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Physicochemical Characteristics and Occurrence of Aflatoxin in Groundnut Crude Oil in Ethiopia

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DECLARATION

I, the undersigned, declare that this is an original work and has never been presented in this or any other university, as well as research center previously and all the source materials used for this thesis have been fully acknowledged.

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LIST OF ABBREVIATIONS

AFB1	Aflatoxin B1
AFG1	Aflatoxin G1
AFs	Aflatoxins
DMRT	Duncan's multiple range test
DON	Deoxynivalenol
ES	Ethiopian Standard
EU	European Union
FUMs	Fumonisins
ISO	International Organization for Standardization
MUFA	Monounsaturated Fatty Acid
OTA	Ochratoxin A
PUFA	Polyunsaturated Fatty Acid
RASFF	Rapid Alert System for Food and Feed
SFA	Saturated Fatty Acid
ZEN	Zearalenone

ABSTRACT

Arachis hypogaea L., commonly referred to as groundnut or peanut, is a significant annual monoecious legume that is grown for food and profit in many regions of the world, including Ethiopia. Groundnuts are an important crop in terms of nutrition, primarily employed in the manufacture of oil. Nonetheless, research indicates that aflatoxin contamination occurs across the value chain in the majority of Ethiopian groundnut production. Thus, the purpose of this study was to assess the physicochemical properties of groundnut oil and determine the levels of aflatoxin in groundnut kernels, oil and cake. Five groundnut varieties were collected from Haramaya University (HU) and two groundnut varieties (Bure and Harer) were collected from Addis Ababa (AA) local market. Groundnut oil was extracted using a mechanical press, and its physicochemical properties were assessed using standard techniques. Aflatoxin B1 (AFB1) and total aflatoxin (TAF) concentrations were also assessed in the groundnut kernel, oil, and cake from the three groundnut samples (one from HU and two from AA market). The mean physicochemical characteristics of the groundnut oil ranges from; 81.92- 90.64 gI2/100g oil; 0.19- 0.27 mg/KOH/g oil, 189.29- 191.68 mg KOH/g oil ; 0.91- 0.92 kg/m3; 0.25-0.3%; 5 meq peroxide O2/kg oil; 1.46 and 0.97 for iodine value (IV), acid value (AV), saponification value (SV), density, moisture value, peroxide value, refractive index and specific gravity respectively. The mean value of the groundnut oil's physicochemical properties (IV, AV, SV, and density) varied significantly ($P < 0.05$) among the five verities. AFB1 was detected in all of the samples with levels ranging from 0.3 - 405 µg/kg, and seven out of nine (77.8%) of the samples contained levels above the Ethiopian maximum level (5 µg/kg). TAF was detected with levels ranging from 0.5 - 495 µg/kg, and 7/9 (77.8%) of the samples exceeded the maximum level of the Ethiopian standard (10 µg/kg) for TAFs. The largest concentration was found in the groundnut kernel in both AFB1 and TAF, whereas the lowest concentration was found in the oil. Overall, the groundnut oil from all the groundnut varieties is better for human consumption in terms of the physicochemical characteristics. The level of aflatoxin is decreased significantly through the extraction of groundnut oil. However, further study is required to make the oil free from aflatoxins.

Keywords: Aflatoxin, Groundnuts, Groundnut oil, Ethiopia

1 INTRODUCTION

1.1 Back-ground

Arachis hypogaea L., commonly referred to as groundnut or peanut, is a species within the legume or "bean" family. It is likely that the cultivation and domestication of groundnut began in the Paraguayan valleys. It is a herbaceous annual plant with a height of 30 to 50 cm (1.0 to 1.6 feet). According to Janna et al. (2013), groundnut is an otetraploid legume crop that self-pollinates and is a member of the Fabaceae family. According to Alemayehu et al. (2014) and Ajeigbe et al. (2015), it is regarded as the most significant monoecious annual legume utilized in Sub-Saharan Africa for human food, fodder, and economic purposes. Ethiopians frequently cultivate groundnuts for food, financial gain, and animal feed. Only small-scale holder farmers in the nation's lowland and drought-prone regions cultivate it. Based on 80,841.57 ha of agricultural area, Ethiopia produced an estimated 145,191.45 tons of groundnuts annually (CSA, 2018). Ethiopia's top producing regions were Oromya and Benishangule Gummuz, in that order. Globally, production area, yield, and productivity have been rising at rates of 5.5%, 7.6%, and 2.2%, respectively; in contrast, during the 2019 and 2020 cropping seasons, these rates were 143.74%, 23.5%, and 1.5%, respectively(Yeshiwas, 2023). Groundnut seeds provide 567 calories per 100 g and are high in fat (40–50%), protein (20–50%), carbohydrates (10–20%), vitamins, and minerals (Ahmed et al., 2016). It is an inexpensive, plentiful source of nutrients because of its high calorie content, protein, and minerals.

As the fourth-ranked oilseed crop and the fourteenth food crop globally, groundnuts are both grains and oilseeds (Ahmed et al., 2016). 50–55% of groundnut kernels are made of oil. With glycerides of oleic and linolic acids as its main ingredients and smaller amounts of glycerides of palmitic, stearic, arachidic, behenic, and lignoceric acids, the oil extracted from the kernel has a yellow to greenish yellow tint. Density, viscosity, acidity, iodine, peroxidation, saponification, refractive index, relative density, and moisture contents are the most frequently utilized physiochemical parameters for fats and oils (Pandurangan et al., 2014).

However, aflatoxin contamination in groundnuts as a result of pre- and postharvest storage mold infection is a serious issue in tropical nations like Ethiopia (Bankole & Adebanjo, 2003). These molds create aflatoxins, which are toxic food pollutants that often cause health and economic problems in many countries (Bhatnagar *et al*., 2003). Aspergillus flavus, Aspergillus parasitacus, and Aspergillus nomius are the primary producers of aflatoxin-producing bacteria, which are a class of naturally occurring, extremely carcinogenic chemicals (Strosnider *et al.,* 2006). Aflatoxin B1, B2, G1, and G2 are the most prevalent and significant ones. Of these, aflatoxins are known to be very hazardous, with aflatoxin B1 (AFB1) being the most toxic (IARC, 1993). Food products can have aflatoxins removed or eradicated by using physical, chemical, or biological procedures (Jalili *et al.,* 2010). These levels may be affected and products with lower levels of contamination may result from the techniques used at each stage of the processing to extract the oil and meal from oil seeds. The general consensus is that aflatoxins are not retained in plant oils due to their strong polarity and lipophobicity. Lipophilicity is the main physicochemical element that connects solubility with membrane permeability. Aflatoxin relative insolubility is present in non-polar solutions (Mahoney and Molyneux, 2010). Because more oxidized oils may be more polar substances and may dissolve aflatoxins more readily, this property may be related to the oil's quality. The varying chemical makeup of oils derived from various sources may also have an impact on the toxin's solubility. Aflations are only weakly soluble in water, insoluble in nonpolar solvents, and readily soluble in polar organic solvents, according to MdQuadri et al. (2013).

1.2 Statement of the problem

In terms of production and export, oil seeds rank third among Ethiopia's major commodities. Ethiopia's economy, according to the Central Statistical Agency, is centered on agriculture, which generates 46% of the country's GDP and is responsible for $407,186,091 in exports of coffee and spices, vegetables, and oil seeds in 2022. Nearly four million smallholder producers are involved in these main production areas (CAS, 2022). Sesame, niger seed, and linseed are the principal oil seed crops exported. (Yazdi-Feyzabadi et al., 2021) indicated that 17% of Ethiopia's agricultural exports are made up of sesame, soybean, and Niger seed. Despite this, groundnut oil is not as concentrated in our nation as it should be given its high production rate. When compared to other oil crops in Ethiopia, groundnut output and area coverage exhibited a growing tendency from 2014 to 2020, ranking third after Noug and Sesame (CAS 2021). Ethiopia imports the majority of its

edible oil from other nations. Furthermore, 479,000 metric tons of cooking oil worth approximately $474 million were imported by Ethiopia in the calendar year 2015 (FBPIDI & GAIN, 2018). The cost of edible oils, such as sunflower and palm, has reportedly increased by 20–30% in Ethiopia over the past year. There are several reasons for this, including inflation in the current exchange rates.

As a result, utilizing oils from other sources including groundnut is crucial in our country. Furthermore, there are few studies that assess the aflatoxin contents of groundnut seed (Chala et al., 2013; Mohammed et al., 2016), and groundnut cake (Mohammed et al., 2016). However, there is no study on the groundnut oil. Hence, to use groundnut seed in oil production, it is good to know how much of the aflatoxin moves from the seed to the oil.

Consumers living in the country are negatively affected by the aflatoxin contamination of groundnuts. It has negative impacts on human health that are both immediate and long-term (Gong et al., 2016; IARC, 2016; Reddy et al., 2010). It has also had a negative impact on the nation's export performance, hindering its ability to contribute to annual export revenues and economic growth. Therefore, to use groundnut in Ethiopia's oil processing industry, it is crucial to assess the physiochemical properties and presence of aflatoxin in groundnut oil.

Objective

1.2.1 General objective

- Evaluate physicochemical characteristic and occurrence of aflatoxin in groundnut crude oil in Ethiopia

1.2.2 Specific objectives

- Evaluate the physicochemical characteristics of groundnut crude oil
- Determine the level of aflatoxin in groundnut and groundnut crude oil

1.3 Research question

- Do the physicochemical characteristics of groundnut crude oil vary among groundnut verities?
- Does ground nut and groundnut crude oil have significant difference in the levels of aflatoxin?

1.4 Significancy of the study

The study's findings might be useful in determining which groundnut variety has the optimum physicochemical properties for oil. It could also serve as baseline information to lower the possibility of aflatoxin contamination when producing groundnut oil.

2 LITERATURE REVIEW

2.1 Groundnut and Varieties

Peanuts (Arachis hypogaea L.), a papilionaceous flower that most likely originated in Brazil, are technically beans rather than nuts. The Incas had been cultivating it there since between 3000 and 2000 B.C., so it wasn't until the discovery of America that the rest of the world learned about it. The peanut was swiftly brought to Portuguese colonies in Africa (16th century). It was transported from Cuba by the Spanish to the Philippines, where it thereafter made its way to China. It was initially mentioned by the Spaniard Oviedo, who resided on the island from 1513 until 1524. It didn't start gaining any traction in Europe and North America until the 19th century. Today, peanuts are grown in regions with a median annual temperature of 22°C, mostly in West Africa, India, China, and the southern United States. The plant is between 30 and 60 cm tall. Its blossoms need two to three months to grow. After pollination, the part of the plant bearing fruit bows down to the soil, where it grows into the (preferably sandy) soil. In two months, the fruit fully ripens there. Peanuts thrive in regions with average monthly temperatures of at least 25 °C and cannot withstand frost (Bockisch, 1998).

Several groundnut varieties have been approved or issued as a result of thorough examinations conducted throughout the years. Shulamith, Nc-343, Nc-4x, Roba, and Sedi are the examples. All varieties—aside from Sedi—are large-seeded, long-season Virginia bunch kinds. The length of a crop is between 130 and 160 days. Under irrigation, their yields range from 50 to 70 q ha-1, but when rainfed, they range from 23 to 50 q ha-1. Under Middle Awash conditions, variety Nc-4x has a yield potential of 6000 kg ha1 and is advised for altitudes below 750 m. Shulamith is better than Nc-4x and Nc-343 in low disease pressure conditions at Werer and Babile, although it is still highly vulnerable to cercospora leaf spots and rust. The Nc-343 cultivar exhibits a spreading habit and an excellent output potential in places with marginal rainfall. For the Babile and Bisidimo areas, it is advised to conserve moisture. The best cultivar is Roba, which has a yield potential that is significantly higher than any of the other types. The only short-maturing (90 tollO days) Spanish-valencia (ssp.fastigiata) variety released for arid and short-season regions is called Sedi. It is easily shelled by hand and has favorable attributes including a higher oil content (52%) and pleasant flavor (Weyessa et al., 1997). In addition to this now days there are a lot of new varieties are relasing because of high production and diseases resistant behavior (Kebede *et al*.,2017).

2.2 Groundnut products

One of the most significant crops in the world for providing protein and oil is the peanut. The majority of peanuts planted worldwide are used to make oil, peanut butter, confections, roasted peanuts, snack items, soups, and desserts. They are also utilized as extenders in meat product formulations. They are included in weight-management diets because they are a source of healthy fat, specifically poly and monounsaturated fatty acids, which promote satiety. The process of harvesting peanuts and extracting peanut oil results in a substantial number of by-products. A substantial amount of peanut meals, skins, hulls, and vines are considered agricultural trash. Due to its high nutritional value—it is a great source of protein, in particular—it can be added to a variety of food preparations as a supplemental food or an emergency food that can be given to people who are hungry or malnourished, particularly in developing and underdeveloped nations.

Harvesting is the first step in the processing of peanuts, according to India, the second nation that processes peanut oil. Small-scale growers harvest using the conventional technique, which entails plow work and hand stacking of the plant for field curing. Mature peanut plants are first mowed in order to begin harvesting. Then, using specialized machinery, these are turned inside out into windrows for open-air drying or field curing, with peanut pods on top. One gathers mature peanuts from the windrows. The next step is mechanical drying after harvesting. To avoid the development of aflatoxin, peanut moisture content is typically maintained below 12% (Nautiyal 2002). There are also on-farm dryers, which are often made up of storage bins with air vents and storage trailers with air channels. After drying, peanuts have a moisture content of 7% to 9%. Following further cleaning, storing, and processing for a variety of applications, these peanuts Kochhar & Dhanesh, 2015).Permit me to attempt to explain the common ground nut product types found in Ethiopia.The next step is mechanical drying after harvesting. To avoid the development of aflatoxin, peanut moisture content is typically maintained below 12% (Nautiyal 2002). Additionally, there are on-farm dryers, which are typically made of storage trailers with air tubes along.

2.2.1 Roasted groundnut

Peanuts acquire their distinctive flavor through roasting, which can be accomplished through either dry or oil roasting. Tetrahydrofuran compounds are created during roasting by the reaction of amino acids and carbohydrates (Bansal, 2013).

2.2.2 Groundnut butter

The main uses for groundnut butter are as an icing or frosting, in sandwiches, candies, and as a spread for toast or biscuits. It is a good source of niacin and a fair provider of calcium, iron, thiamine, and riboflavin. Groundnut butter is made by roasting the nuts for 40 to 60 minutes at 160°C to achieve a controlled browning; cooling the nuts to halt the cooking process; blanching the nuts to remove the skins (testa); and grading the nuts to remove light, burnt, or discolored kernels (Chinnan MS, 2011). Before chilling and packaging, salt, stabilizers, and other optional additions, such as sweeteners, are measured and combined with the butter. Additional ingredients include of whey, lecithin, honey, hydrogenated oil, and antioxidants.

2.2.3 Groundnut oil

Herbaceous and woody oil crops are the two primary categories of oil crops. Triglycerides, which are mostly made up of three chains of saturated and unsaturated fatty acids joined by one glycerol molecule, make up the majority of vegetable oil. As Table 1 shows; Lauric, palmitic, stearic, arachidic, oleic, linoleic, linolenic, and erucic acids are some of the primary fatty acids found in vegetable oils and its sources.

