ADDIS ABABA UNIVERSITY
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES
CENTER FOR FOOD SCIENCE AND NUTRITION

ASSESSMENT OF ACRYLAMIDE LEVELS IN COFFEE POWDER, POTATO CHIPS AND FRENCH FRIES IN ADDIS ABABA, ETHIOPIA

By: **Henok Ashagrie**

Advisor: **Dr. Ashagrie Zewdu**

A thesis submitted to Addis Ababa University, College of Natural and Computational Sciences, Center for Food Science and Nutrition in partial fulfilment of the requirement for the Degree of Master of Science in Food Science and Nutrition.

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Addis Ababa, Ethiopia

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DECLARATION

I, the undersigned, declare that this thesis is my original work and that all sources of materials used for the thesis work have been fully acknowledged.

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This thesis has been submitted with my approval as a university advisor.

Name: **Dr. Ashagrie Zewdu**

Signature: __________ **Date:** 30/07/2019

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LIST OF ABBREVIATIONS AND ACRONYMS

ALARA	As Low as Reasonably Achievable
AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of Variance
BMDL	Benchmark Dose Level
CAS	Chemical Abstracts Service
CDS	Chromatographic Data System
CNS	Central Nervous System
CONTAM	The Panel for Contaminants in the Food Chain
CSA	Central Statistical Agency
DAD	Diode Array Detector
DNA	Deoxyribonucleic Acid
EAC	East African Community
EC	European Commission
EFDA	Ethiopian Food and Drug Authority
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
ESTD	External Standard
EU	European Union
FAO	Food and Agriculture Organization
FAS	Foreign Agricultural Service
FDA	Food and Drug Administration
FMoH	Federal Ministry of Health
GAIN	Global Agricultural Information Network
HPLC	High Performance Liquid Chromatography
HMF	5-Hydroxymethylfurfural
IARC	International Agency for Research on Cancer
IBM	International Business Machine
JECFA	Joint Expert Committee on Food Additives
LOD	Limit of Detection
LOQ	Limit of Quantification
LSQ	Least Squares

MOE	Margin of Exposure
NTP	National Toxicology Program
PAH	Polycyclic Aromatic Hydrocarbons
PLC	Private Limited Company
Ppb	Parts Per Billion
RF	Response Factor
Rpm	Revolutions per Minute
RSD	Relative Standard Deviation
SNNPR	Southern Nations, Nationalities and Peoples Region
SPE	Solid-Phase Extraction
SPSS	Statistical Package for the Social Sciences
U.S.	United States
USDA	United States Department of Agriculture

ANNEXES

ABSTRACT

Acrylamide is a processing contaminant with genotoxic and carcinogenic properties and it is formed in food when free asparagine reacts with reducing sugars at high temperature. The present study was designed to evaluate the levels of acrylamide in three commercial heat processed foods (roasted coffee powder, potato chips and French fries) collected from the market in Addis Ababa, Ethiopia. A total of 90 samples were studied for their acrylamide levels using high performance liquid chromatography with a diode array detector. The acrylamide levels obtained ranged from 134.56 µg/kg to 1138.86 µg/kg in roasted coffee (n=30), from 211.09 µg/kg to 3514.60 µg/kg in potato chips (n=30) and from 35.66 µg/kg to 1410.75 µg/kg in French fries (n=30). 43% of the coffee (n=13), 57% of the potato chips (n=17) and 40% of the French fries samples (n=12) showed acrylamide levels higher than the maximum value recommended by the European Commission. Ethiopia is the largest coffee consuming country in Africa and one of the biggest in the world. Nearly half of the coffee collected from Addis Ababa contained levels beyond the European Commission recommended value. The high levels of acrylamide obtained in the present study call for actions when considering large consumptions. These high levels of acrylamide along with the significantly large consumption history make it difficult to consider health risks associated with acrylamide to be low. A knowledge assessment of French fries producing street vendors (n=30) indicated a huge gap in information as none of the interviewed producers knew about acrylamide, its formation, health impacts or foods susceptible to its formation.

Keywords: Acrylamide; Maillard Reaction; Coffee; Potato Chips; French Fries; Carcinogen

1. INTRODUCTION

1.1. Background

Food processing is an important factor with major roles including the improvement of nutritional quality, microbiological safety, sensory aspects and reduction or removal of some compounds with potential health impacts (Gökmen, 2014; Moskowitz *et al.*, 2009). House hold or industrial thermal processing of foods is a longstanding practice that improves the organoleptic properties of foods, microbiological safety and their preservation (Studer *et al.*, 2004).

Thermal food processing can be the source for the formation of some health benefiting compounds like antioxidants (Lingert & Wailer, 1983; Morales *et al.*, 2009). However, it can also be the reason for the formation of heat-induced toxic food contaminants with carcinogenic and genotoxic properties such as heterocyclic amines, acrylamide, furan, furfurals and chloropropanols (Crews *et al.*, 2001; Durling *et al.*, 2009; Husøy *et al.*, 2008; Morales *et al.*, 2009; Skog *et al.*, 1998; Tritscher, 2004)

Foods produced by thermal processing like bakery products, roasted coffee, potato chips, French fries, snack foods and refined oils may result in the formation of heat generated food toxicants mentioned above at varying concentrations as a result of heat-induced conversions of some naturally available compounds in food products. The level of these contaminants in the final food product depends on the concentration of precursors required for their formation as well as the thermal-processing conditions (Gökmen, 2015).

Acrylamide is a thermal processing contaminant which is produced in the Maillard reaction, a series of non-enzymatic reactions between reducing sugars such as glucose and fructose, and free amino acids (Becalski *et al.*, 2003; Mottram *et al.*, 2002; Zyzak *et al.*, 2003). Its presence in food was first reported by scientists from the Swedish National Food Authority and the University of Stockholm in April 2002 when thermally processed carbohydrate-rich foods were analyzed and high acrylamide concentrations were obtained (Tareke *et al.*, 2002).

Acrylamide has been classified as probably carcinogenic to humans (Group 2A) by the International Agency for Research on Cancer (IARC, 1994). It is not found in raw and unprocessed food items like potatoes. It is formed when food containing the precursors is thermally processed at temperatures of 120°C or above, such as encountered during frying, baking, roasting, grilling, and toasting (Tareke *et al.*, 2002). High levels of acrylamide is found in food products derived from plant ingredients, such as potatoes and cereals because of the natural presence of the precursors (reducing sugars and asparagine) required for its formation. Meat products contain little or no acrylamide because of the lack of these important precursors (Lineback *et al.*, 2012).

The roasted coffee, potato chips and French fries have been reported to have high levels of acrylamide by different studies over the years making them some of the major dietary acrylamide sources with levels going beyond the European Commission recommended benchmark levels for theses specific products (Elias *et al.*, 2017; Kotemori *et al.*, 2018; Lineback *et al.*, 2012; Mesias *et al.*, 2018; Mojska & Gielecinska, 2013; Pacetti *et al.*, 2015). The European Commission drafted a regulation on establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food (EC, 2017). To my knowledge, during the time this study was conducted there are no standards, benchmark levels, guidelines, mitigation toolboxes drafted in Ethiopia associated with acrylamide.

1.2. Statement of the Problem

Coffee is an important part of Ethiopian tradition and culture. Ethiopia is Africa's largest coffee producer accounting for nearly 40% of the continent's coffee production and the world's sixth largest producer. The United States Department of Agriculture estimated Ethiopia's coffee production at 423,300 metric tons and nearly half of the production (approximately 187,000 metric ton) is consumed locally (GAIN, 2018). Different studies reported high levels of acrylamide in coffee powder making it one of the main dietary acrylamide exposure sources (Bertuzzi *et al.*, 2017; EFSA, 2015; Gökmen, 2015; Mojska & Gielecinska, 2013).

On the other hand, the number of street vendors selling French fries and food processors supplying potato chips to supermarkets is rapidly growing in Addis Ababa. Street vended foods are becoming a dish for several people. Selling street food has become the source of employment for many producers and it's not unusual to observe street food vendors and consumers in every place in the city (Temesgen, 2015). The main consumers for these two products are usually children. Many studies have shown that fried products like French fries and potato chips are higher in the list of major dietary acrylamide exposure sources due to the natural availability of the necessary precursors for its formation during thermal processing (EFSA, 2015; Petersen & Tran, 2005; Tareke *et al.*, 2002).

There are many researches done on acrylamide levels worldwide but it is barely possible to find published acrylamide related researches done in Ethiopia with a recently published work by Kumela *et al.* being the exception. Their work focused on acrylamide occurrence in Keribo, an Ethiopian traditional fermented beverage which reported levels of acrylamide going as high as 3440 μg/kg (Kumela *et al.*, 2018).

Dietary exposure to acrylamide is a function of the food's acrylamide content and the amount consumed. The high consumption of these products and the fact that they are higher in the list of potential dietary acrylamide exposure sources creates a serious health concern. Since acrylamide levels in these products sold in Addis Ababa hasn't been studied and documented yet, it is difficult to have an estimate of the exposure. And without the understanding of levels of exposure, it is difficult to propose mitigation practices or set regulatory policies.

1.3. Significance of the Study

The study aims to provide a base line data on the levels of acrylamide in these three major dietary contributors; roasted coffee, potato chips, and French fries sold in Addis Ababa. It can also serve as a benchmark for further studies on related or different food products prone to acrylamide contamination and possible mitigation practices.

The outcomes of this study can be used for creating awareness of consumers on major dietary acrylamide sources, the health impacts associated with acrylamide, and the levels of exposure from these three major dietary acrylamide sources.

The outputs of this study may also help regulatory bodies like the Federal Ministry of Health (FMoH), Ethiopian Food and Drug Authority (EFDA), and Ethiopian Standards Agency in setting regulatory limits on acrylamide level in these food products or developing necessary policies to prevent the consumer from health impacts associated with acrylamide. This study can contribute to filling the existing gap in availability of documented literatures on acrylamide levels in foods in Ethiopian context.

1.4. Objectives

1.4.1. General Objective

To determine the levels of acrylamide in French fries, potato chips, and coffee powder being sold in Addis Ababa, Ethiopia.

1.4.2. Specific Objectives

- To determine levels of acrylamide in coffee powder, potato chips and French fries sold in Addis Ababa, Ethiopia
- Evaluating levels of acrylamide in these food products against recommended benchmark levels
- Assessing the knowledge of street vendor producers about heat generated food toxicants

2. LITERATURE REVIEW

2.1. Heat Generated Food Toxicants

Thermal processing is a crucial factor for food preservation for the production of shelf-stable foods, nutritional quality improvement and reduction or removal of toxic components (Gökmen, 2014). When food is processed at high temperature it induces chemical changes and a large amount of new molecules are generated, of which some have been claimed to have positive health effects such as acting as health-promoting antioxidants and antimutagens (Studer *et al.*, 2004). However, thermal processing can also be the reason for the formation of heat generated toxic compounds with carcinogenic or mutagenic properties such as polycyclic aromatic hydrocarbons, hetrocyclic amines, acrylamide, furan, and furfurals (Capuano & Fogliano, 2011; Crews & Castle, 2007; Gökmen, 2015; Maga & Katz, 1979; Skog *et al.*, 1998; Tareke *et al.*, 2002).

5-Hydroymethyl-2-furfural (HMF) is a heat generated heterocyclic contaminant produced when foods containing asparagine and carbohydrates are thermally processed (Capuano & Fogliano, 2011; Morales, 2008; Murkovic & Pichler, 2006). It has been reported in many food products including honey, citrus juice, tomato paste, syrups, jams, coffee, bakery products and bread (Makawi *et al.*, 2009; Murkovic & Pichler, 2006). Its mutagenic properties have been confirmed through animal studies (Glatt *et al.*, 2005; Sommer *et al.*, 2003).

