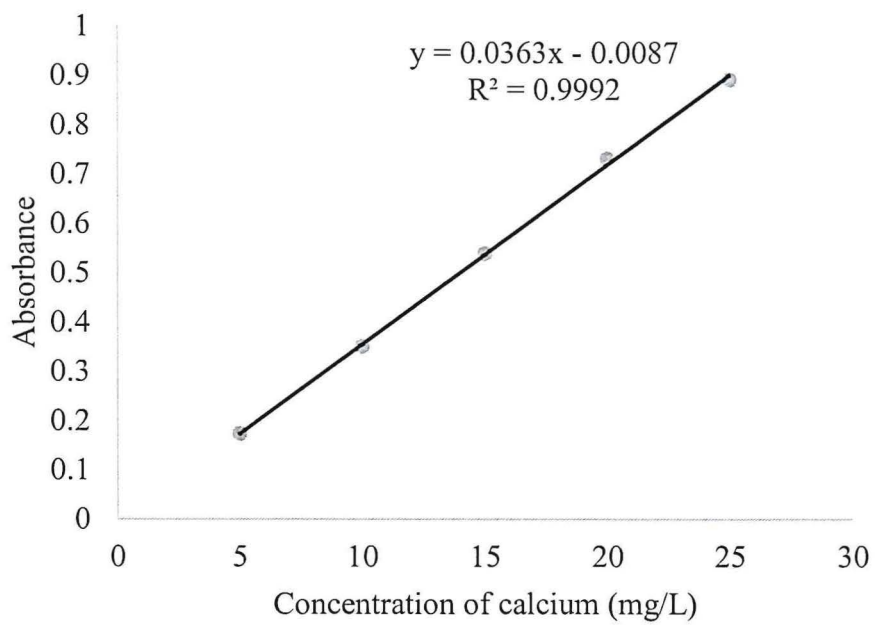
**APPENDIX VI: Standard curve used to analyze iron**



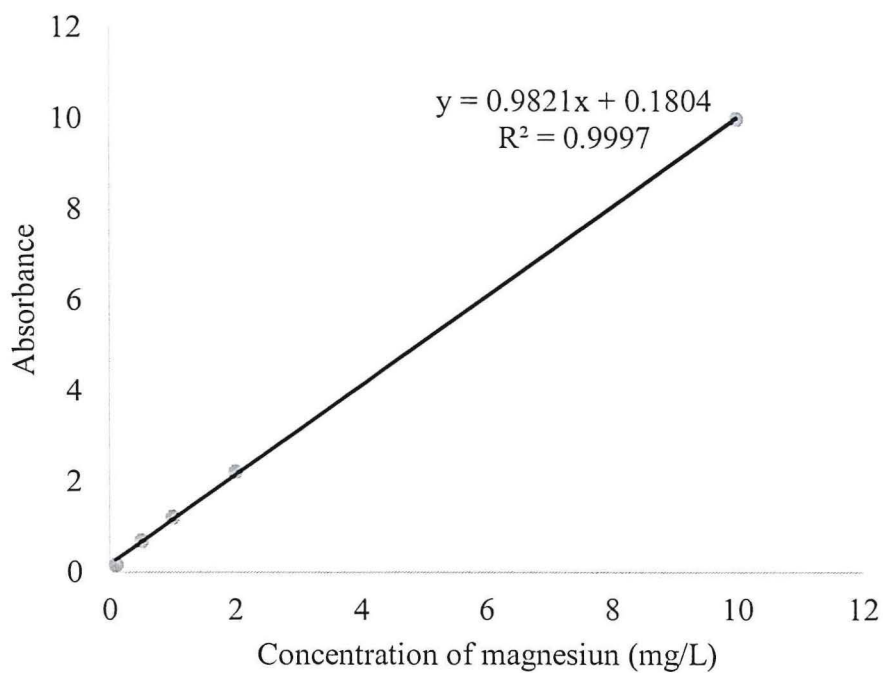
**APPENDIX VII:** Standard curve used to analyze copper



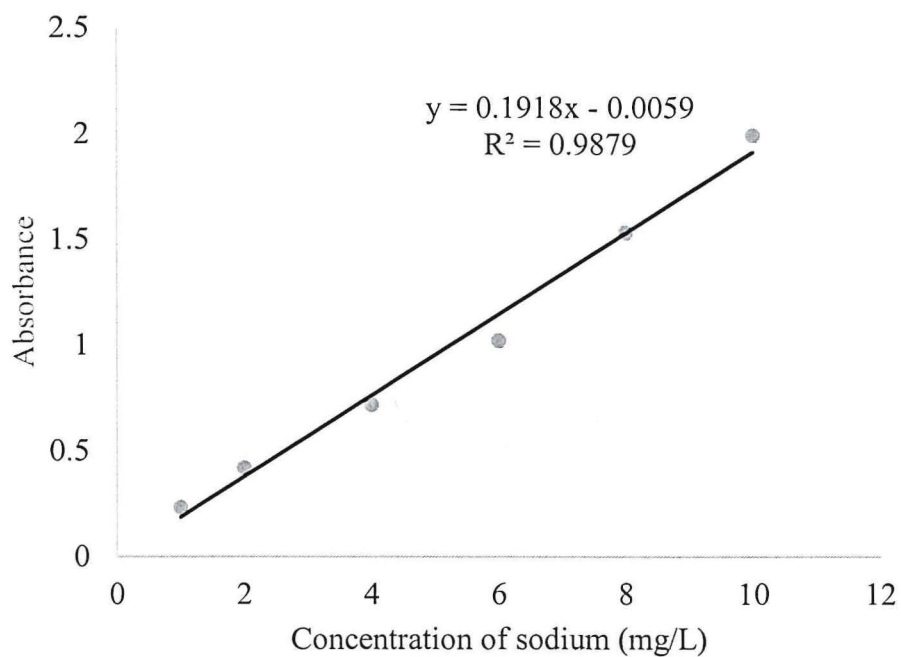
**APPENDIX VIII:** Standard curve used to analyze calcium