Table 1. Oil and fat classification

Classification	Examples
Pulp Oils	Palm oil, olive oil, avocado oil
Lauric Oils	Coconut oil, palm kernel oil, babassu oil, laurel oil, nutmeg oil, dika butter
Fats rich in Palmitic and Stearic Acid	Cocoa butter, illipe butter, mowrah butter, shea butter, borneo tallow
Seed Oils rich in Palmitic Acid	Cottonseed oil, cereal germ oils, corn oil, pumpkin oil
Seed Oils rich in Oleic and Linoleic Acid	Sesame oil, sunflower oil, safflower oil, niger oil, linseed oil, poppy-seed oil, grape-seed oil, walnut oil, fruit-seed oils, berry-seed oils, tea-seed oil
Oil Is of Leguminosae	Peanut oil, soybean oil, lupine oil
Oil Is of Cruciferae	Rapeseed oil, mustard-seed oil

Source, Wen *et al*., 2023

The Organization The nut's kernels, which make up roughly 55% of the nut and have 45% fat, contribute somewhat less than 25% of the nut's overall fat content. Delivered is 5.8 kcal/g (24.3 kJ/g). Peanut oil is rich in oleic acid, a kind of monounsaturated fatty acid, and low in saturated fatty acids. Its low solidification point is hence marginally below O'C. The range of iodine values is 80–106. A little proportion of high-melting triglycerides provides the oil a gel-like look at temperatures below 5 °C. These triglycerides disappear after winterization. In addition to the major fatty acids, peanut oil may include up to 2.4% palmitic acid and up to 0.5% myristic acid. Furthermore, 1.2% eicosenoic acid and 1.2% linolenic acid genotypes are known to exist. The main triglycerides are those that include oleic acid, as may be deduced from the 50% oleic acid concentration. Over 50% of triglycerides have four or more double bonds. The nonfat part of the kernels is fed to animals. If handled properly, it can be used as nourishment for humans. The Oil Mill Gazette published a description of the extensive processing of peanuts in 1980 with the intention of creating peanut meal for human use. After drying the nuts to 12% humidity, they are

roasted to 82°C and left there for 30 minutes. Finally, they are dried again to 6% humidity. The oil is extracted after flocculation. The result is a white meal that contains 65% protein.

The main uses of groundnut oil are in salad dressings and cookery. A great fat for pan-frying and deep-fat frying is groundnut oil. This mild vegetable oil is easily used to make salad dressing, mayonnaise, pastry shortening, oleomargarine, and other food products (Desai et al., 1999). It should keep its original yellow color when used in mayonnaise, but it should be colorless when used in oleomargarine, and it should have an antioxidant when used in shortening and other plastic fats. Along with other additives, peanut oil has also been tried with in weaning diets (Chinnan MS, 2011). Patients with polio are often massaged with groundnut. In addition, it serves as an adrenaline carrier for treating asthma.

Groundnut oil health benefit

- Peanut oil is calorically dense, offering 884 calories per 100 grams.
- It boasts a high smoke point of 450°F, making it suitable for deep-frying at elevated temperatures.
- With its lipid composition comprising saturated, monounsaturated, and polyunsaturated fats in balanced proportions (SFA: MUFA: PUFA = 18: 49: 33), peanut oil presents a favorable lipid profile.
- Renowned for its stability, peanut oil exhibits a lengthy shelf life, making it a reliable choice for cooking purposes..

Among the mechanical extraction methods is cold press extraction, which is also environmentally friendly and uses less energy than other methods of extracting oil. It is produced mostly during the oilseed oil extraction process and is used to extract oil from a range of matrices. The cold press method, which involves producing oils at low temperatures, yields high-quality oils. Its application is environmentally friendly and does not require solvents. Put another way, the cold-press extraction method does not involve the use of heat or chemical extraction. The oils from soybean, sunflower, rapeseed, corn, grape seed, hemp, flaxseed, rice bran, olive, and pumpkin were extracted using the cold press method. Additionally, consumers find these oils intriguing because they are safe, natural, and help avoid specific conditions (Cakaloglu *et al*., 2018).

2.2.4 Physicochemical characteristics of groundnut oil

Several physical and chemical features are used to observe the compositional quality of edible oils (Ceriani et al., 2008, Mousavi et al., 2012). These physical-chemical characteristics include density, refractive index, specific gravity, conductivity, acid value, viscosity, peroxide value (PV), iodine value (IV), saponification value (SV), and optical reference. The majority of the quality parameters are explained in the following paragraphs.

The acid value (AV) is one characteristic that is frequently utilized in the specification of fats and oils. It is a measurement of the amount of free fatty acids (FFA) in the fat or oil and is defined as the weight of KOH in milligrams required to neutralize the organic acids present in 1g of fat. Triglyceride (TG) mixes make up oils, and the type of TGs in an oil will determine how viscous it is. Viscosity was changed as a result of the fatty acids' new arrangement on the triglyceride molecule's glycerol backbone. Thus, viscosity is influenced by the chemical properties of oils, including chain length and saturation/unsaturation. The viscosity of oils is inversely correlated with temperature, just like other substances. Additionally, it inversely correlates with the esterified fatty acids' total amount of double bonds. The amount of rancidity reactions that an oil or fat has had during storage is measured by its peroxide value (PV), which is a measure of the product's stability and quality (Ekwu & Nwagu, 2004). Furthermore, it was shown that temperature, air contact, and the length of storage all enhanced the oil sample's peroxide value (Knothe & Dunn, 2003). The amount of rancidity in the oil is indicated by the peroxide value.

A measure of the average fatty acid molecular mass in an oil sample is called the saponification value (SV). A smaller mean molecular weight of fatty acids or fewer ester connections are suggested by the lower saponification values. This implies that there was no mutual reaction between fat molecules (Denniston et al., 2004). An alkali is used in the saponification process to break down or degrade a neutral fat into fatty acids and glycerol. The saponification number (value) is the milligrams of potassium hydroxide (KOH) required to saponify one gram of fat. It is a measurement of the average molecular weight of the triacylglycerols in the sample. Triacylglycerol molecular weight should be divided by three to approximate the average molecular weight of the fatty acid present. Kirk and Sawyer suggest that the strong saponification characteristics of fats and oils are partly due to a large concentration of fatty acids with shorter carbon chains. Nielson's finding that the average fatty acid chain is longer when the saponification

values are lower supports this claim. If the fatty acids in the glycerides have a low molecular weight (short-chain acids) as opposed to a high molecular weight (long-chain acids), there will be more glycerides molecules per gram of fat. Because each glyceride molecule requires three molecules of potassium hydroxide to be saponified, fats containing low molecular weight glycerides have higher saponification values. There have been reports indicating an inverse relationship between the saponification values and the average molecular weight of the fatty acids in the oil fractions. When paired with acid values, measurements for saponification can be used to determine the type, concentration, and average weight of acid in a given sample. It's critical to understand an oil's saponification value if it's meant for industrial use. Additionally, the saponification value is used to verify adulteration. The saponification number of the oil indicates how much more capable it is of making soap. The saponification value of unrefined vegetable oils may also be influenced by the ingredients in the nonsaponifiable fraction (Odoom & Edusei, 2015).

The iodine value of a fat or vegetable oil indicates how unsaturated it is. It determines an oil's ability to withstand oxidation and makes it possible to assess the fat's overall unsaturation qualitatively (AOCS, 1993, Asuquo et al., 2012). Oil can be identified by its iodine value. The iodine value of an oil or fat is expressed in grams of iodine absorbed by a 100g sample. The iodine value, often known as the iodine number, is a commonly used metric to describe the degree of unsaturation and the amount of carbon-carbon double bonds in fats or oils. This statistic, which shows how vulnerable the oil is to oxidation, can be used to calculate the number of double bonds present in the oil. When categorizing oils and fats, the iodine value must be determined. The following is a classification of oils and fats as drying, semi-drying, and non-drying: IV 200–130 for drying oils, IV 130–100 for semi-drying oils, and IV less than 100 for non-drying oils. Compared to glycerides, free fatty acids have a greater IV. The IV rises by 0.00045×IV for every percentage of FFA. The higher the iodine value, the higher the unsaturation level because more iodine is absorbed at higher unsaturation levels. High iodine values indicate increased unsaturation of fats and oils, whereas low-IV oils are more saturated with fewer double bonds. Furthermore, higher iodine concentrations indicate that the oils may have nutritional as well as cosmetic applications (cosmetics, oil paintings, varnish) (Odoom & Edusei, 2015).

The refractive index of a substance is the relationship between the speed of light in vacuum and the speed of light within the substance. Liquids are generally described using the refractive index. Refractive index is a useful tool for identifying materials by comparing their values to known values, figuring out how much of a known substance is in a solution, and assessing a substance's purity by comparing their values to a standard value. The refractive index also tells us how polarizable a substance is. The three main factors that affect refractive index measurement are temperature, the wavelength of the light being used, and the sample concentration. Temperature and the wavelength of the light have an impact on a substance's refractive index. Unless otherwise stated, the speed of light in air is typically substituted for the speed in a vacuum.

The definition of density as a physical attribute of matter is "its mass per unit volume." It describes an object's weight or the degree of packing between its constituent parts. Vegetable oil's density is sometimes compared to that of water (1.00g/ml), despite the fact that all oil is less dense. Each variety and temperature of vegetable oil has a different density. Between 15 °C and 25 °C, the range is 0.91 and 0.93 g/cm3. Compared to water, which has a density of 1 g/ml, cooking oil is less dense. The oil expands as the temperature rises, resulting in a reduction in density. Therefore, it is required to express the oil's density in proportion to temperature.

Peanut-based products often have high protein and amino acid content and can be a great source of nutrition. According to Gulluoglu et al. (2016), peanut seeds have between 44 and 56% oil, 22 and 30% protein, and 9.5 and 19.0% carbs by dry weight. It is also a good source of minerals (phosphorus, calcium, magnesium, and potassium) and vitamins (E, K, and B group). The fatty acid composition of peanuts greatly influences their nutritional value and capacity for storage (Shasidhar et al., 2017). Table 2 and Table 3 showed that different physiochemical parameters of ground nut oil parameters and this are Acid value saponification value, moisture level, iodine, peroxide value and others. Peanut oil contains both saturated (SFA) and unsaturated (UFA) fatty acids. The percentage of SFA and UFA fatty acids in peanut oil varies (about 11–17% and 81–94%, respectively). Oleicacid (C18:1) and linoleic acid (C18:2) concentrations in peanut genotypes can vary from 2 to 43% and 2 to 85%, respectively. Shad et al. (2012) showed that the oil had a specific gravity of between 0.915 and 0.918, an acid value of between 3.96 and 4.95 mg/g, a saponification value of between 226.40 and 246.56 mg/g, and an unsaponifiable matter of between 3.20 and 4.20 g 100g-1.

Table 2. Physiochemical parameters values of groundnut oil

Physiochemical parameters values of peanut oil	**Amount**
Calories	899 kcal
Saturated Fat	22gm
Mono Unsaturated Fat	44gm
Poly Unsaturated Fat	34 gm
Trans Fat	0 gm
Total Carbohydrate	0 gm
Sodium	9 mg
Dietary Fibre	0 gm
Total fat	100gm
Sugar	0 gm
Protien	0 gm

Source,Sandefur et al., 2017

Table 3. Physicochemical parameters of peanut oil

Characteristic	Value
Acid Value (Refined)	0.6 to 0.99 mg KOH/g oil
Iodine Value	86 to 107
Peroxide Value	1.0 meq peroxide O^2/kg oil
Melting Point	0 to 30°C
Melting Point of Fatty Acids	22 to 30°C
Moisture and Volatiles	0.23%
Refractive Index (40°C)	1.46 to 1.465
Saponification Number	187 to 196
Smoke Point	226.4°C
Surface Tension	35.6 Nm/m
Color (Lovibond, Maximum)	Yellow 16 to 25; Red ≤ 2.0
Color (Visual)	Light yellow

Source, Akhtar *et al*., 2014

2.3 **Mycotoxin**

Mycotoxin contamination in goods like maize, groundnuts, sorghum, sweet potatoes, and spices has been reported to be greater in several African studies (Darwish et al., 2014). Eating a diet high in mycotoxin has much more detrimental effects on health. According to Liu et al. (2010), almost 40% of liver cancer cases in Africa were linked to dietary exposure to AFs. Africa has suffered significant economic losses as a result of mycotoxins; for example, the annual cost incurred by AFs-contaminated crops is estimated to be more than USD 750 million. Ignorance, subpar farming methods, improper pre- and post-harvest handling, and climate change have all been linked to the substantial impact of mycotoxins in Africa (Paterson et al., 2010). Darwish et al. (2014). Inadequate marketing and transportation networks, as well as the lack of contemporary infrastructures like drying and storage facilities, have exacerbated the effects of these issues (Beyene et al., 2016). Moreover, mycotoxin contamination of African crops is widespread due to a lack of implementation ability, a deficient surveillance system, and insufficient rules and regulations (Udomkun et al., 2017).

2.3.1 Mycotoxins and their economic impacts in Ethiopia

Numerous studies have documented the presence of mycotoxigenic fungus and their toxins in important cereals and other agricultural products in Ethiopia. The presence of toxins that surpass the upper limits established by EU nations, Ethiopia's primary trading partners, has received particular attention. Major peanut-producing regions in eastern Ethiopia have shown higher Aspergillus spp. occurrences (70-100%); A. flavus, A. parasiticus, A. caelatus, A. niger, A. tamarii, and A. ochraceus were observed as co-occurring fungus species (Mohammed et al.,2016). In addition, AFs were detected in 33.8% of the total samples (160 peanuts and 50 peanut cakes). Likewise, other related studies have reported higher incidences of different mycotoxigenic fungal genera such as *Aspergillus* (*A. flavus*, *A. parasiticus*, *A. ochraceus*, *A. tamarii*,and *A. niger)* and *Penicillium* in groundnut collected from eastern Ethiopia (Assaye et al.,2016. For instance, research by (Chala et al., 2013) showed that 93% of peanut samples from eastern Ethiopia had total AFs levels (15-11, 900 g/kg) above the EU maximum limit (4 g/kg) for groundnuts intended for direct human consumption (EC, 2010).

It is not well understood how much Ethiopia's mycotoxin contamination has damaged the country's economy. However, reports that are now available show how mycotoxin contamination affects Ethiopia's export market. From February to December 2015, the EU Rapid Alert System for Food and Feed (RASFF) sent out four notifications for OTA levels (92.5-139 g/kg) in a variety of spice commodities from Ethiopia; the products were turned away three times at the German and British borders (Malir et al., 2016). As a result, exporters have moved their operations to other nations with less stringent regulations regarding mycotoxin levels but still relatively inexpensive markets. Ethiopia's exports to the EU have consequently decreased significantly in volume. Additionally, mycotoxin contamination has an impact on regional food processing businesses that source their raw materials from regional farmers and producers. Ethiopian companies have begun importing premium agricultural raw materials because one of the barriers to local products' commercialization is the presence of dangerously high levels of mycotoxins in them. But the country's main problem, the growing cost of foreign exchange, can also be impacted by the import of raw commodities (Lloyd et al., 2016). All things considered, Ethiopia's economy may suffer from the direct and indirect effects of AFs pollution. It may also have an influence on smallholder farmers' livelihoods by lowering the quality of their crops, which may lead to price reductions or export restrictions.

2.3.2 Aflatoxin in groundnut products

According to Galvano et al. (2005), animal feeds, peanuts, cottonseed meal, corn, and other grains are among the many foods that frequently contain the toxin aflatoxin B1 (Azab et al., 2005). Physical factors like pH, light, moisture, temperature, water, relative humidity, and air gasses can all lead to aflatoxin contamination. Aflatoxin contamination is always more likely in environments with high moisture content since moist conditions are good for fungal development. According to Ding et al. (2015), aflatoxin synthesis is most effective at 85% relative humidity, but it also increases dramatically to a significant level at 95% relative humidity. However, there is no documented relationship between water content and aflatoxin contamination. While Aspergillus flavus can grow in temperatures ranging from 12 °C to 48 °C and has good survival rates, its optimal growth range is between 28 °C and 37 °C (Hawkins et al., 2005). While aflatoxin formation can occur at a number of temperatures, the optimal range is 25–35 °C (Siciliano et al., 2017). The best temperature range for AFG growth is when both AFG and AFB production are equal, however AFG production is usually larger at high temperatures than AFB production (Matumba et al., 2015). The availability of O2 and CO2 also affects aflatoxins' capacity to develop. A higher concentration of CO2 and a lower concentration of O2 prevent the growth of fungi and aflatoxins, respectively. The availability of O2 and CO2 also affects aflatoxins' capacity to develop. A higher concentration of CO2 and a lower concentration of O2 prevent the growth of fungi and aflatoxins, respectively (Mahbobinejhad et al.2019).