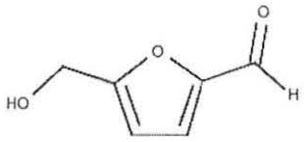
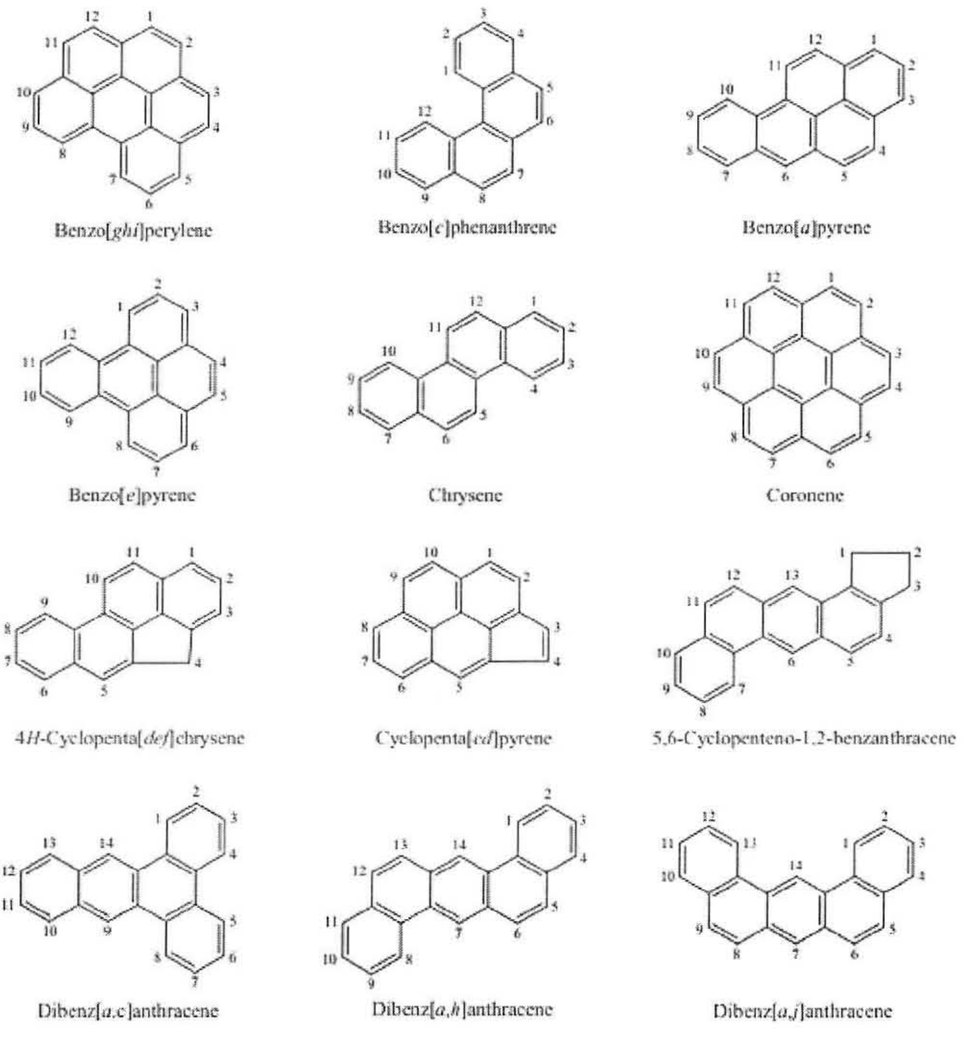


Figure 1. Structure of 5-Hydroymethyl-2-furfural (HMF) (Severin *et al.*, 2010)

Polycyclic aromatic hydrocarbons (PAHs) on the other hand are group of hydrophobic compounds that contain only carbon and hydrogen and are comprised of two or more fused aromatic rings. In food, PAHs are formed during processing and food preparation, especially during the processes of smoking, drying and cooking as a result of incomplete combustion or pyrolysis of organic materials (Badolato *et al.*, 2006; Zhang *et al.*, 2009). Some of these polycyclic aromatic hydrocarbons include Benz[a]anthracene, Benzo[a]pyrene, and Dibenz[a,h]anthracene which are categorized as "probably carcinogenic to humans (Group 2A)" by the International Agency for Research on Cancer (IARC, 2010).



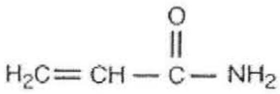
Benzo[*ghi*]perylene
Benzo[*c*]phenanthrene
Benzo[*a*]pyrene
Benzo[*e*]pyrene
Chrysene
Coronene
4*H*-Cyclopenta[*def*]chrysene
Cyclopenta[*cd*]pyrene
5,6-Cyclopenteno-1,2-benzanthracene
Dibenz[*a,c*]anthracene
Dibenz[*a,h*]anthracene
Dibenz[*a,j*]anthracene

Figure 2. Structure of some polycyclic aromatic hydrocarbons (IARC, 2010)

Acrylamide is a processing contaminant that is produced via the Maillard reaction, a series of non-enzymatic reactions between reducing sugars such as glucose and fructose, and free amino acids (Halford *et al.*, 2011; Mottram, 2007; Nursten, 2005). Acrylamide is found in a wide range of heat-treated foods, prepared commercially or cooked at home, including bread, crisp bread, bakery wafers, breakfast cereals, potato products (crisps, French fries), chocolate and coffee (Stadler & Lineback, 2008).

2.2. Properties of Acrylamide

Acrylamide (2-propenamide, CAS Number: 79-06-1) is an odorless white crystalline solid. It has a molecular weight of 71.08 g/mol. Acrylamide is highly soluble in water. Acrylamide has a melting point of 84.5°C and a boiling point of 192.6 °C at atmospheric pressure. Acrylamide is a reactive chemical, which is used as a monomer in the synthesis of polyacrylamides used for example, in the purification of water and in the formulation of grouting agents. Water-soluble polyacrylamides are the largest application of acrylamide and they are used to enhanced oil recovery as mobility control agents in water flooding, additives for oil well drilling fluids, and aids in fracturing, acidifying and other operations. Acrylamide is primarily reactive through its ethylenic double bond. Polymerization of acrylamide occurs through radical reactions with the double bond. Acrylamide is not known to occur as a natural product (IARC, 1994; Lingnert *et al.*, 2002; Smith *et al.*, 1997).



$$H_2C{=}CH-\overset{\overset{\displaystyle O}{\|}}{C}-NH_2$$

Figure 3. Structure of acrylamide (IARC, 1994)

2.3. Mechanism of Acrylamide Formation in Food

Since acrylamide has not been found in raw and unheated foods, its formation is associated with heating at high temperatures (Becalski *et al.*, 2003; Stadler *et al.*, 2002). The non-enzymatic Millard reaction has been reported by many studies to be the primary route for acrylamide formation. At temperatures above 120 °C free amino acids will react with reducing sugars to generate reactive monocarbonyl and dicarbonyl compounds that are proposed to be responsible for the formation of color, flavor, and aroma. The mechanism of acrylamide formation involves reaction of a carbonyl compound with asparagine (Feather, 1994; Mottram *et al.*, 2002; Stadler *et al.*, 2002).

The mechanism for acrylamide formation involves a series of steps. The initial step involves the formation of Schiff base between the carbonyl formed by the Maillard reaction and the R-amino group of asparagine. Under high temperatures, the Schiff base decarboxylates (facilitated by delocalization of negative charge, which Schiff base formation allows), forming a product that can react in one of two ways. In the first way, it can undergo hydrolysis to form 3-aminopropionamide which further decomposes via elimination of ammonia at high temperatures forming acrylamide. In the second way, the decarboxylated Schiff base can form acrylamide via the elimination of an imine (Zyzak *et al.*, 2003).

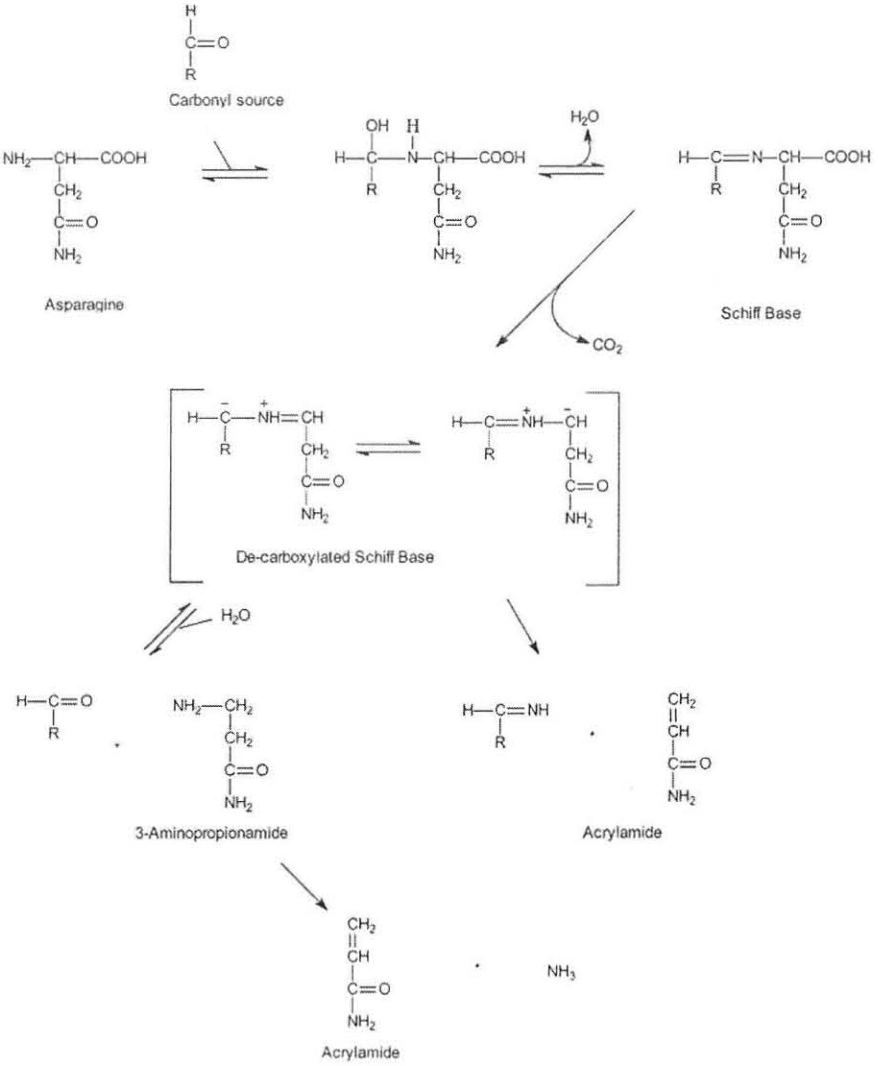


Figure 4. Acrylamide formation in heated foods (Borda & Alexe, 2011; Zyzak *et al.*, 2003)

2.4. Acrylamide Levels in Foods

As mentioned in the previous section, acrylamide in food is formed when food with free amino acids, mainly asparagine, and reducing sugars are cooked at high temperatures. This is the reason for acrylamides occurrence in a wide range of food products that are commonly consumed worldwide on a daily basis. Dietary exposures from acrylamide is not limited to consumption of one or two food products, instead the cumulative effect of exposure from a wide variety of foods is the problem (Becalski *et al.*, 2003; Mottram *et al.*, 2002; Petersen & Tran, 2005).

Dietary exposure is a function of the acrylamide content of the food products and the amounts consumed. A food can still act as a major contributor even when it has low acrylamide content, if consumption of that food item is large, e.g., coffee. Sources of acrylamide in the diet include foods/food products made at home, in restaurants, commercially, and/or by catering services (Lineback *et al.*, 2012).

Since the discovery of acrylamide in food items, several studies have been and are being conducted worldwide on the levels of acrylamide in variety of foods. A lot of studies have confirmed the presence of acrylamide in a wide variety of heat treated foods like in deep fried snacks in India (Shamla & Nisha, 2014); in roasted coffee, barley and potato crisps in Italy (Bertuzzi *et al.*, 2017); in potato chips, snacks, vegetable-based non-cereal foods and in process-based cereal infant foods in Estonia (Elias *et al.*, 2017); in coffee qahwa, coffee and tea (Khan *et al.*, 2017); in baked and deep fried foods in China (Wang *et al.*, 2013); in infant formula, coffee, chocolate, bakery products and potato products in Colombia (Pacetti *et al.*, 2015); in French fries, potato chips, bread, crackers, breakfast cereals, coffee and beer in Brazil (Arisseto *et al.*, 2007); in processed potato products, bread, breakfast cereals, biscuits, cookies, snacks and coffee in Sweden (Svensson *et al.*, 2003); and in French fries in Spain (Mesias *et al.*, 2018).

