KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF SCIENCE

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

PROBABILISTIC EXPOSURE ASSESSMENT OF ACRYLAMIDE IN OILS

BY

ACHEAMPONG SERWAA ZEINAB

A THESIS SUBMITTED TO THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY, COLLEGE OF SCIENCE IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MSc FOOD QUALITY MANAGEMENT

JUNE 2017

DECLARATION

I hereby declare that this submission is my own work towards the MSc and that, to the best of my knowledge, it contains no material previously published by another person, nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

Certified by:

……	……
Zeinab Serwaa Acheampong	Isaac Williams Ofosu
Student	Supervisor
……	……
Signature	Signature
……	……
Date	Date

Certified by:

……

John Barimah

Head of Department

……

Date

DEDICATION

This work is dedicated to the ever-loving memory of Maulvi Dr. Abdul Wahab Bin Adam. May the Almighty Grant him a lofty place in paradise.

ACKNOWLEDGEMENT

I am grateful to the Almighty Allah for His strength, grace, mercy, favour, love and protection throughout my education. I am greatly appreciative to my supervisor, Mr Isaac Ofosu Williams firstly for his patience, tolerance and secondly for his constructive suggestions and criticisms in the preparation of this report.

My gratitude also goes to my ever-supportive husband, Saeed Kofi Sam and my daughter Hannah Otuwaah Sam. To my parents, Mr. and Mrs. Acheampong and all my siblings. God richly bless you Finally, I will like to thank my good friend Barry Whyte and William Ofori Appaw for their support in diverse ways to make this project work a success.

ABSTRACT

There is an increasing trend of high fat in Ghanaian diet. These fats (cooking oils) produced locally and some imported into our country are partly regulated for some chemical hazards such as acrylamide. In our local oil industry, it has been observed that, the processing methods used are highly questionable. Thus, acrylamide a known hazard produced during processing could be present in substantial quantities. The objective of this work was to determine the probabilistic exposure assessment of acrylamide in locally produced oils and other oils being sold on our local market. Crude palm oil, groundnut oil and refined palm oil were purchased from retailers in the Kumasi metropolis. Consumers of these oils were identified and made to fill a dietary and lifestyle questionnaire. Using HPLC the concentration of Acrylamide in the various oils was determined. Palisade @ Risk (2014) Software at 10,000 iterations was used to determine the probabilistic exposure of acrylamide in the population of study. The acrylamide concentrations determined were 0.844 mg/g, 0.374 mg/g and 0.172 mg/g for Groundnut oil, Crude palm oil and refined palm oil respectively. Groundnut oil recorded the highest value for chronic daily intake when compared to crude palm oil and refined palm oil. Results for hazard quotient conclude that consuming groundnut oil puts the population at higher risk of ingesting a probable carcinogen as compared to crude palm oil and refined palm oil.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS/ACRONYMS

AT	Averaging Time of the CDI
AA	Acrylamide
BW	Body Weight
CDI	Chronic Daily Intake
CR	Consumption Rate
DF	Degree of Freedom
EFSA	The European Food Safety Authority
EPA	The United States Environmental Protection Agency
GC-MS/MS	Gas Chromatography Tandem Mass Chromatography
HC	Hazard Concentration
HI	Hazard Index
HPLC	High Performance Liquid Chromatography
HQ	Hazard Quotient
IARC	The International Agency for Research on Cancer
IRMM	The European Community Institute for Reference Materials and Measurements
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MoFA	The Ministry of Food and Agriculture, Ghana
PF	Potency Factor also known as slope factor
RfD	Reference Dose
SE	Standard of Error
SEM	Standard Error of the Mean

CHAPTER ONE

INTRODUCTION

BACKGROUND

Edible vegetable oils such as soya bean oil, palm oil, rapeseed oil and sunflower oil are mainly used globally in preparing our foods (Rosillo-Calle *et al*., 2009). However, groundnut oil, coconut oil, sheabutter cooking oil and crude palm oil are mostly found on the Ghanaian market. During frying processing, chemical, physical, sensory and nutritional parameters are affected during frying at elevated temperatures (Singh, 1995). Research has demonstrated that the mutagens are formed as a result of heating process thus resulting in changes in the DNA structure or changes in chromosomes which is likely to lead to carcinogenesis (Wu and Yen, 2004; Dung *et al.,* 2006). However epidemiological studies in Ghana show a steady increase in prevalence rates of non-communicable diseases since the 1950's (Agyei-Mensah and de-Graft Aikins, 2010). Nelson *et al.,* (2003), linked western diet of too high animal fats and insufficient fruits and vegetables as well as the type of food processing, that is, subjecting foods to high heat from processing methods such as frying and grilling enable the formation of heterocyclic amines and polycyclic aromatic hydrocarbons which are mutagenic agent at high temperatures above 120 ^{o}C. According to IARC report 2010, cooking oil has little mutagenic potential when heated below 100 ^{o}C and high mutagenic potential above 230 ^{o}C .

In 2002, acrylamide a processing contaminant was discovered (Eriksson, 2005). Foods especially plant commodities that are high in carbohydrates and low in protein during processing at temperatures greater than 120 °C were found to be high in acrylamide contaminant after processing. A recent concern about their possible public health risks which is associated with dietary exposure has drawn attention to them in

the international arena. (Eriksson, 2005; Friedman, 2003). The toxic nature of acrylamide has been extensively investigated. Findings of studies on acrylamide indicated that it has a neurotoxic effect in both animals and humans. In rodents, it has been found to be a reproductive and developmental toxic agent and carcinogen (Cuciureanu *et al.*, 2005; Dybing and Sanner, 2003; Centre for the Evaluation of Risks to Human Reproduction (CERHR), 2005).