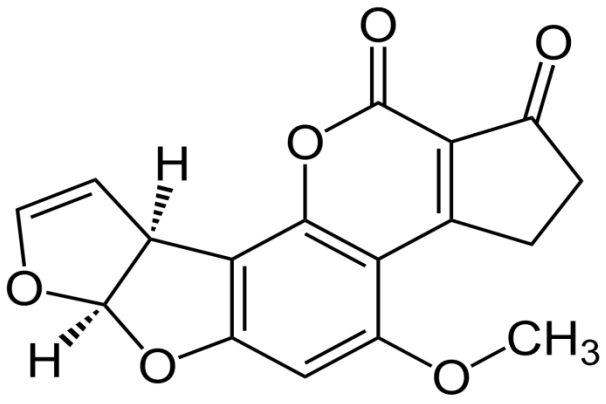


Figure 1. Aflatoxin B1

The most realistic strategy is through sound agricultural practices, such as "fungal bio-competition" and "plant breeding" to create crops resistant to mold. Toxigenic fungal colonization in later years is reduced when non-toxigenic strains of Aspergillus flavus and Aspergillus parasiticus are applied to the soil in maize plots. Because the toxic isolates are outcompeted by the non-toxic, biocompetitive Aspergillus strains, there is less aflatoxin pre-harvest contamination in cotton and groundnuts. According to Devegowda et al. (2019), the storage under management should be:

- Ensure that raw materials are promptly dried after harvesting until their moisture content is below 13%.
- Refrain from utilizing damaged or broken grains, which are prone to fungal proliferation.
- Avoid the inclusion of grains damaged by insects, as they are also susceptible to fungal growth.
- Before storage in silos or bags, pre-clean and thoroughly dry the grains.
- Employ mold inhibitors if the moisture content of the raw materials exceeds 13% to mitigate fungal growth.
- Store the raw materials on wooden pallets or crates, positioned away from walls to prevent moisture migration from the floor and walls.

HPLC is one of the most popular techniques for identifying and measuring aflatoxins in food, and it is the method employed for analysis in this study. It has been combined with methods including mass spectrometry, fluorescence, UV absorption, and amperometric detectors. According to Elizalde-González et al. (1998), aflatoxins B1, B2, G1, and G2 can be detected at 5 ng using amperometric and HPLC methods of analysis.

Aflatoxin in food can be analysed using chromatography in a number of ways (principally in milk, cheese, maize, peanuts, almonds). A fluorescence detector, which uses the aflatoxins' fluorescence properties at specific wavelengths, is typically used to quantify aflatoxins. Therefore, in order to create more sensitive techniques than those that have been widely employed thus far, researchers have concentrated on enhancing these fluorescent qualities. Nowadays, methods like post- and pre-column derivatization are frequently employed to enhance the fluorescence features of aflatoxins. In order to obtain a more pure sample and enable better measurement, they also feature a clean-up stage. Among the frequently employed techniques during the cleanup step are solid phase

extraction and immunoaffinity columns. The maximal absorption of all aflatoxins is approximately 360 nm (Akbas and Ozdemir, 2006). The letters "B" and "G" on aflatoxins stand for the blue (425 nm) and green-blue (450 nm) fluorescence colors that these substances emit when exposed to ultraviolet (UV) light. The most prevalent aflatoxin is AFB1, which is followed by AFB2. AFG is not very common. According to Alcaide-Molina et al. (2009), the fluorescence emission of the G toxin is more than ten times higher than that of the B toxin.

3 MATERIALS AND METHODS

3.1 Sampling area and sample collection

Five varieties of groundnut samples (5 kg each) such as Babile1, Babile 5, Werer 961, Bahaa jiduu, and Bahaa-guda) were collected from Haramaya University and two groundnut samples such as Bure and Harer variety were collected from Addis Ababa local market. The samples were shipped to the Center for Food Science and Nutrition laboratory in plastic bags, where they were kept at room temperature until analysis. Eastern Ethiopia produces the majority of Ethiopia's groundnut that is why we have collected the samples in this area for this study. And werer 961 chooses for afaltoxin analysis through lottery method. That means random starting point within the population was selected, often using a random number generator.

3.2 Experimental design

For the laboratory analysis, a completely randomized design (CRD) was used. Each laboratory analysis was conducted in duplicate and in triplicate for this experiment. The Center for Food Sciences and Nutrition research laboratories at Addis Ababa University, the food engineering department at Bahir Dar University (BDU), and Ethiopian Conformity Assessment Enterprise (ECAE) all hosted the experiment.

3.3 Oil extraction

The oil extraction from the groundnut was performed at the department of food engineering BDU. The oil extraction was done using cold press squeezing method (Ghiasi et al., 2022) for all variety of groundnut seeds using mechanical presser (CA 59 G,IBG and Germany). The groundnut samples were manually cleaned and dehulled prior to oil extraction and the equipment was carefully cleaned using water after each variety oil extraction. Then the seed added to the machine so the machine itself pressed the seed and extact the oil. Screw oil used for extract the oil that is cold presser. By altering the pressing screw pitch size and screw thread depth, the presser reduces the volume between the screw pitch and the pressing chamber, gradually increasing the pressure on the oil materials and forcing the oil out of them. High temperature is produced in the meantime by the chamber's high pressure and vigorous friction, which might increase the oil yield. The product line was called Made in Germany Monforts.

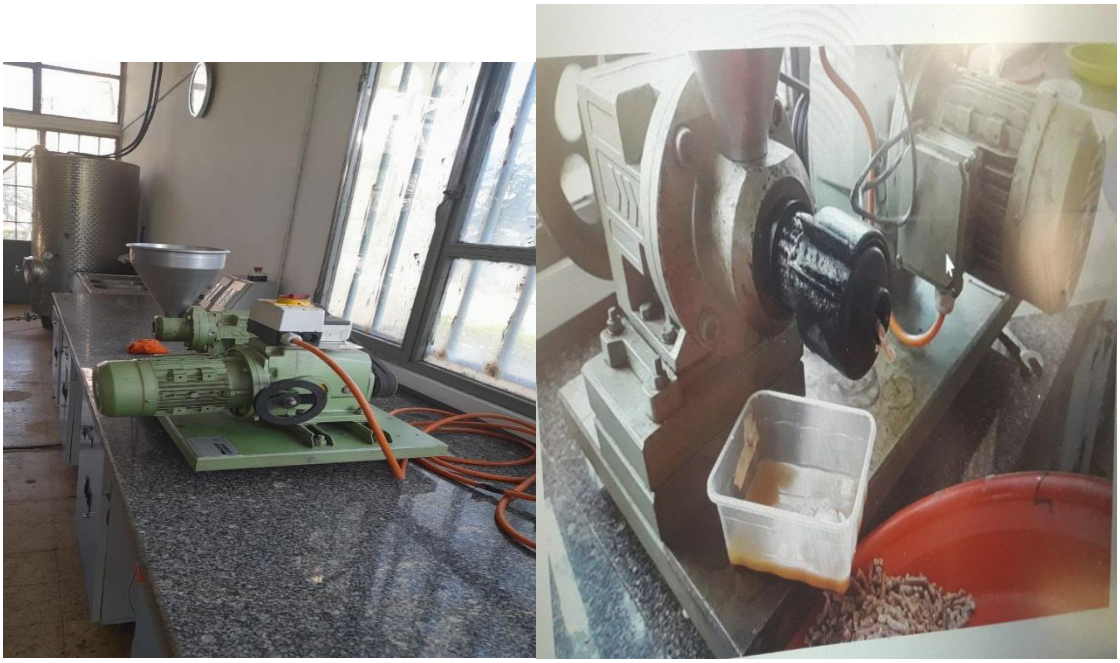


Figure 2. Screw oil presser

3.4 Physicochemical analysis of groundnut oil

The analysis of all parameters was conducted in accordance with the standard methods for oil analysis specified by ES ISO (2023).

3.4.1 Moisture content

A moisture analyzer was used to weigh five grams of oil sample, heat it to dry it, and then weigh it once again (Negash et al., 2019).

$$\text{Moisture content (\%)} = \frac{W1 * 100\%}{W2}$$

- ✓ Where: W1 is weight loss upon drying

  W2 weight of the oil sample

3.4.2 Specific gravity

With the use of a dry pyrometer, the specific gravity was determined. Specific gravity was calculated using the oil to water ratio. The pycnometer was filled with distilled water, and the results were measured using an electronic balance. Also, the weight of the oil was calculated. To keep air leaks from entering the pycometer, an attempt was made. Negash et al. (2019) state that the specific gravity value was determined using the following formula:

Specific gravity = Weight of the oil(g)

Weight of distilled water (g)

3.4.3 Peroxide value

A five-gram sample of oil was dissolved in thirty milliliters of acetic acid/chloroform solution. One more reaction was performed on this solution using 0.5 mL of 15% potassium iodide (KI). Using 0.5 mL of starch as an indication, the released iodine was titrated with 0.1 N sodium-thiosulphate. (Al Fatlawi & Abbas, 2010).

The titration was done in blank. This is how the peroxide value was computed

Peroxide value (mill equivalent peroxide/Kg oil)= S*M*1000,

g sample

Where =

S = ml of Na2S2O3 (blank corrected)

M= molarity of Na2S2O3 solution

g = mass of the sample

3.4.4 Acid value

The contents of a mixture comprising 20g of oil sample and 50 mL of ethyl alcohol with 0.5 ml of phenolphthalein indicator were heated to boiling point. When the temperature exceed 70^0C, neutralized it carefully with a solution of 0.1M sodium hydroxide. Using phenolphthalein as the endpoint indicator, a 15% KOH solution was then used to titrate. The acid value was computed

Acid value = [56.1 *V*C]/m

Where =

V- the volume in milliliters of the sodium hydroxide standard volumetric solution

C- is the precise concentration of sodium hydroxide in a standard volumetric solution expressed in moles per liter.

m- is the test portion's mass in grams.

3.4.5 Iodine value

After dissolving 18.2 g of elemental iodine in 1L of glacial acetic acid and adding 3 ml of bromine water to increase the halogen content, a mixture of 0.25 g of oil sample and 10 mL chloroform was added to 30 ml of Hanus solution. The mixture was left for 30 minutes to allow the iodine and the unsaturated bonds of oils to fully react. To protect the flask from light exposure, aluminum foil was placed over it. The remaining iodine was then converted to iodide by adding 100 mL of distilled water and 10 mL of 15% aqueous KI. Using starch as an indicator, 0.1 N sodium-thiosulphate (Na2S2O3) solutions were used to titrate the final concentration. This is how the value of iodine was calculated (ES ISO 3961) (Negash et al., 2019):

$$\text{Iodine value} = \frac{((B-S) \times N \text{ of } Na^2S^2O^3 \times 0.127 g/meq \times 100) \text{ volume for sample}}{\text{Weight of the sample (g)}}$$

Where

- ✓ B Volume ml of $Na^2S^2O^3$ volume for sample
- ✓ S Volume ml of $Na^2S^2O^3$ volume for sample

3.4.6 Relative density

Relative density done by Densitometer at 20^0c (Anton peer MDA 5000M Digital densitometer).

3.4.7 Refractive index

Before doing the measurement, it was important to make sure the refractive index of the glass plate was calibrated according to the manufacturer's instructions or by finding the refractive index of ethyl Laureate. At a temperature of 400 degrees Celsius, the sample's refractive index was measured. By running water from the water bath through the instrument, the refractometer's prism temperature was kept at the necessary constant value. It was required to keep an eye on the water's temperature after it was discharged from the refractometer using a sufficiently accurate

thermometer. Just prior to the measurement, the prism's movable element was subsequently lowered to a horizontal position. The prism's surface was then cleaned using a gentle cloth and a cotton wool piece that had been wet with a few drops of the solvent. Measure as directed by the instrument's operating handbook once it has had time to dry. Record the temperature of the instrument's prism and read the refractive index as an absolute value to the closest 0.001. Following the measurement, clean the prism's surface with a soft cloth and then a piece of cotton wool that has been saturated with a few drops of solvent. After letting it dry, take two more readings of the refractive index, compute the arithmetic mean of the three readings, and record the result as the test's outcome (Kumar et al., 2018).

$$n^{t}_{d} = n^{t1}_{d} + (t1-t)F$$

Where;

- ✓ t1-the temperature in degrees Celsius
- ✓ t- the temperature in degrees Celsius

At $t=40^{0}c$, the factor F is equal to 0.00036.

3.4.8 Saponification value

A conical flask was filled with two (2g) grams of oil. Next, 25 milliliters of the ethanolic potassium hydroxide solution were pipetted into the oil. After attaching the reflux condenser, place the flask on the heating apparatus and let it to gently boil for two hours while shaking it periodically. The heated solution was mixed with one milliliter of the phenolphthalein solution. After that, the standard volumetric hydrochloric acid solution was titrated until the indicator's pink tint almost completely vanished. Next, carry out a blank test with an additional 25 milliliters of the ethanolic potassium hydroxide solution.

$$\text{Saponification Value} = \frac{(Vo-V1)*c*56.1}{m}$$

Where

V1 is the volume of the standard volumetric hydrochloric acid solution used for the determination; c is the precise concentration of the standard volumetric hydrochloric acid solution, expressed in moles per liter; and

Vo is the volume, expressed in milliliters, of the standard volumetric hydrochloric acid solution used for the blank test.

m represents the test portion's mass in grams.

3.5 Aflatoxin analysis

Chemical and reagents

Acetonitrile, n-hexene, distilled water, methanol, and HPLC mobile phase (methanol/water/acetonitrile, 15/60/30, v/v) were the chemicals and reagents utilized for the aflatoxin analysis. Every chemical was bought from Sigma Aldrich in the United States.

Extraction and analysis

ES ISO 16050 was followed in the determination of aflatoxin concentrations from groundnut seed, oil, and cake using Alliance® high-performance liquid chromatography (HPLC) - e2695 Separations Module by Waters HPLC apparatus (USA) and 2475 fluorescence detector.

Figure 3. Extracted Groundnut seed oil and cake

The groundnut seed and cake samples were grounded to extract the afflation in addition to the oil sample (**Figure 3 and 4**). A polytron homogenizer was used to combine twenty-five (25) g of the

sample with 125 ml of solvent (70% methanol and 30% water) for ten minutes at 6000 rpm in a 250 ml baker. Extraction was done in duplicate. After each mixing there was clean up of the machine by water and methanol and then filtered by fast fluted filter paperAfter that 15 ml of the extracted sample was mixed with 30 ml of distilled water and then, 15 ml of the diluted extract was passed through immune affinity column (IAC) at a speed of 4 ml/min.

The column was then cleaned by distilled water if there any sample adheres to the tube after this eluted with methanol and waited for 10 minutes to create contact time. After evaporated using nitrogen at 40^0c added 1ml of methanol then mixed with vortex that used to create a fluid if it is dried. Then 50µL litter injected to HPLC-FLD and the excitation and emission rate is 360nm and 440 nm respectively transferred to HPLC that have Florence detector. Its mobile phase was 60 de-ionized water, 25% acetonitrile and 15% methanol. Type of column was Eclipsed plus C18, 5um, 4.6*250mm and its temperature was 35^0c; its flow rate was 1ml/min and run time 15min (ES ISO 2023).

For the preparation of spike samples and device calibration, a standard aflatoxin solution (Standard sigma Aldrich, USA) was utilized. The aflatoxin stock standard is offered for sale in a methanol solution at a concentration of 1000 µg/L. There are 250 µg/L of aflatoxins of the AFG1, G2, B1, and B2 types in it. A standard solution of aflatoxin (containing 2600 µg/L; AFG1, AFB1 = 1000 µg/L; AFG2, AFB2 = 300 µg/L) was dissolved in methanol and utilized in conjunction with this standard in the calibration curve procedures. For analysis, 60% water, 15% methanol, and 30% acetonitrile were employed in an HPLC gradient grade. For drawing standard calibration curve of AFB1 and Total Aflatoxin (TAF) used 7 different concentration (0.5ppb, 1ppb, 2ppb, 3ppb,5ppb,7ppb and 10ppb).