Acrylamide levels of most abundant food items and food groups collected between 2002 and 2006 in European Union member states was summarized by Wenzi & Anklam, 2007 and is presented in Table 1.

Table 1. Overview on most abundant food items/food categories in the European Union database on acrylamide levels in foods (Wenzi & Anklam, 2007)

Food	n	Acrylamide (µg/kg)		
		Minimum	**Median**	**Maximum**
Potato Chips (French Fries)	1,408	5	186	4,653
Potato Crisps	843	5	528	4,215
Bakery Ware, Biscuits	1074	4	145	3,324
Ginger Bread	1007	5	303	7,834
Crisp Bread	455	5	244	2,838
Bread and Toast	151	5	50	1,987
Diabetics Cakes and Biscuits	402	5	230	3,044
Snacks, Popcorn, Salt Crackers	112	5	145	3,100
Cereals	274	5	70	1,649
Roasted Coffee Beans	291	79	320	1,188
Baby Food	191	5	67	432
Potato Pancake	118	10	385	3,072
Infant Biscuits	157	5	79	910
Coffee Substitutes	108	116	773	2,955

2.4.1. Acrylamide in Coffee

Coffee has been reported to be one of the highest dietary acrylamide sources to adults and high levels have been reported in many studies (Arvanitoyannis & Dionisopoulou, 2014; Bertuzzi *et al.*, 2017; EFSA, 2015; Gökmen, 2015; Kotemori *et al.*, 2018; Lineback *et al.*, 2012; Mesias *et al.*, 2018; Mojska & Gielecinska, 2013; Sirot *et al.*, 2012). A study conducted on roasted coffee powder in Saudi Arabia reported acrylamide levels ranging between 152 and 682 µg/kg (Khan *et al.*, 2017). A Romanian study on 50 roasted coffee powder showed acrylamide levels ranging between 48 and 6968 µg/kg (Oroian *et al.*, 2015). The EFSA Panel on Contaminants in the Food Chain (CONTAM) evaluated over 1500 coffee powder samples collected and analyzed between 2010 and 2013 showed an average acrylamide level of 578 µg/kg (EFSA, 2015).

Acrylamide levels higher than the European Commission recommended level of 400 µg/kg have been reported in roasted coffee powder by different researchers over the years (Bagdonaite *et al.*, 2008; Bertuzzi *et al.*, 2017; Mojska & Gielecinska, 2013; Oroian *et al.*, 2015; Pacetti *et al.*, 2015).

An experiment done to examine the effect of brewing on acrylamide levels showed that all the acrylamide present in the coffee powder was transferred to the coffee brew as a result of acrylamide's high solubility in water and the analysis of the brewed coffee powder indicated that no acrylamide was detected (Andrzejewski *et al.*, 2004; Bagdonaite *et al.*, 2008).

Coffee is roasted at high temperatures usually ranging between 240 °C and 300 °C (Cirilo *et al.*, 2003). Emphasis on acrylamide levels of coffee is important as a research because of its high consumption practices in some countries which in turn can and therefore will have possible hazardous influence on human health (Bagdonaite *et al.*, 2008).

The significant variation in acrylamide levels between coffee powders could be associated to different factors including the roasting time, the temperature applied, concentration of precursors and the difference in coffee bean species. Amount of asparagine in the green coffee is the limiting factor for the formation of acrylamide in coffee. Studies also showed that acrylamide level reaches maximum during the beginning of the roasting process (as high as 7 mg/kg) and decreases shortly resulting in high concentration of acrylamide in light roasted coffee than darker ones. The study results also showed that Robusta coffee contained higher level of acrylamide than Arabica coffee because of its lower sucrose content and higher asparagine content (Bagdonaite *et al.*, 2008; Lantz *et al.*, 2006).

Following the various studies on reduction of acrylamide, FoodDrinkEurope developed the acrylamide toolbox with an aim to provide manufacturers, local authorities and other interested parties with information about intervention steps that can help prevent or reduce the formation of acrylamide in specified products and processes. This toolbox can also help the food processing business to comply with the European Union requirements of the regulation (EU) 2017/2158. Mitigation results on coffee have been evaluated and they led to the conclusion that there are very limited process options available to lower acrylamide contents of coffee without affecting its sensorial quality and consumer acceptance. The acrylamide toolbox recommends optimizing roasting conditions to reduce acrylamide levels in coffee since darker roasting and applying low temperatures and extended roasting time tend to reduce acrylamide levels; (FoodDrinkEU, 2019).

2.4.2. Acrylamide in Potato Chips and French Fries

Potato products are highly susceptible to the formation of acrylamide as it contains the precursors required for its formation and that processing techniques associated with this product involve high temperature as in frying which favors the Millard reaction, a reaction linked to the formation of acrylamide (Vinci *et al.*, 2012).

Potato chips and French fries are of the major dietary acrylamide exposure sources and high levels of acrylamide in these products has been reported in many studies (Arvanitoyannis & Dionisopoulou, 2014; Elias *et al.*, 2017; Gökmen, 2015; Mojska & Gielecinska, 2013; Pacetti *et al.*, 2015; Tareke *et al.*, 2002; Tateo *et al.*, 2007). A study on 20 potato chips samples in India showed acrylamide levels that ranged between 82 and 4245.5 μg/kg (Shamla & Nisha, 2014). Another study conducted in Estonia reported mean acrylamide levels of 529 and 299 μg/kg for potato crisps and French fries respectively (Elias *et al.*, 2017).

Acrylamide levels as high as 1000 μg/kg and above were reported in processed potato products by many researchers over the years (Chen *et al.*, 2008; Claeys *et al.*, 2016; Freisling *et al.*, 2013; Mesias *et al.*, 2018; Pacetti *et al.*, 2015; Tareke *et al.*, 2002; Tateo *et al.*, 2007; Wenzi & Anklam, 2007).

There are several factors that affect the levels of acrylamide in potato products including processing conditions, the content of precursors and moisture content (Elmore *et al.*, 2005). In potato chips and French fries, the reducing sugar content of the potatoes used is the limiting factor for the formation of acrylamide as asparagine exists in high concentrations (Amrein *et al.*, 2004; Becalski *et al.*, 2004; De Wilde *et al.*, 2005; Mesias *et al.*, 2018). A study on the effect of soil types on the acrylamide forming capacity of 16 varieties of potato grown in two different types of soil (clay and sandy loam) indicated that there was insignificant variations between acrylamide levels associated with soil type (De Wilde *et al.*, 2006). Since acrylamide is formed on the upper layer of the potato product during frying, the surface-to-volume ratio of the final cut will influence acrylamide levels and accordingly thinner and smaller sizes can lead to an increased level of acrylamide in the final fried potato product (Mathau¨s *et al.*, 2004).

Blanching has also been reported to contribute to the reduction of acrylamide in potato products as it leads to the leaching out of reducing sugars (Pedreschi *et al.*, 2007; Vinci *et al.*, 2010). Drying of potato products after blanching and before frying also reduces formation of acrylamide as the duration of frying required is shorter (Gökmen V, 2006). A pilot study on levels of acrylamide in French fries prepared at home conditions by having five volunteers prepare the fries using the same ingredients but just as they would at home resulted in a significant variations in acrylamide levels where it was between 24 and 3641 µg/kg indicated that processing conditions have significant effects on levels of acrylamide (Mesias *et al.*, 2018).

2.5. Toxicity of Acrylamide

Acrylamide is classified as "probably carcinogenic to humans (Group 2A)" by the International Agency for Research on Cancer as (IARC, 1994). The U.S. Environmental Protection Agency has also classified acrylamide as "probable human carcinogen (Group B2)" (EPA, 2011a). The U.S. National Toxicology Program's (NTP) additionally reported acrylamide as "reasonably anticipated to be a human carcinogen" (NTP, 2011a). Acrylamide's pressuring health concern is associated with its widespread occurrence in foods and its carcinogenic properties (JECFA, 2011).

Exposure to acrylamide can be a result of diet, smoking, drinking water, occupational conditions, etc. Coffee, potato chips and French fries are generally considered the more commonly consumed dietary sources of acrylamide in the world (Gökmen, 2015).

Animal studies have shown the carcinogenic and genotoxic properties of acrylamide and its metabolite glycidamide. Effects of acrylamide on animals administered with acrylamide includes incidence of tumors in mammary and clitoral glands; gene mutation; increased incidence of CNS tumors; DNA damage; decreased sperm concentration; morphological defects of sperm; deterioration in the germ cells of the seminiferous epithelium; increased incidence of cancer in the pancreatic islets and of malignant mesotheliomas; and increased incidence of thyroid gland adenoma or adenocarcinoma (M. A. Friedman *et al.*, 1995; Johnson *et al.*, 1986; Mojska *et al.*, 2010; NTP, 2012; Yang *et al.*, 2005).

The primary metabolism of acrylamide is through the action of cytochrome P450-2E1 to form reactive glycidamide via epoxidation. Glycidamide, the metabolite of acrylamide is anticipated to play a significant role in the genotoxicity of acrylamide by attaching itself to DNA and hemoglobin and forming adducts. Acrylamide undergoes detoxification by direct conjugation with glutathione through enzymatic action of glutathione-S-transferase further metabolized to mercapturic acids, and it is detected in the urine as cysteine metabolites (Doerge *et al.*, 2007; Doroshyenko *et al.*, 2009; Fennell *et al.*, 2004; Katen & Roman, 2015; Kopp & Dekant, 2009).

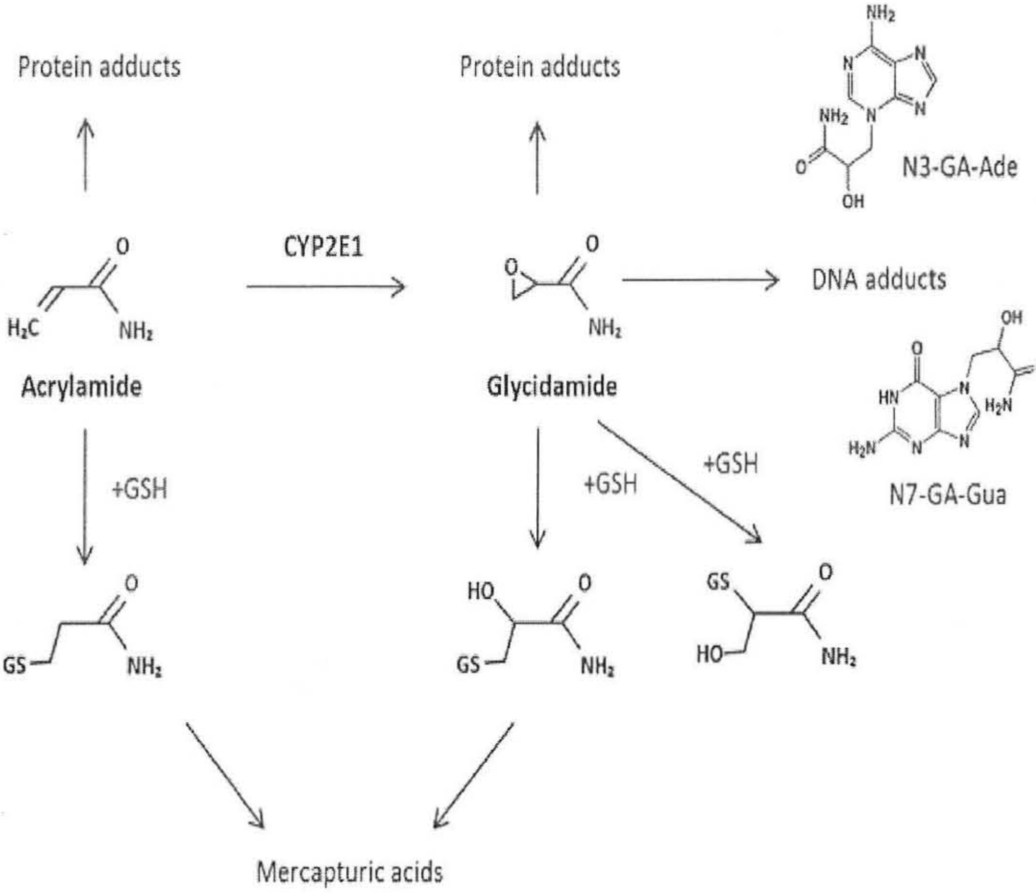


Figure 5. Metabolism of acrylamide in the body by cytochrome P450-2E1 (Fuhr *et al.*, 2006; Katen & Roman, 2015; Martins *et al.*, 2006)

According to studies, acrylamide can be found in the fetus of pregnant animals and in breast milk since it is distributed rapidly to all tissues without evidences of accumulation (JECFA, 2011).