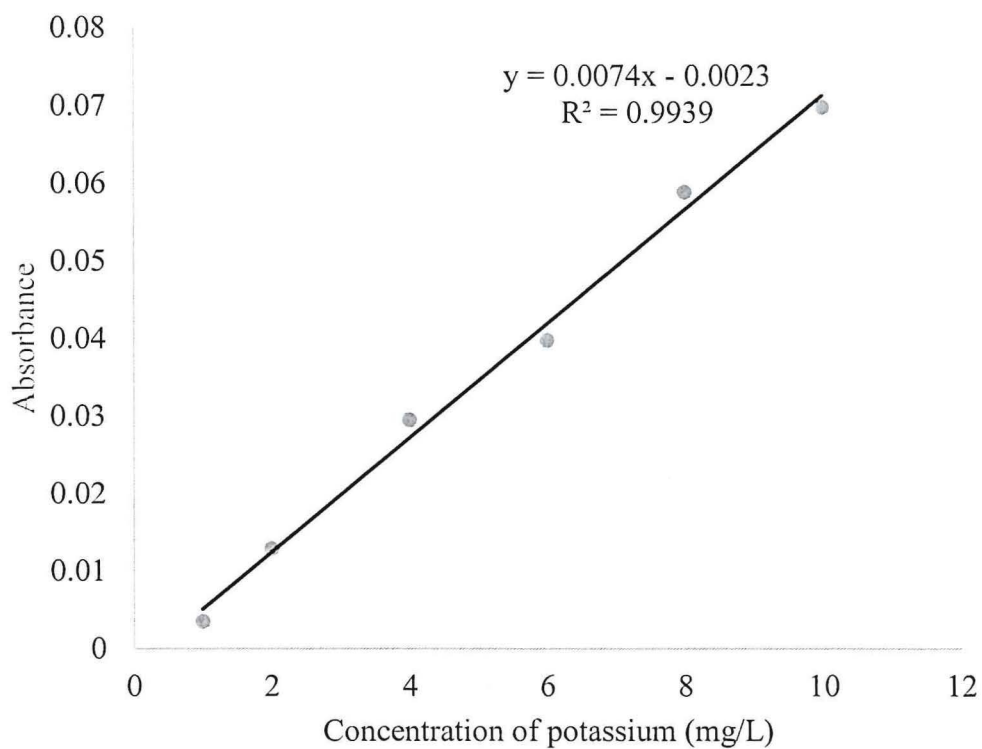
**APPENDIX IX:** Standard curve used to analyze magnesium



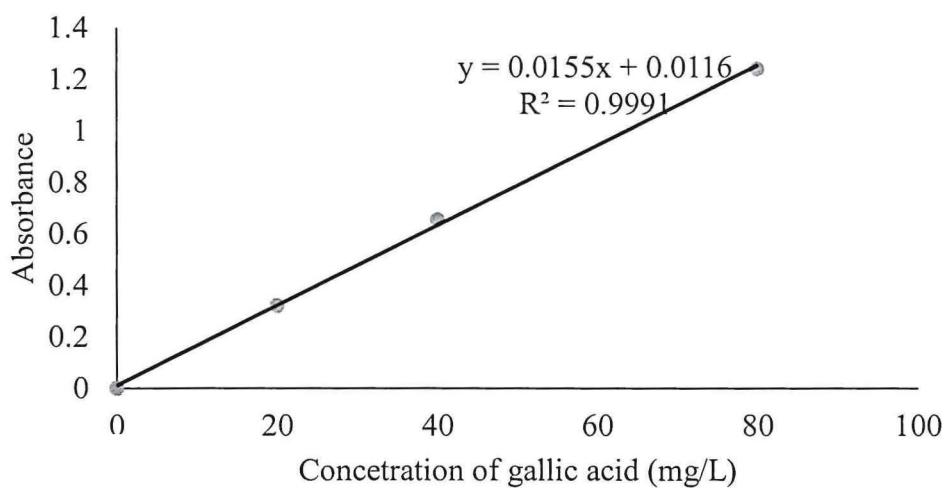
**APPENDIX X:** Standard curve used to analyze sodium



**APPENDIX XI:** Standard curve used to analyze potassium



**APPENDIX XII:** Standard curve used to determine the total polyphenol content



**APPENDIX XIII:** Standard curve used to determine the total flavonoid content