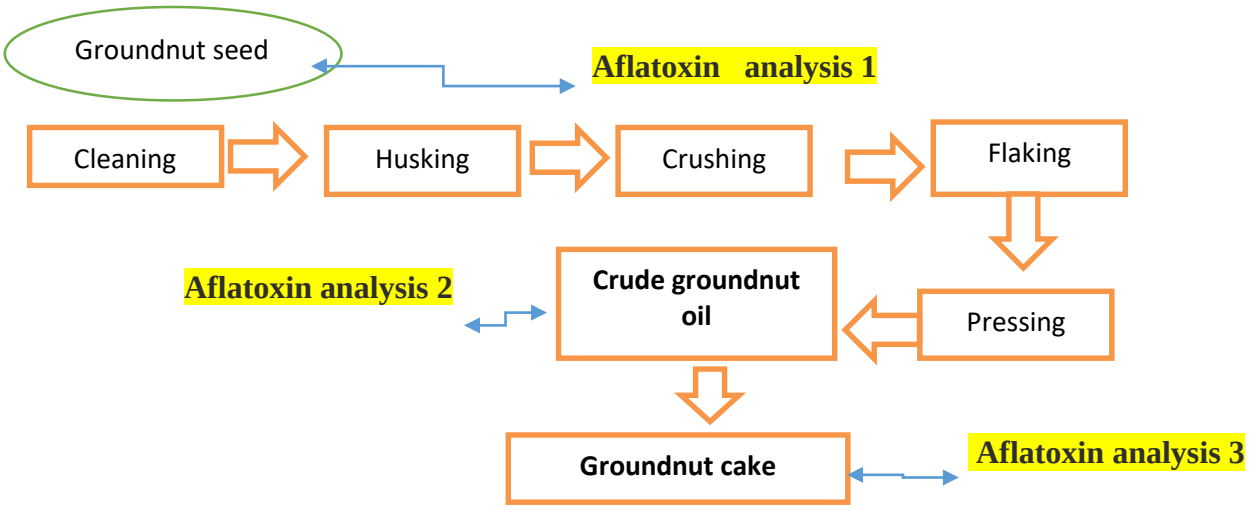


Figure 4. Flow chart of groundnut oil processing and aflatoxin analysis

3.5 Statistical analysis

Version 20 of the SPSS statistical program was used to conduct the statistical analysis. The means ± standard deviations (SD) of the data were displayed. ANOVA was employed to assess the aflatoxin levels and physicochemical properties among the groundnut, cake, and oil samples. When P values ($P<0.05$) were determined to be significant, mean separation was performed using Tukey.

4 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Physicochemical characteristics of groundnut oil

4.1.1.1 Peroxide value

The five groundnut varieties' peroxide values satisfy the Codex requirement (Codex, 2001). The mean for the five tested verities was 5 milliequivalents of active oxygen/kg, compared to the standard of up to 15 milliequivalents of active oxygen/kg oil (Table 4). In the Ethiopian standard, the peroxide value for crude oils is not specified.

4.1.1.2 Moisture content

It was found that the average moisture content of the different types of groundnut oil was 0.22 + 0.057. Table 4 indicates that there is no statistically significant variation ($P < 0.05$) in the moisture content amongst the various groundnut oils.

Table 4 Moisture content of groundnut oil produced from different varieties of groundnut seed collected from Haramaya University, 2023.

Moisture content	
Ground nut Oil varietes	**Moisture content(%)**
babile 5	0.25±0.05
bahaa guda	0.25±0.05
baha jidu	0.25±0.05
babile 1	0.3±0.05
werer	0.3±0.05

* Means with different letters across column are significantly different.

4.1.1.3 Acid value

The average acid values for five varieties of groundnut oil were found to be 0.193±0.006, 0.275±0.003, 0.253±0.012, 0.253±0.003, and 0.237±0.009 for Babile 1, Werer 961, Bahaajiduu, Babile 5, and Bahaa-guda respectively (as shown in Table 4). There was a notable distinction ($P < 0.05$) observed among the acid values of these different groundnut varieties' oils.

4.1.1.4 Iodine value

The average iodine values were recorded as 81.92±1.107, 90.64±0.756, 86.14±0.126, 89.16±0.934, and 84.4±1.117 for Babile 1, Werer 961, Bahaajiduu, Babile 5, and Bahaa-guda respectively, as indicated in Table 4. There was a notable difference ($P<0.05$) observed in the iodine values among the various groundnut oil varieties.

4.1.1.5 Saponification Value

The average saponification values for Babile 1, Werer 961, Bahaajiduu, Babile 5, and Bahaa-guda were recorded as 190.25±0.145, 191.68±0.062, 191.68±0.038, 190.32±0.049, and 189.29±0.503 respectively, as presented in Table 4. There was a significant discrepancy ($P<0.05$) observed among the saponification values of the different groundnut oil varieties. Specifically, Werer 961 exhibited the highest saponification value, whereas Bahaa-guda demonstrated the lowest.

4.1.1.6 Refractive index

The mean refractive index for the five groundnut oil varieties was 1.46±0.0 (Table 4). The refractive indices of the five different types of groundnut oil did not significantly differ from one another.

4.1.1.7 Density

The mean relative density for Babile 1 was 0.916±0.003, Werer 961and Bahaa-guda test result the same that was 0.910±0 for and Bahaajiduu and Babile 5 was 0.92±0. The relative densities of the five different groundnut oils vary significantly ($P < 0.05$). . Werer 961 groundnut variety oil had the lowest relative density, while Babile 5 had the lowest.

4.1.1.8 Specific gravity

Each of the five groundnut oils had a mean specific gravity of 0.92 (Table 4). There was no discernible difference in the specific gravity of the five types of groundnut oil.

Table 5. The physicochemical characteristic of groundnut oil produced from different varieties of groundnut seed collected from Haramaya University, 2023.

Ground nut Variety	**Physicochemical characteristics**						
	Peroxide value (mEq oxygen/ kg oil)	**Iodine value (g I_2/100 g oil)**	**Acid value (mg KOH/g oil)**	**Saponification value (mg KOH/g oil)**	**Refractive index**	**Density (kg/m^3)**	**Specific gravity**
Babile 1	5.00±0.2 a	81.92±1.9 1a	0.19±0.0 1a	190.25±0. 25a	1.46±0.0 1a	0.92±0.0 1b	0.92±0.0 1a
Werer 961	5.00±0.3 a	90.64±1.3 0d	0.27±0.0 0c	191.68±0. 1b	1.46±0.0 1a	0.91±0.0 0a	0.92±0.0 0a
Bahaajid uu	5.01±0.0 1a	86.14±0.2 1bc	0.25±0.0 1bc	191.68±0. 06b	1.46±0.0 0a	0.92±0.0 0b	0.92±0.0 0a
Babile 5	5.00±0.0 1a	89.16±1.6 1cd	0.25±0.0 0bc	190.32±0. 08a	1.46±0.0 1a	0.92±0.0 0b	0.92±0.0 0a
Bahaa-guda	5.01±0.1 a	84.40±1.9 3ab	0.23±0.0 1b	189.29±0. 87a	1.46±0.0 2a	0.91±0.0 0a	0.92±0.0 1a

* Refractive index and specific gravity is unit less. mEq: milliequivalents. Means with different letters across column are significantly different.

4.1.2 Ocurrence of aflatoxin

Aflatoxin levels in the oil, cake, and groundnut seed samples were measured after each groundnut sample had its oil extracted. Aflatoxin levels in each sample are displayed in (Table 5).

Out of the nine groundnut samples that were tested for aflatoxins, all of them had levels of AFB1 contamination ranging from 0.3 to 405 µg/kg and TAF contamination ranging from 0.5 to 495.9 µg/kg. Examining the data reveals that when the oil was extracted from the groundnut samples, aflatoxin was transferred to it at a very low level. Total aflatoxin (TAF) (495.9 (µg/kg)) from the Bure groundnut seed variety, which was purchased at the AA local market, had the highest aflatoxin concentration found. The maximum AFB1 concentration found in the groundnut oil was 38.65 (µg/kg), but the TAF concentration was 79.7 (µg/kg). The average concentrations of AFB1 and TAF in the samples of groundnut seed, oil, and cake were The mean AFB1 and TAF concentration from the groundnut seed, oil and cake samples were significantly different ($P < 0.05$) among all the samples. It was also observed that aflatoxin remained in cake at a higher level.

Method validation

For the analysis, the limit of detection (LOD) was 0.165, 0.066, 0.165, and 0.066 G1, G2, B1, and B2, respectively. The LOD is the lowest concentration at which the method can identify the analyte within the matrix with a certain degree of confidence. The lowest concentration of the analyte that could be reliably measured by the method was known as the limit of quantitation (LOQ), and it was 0.05, 0.02, 0.05, and 0.02 for G1, G2, B1, and G2, respectively. Additionally, the percentage of the analyte that was added to or present in the analytical portion of the test material that was extracted and submitted for measurement is represented by the recovery values of 85%, 75%, 75%, and 85% for G1, G2, B1, and B2, respectively.

Table 6. The level of aflatoxin in groundnut seed, oil and cake samples collected from Addis Ababa local market and Haramaya University, 2023.

No.	Types of groundnut seed	AFB1 (µg/kg)	TAF (µg/kg)
1	Groundnut variety collected from Addis Ababa (AA) local market	405.00 ± 0.00 c	495. 9 ± 0.00 b
1	Groundnut oil	38.65 ± 0.35 a	79.7 ± 1.69 a
1	Groundnut cake	305.00 ± 0.00 b	490 ± 14.14 b
2	Groundnut variety collected from AA local market	75.00 ± 2.82 c	335.00 ± 0.00 c
2	Groundnut oil	4.80 ± 0.00 a	15.75 ± 0.21 a
2	Groundnut cake	23.00 ± 0.00 b	25.6 ± 0.14b
3	Werer 961 groundnut variety collected from Haramaya University	11.15 ± 0.07 b	24.50 ± 0.00 c
3	Groundnut oil	0.30 ± 0.00 a	0.50 ± 0.00 a
3	Groundnut cake	0.30 ± 0.00 a	1.00 ± 0.14 b

*TAF: Total afaltoxin. Means with different letters across column among each groundnut seed types are significantly different.

Table 7 The level of aflatoxin B2,G1 and G2 in groundnut seed, oil and cake samples collected from Addis Ababa local market and Haramaya University, 2023.

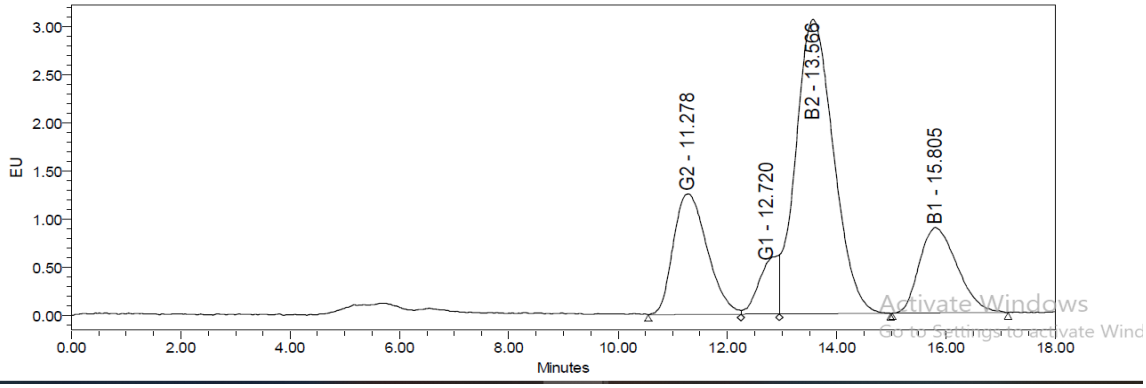
No.	Types of groundnut seed	AFB2 (µg/kg)	AFG1 (µg/kg)	AFG2 (µg/kg)
1	Groundnut variety collected from Addis Ababa (AA) local market Bure	15± 0.00 b	2.5±0.15b	1.90±0.20a
1	Groundnut oil	6.5±0.00a	0.8±0.01a	0.00±0.00a
1	Groundnut cake	15±00b	20.00±0.23c	0.00±0.00a
2	Groundnut variety collected from AA local market Harer	20.0±0.02c	215.00±1.34c	215.00±1.23c
2	Groundnut oil	2.50±0.12b	0.00±0.02a	0.00±0.02a
2	Groundnut cake	0.80±0.02a	9.45±0.34b	9.45±0.34b
3	Werer 961 groundnut variety collected from Haramaya University	1.90±0.35b	9.950±0.00b	1.50±0.43b
3	Groundnut oil	000±0.00a	0.20±0.01a	0.00±0.00a
3	Groundnut cake	000±0.00a	0.60±0.02a	0.10±0.00a

*Means with different letters across column among each groundnut seed types are significantly different.

Table 8 Chromatogram for Aflatoxin analysis

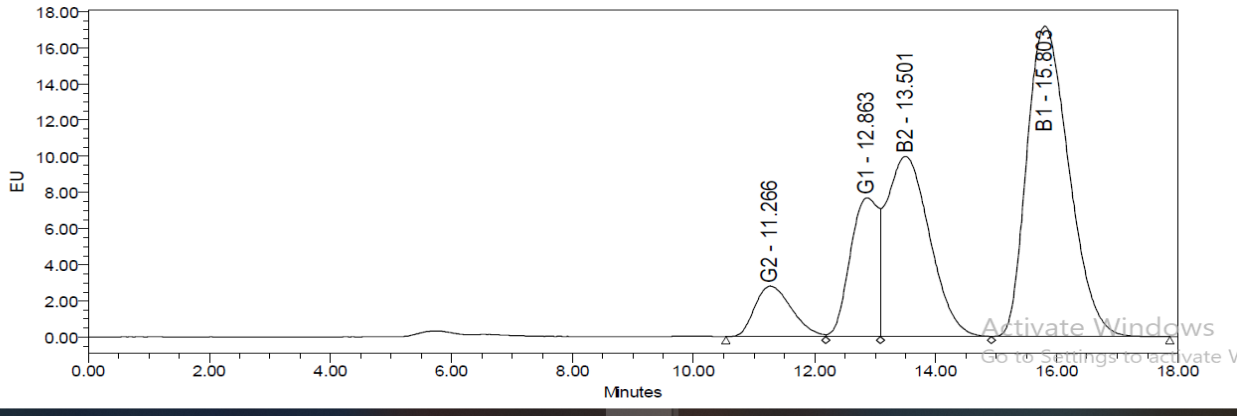
SAMPLE INFORMATION

Sample Name:	CCV 2 PPB	Acquired By:	ECAE
Sample Type:	Control	Sample Set Name:	230523 Afla in ground nut oil
Vial:	2	Acq. Method Set:	Aflatoxin Analysis method
Injection #:	1	Processing Method:	230517 Aflatoxin
Injection Volume:	10.00 ul	Channel Name:	2475ChA ex360/em440
Run Time:	18.0 Minutes	Proc. Chnl. Descr.:	2475ChA ex360/em440
Date Acquired:	5/23/2023 2:18:25 PM EAT		
Date Processed:	5/24/2023 11:14:53 AM EAT		



SAMPLE INFORMATION

Sample Name:	15251038	Acquired By:	ECAE
Sample Type:	Unknown	Sample Set Name:	230523 Afla in ground nut oil
Vial:	3	Acq. Method Set:	Aflatoxin Analysis method
Injection #:	1	Processing Method:	230517 Aflatoxin
Injection Volume:	10.00 ul	Channel Name:	2475ChA ex360/em440
Run Time:	18.0 Minutes	Proc. Chnl. Descr.:	2475ChA ex360/em440
Date Acquired:	5/23/2023 2:37:09 PM EAT		
Date Processed:	5/24/2023 11:14:53 AM EAT		



SAMPLE INFORMATION

Sample Name:	15251038	Acquired By:	ECAE
Sample Type:	Unknown	Sample Set Name:	230523 Afla in ground nut oil
Vial:	3	Acq. Method Set:	Aflatoxin Analysis method
Injection #:	2	Processing Method:	230517 Aflatoxin
Injection Volume:	10.00 ul	Channel Name:	2475ChA ex360/em440
Run Time:	18.0 Minutes	Proc. Chnl. Descr.:	2475ChA ex360/em440
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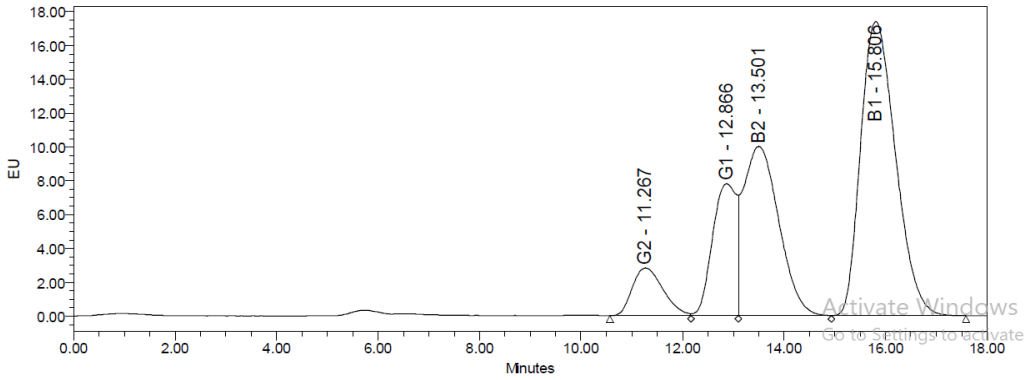