Acrylamide's neurotoxicity, reproductive toxicity and carcinogenic properties have been confirmed through studies (Capuano & Fogliano, 2011; LoPachin, 2004). Acrylamide's neurotoxicity is induced through a mechanism that involves the inactivation of kinesin and dyneine, proteins that are involved in trans-axonal transport. This will result in an interference with trans-axonal transport where transport of nerve growth factor from the distal axon is inhibited. This will hinder transport of organelles and nutrients from the cell body to the distal axon causing nerves to die (Tyl & Friedman, 2003). Acrylamide also degenerates central and peripheral nerve terminals, damages cerebral cortex, causes myodegeneration of muscles and interferes with kinesin motor protein functions and trans-axonal transport (Capuano & Fogliano, 2011). Earlier concerns to acrylamide exposure were typically associated with occupational exposure, but animal studies showed the contribution of dietary exposure (M. Friedman & Levin, 2008).

Margin of Exposure (MOE) approach was chosen by JECFA to evaluate human health risks associated with acrylamide exposure. MOE estimates is the ratio between the dose resulting in a low but defined cancer incidence to human acrylamide intake. In its recent evaluation, the EFSA CONTAM panel chose two points for characterizing health impacts associated with acrylamide based on NTP's study on F344 rats exposed to acrylamide in drinking water for 2 years (NTP, 2012). A benchmark lower dose level or $BMDL_{10}$ of 0.43 mg/kg body weight per day for induction of mammary tumors in rats and a value of 0.17 mg/kg body weight per day for Harderian gland adenomas and adenocarcinomas in mice were selected as a reference point for non-neoplastic and neoplastic effects respectively (EFSA, 2015).

MOE value above 100 gives no reason for public health concern as long as there are no large gaps concerning toxicity data. MOE of 100 includes the uncertainties and variability associated with both kinetic and dynamic differences between study animals and humans, as well as within a population. For substances that have bot genotoxic and carcinogenic properties, MOE values of 10000 or higher would be of a low health concern from the view point of the public. MOE values lower than 10000 indicate a concern associated with neoplastic effects (EFSA, 2012).

2.6. Legislations Associated with Acrylamide Levels in Foods

Countries are drafting codes of practice, guidance and minimization concepts for the industry to encourage minimization of acrylamide levels in foods using the ALARA (as low as reasonably achievable) approach (Kliemant & Göbel, 2007).

The European Commission drafted a regulation on establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food. Benchmark levels are performance indicators that can be used to verify the effectiveness of the mitigation measures and are based on experience and occurrence for broad food categories. They are established at a level as low as reasonably achievable with the application of all relevant mitigation measures (EC, 2017). The benchmark levels for the presence of acrylamide in foodstuffs as set by the European Commission Regulation are selectively summarized in Table 2.

Table 2. European Commission benchmark levels for acrylamide in foodstuffs (EC, 2017)

Food	Benchmark Level (μg/kg)
French Fries	500
Potato Chips	750
Soft Bread from Wheat Soft Bread Other than wheat	50 100
Breakfast Cereals Bran Products and Whole Grain Cereals Wheat and Rye Based Maize, Oat, Spelt, Barley, and Rice Based	 300 300 150
Roasted Coffee	400
Instant (Soluble) Coffee	850
Processed Cereal Foods for Infants and Young Children Excluding Biscuits and Rusks	40
Biscuits and Rusks for Infants and Young Children	150

Codex Alimentarius on its document numbered CAC/RCP 67-2009 gives codes of practice for the reduction of acrylamide levels in foods with possible mitigation practices that can benefit producers (Codex, 2009). An East African standard developed for potato chips by East African Partner States under the East African Standardization, Quality Assurance, Metrology and Test Act, 2006 enactment sites the above mentioned Codex document as a requirement for this potato product to ensure reduced acrylamide levels (EAC, 2010).

A toolbox and a guidance for industry have been developed by Food Drink Europe and the U.S. Food and Drug Administration in order to provide the manufacturing sector with mitigation options to lower acrylamide levels (FDA, 2016; FoodDrinkEU, 2019).

2.7. Coffee Production in Ethiopia

Ethiopia is the birthplace of coffee Arabica (Daviron & Ponte, 2005; Jena *et al.*, 2012). Coffee is grown by over 4 million smallholder farmers and employs nearly 15 million people, or roughly 15% of the country's population at different points along the value chain. Around 95% of the coffee is cultivated on small plots. Smallholder farmers do not typically use pesticides and fertilizer and harvesting is done by hand. Ethiopia is the world's sixth largest coffee producer, accounting for 4% of production and the largest producer in Africa, accounting for about 40% of continental production (GAIN, 2018).

Oromia and Southern Nations, Nationalities and Peoples Region account for nearly 97 percent of Ethiopia's coffee production making them the two major coffee production regions. The southern part of Ethiopia including Sidama, Wolaita and Yirgacheffe produce washed coffee and accounts for 22 percent of the total coffee production with. The southwest part of Ethiopia which includes Jimma, Illubabor, Kaffa, Kelem, Bench Maji, Shaka, and Wollega produce dry coffee accounting nearly 70 percent of the coffee production. The eastern part of Ethiopia including East and West Hararghe (Harar) produces both dry and washed coffee beans accounting for nearly 8 percent of the total national coffee production (Hernandez *et al.*, 2015).

The United States Department of Agriculture (USDA) Foreign Agricultural Services (FAS) on its report approved by the World Agriculture Outlook Board summarized Ethiopia's coffee production between the years 2013 and 2018 is given in Table 3.

Table 3. Ethiopia's coffee production data (FAS, 2018)

	Production (60 kilogram bags)
2013/14	6,345
2014/15	6,475
2015/16	6,510
2016/17	6,943
2017/18	7,055
2018/19	7,100

Share of coffee production that is exported was summarized by USDA FAS is given in Table 4.

Table 4. Share of coffee production in Ethiopia that is exported (FAS, 2018)

	Production (60 kilogram bags)			**% Locally**
	Production	**Export**	**% Exported**	**Consumed**
2014/15	6,475	3,500	54	46
2015/16	6,510	3,405	52	48
2016/17	6,943	3,853	55	45
2017/18	7,055	3,950	56	44
2018/19	7,100	3,980	56	44

Coffee consumption is growing slowly as the population expands. Coffee is an important part of the country's tradition, culture, and religion. Families roast, prepare, and drink coffee at home as part of a traditional ceremony as many as three times a day (GAIN, 2018).

2.8. Potato Production in Ethiopia

Potato (*Solanum tuberosum L.*) is one of the world's major staple food crops (Pedreschi *et al.*, 2007). According to Food And Agriculture Organization of the United Nations, potatoes were among the top five items produced in 2013 with an amount of over 368 million tons (FAO, 2015). Potato is easy to grow and relatively not that expensive to buy. Potato can provide stable yield in conditions where other crops find it difficult to grow (Lutaladio & Castaldi, 2009).

In terms of volumes of production, potato ranked fourth in the world after rice, wheat and maize (Hirpa *et al.*, 2010). Potato's yielding capacity and flexibility when it comes to environmental conditions make it one of the best crops for food security (Kyamanywa *et al.*, 2011). It is this ability to grow in a range of climatic conditions and acceptance by a wide range of cultures which resulted in an increased potato consumption worldwide (King & Slavin, 2013).

Potato has been produced in Ethiopia for over 150 years (Dersseh *et al.*, 2016). It is produced in Ethiopia mainly for local market and consumption (Gebru *et al.*, 2017). In its 2017/18 Agricultural Sample Survey Report, the Ethiopian Central Statistical Agency reported that there were around 1,127,467 farmers involved in the production of potato in Ethiopia cultivating potatoes in around 69,610 hectares of land which was higher by 4 percent than the previous production year (CSA, 2018).

In Ethiopia, potatoes are grown in three major regions: Oromia, Amhara and Southern Nations Nationalities and Peoples Region. This three regions account for 99 percent of the country's potato production. In the 2017/18 production year, Ethiopia's potato production was around 968,969 tonnes which was higher by 5 percent from the previous production year. Around 50 percent of the potato production was from Oromia region with a potato productivity of about 12.46 tonnes/hectare. Nearly 30 percent of the country's potato production was obtained from the Amhara region with a productivity of 14.99 tonnes/hectare. The remaining about 20 percent of the national potato production was obtained from the Southern Nations Nationalities and Peoples Region with a productivity of around 17.58 tonnes per hectare (CSA, 2018). Table 5 summarizes the potato production in Ethiopia.

Table 5. Ethiopia's 2017/18 potato production data (CSA, 2018)

Region	Area (hectares)	Production (tonnes)	Yield in (tonnes/hectares)
Amhara	19,199.47	287,801.92	14.99
Oromia	38,925.67	484,831.16	12.46
SNNPR	10,771.22	189,378.39	17.58
Tigray	547.25	4,235.30	7.74

3. MATERIALS AND METHODS

3.1. Location of the Study

The study was conducted in Addis Ababa, the capital city of Ethiopia. Addis Ababa city is comprised of 10 sub cities. The study covered samples collected from all 10 sub cities of Addis Ababa.

3.2. Study Design

A cross-sectional study design was used for the evaluation of acrylamide levels in roasted coffee, potato chips and French fries. All analytical laboratory works were carried out in Bless Agri Food Laboratory Services PLC, the largest private food and agricultural testing laboratory located in Lege Tafo, Oromia, Ethiopia.

3.3. Knowledge Assessment of French Fries Producers

A structured questionnaire was developed and used to evaluate the knowledge of street vendors producing and selling French fries about acrylamide. A total of 30 people from the street vendors were interviewed.

3.4. Estimation of Exposure to Acrylamide from Consumption of French Fries

A questionnaire was developed and used to have an estimate of French fries consumption which later was used to estimate levels of exposure to acrylamide. A total of 30 French fries consumers were interviewed for this purpose.

3.5. Sample Collection

A total of 90 samples (30 roasted coffee powder, 30 potato chips and 30 French fries) were collected from Addis Ababa. The roasted coffee powder and potato chips samples, commercially packaged and produced in Ethiopia, were collected from supermarkets while the French fries were collected from street vendors with priorities given to those located near primary and secondary schools.

Figure 6. Samples collection activities

3.6. Sample Handling

100 grams of commercially packed potato chips and 250 – 500 grams of roasted coffee powder samples were collected and transported to the laboratory for analysis. About 100 grams of French fry samples were collected and transported to the laboratory for analysis in sealed plastic sample bags. The samples were stored in the refrigerator at 4 °C until the time of analysis.

The French fry and potato chips samples were ground using a high speed multi-functioning laboratory grinder (RRH-500A, Zhejiang Winki Plastic Co. Ltd, Wuyi, Jinhua City, Zhejiang China).

Figure 7. French fries sample preparation at the laboratory

3.7. Analysis of Acrylamide

3.7.1. Chemicals and Reagents

Chemicals, reagents and analytical standards used in this study were; Acrylamide Analytical Standard (≥99% assay, Sigma Aldrich, Germany, p/n 23701), n-Hexane (HPLC Grade, ≥97%, Sigma Aldrich, Germany, p/n 34859), Water (HPLC Grade, Sigma Aldrich, Germany, p/n 270733), Acetic Acid (Extra Pure, ≥99% assay, Sigma Aldrich, Germany, p/n A6283), Potassium Hexacyanoferrate (≥99.50% assay, Sigma Aldrich, Germany, p/n 60279), Zinc Sulfate (99% assay, Sigma Aldrich, Germany, p/n 221376), Agilent and Waters SPE Cartridges.

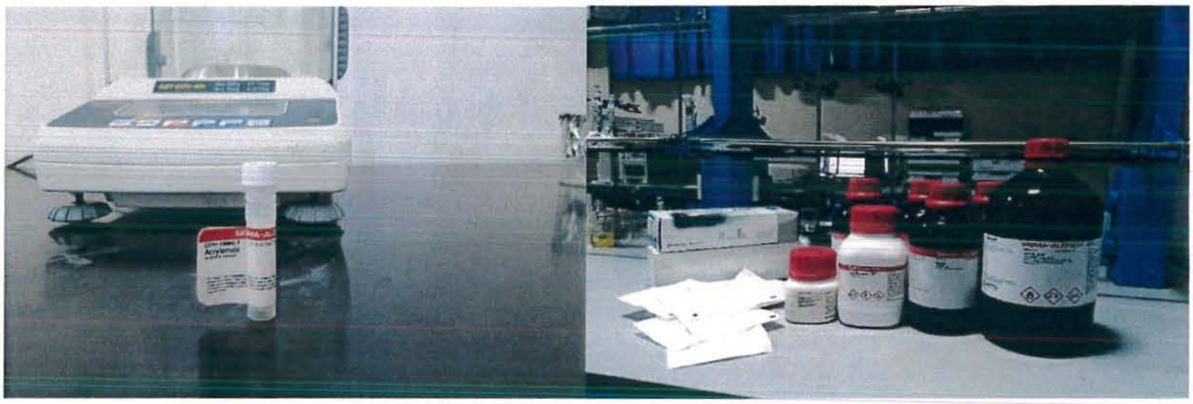


Figure 8. Chemicals and analytical standard used for the determination of acrylamide

3.7.2. Analytical Quality Assurance

3.7.2.1. Limit of Detection

The limit of detection was determined by comparing measured signals of known low concentration analyte to the signal of a blank sample and measuring signal-to-noise ration. The minimum concentration of the analyte that gives a signal-to-noise ratio of 3 was taken as the limit of detection (FDA, 2010).

3.7.2.2. Limit of Quantification

The limit of quantification was determined by comparing measured signals of known low concentration analyte to the signal of a blank sample and measuring signal-to-noise ration. The minimum concentration of the analyte that gives a signal-to-noise ratio of 10 was taken as the limit of quantification (FDA, 2010).

3.7.2.3. Accuracy

Accuracy refers to the closeness of test results to the "true" or accepted value. The accuracy of the method was determined by spiking samples with acrylamide, analyzing in replicates (n=6) and evaluating the recovery against the AOAC guideline for the respective concentration value (Horwitz, 2002).

The % recovery was calculated using the following formula;

$$\%Recovery = \frac{C_s - C_O}{C_A} \; x\ 100$$

Where; C_s is the concentration of acrylamide in the spiked sample in µg/kg,

C_o is the concentration of acrylamide in the original sample in µg/kg, and

C_a is the amount of acrylamide added to the original sample in µg/kg.

3.7.2.4. Precision

The precision of the method was evaluated by analyzing samples spiked with acrylamide in replicates (n=6) and evaluating the relative standard deviation of the measurements against AOAC guideline for the respective concentration value (Horwitz, 2002).

3.7.2.5. Linearity

The linearity of the calibration curve was evaluated by plotting peak area as a function analyte concentration and using goodness of fit of the linear regression line (FDA, 2010).

3.7.3. Extraction and Cleanup

Water extraction with SPE cleanup was employed for the determination of acrylamide (Gökmen *et al.*, 2005; Oroian *et al.*, 2015; Wang *et al.*, 2013).

2.5 grams of well ground sample was weighed in a 50 ml centrifuge tube. 10 ml of water was added to the tube. The tube was mixed vigorously for 5 minutes using a vortex mixer. 5 ml of n-hexane was then added to the contents of the tube in order to remove fat. The tube was mixed vigorously for 5 minutes and centrifuged at 4000 rpm for 5 minutes. The upper hexane layer was then discarded. 1 ml of Carrez I (prepared by dissolving 15 grams of potassium hexacyanoferrate in 100 ml of water) and 1 ml of Carrez II (prepared by dissolving 30 grams of zinc sulfate in 100 ml of water) solutions were added to the content of the flask to remove the protein and to purify the water layer. The tube was mixed vigorously for 5 minutes and centrifuged at 4000 rpm for 5 minutes. The clear layer was again purified by adding 1 ml of Carrez I and 1 ml of Carrez II solutions. The tube was mixed vigorously for 5 minutes and centrifuged at 4000 rpm for 5 minutes. The clear layer was transferred to an SPE column previously conditioned with 1 ml of methanol and 1 ml of water. The extract was passed through the cartridge. The first five drops were discarded. 4 ml filtrate was collected and evaporated to dryness. The residue was reconstituted using 2 ml water. It was then filtered using 0.20 μm filter disk and transferred to auto-sampler vials for HPLC-DAD determination of acrylamide.

A flow diagram for the extraction of acrylamide is given in Figure 6.

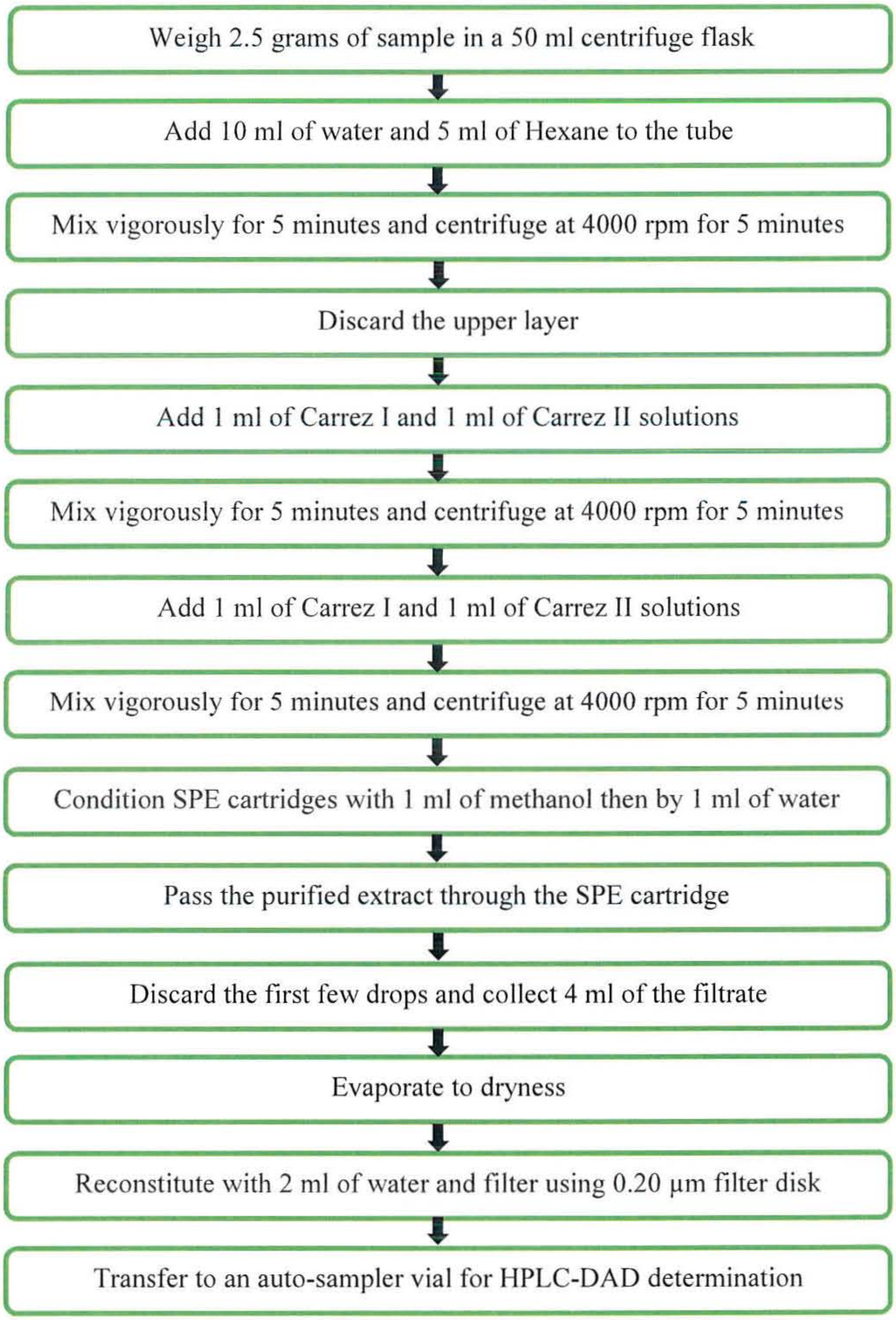


Figure 9. Flow chart for the extraction and cleanup of acrylamide

Figure 10. Extraction of acrylamide in the laboratory

3.7.4. Chromatographic Conditions

The flow rate was set at 0.50 ml/min and the column oven was fixed at 30 °C. The total run time was 15 minutes with 10 minutes for acquisition and 5 minutes for post-run. The injection volume was set at 20 µl. The mobile phase used was 0.1% Acetic Acid solution. Acrylamide was detected using a Diode Array Detector set at 210 nm.

3.7.5. Equipment

The identification and quantification of acrylamide was performed using an Agilent 1260 Infinity HPLC equipped with quaternary pump and Diode Array Detector set at 210 nm. Separation of the compounds was achieved by using an Agilent ZORBAX Eclipse Plus column (4.6 mm x 100 mm, 3.5 µm). The data was processed by an Agilent OpenLab CDS software.

Other instruments used in the analysis of acrylamide included centrifuge (Universal 320, Andreas Hettich GmbH & Co.KG, Tuttlingen, Germany), analytical balance (ABT 220-4M, KERN & SOHN GmbH, Balingen, Germany), vortex mixer (GENIUS 3, IKA – Werke GmbH & Co.KG, Germany), ultrasonic bath (5510, Branson Ultrasonics, Danbury, USA).

3.7.6. Identification of Acrylamide

Acrylamide was identified based on retention time by injecting a blank sample and a pure acrylamide standard solution using the specified chromatographic conditions. The injection repeatability and retention time precision was evaluated by injecting a pure acrylamide standard solution six times over a range of ten days. A relative standard deviation of 2% or lower was taken as an acceptable limit (Sistla *et al.*, 2005).

3.7.7. Calibration of the HPLC

Standard stock solution of 1000 mg/l acrylamide was prepared by dissolving 10 mg of acrylamide analytical standard in 10 ml of water. An intermediate stock solution of 10 mg/l acrylamide was then prepared by taking 1 ml of the standard stock solution in 100 ml one-mark volumetric flask and diluting it to the mark with water. Eight calibration solutions at acrylamide concentrations of 50, 100, 250, 500, 750, 1,000, 1,500, and 2,000 μg/l were prepared by diluting the intermediate stock solution with HPLC grade water and injected to the HPLC for calibration.

Figure 11. Identification of acrylamide using HPLC-DAD in the laboratory

3.7.8. Calculation

Acrylamide in the sample was calculated using the following formula;

$$\text{Acrylamide } (\mu g/kg) = n\ x\ \left(\frac{V_e}{V_I}\right) x\ (\frac{1}{M_e})$$

Where; n is nanogram of acrylamide obtained from HPLC,

V_e is eluate volume in µl,

V_i is injection volume in µl, and

M_e is the mass of sample in grams represented by the final extract.

3.7.9. Statistical Data Analysis

Microsoft Excel 2016 and IBM SPSS Statistics Version 20 were used for data analysis. One-way analysis of variance was used and $\rho\text{-value} < 0.05$ was considered statistically significant. Duncan Multiple Range Test was used for separation of means.

4. RESULTS AND DISCUSSION

4.1. Identification of Acrylamide

The retention time for acrylamide was found to be 6.99 minutes. The instruments injection repeatability and retention time precision were demonstrated (Table 6) as the obtained relative standard deviation was below the specified limit of 2%.