SAMPLE INFORMATION

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Sample Type:	Unknown	Sample Set Name:	230523 Afla in ground nut oil
Vial:	4	Acq. Method Set:	Aflatoxin Analysis method
Injection #:	1	Processing Method:	230517 Aflatoxin
Injection Volume:	10.00 ul	Channel Name:	2475ChA ex360/em440
Run Time:	18.0 Minutes	Proc. Chnl. Descr.:	2475ChA ex360/em440
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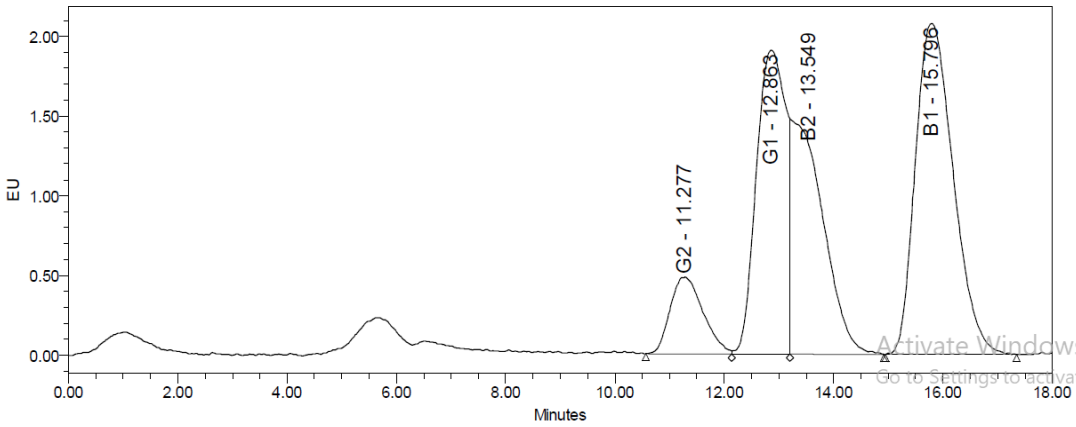

SAMPLE INFORMATION

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Injection Volume:	10.00 ul	Channel Name:	2475ChA ex360/em440
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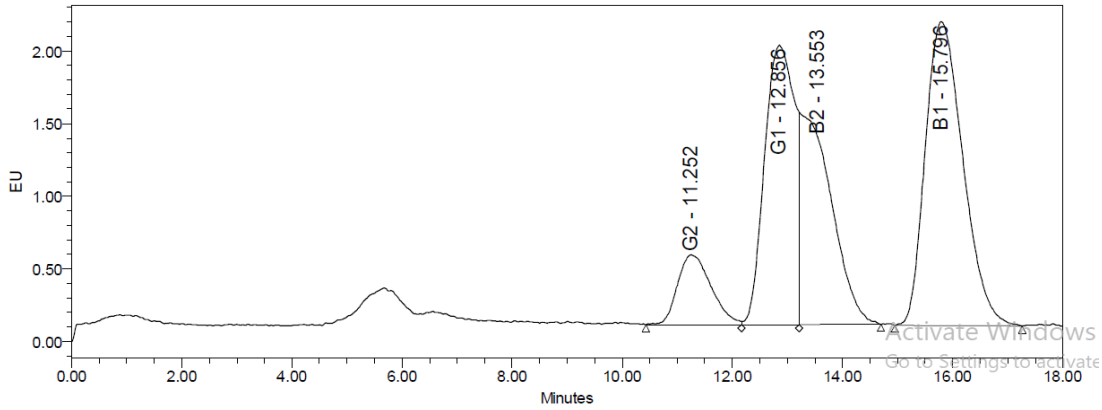

SAMPLE INFORMATION

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Injection #:	1	Processing Method:	230517 Aflatoxin
Injection Volume:	10.00 ul	Channel Name:	2475ChA ex360/em440
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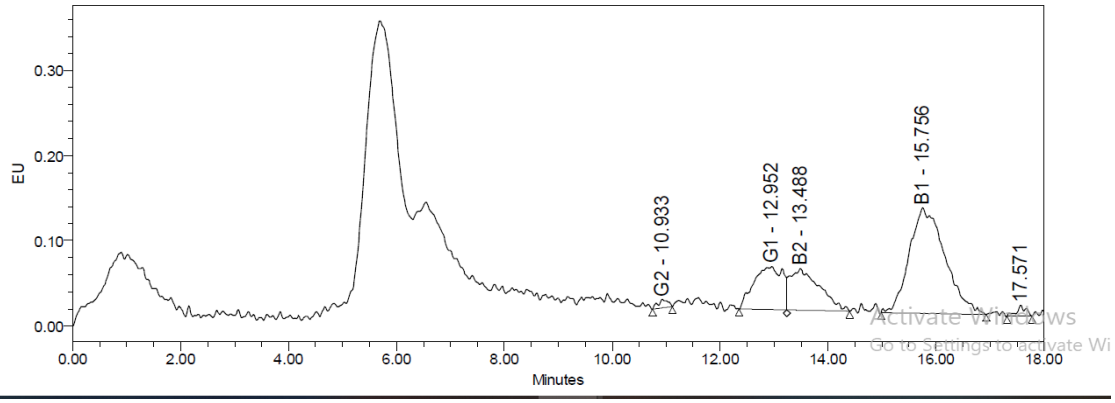

SAMPLE INFORMATION

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Injection #:	1	Processing Method:	230517 Aflatoxin
Injection Volume:	10.00 ul	Channel Name:	2475ChA ex360/em440
Run Time:	18.0 Minutes	Proc. Chnl. Descr.:	2475ChA ex360/em440
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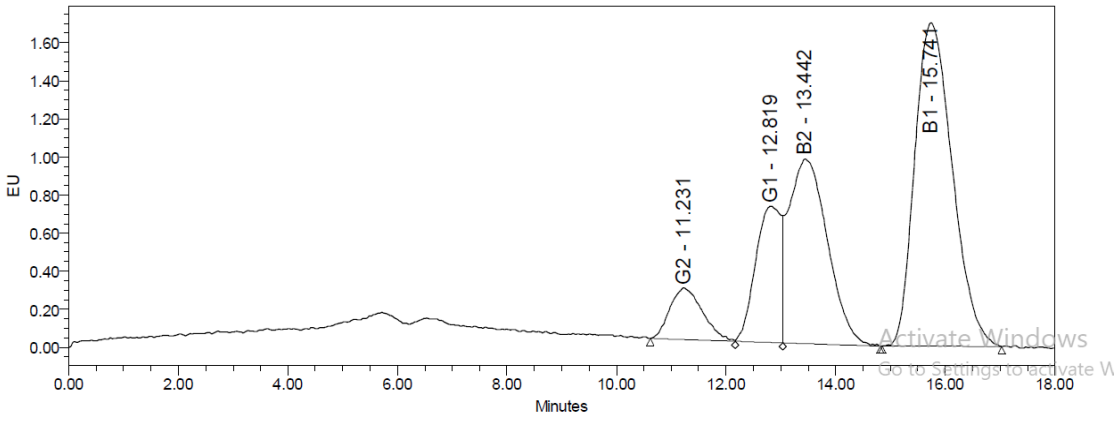

SAMPLE INFORMATION

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Sample Type:	Unknown	Sample Set Name:	230523 Afla in ground nut oil
Vial:	5	Acq. Method Set:	Aflatoxin Analysis method
Injection #:	2	Processing Method:	230517 Aflatoxin
Injection Volume:	10.00 ul	Channel Name:	2475ChA ex360/em440
Run Time:	18.0 Minutes	Proc. Chnl. Descr.:	2475ChA ex360/em440
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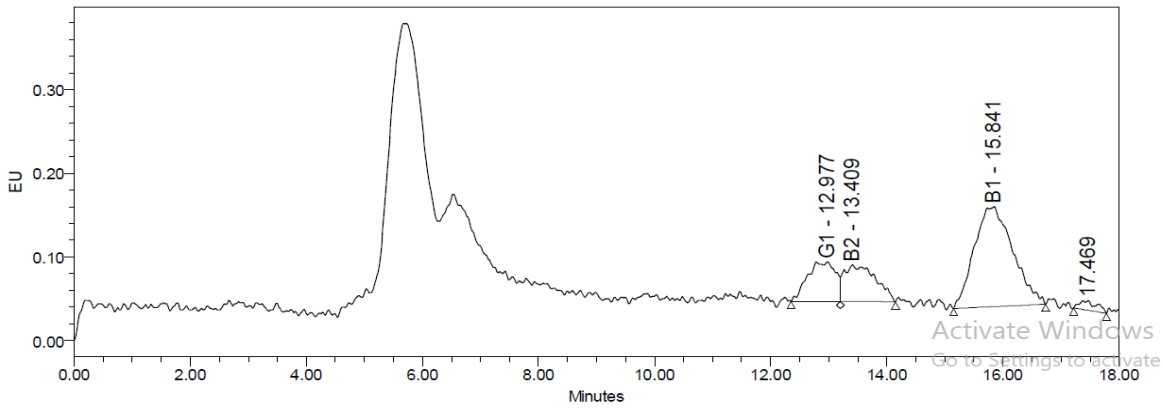

SAMPLE INFORMATION

Sample Name:	15251038 dil 10*	Acquired By:	ECAE
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Vial:	3	Acq. Method Set:	Aflatoxin Analysis method
Injection #:	2	Processing Method:	230517 Aflatoxin
Injection Volume:	10.00 ul	Channel Name:	2475ChA ex360/em440
Run Time:	18.0 Minutes	Proc. Chnl. Descr.:	2475ChA ex360/em440
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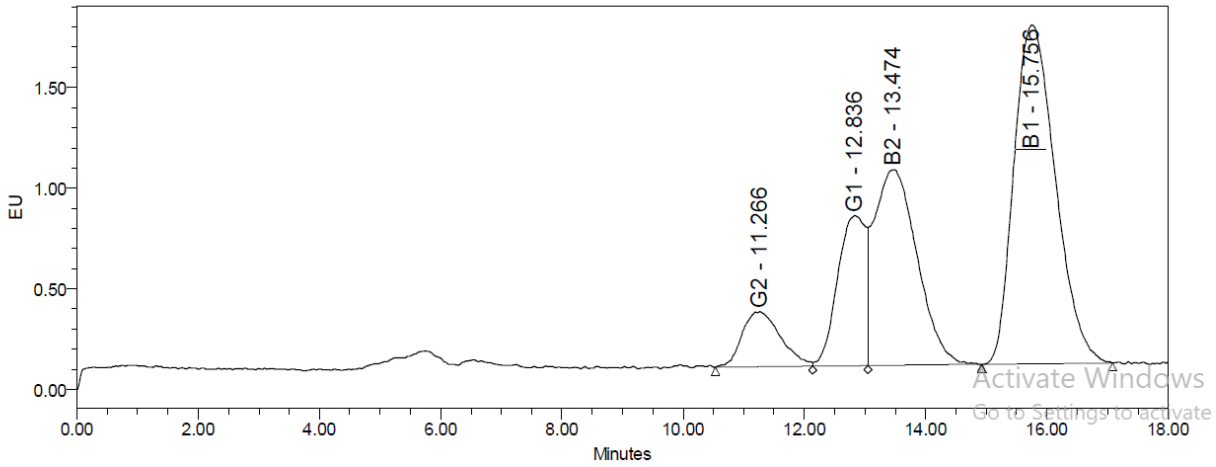

4.2 Discussion

Edible oils with moisture contents between 0.05 and 0.3 indicate a high risk of rancidity according to the Codex Alimentarius. Previous research (Al-Fatlawi & Abbas, 2010; Okechalu et al., 2011) shown that oils produced with inferior technology had a greater moisture content. The mean moisture level in our sample was 0.22, which is more than the 0.05% Ethiopian standard maximum limit and suggests that the groundnut oil may undergo rancidity (ES ISO, 2023). The major purpose of determining an oil's iodine value was to identify the varieties of oil that are more saturated. The amount of iodine needed to saturate the fatty acids in 100 grams of oil is equal to the iodine value. Iodine values are low in oils rich in saturated fatty acids and high in oils rich in unsaturated fatty acids (Bennion, 1995). An essential feature of oils is their iodine value, which shows how much unsaturated fatty acid is there. In our investigation, we discovered that the groundnut oil's iodine values ranged from 81.92 to 90.64g I2/100g. The iodine readings of 95.87±0.15g I2/100g obtained by Sulaiman *et al*. (2012) are higher than these values. Furthermore, Tupe & Shewale (2022) studied 90 I2/100g, which was less than this trial. Therefore, all detected groundnut varieties (Ethiopian meat standards for oil consumption range from 71 to 107) have lower iodine values and are not advised for ingestion. It is advised by studies to move from saturated to unsaturated. Comparing the experiment's results to the Ethiopian standard (2023) for edible oil reveals that the iodine values obtained were within the regulatory norm and did not surpass the allowable limit; the range is 77-107. Werer 961 oil extract has the highest iodine value of 90.64±1.3 g/100g, suggesting that unsaturated fatty acids, particularly oleic oil, are present. This is a measurement of the stability and oxidation resistance of fat or oil. This significant variation in iodine value results from a variable fatty acid arrangement (Musa et al., 2012).

Through oxidative deterioration, edible oil's peroxide value (PV) serves as a freshness indicator. Our study's PV was less than the WHO/FAO guidelines, which are 10 milliequivalents of active oxygen per kilogram of oil. However, PV of our study results was higher compared to a previous study by Island & Harcourt (2015) that reported 1.8 mEq/kg on fresh extracted groundnut seed oil This might be a cause of oxidation during storage and radiation after extraction of oil (Alem & Taufiq , 2004). As Maduelosi (2012) indicated the effect of light increase peroxide value of groundnut crude oil. The oils' higher saturation, which prevents oxidation and satisfies Ethiopia's

peroxide value criterion of a maximum of 15 milliequivalents of active oxygen/kg of oil, is probably the cause of their lower peroxide values.

Acid value (AV), which quantifies free fatty acids, is typically regarded as one of the key indicators of groundnut oil quality, refining level, and quality deterioration over storage. The acceptable limit (AV) for cold-pressed groundnut oil (below 1 mg KOH/g) is met by the study's AV, which ranged from 0.19 to 0.28 mg KOH/g. Our research findings were consistent with those of Huang et al.'s (2022) earlier investigation, which found that cold-pressed groundnut oil contained 0.11–2.74 mg KOH/g AV. However, when compared to a prior study (Rao et al., 2009), our results were lower. Lower AV value of edible oil has significantly good nutritional value because there is no degrading of essential fatty-acids and nutrients (Li *et al.*, 2015). All results for the acid value fell under the maximum 4.0 mg KOH/g for cold-pressed oil allowed by Ethiopian standards. Determining the acid value of an oil is frequently used to provide a broad indication of its state and edibility. This is because, depending on the variety, an increase in acid value is associated by the development of unpleasant flavors and odors (Nkafamiy *et al*., 2006).

The density of vegetable oil depends on the amount of fatty acids. The mass of a substance per unit volume is its density. The concept of relative density can be used to measure density.
The refining and upgrading procedure may have an impact on the relative density value. The density of groundnut oil was tested by Briggs & Victor (2014), and their results, which were comparable to our study's, were 0.920 kg/m3. The improper upgrading and refining procedure may be the cause of the specific gravity variation (Australian Oilseeds Federation Inc, 2011). The findings of our investigation were consistent with those of Briggs & Victor's (2014) earlier study, which found a specific gravity of 0.914 kg/m3. Our results meet the Ethiopian standard for the Relative Density range of 0.912-0.920. Research has demonstrated that the molecular weights of various oils vary with their specific gravities, which are influenced by refining procedures and provide non-invasive access to molecular information (Nkafamiy et.al 2006).