Table 6. System suitability data for the HPLC-DAD determination of acrylamide

	Acrylamide (500 µg/l)	
	Retention Time (minutes)	**Peak Area**
Mean (n = 6)	7.01	80833018
SD	0.13	1287759
%RSD	1.81	1.59

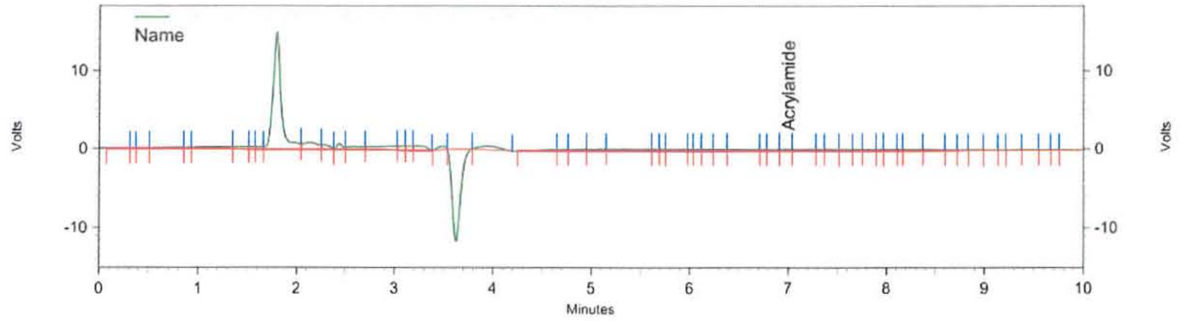


Figure 12. Chromatogram for blank

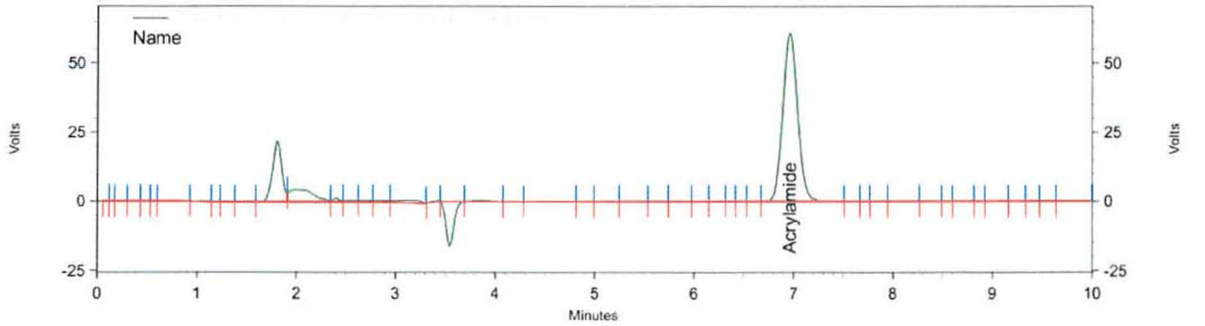


Figure 13. Chromatogram for 500 µg/l acrylamide standard

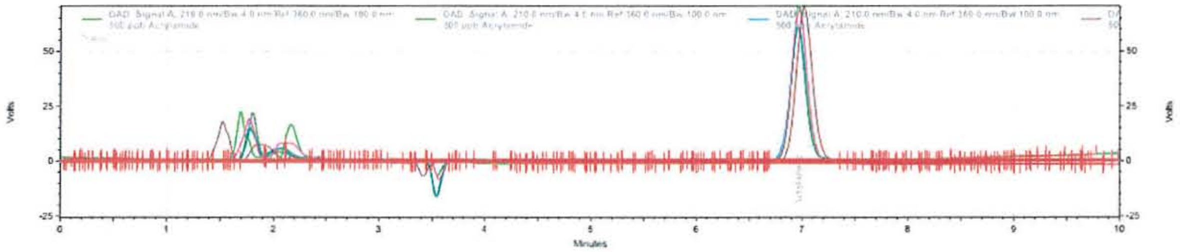


Figure 14. Overlaid chromatogram of 500 µg/l acrylamide solution system suitability runs

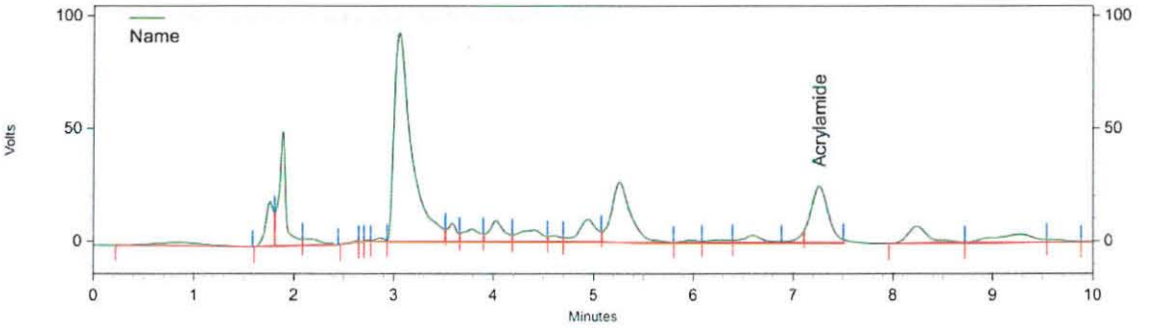


Figure 15. Chromatogram for sample

4.2. Preparation of Calibration Curve

An eight-point linear calibration curve (Figure 10) was constructed by plotting peak area against concentration of acrylamide using the data obtained (Table 7) from replicate (n=3) injections of each concentration level. The calibration curve obtained (Figure 13) demonstrated linearity with a correlation coefficient value of (r^2) 0.9997.

Table 7. Calibration data for the analysis of acrylamide

Acrylamide Concentration (µg/l)	Peak Area			
	Run 1	Run 2	Run 3	Average
50	9504289	9564181	9786661	9618377
100	17060983	16681828	17049356	16930722
250	43846683	44165050	43769410	43927048
500	85557163	81763535	81247079	82855926
750	126066147	125296758	124167631	125176845
1000	163050564	163603710	164178049	163610774
1500	245303794	245236627	244972019	245170813
2000	322798509	322848925	323257916	322968450

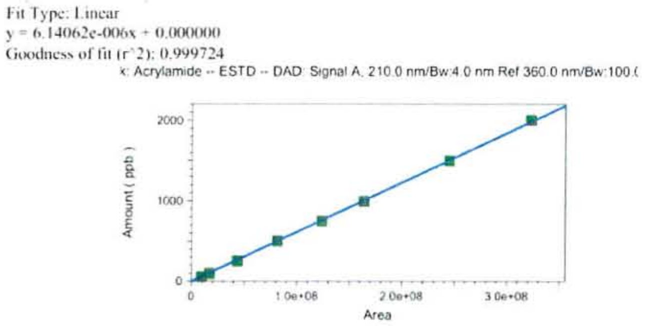


Figure 16. Calibration curve for the analysis of acrylamide

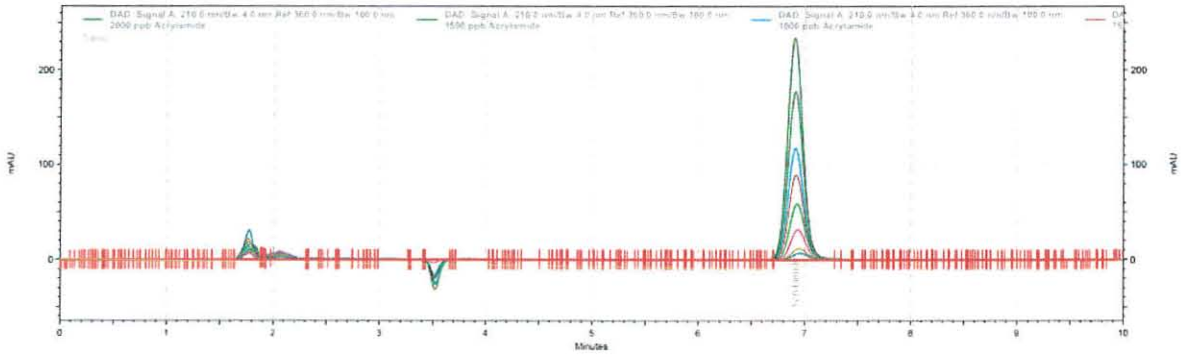


Figure 17. Overlaid chromatogram of the calibration series

4.3. Analytical Quality Assurance

4.3.1. Limit of Detection (LOD)

The limit of detection was found to be 5 μg/kg.

4.3.2. Limit of Quantification (LOQ)

The limit of quantification was found to be 20.40 μg/kg.

4.3.3. Accuracy and Precision

The recoveries obtained (Table 8) ranged between 85% and 108% and the %RSD obtained was 2.63% for the coffee powder. The method was found to be both accurate and precise for its intended use as the recovery was between 70% and 125%; and the %RSD was less than 15% as specified in AOAC Guidelines for Single Laboratory Validation of Chemical Methods (Horwitz, 2002) for this concentration level.

For the potato chips sample, the recoveries obtained ranged between 83% and 100% and the %RSD obtained was 6.20%. This shows the method's accuracy and precision as the recovery was between 75% and 120%; and the %RSD was less than 8% as specified in AOAC Guidelines for this concentration level. The method was also found to be accurate and precise for the determination of acrylamide in French fries as the obtained recovery was between 70% and 125%; and the %RSD was less than 15% as specified in AOAC Guidelines for Single Laboratory Validation of Chemical Methods (Horwitz, 2002) for this concentration level.

Table 8. Accuracy and precision data for acrylamide analysis

Matrix	Acrylamide (µg/kg)	Acrylamide Spiked (µg/kg)	Acrylamide Obtained (µg/kg)	%Recovery (n = 6)	%RSD (n = 6)
Coffee Powder	270.34 ± 7.10	100	365.07 ± 9.62	94.73	2.63
Potato Chips	247.39 ± 7.52	1000	1184.65 ± 73.42	93.73	6.20
French Fries	363.91 ± 10.51	500	813.11 ± 34.20	89.84	4.21

4.4. Acrylamide Levels in Coffee Powder

The acrylamide content of roasted coffee powders collected from Addis Ababa ranged between 134.56 µg/kg and 1138.86 µg/kg (Table 9). The mean acrylamide content of roasted coffee powders was 420.48 µg/kg.

Different studies on acrylamide levels in roasted coffee were conducted in different parts of the world. In Italy, 66 coffee samples showed a mean acrylamide level of 465 µg/kg (Bertuzzi *et al.*, 2017) which is similar to the reports of this study. In Poland, 17 roasted coffee samples showed an acrylamide level ranging between 17.7 µg/kg and 776.1 µg/kg (Surma *et al.*, 2017) where both lower and higher ranges were significantly lower than what is observed in this study. 291 roasted coffee samples analyzed in Europe showed an acrylamide concentration ranging between 79 µg/kg and 1188 µg/kg (Wenzi & Anklam, 2007) where both lower and higher values were relatively similar to the outputs of this study.

The significant variation in acrylamide level between the samples may be associated with roasting conditions including factors like roasting temperature, duration and level of roasting. Different studies show that during the beginning of roasting the amount of acrylamide formed increases drastically, attains a maximum point, and then lowers quickly (Bagdonaite *et al.*, 2008; Borda & Alexe, 2011; Lantz *et al.*, 2006; Şenyuva & Gökmen, 2005). Coffee species also has an impact on the level of acrylamide formed during roasting since Robusta coffee contains higher amount of asparagine, which is the limiting factor on the formation of acrylamide in coffee, than Arabica coffee (Bagdonaite *et al.*, 2008; Lantz *et al.*, 2006). Since Ethiopia only produces Arabica coffee (GAIN, 2018), the variations in this study couldn't have been a result of variations in species.

Table 9. Acrylamide levels in roasted coffee powder collected from Addis Ababa

		Acrylamide Content (μg/kg)			
Collection Site	**N**	**Minimum**	**Maximum**	**Mean**	**Median**
Site 1	3	134.56	517.78	389.56	516.35
Site 2	3	252.47	541.81	392.47	383.14
Site 3	3	199.10	517.69	329.17	270.72
Site 4	3	238.47	565.38	407.75	419.40
Site 5	3	333.64	768.69	478.94	334.49
Site 6	3	492.51	851.58	646.90	596.62
Site 7	3	278.42	1138.86	598.09	376.98
Site 8	3	311.52	535.71	410.20	383.38
Site 9	3	181.94	302.16	238.62	231.77
Site 10	3	193.84	516.04	313.10	229.43
TOTAL	30	134.56	1138.86	420.48	380.06

The benchmark level for acrylamide in roasted coffee powder set by the European Commission is 400 μg/kg (EC, 2017). Out of a total of 30 roasted coffee powder samples collected from the 10 sub cities in Addis Ababa, 13 samples (43%) had acrylamide levels higher than the recommended 400 μg/kg benchmark level set by the European Commission (Figure 15). Acrylamide concentration as high as 1138.86 μg/kg was found in a roasted coffee powder in one of the collection sites.

Since studies show that brewing effectively transfers all the acrylamide to the coffee brew due to acrylamide's high solubility in water (Andrzejewski *et al.*, 2004; Bagdonaite *et al.*, 2008), the fact that 43% of the collected coffee powder contained acrylamide beyond the EC recommended level raises a serious concern to the consumer.

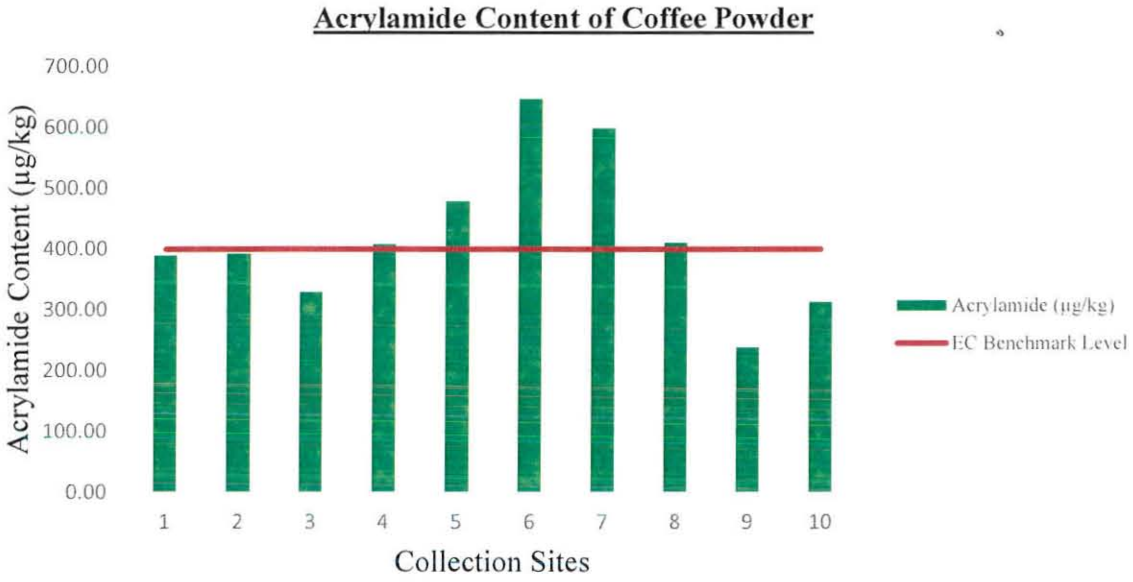


Figure 18. Graphical presentation of acrylamide levels in roasted coffee powder collected from Addis Ababa

4.5. Acrylamide Levels in Potato Chips

The acrylamide content of the potato chips collected from Addis Ababa ranged between 211.09 μg/kg and 3514.60 μg/kg (Table 10). The mean acrylamide content of the potato chips was found to be 1298.28 μg/kg.

Various studies on acrylamide levels in potato chips were conducted in different parts of the world. Acrylamide concentrations ranging between 150 μg/kg and 4000 μg/kg were reported by Tareke *et al.* (2002) in carbohydrate-rich foods including potato, beetroot and selected commercial potato products for the first time which were relatively similar to the findings of this study. In Romania, 50 potato chips samples showed acrylamide concentrations ranging up to 1782 μg/kg (Oroian *et al.*, 2015). A Chinese study reported acrylamide concentrations as high as 3016 μg/kg in potato crisps (Chen *et al.*, 2008) which is in agreement with the highest values found in this study. 1408 potato chips samples analyzed in Europe showed an acrylamide concentration ranging between 5 μg/kg and 4653 μg/kg (Wenzi & Anklam, 2007). The lower acrylamide content reported in this study was significantly higher while the maximum acrylamide content was significantly lower than what is reported by Wenzi & Anklam in 2007.

The high content of acrylamide and the significant variation between samples can be a result of many factors. The concentration of the precursors required for the formation of acrylamide may significantly affect the rate of acrylamide formation (Elmore *et al.*, 2015; Mottram *et al.*, 2002; Rydberg *et al.*, 2003; Tareke *et al.*, 2002).

The reducing sugar content of potatoes being the limiting factor for the formation of acrylamide, the variation in levels of acrylamide in the potato chips samples may be associated with the reducing sugar content of the raw potatoes (Amrein *et al.*, 2004). The variation can also be due to variations in processing conditions since time and temperature play a crucial role in the formation of acrylamide (Becalski *et al.*, 2003; Mesias *et al.*, 2018; Mottram *et al.*, 2002; Tareke *et al.*, 2002).

Table 10. Acrylamide levels in potato chips collected from Addis Ababa

		Acrylamide Content (μg/kg)			
Collection Site	**N**	**Minimum**	**Maximum**	**Mean**	**Median**
Site 1	3	552.25	1906.43	1168.65	1047.28
Site 2	3	320.83	1967.39	1322.71	1679.90
Site 3	3	226.97	2631.40	1028.55	227.28
Site 4	3	1590.43	2203.85	1936.17	2014.23
Site 5	3	211.09	930.03	479.24	296.59
Site 6	3	676.14	2522.93	1329.91	790.67
Site 7	3	340.81	3514.60	1458.66	520.58
Site 8	3	1258.92	2835.61	1906.91	1626.19
Site 9	3	319.97	1472.30	790.15	578.17
Site 10	3	468.90	2360.43	1561.85	1856.23
TOTAL	30	211.09	3514.60	1298.28	1153.10

The benchmark level for acrylamide in potato chips in a regulation drafted on establishing mitigation measures and reduction of the presence of acrylamide in food set by the European Commission is 700 μg/kg (EC, 2017). Out of the 30 potato chips, 17 samples (57%) had acrylamide levels higher than the 700 μg/kg benchmark level set by the European Commission (Figure 16). Acrylamide concentration as high as 3514.60 μg/kg was found in one of the sample sites.

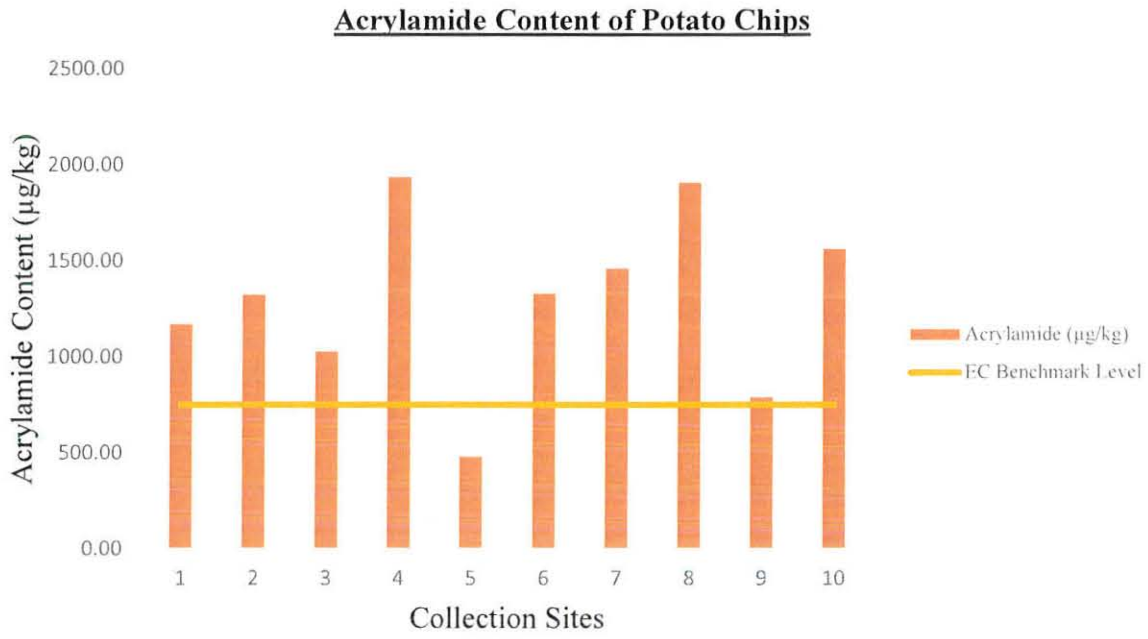


Figure 19. Graphical presentation of acrylamide levels in potato chips collected from Addis Ababa

4.6. Acrylamide Levels in French Fries

The acrylamide content of the French fries collected from Addis Ababa ranged between 35.66 µg/kg and 1410.75 µg/kg (Table 11). The mean acrylamide content of the French fries was 614.84 µg/kg.

Elias *et al.* (2017) reported acrylamide levels up to 750 µg/kg in French fries in Estonia and a mean acrylamide content of 299 µg/kg which is significantly lower than both the highest amount and mean acrylamide content found in French fries in this study. 10 homemade and 15 fast food type French fries samples collected from the Romanian market showed acrylamide levels ranging between 210 and 2922 µg/kg (Oroian *et al.*, 2015). Both higher and lower values reported by Oroian *et al.* (2015) were significantly higher than the findings of this study.

The variations in acrylamide levels between the samples can be a result of different factors. One of the reasons for this variation can be the way the potatoes were sliced before frying as surface-to-volume ratio plays a role in the formation of acrylamide. Small size cuts can lead to an increased level of acrylamide since it is formed on the upper layer of the fried potato (Mathau¨s *et al.*, 2004).

Pretreatments of the raw potato by blanching can lead to a reduced acrylamide formation as reducing sugars can be leached out (Pedreschi *et al.*, 2007; Vinci *et al.*, 2010). Frying temperature and time also significantly influence the formation of acrylamide and high temperature and longer frying time can result in the formation of higher acrylamide levels. That is why it is essential to determine proper end point of the frying process since acrylamide formation significantly increases approaching the end of the frying process (Franke, 2009; Grob & Pfefferle, 2003).

Table 11. Acrylamide levels in French fries collected from Addis Ababa

		Acrylamide Content (μg/kg)			
Collection Site	**N**	**Minimum**	**Maximum**	**Mean**	**Median**
Site 1	3	163.97	1041.36	487.70	257.77
Site 2	3	457.22	744.77	619.92	657.78
Site 3	3	129.15	1410.75	897.50	1152.61
Site 4	3	315.74	1406.42	837.50	790.34
Site 5	3	639.38	1196.87	830.40	654.96
Site 6	3	86.61	1046.45	569.29	574.80
Site 7	3	35.66	889.47	457.47	447.28
Site 8	3	290.77	789.93	485.53	375.88
Site 9	3	242.56	763.84	528.50	579.11
Site 10	3	146.29	948.22	434.63	209.37
TOTAL	30	35.66	1410.75	614.84	609.24

The benchmark level for acrylamide in French fries as set by the European Commission is 500 μg/kg (EC, 2017). 12 French fries' samples (40%) out of the 30 analyzed had acrylamide levels higher than the 500 μg/kg benchmark level set by EC (Figure 17).