The ratio of light speed in a vacuum to light speed through a specific substance is known as the refractive index, or RI. This physical characteristic has been widely applied to the identification and characterization of materials, including lipids. For a long time, it has been known that fatty

acid chemistry, such as chain length and degree of unsaturation, and RI are related. The RI of a given oil will rise as unsaturated fatty acid contents rise, as often shown by the iodine value (Majors *et al.,* 1939). The appropriate range of 1.46 to 1.465 is occupied by the RI of the groundnut oil utilized in our investigation. Our findings concurred with those of a prior study conducted in 2015 by Island & Harcourt, which found that cold-pressed groundnut oil had 1.464 RI. Our results are within the 1.460–1.465 Ethiopian standard.

Studies have demonstrated that RI rises in proportion to the number of double bonds present in a lipid molecule's fatty acids (Ermosele & Pascal, 2003). In order to determine the oil's relative reflectance (RI), Pearson (1991) states that the number of impurities in the oil has an impact on the degree of reflection a light ray causes.

The type of fatty acid that is present in the fat determines the saponification value (SV). The SV for groundnut oil in our study is between 189.29 and 191.68 mg KOH/g, which is consistent with a prior work by Siddeeg & Xia (2015) that found an SV of 190 mg KOH/g. High SV (192 mgKOH/g oil) has been recorded, which is in contrast to our findings (Aluyor et al., 2009). The neutralization of fatty acids that may have come from the oil's hydrolysis is widely thought to be the cause of this drop. The amount of potassium hydroxide (KOH) milligrams needed to neutralize the fatty acids indicates the oil sample's degree of hydrolysis (Aluyor et al., 2009). The results of this investigation into the several types of ground nut oil obtained were all within the acceptable range (187–196 mg KOH/g oil) and the legal Ethiopian requirement. According to Denniston et al. (2004), a high saponification value suggested that there were more ester linkages present and that the fat molecules were intact. However, calculating the saponification value is a valid way to characterize fat because each fat has a constant fatty acid content within the bounds of biological fluctuation (Musa et al., 2012).

Aflatoxin was found in samples of groundnut seed, oil, and cake from each of the various groundnut cultivars. Aflatoxin levels ranged from 0.5 - 495.9 µg/kg (TAF) and 0.3 - 405 µg/kg (AFB1), respectively, in all the samples. The quantities of AFB1 and TAF in the peanut kernel were higher than those in the cake and oil samples. In this study, following pressing and extraction, 2.02–16% of the TAF and 2.7-9.5% of the AFB1 found in the groundnut kernel were transported to the extracted oil, respectively. This could be because aflatoxins are mostly attached to proteins

and are eliminated together with the cake (van Duijn, 2023). Depending on the type of oil and the refinement method used, aflatoxin levels can be reduced by extracting and refining edible vegetable oils (Bordin et al., 2014).

The groundnut seed had greater levels of aflatoxin, according to our study's findings. Seven out of nine (77.8%) AFB1 positive samples had levels over Ethiopia's upper threshold of five micrograms per kilogram. In a similar vein, the maximum amount of total aflatoxins in 7 out of 9 (77.8%) of the samples was higher than the Ethiopian standard (10 µg/kg). Our findings, however, agreed with those of a prior investigation conducted by Ayalew et al. (2006), which found that groundnut seeds contained 5–250µg/kg of total aflatoxin. Total aflatoxin concentrations in groundnut seed were found to range from 15 to 11,900 µg/kg in another study by Chala et al. (2013). Total aflatoxin concentrations in Ethiopian groundnut seed samples were also found to range from 786 to 31,35 µg/kg in another study by Mohammed et al. (2016).

Aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), and G2 (AFG2) study was carried out in 76 edible oil samples (peanut oil, soybean oil, corn embryo oil, and blended oil) by Yang *et al*., 2011. Oil sample AFB1 and AFB2 levels ranged from 0.14 to 2.72 µg kg−1 and 0.15 to 0.36 µg kg−1, respectively. AFG1 levels were lower, ranging from 0.01 to 0.02 µg kg−1, and no AFG2 contamination was found in any of the samples. This result correspondence with our investigation range with 6.5 to 0.00 µg kg−1 for AB2 and 0.8 to 0.00 µg kg−1 for AG1 alike our AFG2 is not present in our extracted ground nut oil . As mention on perivious paragraph the aflatoxin lavel decreses from the seed to oil in case of AF B2 G1G2 similarly.

Peanut oil AFB2 average is 0.89 ng/g compared to 6.5 ng/g ($P<0.05$). The maximum level of our result was 0.8 ($P<0.05$), whereas the AFG1 average for peanut oils is 0.54 ng/g. In contrast to our study, the AFG2 average of peanut oils was 0.66 ng/g, meaning that in this finding, AFG2 had the highest proportion of all oils, but not in our study.

5 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Groundnut seed extracted its crude oil to measure the physicochemical parameter that of crude oil and aflatoxin level. The results showed for all tested Groundnut crude oil varieties has acceptable and meet the physicochemical parameter that were acid value, peroxide value, iodine value, saponification value, relative density, specific gravity and referactive index of edible oil according to Ethiopian standard also Codex (2023). But the moisture content in oil was above the standards that might be one factor for aflatoxin presence in the groundnut oil as it known one is major factor for aflatoxin development is availability of moisture. As previously mentioned in the literature, there is a strong likelihood that aflatoxin is present in large quantities in the groundnuts that are readily available in the market due to several factors, including storage issues. This study likewise comes to this conclusion. However, the analysis's findings indicate that aflatoxin is only very weakly transferred to crude oil. Even while aflatoxin accumulates in the oil cake and is transferred to oil at a slower pace than it is in seeds, crude oil still doesn't fulfill standards. This reveals oil extraction lower the aflatoxin transmission but attention to be taken at first stage on the production of groundnut and postharvest mechanism. The oil cake shows highest Aflatoxin presence this may because high nutrition found on it.

5.2 Recommendation

Following the analysis conducted, it is highly recommended to advance towards refining the crude oil process as the subsequent step in our course of action. Through refining the crude oil, we foresee a potential reduction or stabilization in aflatoxin levels. This refining process holds paramount importance in guaranteeing the quality and safety of the eventual product. Therefore, it is imperative to implement measures aimed at enhancing the refining process to effectively mitigate the risks of aflatoxin contamination and maintain product standards at their highest level.

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7 Annexes

Annex 1: ANOVA table for the physicochemical characteristcs of groundnut oil and the levels of aflatoxin in the groundnut seed, cake and oil

A. Physicochemical characteristcs of groundnut oil

Groundnut Variety	**Physicochemical characteristics**						
	Peroxide value (mEq oxygen/kg oil)	**Iodine value (g I_2/100 g oil)**	**Acid value (mg KOH/g oil)**	**Saponification value (mg KOH/g oil)**	**Refractive index**	**Density (kg/m^3)**	**Specific gravity**
Babile 1	5.00±0.2a	81.92±1.91a	0.19±0.01a	190.25±0.25a	1.46±0.01a	0.92±0.01b	0.92±0.01a
Werer 961	5.00±0.3a	90.64±1.30d	0.27±0.00c	191.68±0.1b	1.46±0.01a	0.91±0.00a	0.92±0.00a
Bahaajiduu	5.01±0.01a	86.14±0.21bc	0.25±0.01bc	191.68±0.06b	1.46±0.00a	0.92±0.00b	0.92±0.00a
Babile 5	5.00±0.01a	89.16±1.61cd	0.25±0.00bc	190.32±0.08a	1.46±0.01a	0.92±0.00b	0.92±0.00a
Bahaa-guda	5.01±0.1a	84.40±1.93ab	0.23±0.01b	189.29±0.87a	1.46±0.02a	0.91±0.00a	0.92±0.01a

B. The levels of aflatoxin in the groundnut seed, cake and oil for the three samples

No.	**Types of groundnut seed**	**AFB1 (µg/kg)**	**TAF (µg/kg)**

1	Groundnut variety collected from Addis Ababa (AA) local market	405.00 ± 0.00 c	495. 9 ± 0.00 b
1	Groundnut oil	38.65 ± 0.35 a	79.7 ± 1.69 a
1	Groundnut cake	305.00 ± 0.00 b	490 ± 14.14 b
2	Groundnut variety collected from AA local market	75.00 ± 2.82 c	335.00 ± 0.00 c
2	Groundnut oil	4.80 ± 0.00 a	15.75 ± 0.21 a
2	Groundnut cake	23.00 ± 0.00 b	25.6 ± 0.14b
3	Werer 961 groundnut variety collected from Haramaya University	11.15 ± 0.07 b	24.50 ± 0.00 c
3	Groundnut oil	0.30 ± 0.00 a	0.50 ± 0.00 a
3	Groundnut cake	0.30 ± 0.00 a	1.00 ± 0.14 b

Annex 2: Chromatogram for aflatoxin analysis

SAMPLE INFORMATION

Sample Name:	CCV 2 PPB	Acquired By:	ECAE
Sample Type:	Control	Sample Set Name:	230523 Afla in ground nut oil
Vial:	2	Acq. Method Set:	Aflatoxin Analysis method
Injection #:	1	Processing Method:	230517 Aflatoxin
Injection Volume:	10.00 ul	Channel Name:	2475ChA ex360/em440
Run Time:	18.0 Minutes	Proc. Chnl. Descr.:	2475ChA ex360/em440
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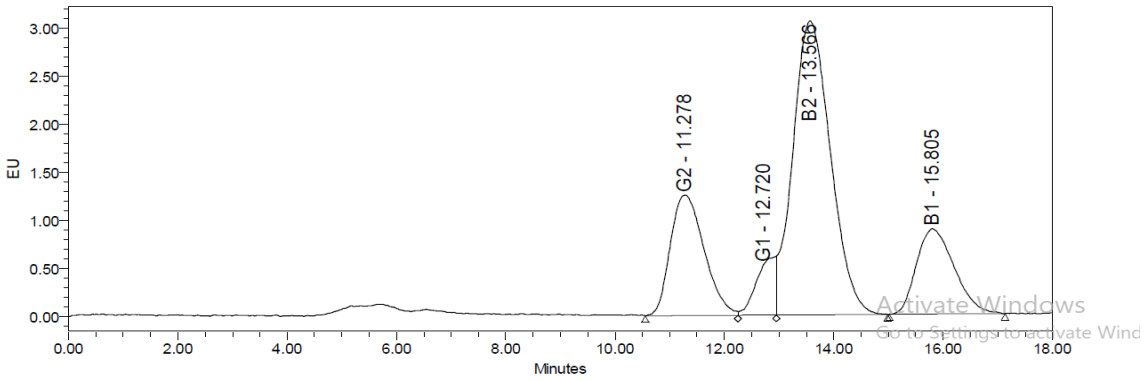

SAMPLE INFORMATION

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Sample Type:	Unknown	Sample Set Name:	230523 Afla in ground nut oil
Vial:	3	Acq. Method Set:	Aflatoxin Analysis method
Injection #:	1	Processing Method:	230517 Aflatoxin
Injection Volume:	10.00 ul	Channel Name:	2475ChA ex360/em440
Run Time:	18.0 Minutes	Proc. Chnl. Descr.:	2475ChA ex360/em440
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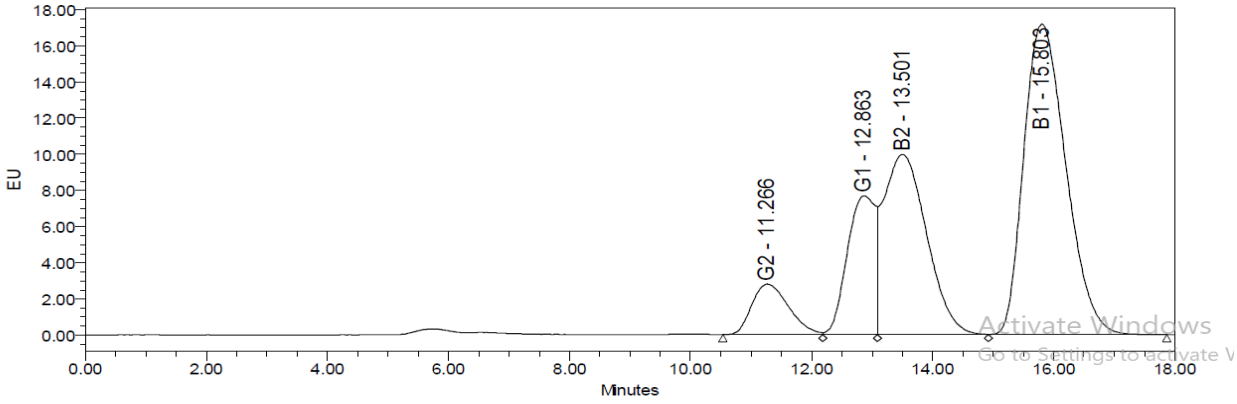

SAMPLE INFORMATION

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Sample Type:	Unknown	Sample Set Name:	230523 Afla in ground nut oil
Vial:	3	Acq. Method Set:	Aflatoxin Analysis method
Injection #:	2	Processing Method:	230517 Aflatoxin
Injection Volume:	10.00 ul	Channel Name:	2475ChA ex360/em440
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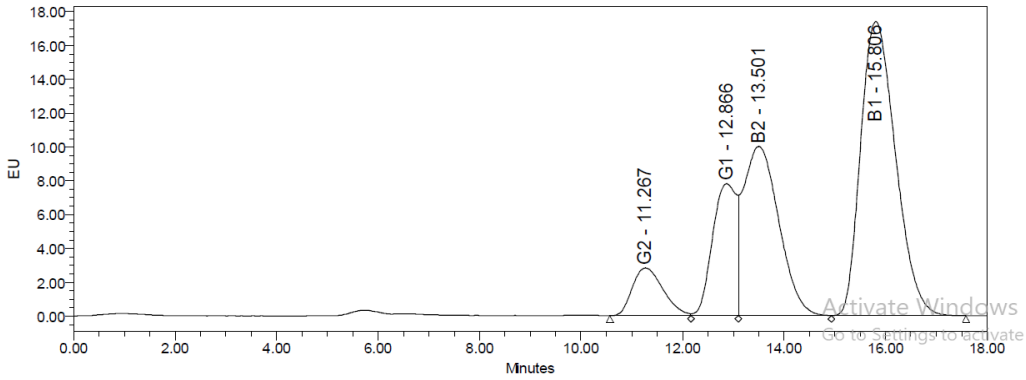

SAMPLE INFORMATION

Sample Name:	15251039	Acquired By:	ECAE
Sample Type:	Unknown	Sample Set Name:	230523 Afla in ground nut oil
Vial:	4	Acq. Method Set:	Aflatoxin Analysis method
Injection #:	1	Processing Method:	230517 Aflatoxin
Injection Volume:	10.00 ul	Channel Name:	2475ChA ex360/em440
Run Time:	18.0 Minutes	Proc. Chnl. Descr.:	2475ChA ex360/em440
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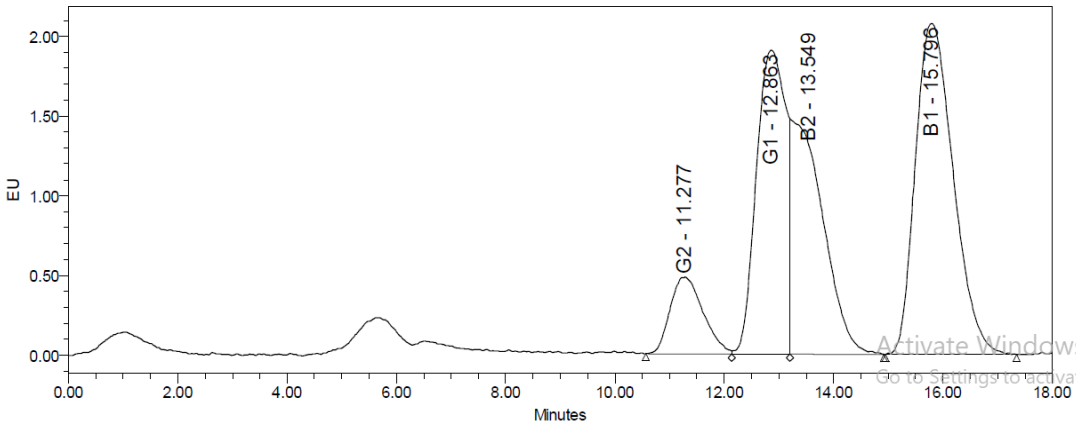