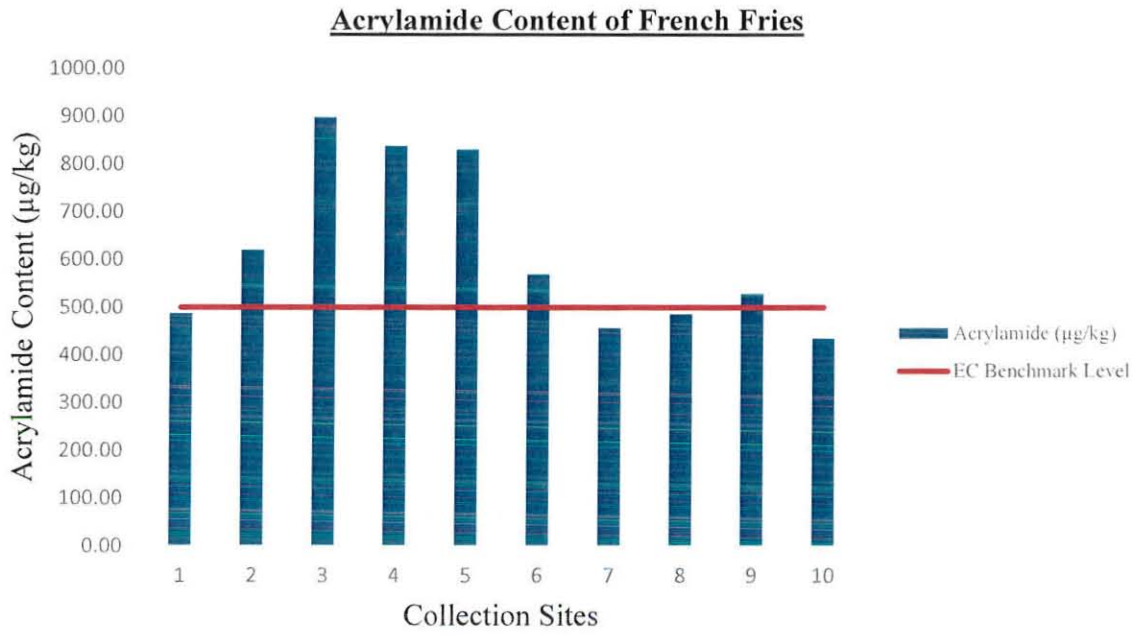


Figure 20. Graphical presentation of acrylamide levels in French fries collected from Addis Ababa

4.7. Comparison of Acrylamide Levels Between the Food Items

The significance of the differences in acrylamide levels between roasted coffee, potato chips and French fries collected from Addis Ababa was evaluated using analysis of variance (Table 12).

While the difference between the acrylamide content of roasted coffee and French fries was not significant, the potato chips exhibited a significant difference in acrylamide content. The relatively high natural availability of the reactants required for the formation of acrylamide, mainly the reducing sugars, is considered the main reason for the higher levels of acrylamide in heat processed potato products (Lineback *et al.*, 2012; Studer *et al.*, 2004; Williams, 2005).

The reason why potato chips contained high levels of acrylamide when compared to French fries while potatoes are the main ingredient can be associated to the surface-to-volume ratio of the potato cuts. Smaller and thinner potato cuts can result in a higher level of acrylamide since it is formed on the upper layer of the product during frying (Mathau"s *et al.*, 2004). Since potato chips have larger surface area when compared to volume, there is a higher chance of acrylamide formation when compared to French fries.

Table 12. Comparison of acrylamide levels between roasted coffee, potato chips and French fries

Food Type	**N**	**Mean Acrylamide Level (μg/kg)**
Roasted Coffee	30	420.48^{a}
Potato Chips	30	1298.28^{b}
French Fries	30	614.84^{a}

$^{a-b}$ Different alphabate superscripts between food types denote significant difference ($p<0.05$) using Duncan Multiple Range Test.

Although the risk of exposure to acrylamide is a function of the foods acrylamide content to the amount of consumption, of all the three products investigated in this study, potato chips can introduce a higher level of acrylamide to the consumer when compared to roasted coffee and French fries.

4.8. Knowledge Assessment of French Fries Producers

A total of 30 French fries producing street vendors from Addis Ababa were interviewed using the annexed interview format. Out of the 30 interviewees 26 (87%) were females and 4 (13%) were males. The interviewees were between the ages of 15 and 42 (Table 13).

None of the interviewed French fries' street vendors knew what acrylamide was or even heard of acrylamide before. None of them knew how it is formed in food or its health impacts. All of the producers were not familiar with the types of food susceptible to the formation of acrylamide. None of the interviewed street vendors had their French fries tested by a laboratory before.

The overall outcomes of the interview indicate that the knowledge of people involved in the street production of French fries in Addis Ababa about acrylamide, its formation, and associated health impacts is nonexistent.

Table 13. Summary on knowledge of French fries producers about acrylamide

Questions	Response	Respondents
Do you know what acrylamide is?	Yes	0 (0%)
	No	30 (100%)
Have you ever heard of acrylamide before?	Yes	0 (0%)
	No	30 (100%)
Do you know how acrylamide is formed in food?	Yes	0 (0%)
	No	30 (100%)
Are you familiar with health associated impacts of acrylamide?	Yes	0 (0%)
	No	30 (100%)
Do you know what food types are mainly susceptible for the formation of acrylamide?	Yes	0 (0%)
	No	30 (100%)
Do you frequently consume fried products?	Yes	27 (90%)
	No	3 (10%)
Do you reuse frying oil?	Yes	30 (100%)
	No	0 (0%)
Have you ever had your food products tested?	Yes	0 (0%)
	No	30 (100%)

4.9. Estimation of Exposure to Acrylamide from Consumption of French Fries

A total of 30 French fries' consumers from all 10 sub cities of Addis Ababa were interviewed using the annexed interview format at point of purchase in order to obtain an estimation about the frequency of French fries consumption. Out of the 30 interviewees 11 (37%) were females and 19 (63%) were males. The interviewees were between the ages of 7 and 16.

Out of the 30 children interviewed, 16 (53%) consume French fries at least 3 times in a week. Out of the 30 children interviewed, 23 children (77%) take more than 100 grams of fries during a single consumption (Table 14). The amount of single dose consumption was estimated by associating the money paid for one-time consumption with the relative weight of the product in the market for that paid amount.

Considering a 100 grams of French fries' consumption 3 times a week, the average child consumes about 15.60 kg of French fries in a year. This value is equivalent to a daily consumption of 42.74 grams of French fries. Taking 614.84 μg/kg mean acrylamide content obtained in this study, the exposure from consumption of 42.74 grams of French fries per day becomes 26.28 μg.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommends the Margin of Exposure (MOE) approach to evaluate the health risks associated with exposure to acrylamide by estimating the ratio between the dose resulting in a low but defined cancer incidence to acrylamide intake (EFSA, 2015).

Considering a body weight of 40 kg, the daily consumption of acrylamide can be calculated to 0.66 μg/kg body weight/day. Using the JECFA approach and the two reference points selected by the EFSA CONTAM panel (0.43 mg/kg body weight/day for non-neoplastic effects and 0.17 mg/kg body weight/day for neoplastic effects) the margin of exposure was calculated to be 652 for non-neoplastic effects and 258 for neoplastic effects. These values indicate that there is no risk of non-neoplastic health effects from exposure to acrylamide from French fries alone as the value is higher than 100 giving no reason for public health concern. However, the MOE value for neoplastic effects is significantly lower than the theoretical maximum of 10000 indicating risk of neoplastic health effects (characterized by the incidence of Harderian gland adenomas and adenocarcinomas in animal studies) from exposure to acrylamide from the consumption of French fries considering the frequency and volume of consumption stated above.

Table 14. Summary of interview outcomes on French fries consumption frequency & amount

Questions	Response	Respondents
How many times do you consume French fries in a single week?	1 time	6 (20%)
	2 times	8 (27%)
	3 times	9 (30%)
	4 times	5 (17%)
	5 times	2 (6%)
	6 times	0 (0%)
	7 times	0 (0%)
On average, how much French fries do you consume at a single time?	50 grams	7 (23%)
	100 grams	11 (37%)
	150 grams	7 (23%)
	200 grams	5 (17%)

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

Acrylamide was detected in all coffee powder, potato chips and French fries investigated in this study. According to the outcomes of this study, 43% of the coffee powders contained acrylamide higher that the recommended European Commission 400 μg/kg benchmark level. Ethiopia being the largest coffee consuming country in Africa, risk of exposure is not something that should be taken lightly considering nearly half of the samples contained acrylamide above the recommended benchmark level.

Acrylamide levels higher than the maximum benchmark levels recommended by the European Commission were observed in 57% of the potato chips and 40% of the French fries analyzed in this study. From the three food types evaluated in this study, potato chips comparatively had higher mean acrylamide content than coffee powder and French fries. The outcomes of this study indicate that these food products can be major dietary acrylamide sources as nearly half of the food items analyzed showed acrylamide levels beyond the recommended benchmark values. Accordingly, the health risks associated with these products high acrylamide levels is not something that can be considered insignificant.

The outcomes of this study also showed that there is a huge knowledge gap in the street vendors involved in the production of French fries when it comes to acrylamide.

The estimation of exposure to acrylamide and its associated health impacts from the consumption of French fries indicates a health risk that requires a serious attention especially when considering there are many additional dietary acrylamide sources in addition to French fries that a consumer can be exposed to.

5.2. Recommendation

- Since these study was aimed to provide a baseline data on levels of acrylamide in coffee powder, potato chips and French fries; pilot scale researches focusing on lowering acrylamide levels by working on processing temperature, time, technology, pretreatments and other parameters that have impacts on formation of acrylamide should be conducted.
- Ethiopia is country rich in culture where there are different traditional foods and beverages are produced and consumed. Researches should focus on studying other heat processed traditional foods and beverages with the necessary precursors for the formation of acrylamide
- Regulatory authorities should focus on creating awareness of consumers on acrylamide, its health risks and major dietary sources as it is evident from this study that consumers could be exposed to high levels of acrylamide from consumption of these food items. This will help consumers make an informed decision about consuming foods with high level of acrylamide.
- The large variations in acrylamide levels within the same food item as evident by the outcomes of this study indicates that process optimization could be a critical factor. Regulatory authorities should work hand in hand with universities, researchers and processors for process optimization to identify, implement and monitor appropriate mitigation practices.
- Adopting benchmark levels on acrylamide levels based on researches and help street vendors and processors in implementing interventions in order to lower acrylamide levels to achieve the values set.

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ANNEX-1

INTERVIEW FORM FOR FRENCH FRIES VENDORS

Sex: ☐ Male ☐ Female

Age: ________________

Date: ________________

	Yes	No
Do you know what acrylamide is?		
Have you ever heard of acrylamide before?		
Do you know how acrylamide is formed in food?		
Are you familiar with health associated impacts of acrylamide?		
Do you know what food types are mainly susceptible for the formation of acrylamide?		
Do you frequently consume fried products?		
Do you reuse frying oil?		
Have you ever had your food products tested by a laboratory?		

ANNEX-2

INTERVIEW FORM FOR FRENCH FRIES CONSUMERS

Sex: ☐ Male ☐ Female

Age: ________________

Date: ________________

Consumption Frequency

1- How many times do you consume French fries in a single week?

1 time 2 times 3 times 4 times 5 times 6 times 7 times

2- On average, how much French fries do you consume at a single time?

50 grams 100 grams 150 grams 200 grams

ANNEX-3

ANOVA AND MEAN SEPARATION

Descriptive Statistics

Acrylamide Content (μg/kg)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Roasted Coffee	30	420.4817	220.20282	40.20335	338.2566	502.7068
Potato Chips	30	1298.2800	929.08827	169.62753	951.3527	1645.2073
French Fries	30	614.8443	396.47013	72.38521	466.8000	762.8887
Total	90	777.8687	701.13482	73.90610	631.0188	924.7185

	Minimum	Maximum
Roasted Coffee	134.56	1138.86
Potato Chips	211.09	3514.60
French Fries	35.66	1410.75
Total	35.66	3514.60

ANOVA

Acrylamide Content (μg/kg)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12753910.707	2	6376955.353	17.898	.000
Within Groups	30997603.025	87	356294.288		
Total	43751513.731	89			

Acrylamide Content (μg/kg)

	Food Type	N	Subset for alpha = 0.05	
			1	2
Duncan[a]	Roasted Coffee	30	420.4817	
	French Fries	30	614.8443	
	Potato Chips	30		1298.2800
	Sig.		.211	1.000