SAMPLE INFORMATION

Sample Name:	15251039	Acquired By:	ECAE
Sample Type:	Unknown	Sample Set Name:	230523 Afla in ground nut oil
Vial:	4	Acq. Method Set:	Aflatoxin Analysis method
Injection #:	2	Processing Method:	230517 Aflatoxin
Injection Volume:	10.00 ul	Channel Name:	2475ChA ex360/em440
Run Time:	18.0 Minutes	Proc. Chnl. Descr.:	2475ChA ex360/em440
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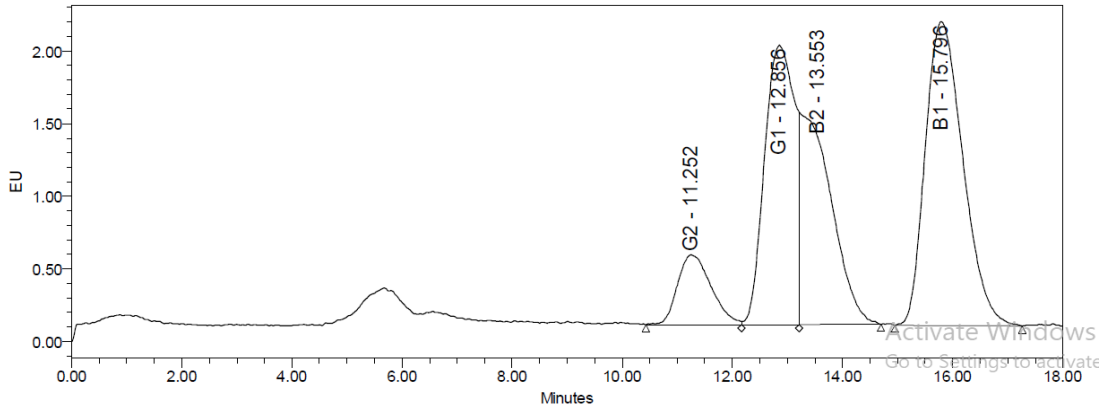

SAMPLE INFORMATION

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Vial:	5	Acq. Method Set:	Aflatoxin Analysis method
Injection #:	1	Processing Method:	230517 Aflatoxin
Injection Volume:	10.00 ul	Channel Name:	2475ChA ex360/em440
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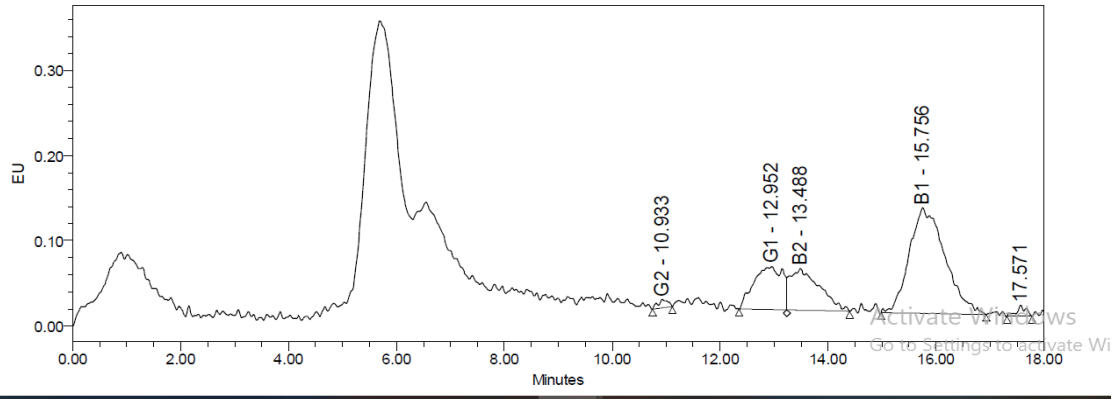

SAMPLE INFORMATION

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Injection #:	1	Processing Method:	230517 Aflatoxin
Injection Volume:	10.00 ul	Channel Name:	2475ChA ex360/em440
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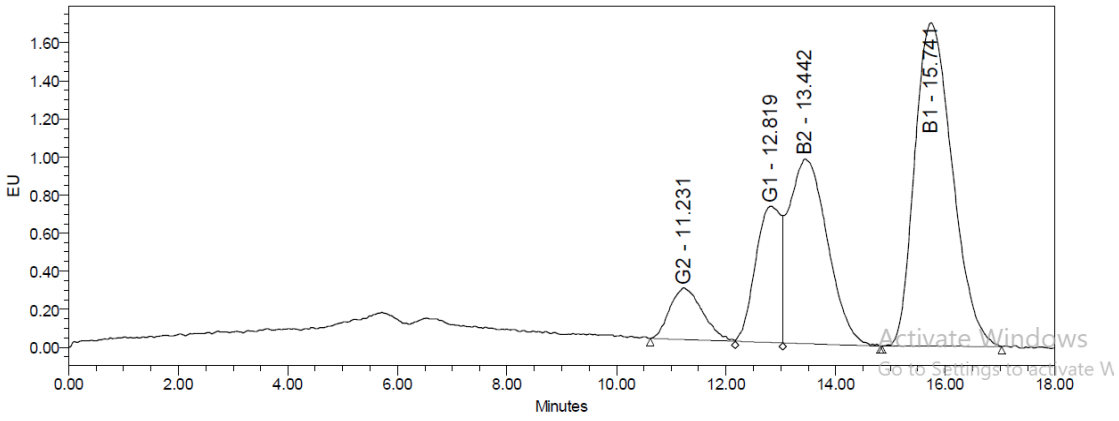

SAMPLE INFORMATION

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Sample Type:	Unknown	Sample Set Name:	230523 Afla in ground nut oil
Vial:	5	Acq. Method Set:	Aflatoxin Analysis method
Injection #:	2	Processing Method:	230517 Aflatoxin
Injection Volume:	10.00 ul	Channel Name:	2475ChA ex360/em440
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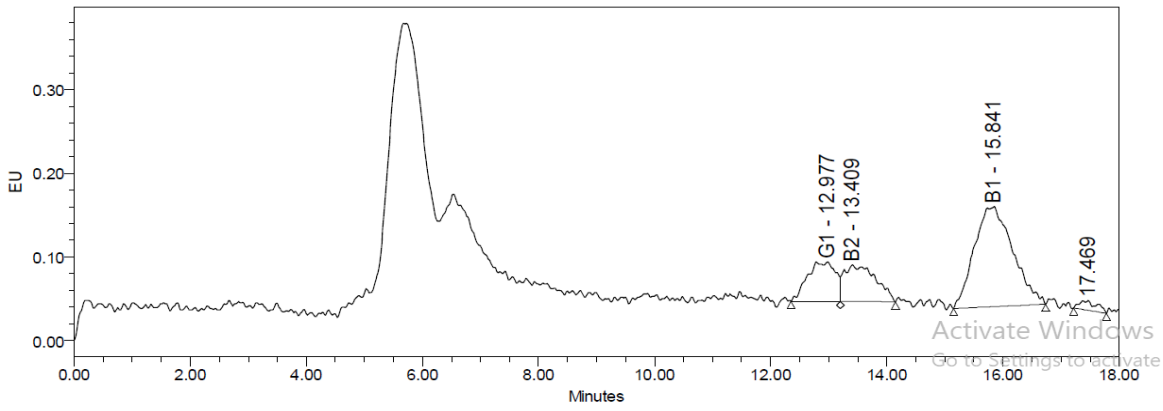

SAMPLE INFORMATION

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Vial:	3	Acq. Method Set:	Aflatoxin Analysis method
Injection #:	2	Processing Method:	230517 Aflatoxin
Injection Volume:	10.00 ul	Channel Name:	2475ChA ex360/em440
Run Time:	18.0 Minutes	Proc. Chnl. Descr.:	2475ChA ex360/em440
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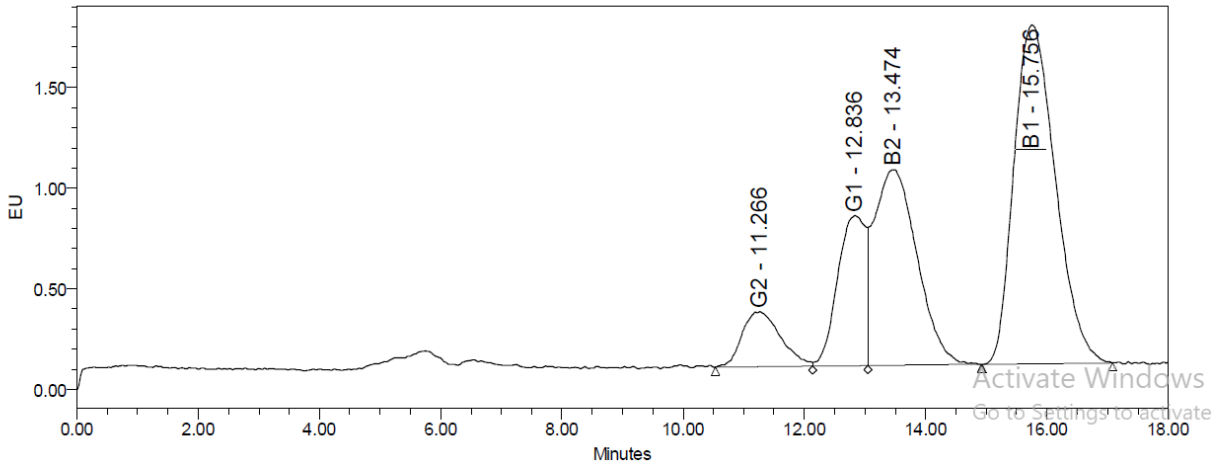
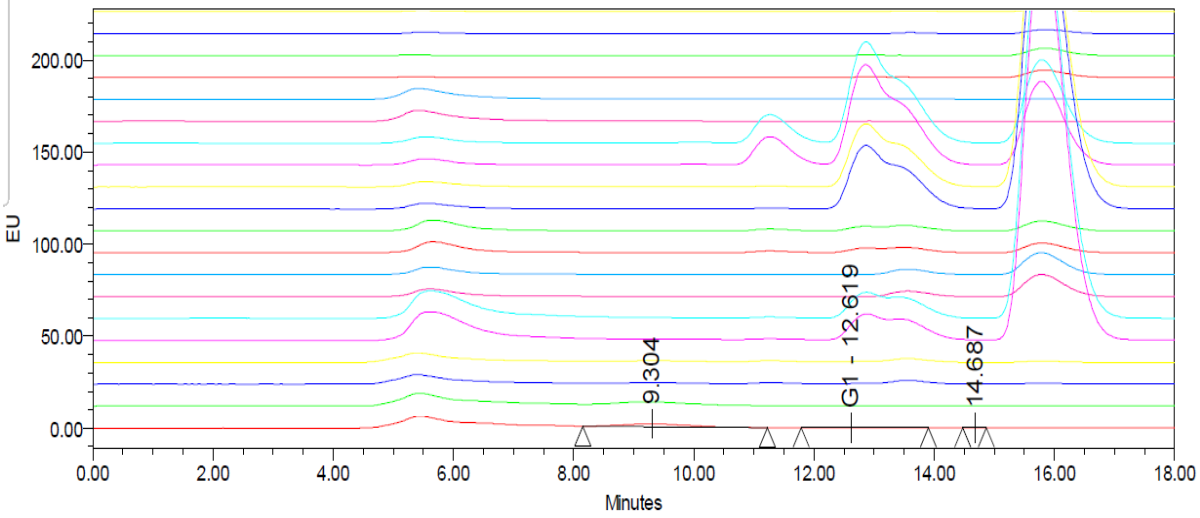
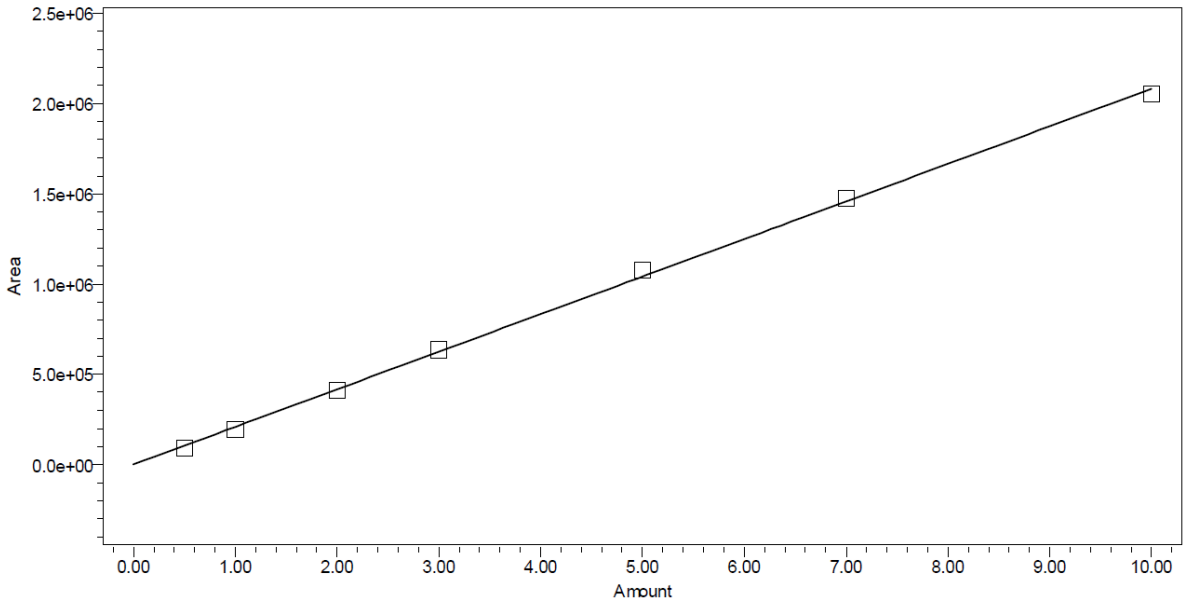


Empower™ 3 SOFTWARE

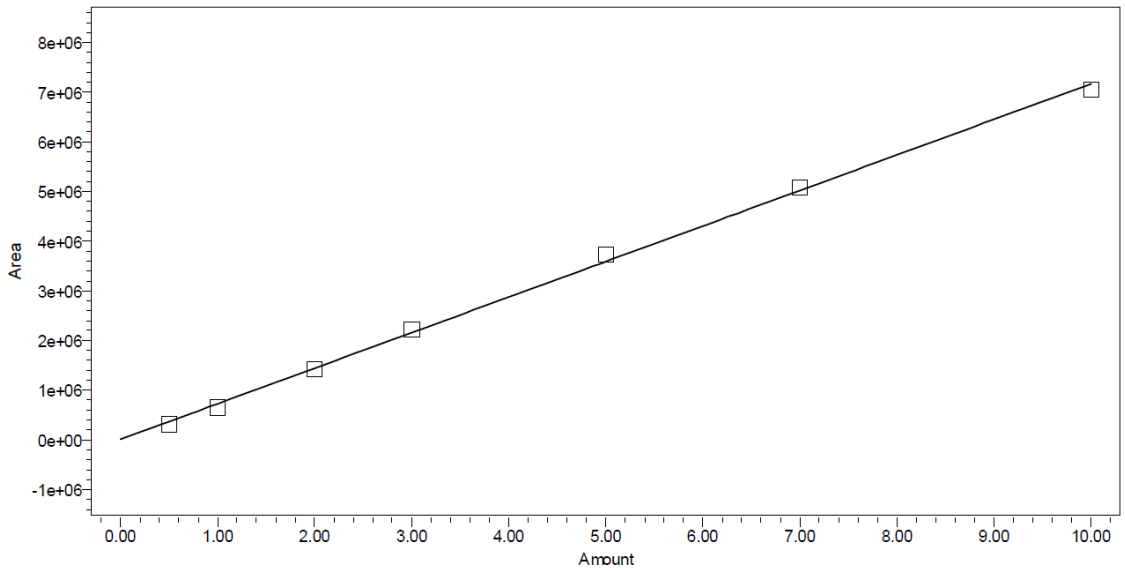
Peak Summary Report



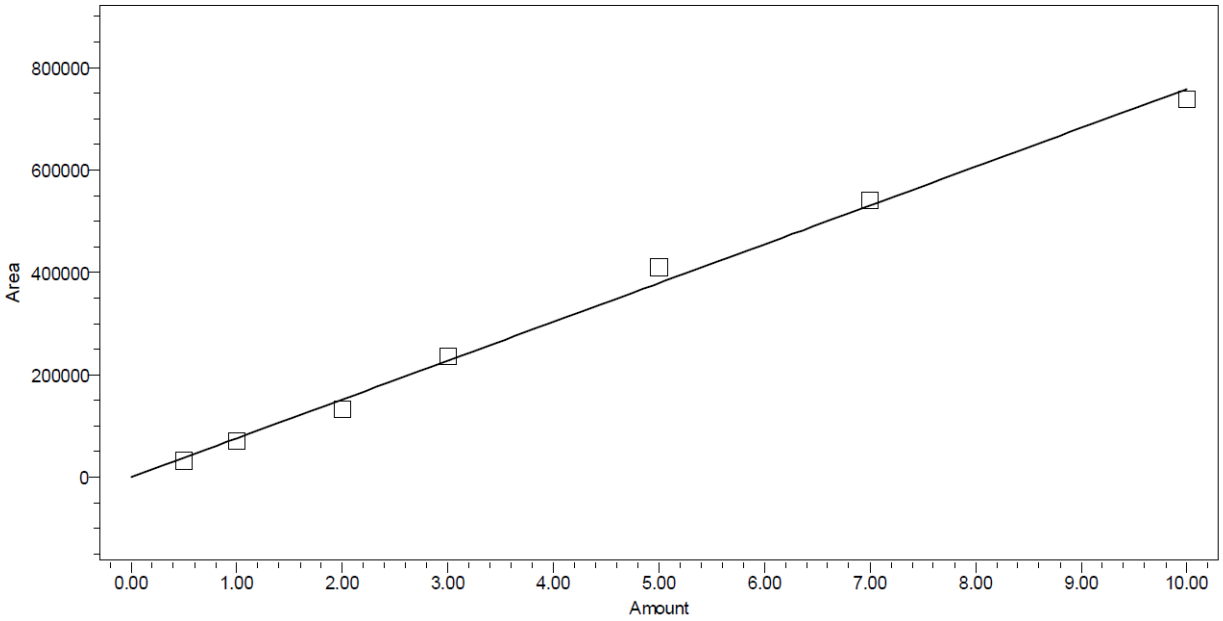
Annex 3: Calibration curve for aflatoxin analysis



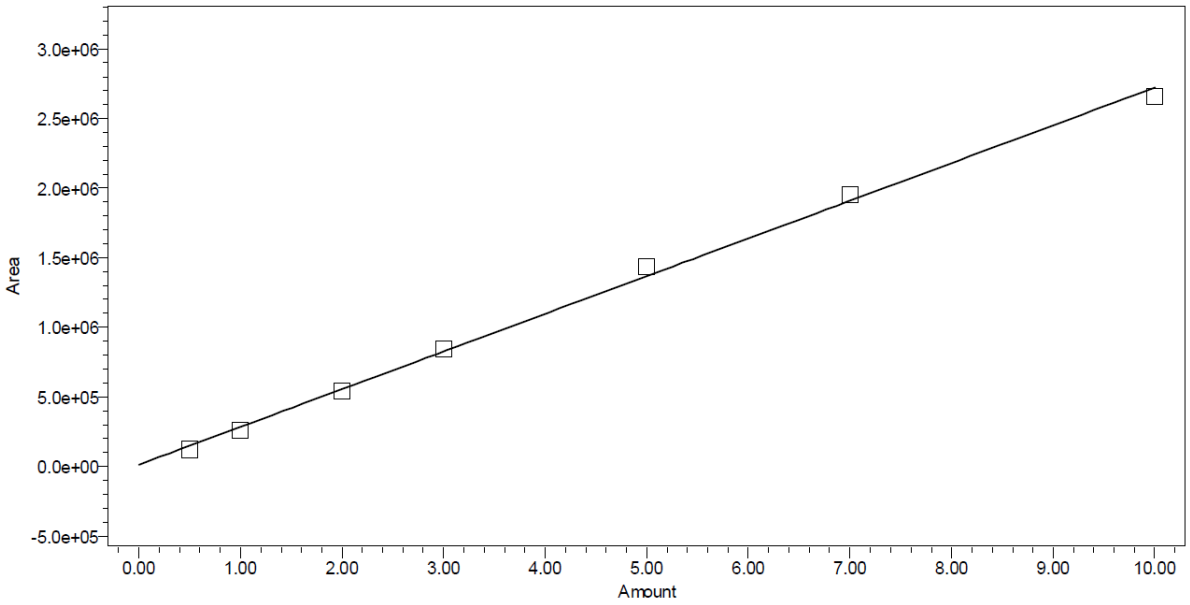
Peak Name: B1; RT: 15.837; Fit Type: Linear (1st Order); Cal Curve Id: 23311; R: 0.999505; R^2: 0.999011; Weighting: None; Equation: Y = 2.08e+05 X + 1.54e+03; Normalized Intercept/Slope: 0.001411; RSD(E): 2.932074



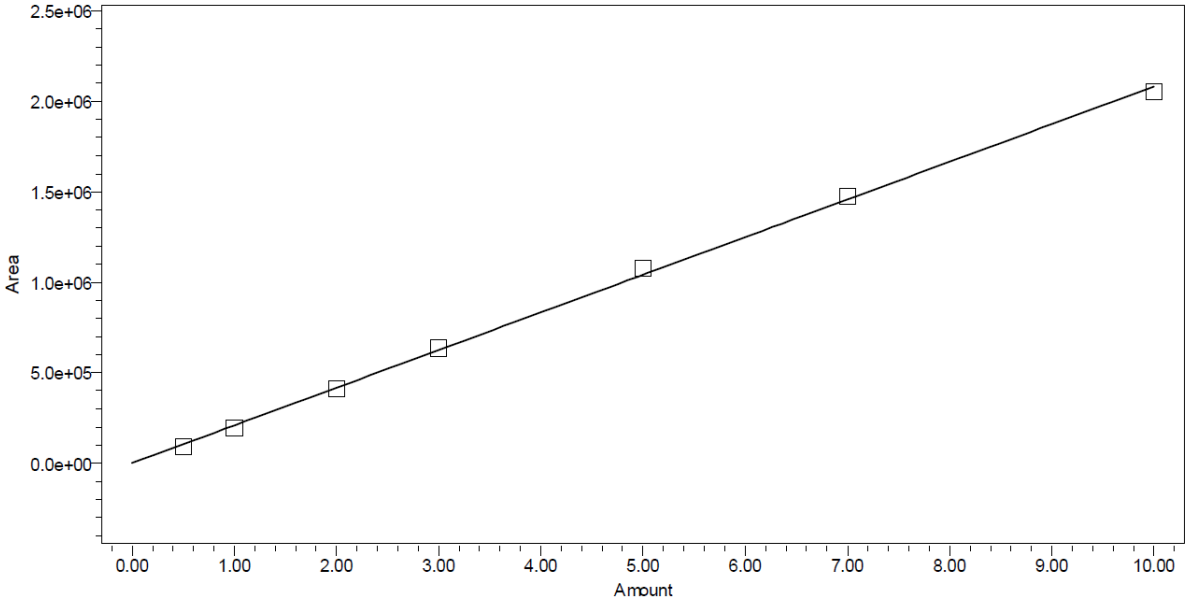
Peak Name: B2; RT: 13.543; Fit Type: Linear (1st Order); Cal Curve Id: 23310; R: 0.999361; R^2: 0.998722; Weighting: None; Equation: Y = 7.16e+05 X + 1.13e+04; Normalized Intercept/Slope: 0.003018; RSD(E): 3.325598



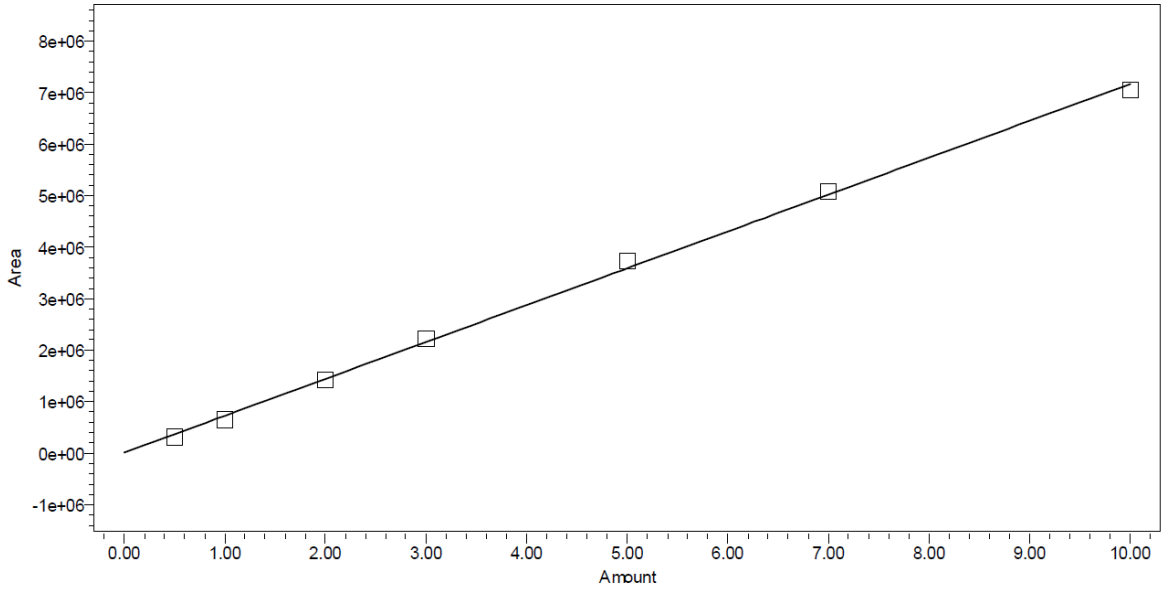
Peak Name: G1; RT: 12.785; Fit Type: Linear (1st Order); Cal Curve Id: 23309; R: 0.997677; R^2: 0.995360; Weighting: None; Equation: Y = 7.59e+04 X - 2.80e+01; Normalized Intercept/Slope: -0.000070; RSD(E): 6.373563



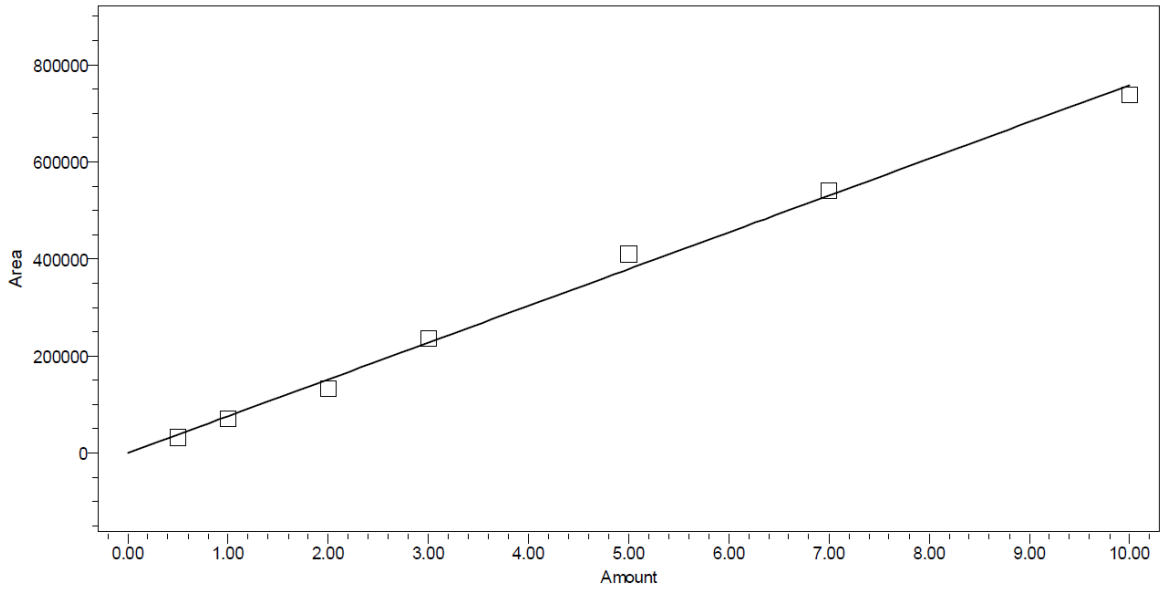
Peak Name: G2; RT: 11.272; Fit Type: Linear (1st Order); Cal Curve Id: 23308; R: 0.998795; R^2: 0.997592; Weighting: None; Equation: Y = 2.71e+05 X + 1.37e+04; Normalized Intercept/Slope: 0.009652; RSD(E): 4.529499



Peak Name: B1; RT: 15.837; Fit Type: Linear (1st Order); Cal Curve Id: 23311; R: 0.999505; R^2: 0.999011; Weighting: None; Equation: Y = 2.08e+05 X + 1.54e+03; Normalized Intercept/Slope: 0.001411; RSD(E): 2.932074



Peak Name: B2; RT: 13.543; Fit Type: Linear (1st Order); Cal Curve Id: 23310; R: 0.999361; R^2: 0.998722; Weighting: None; Equation: Y = 7.16e+05 X + 1.13e+04; Normalized Intercept/Slope: 0.003018; RSD(E): 3.325598



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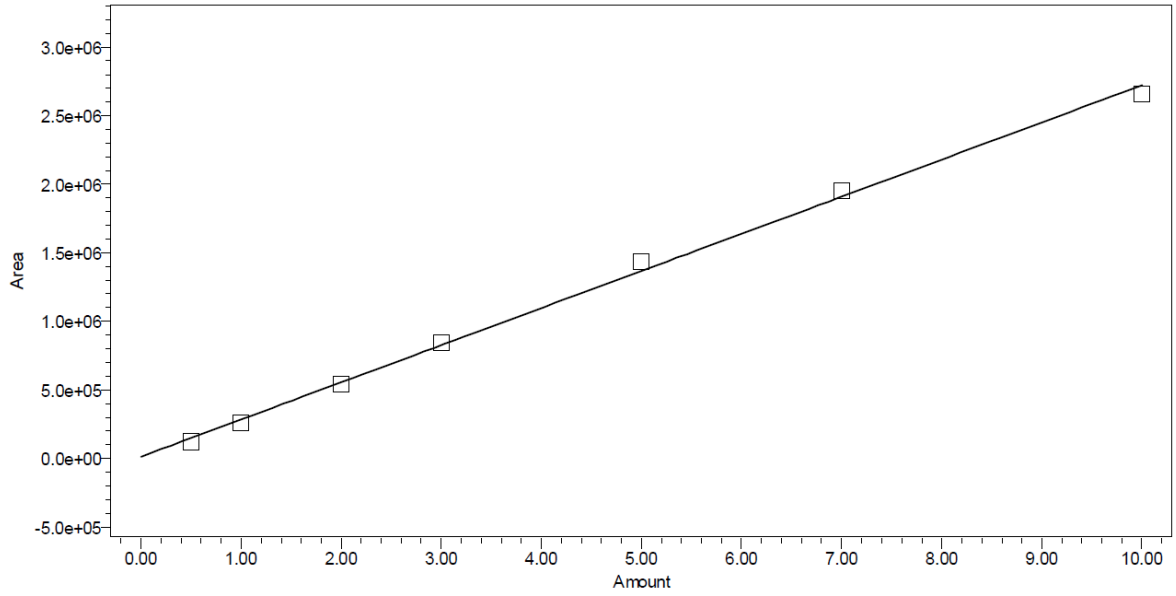

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