In 2005 food researchers evaluated the safety of acrylamide. Their finding was re-evaluated in 2010. A Benchmark Dose Level of lower confidence limit for a 10%. ($BMDL_{10}$) was considered important in assessing the risk of acrylamide exposure in animals.

Although processing of foods at elevated temperatures of greater than 120 ^{o}C increases the production of acrylamide contaminant in foods, the presence or addition of preservatives, flavour enhancers, colour additives do not have any role as far as acrylamide is concerned (Eriksson, 2005).

A major component (30 %) of our daily energy requirements in our diet is supplied by fats and oils (Mahan *et al*., 2012). These oils enhance the nutritive value of food by making it edible and digestible as well as improving the texture and imparting flavour to food. Poverty, illiteracy and ignorance on the part of food processors and consumers alike may increase the risk of exposure to mutagenic compounds formed during oil processing.

1.1 PROBLEM STATEMENTS AND JUSTIFICATION

The increasing trend of non-communicable diseases including cancer has become a public health concern both locally (Ghana) and globally. According to researchers

these non-communicable diseases are mostly linked to our diet. Acrylamide a probable carcinogen and its genotoxic and neurotoxic effect on our health have made it an area of interest for most researchers.

These compounds are the most mutagenic agents formed during cooking and high temperatures induce their formation. According to IARC report in 2010 cooking at elevated temperatures greater than 120 ^{o}C increases the formation of acrylamide in foods and a high mutagenic potential above 230 ^{o}C.

This has raise a concern in our part of the world where oil processing is partly regulated since oils such coconut oil, palm oil, groundnut oil, sheabutter oil are mostly processed by small and medium enterprises whom are partly regulated along the lines of Good Manufacturing Practices(GMP).

It is therefore of public health relevance and dietetic interest to identify these risk factors that may contribute to the increase in exposure to acrylamide, and thus inform health professionals in the education of the populace. In addition, there is little information as to the acrylamide values for such products yam, cocoyam, plantain, fried fish and other staples in less developed countries as Ghana.

1.2 OBJECTIVE

This work was to contribute to safeguard the safety of vegetable oils for consumption. However, the main objective of the work was to determine the probabilistic exposure assessment of acrylamide in cooking oils sold on our local market.

CHAPTER TWO

LITERATURE REVIEW

2.1 ACRYLAMIDE

Acrylamide (AA) which is a chemical substance can naturally be naturally formed high carbohydrate foods heated at elevated temperatures (Madle *et al*., 2003). According to Calleman, (1996) and Segerbäck *et al*., (1995) the reaction between nucleophilic sites in macromolecules and a extremely water-soluble α, β-unsaturated amide in Michael-type additions is defined as Acrylamide. In addition, the reaction between an amino acid and a carbonyl group of a reducing sugar, known as Maillard's reaction is the major pathway of acrylamide formation (Taeymans *et al*., 2005; Claus *et al*., 2008). Non-dietary sources may include cigarette smoke and cosmetics are known (Ötles and Ötles 2004).

2.2 CHEMISTRY OF ACRYLAMIDE

In April 2002, Tareke *et al.,* (2002), Swedish researchers discovered acrylamide in foods. It revealed that high level of acrylamide was formed in starchy foods, such as French fries, Potato chips and bread that has been heated at elevated cooking temperatures like frying or baking. As a chemical, naturally generated as part of a cooking process, researchers around the world have been alarmed to research into both their toxicity and occurrence in a wide variety of foods.

2.2.1. The physical parameters of acrylamide

Acrylamide (CH_2=CH-CO-NH_2; 2-propenamide) is an odorless white crystalline solid formed from the hydration of acrylonitrile with sulphuric acid between the temperatures; 90-100 ^{o}C or by catalytic hydration using copper catalyst. It is soluble

in a number of polar solvents like water, acetone and acetonitrile and very difficult to determine its boiling point at ambient pressure because there is the susceptibility of acrylamide to polymerization during heating (Annon 1999, Eriksson 2005).

Table 1. Physical Parameters of Acrylamide

Parameter	Specification
Chemical Formula	CH_2=CH-CO-NH; propenamide
Molecular Weight	71.08 $gmol^{-1}$
Solubility	216 g/100g water (30 ^oC)
Boiling Point	136 ^oC at 3.3 kPa/25 mmHg
Melting Point	84.5 ^oC
Vapour Density	2.45 (air = 1)
Density/Specific Gravity	1.122 $kgdm^{-3}$ at 30 ^oC
Vapour Pressure	0.007 mm Hg at 25 ^oC

Source (Annon 1999, Eriksson 2005).

2.2.2. Chemical Structure of the Acrylamide Molecule

An amide group and the electron-deficient vinylic double bound that makes it readily available for a wide range of reactions like radical reactions as well as nucleophilic and Diel-Alder reactions which are very important in biological systems are the two functional groups that make acrylamide. Studies by different authors have shown that, the amide group undergoes hydrolysis, alcoholysis, dehydration and condensation reactions with aldehydes (Andrzejewski *et al*., 2004; Taeymans *et al*., 2005; Claus *et al*., 2008). The vinylic double bond on the other hand also reacts with ammonia, aliphatic amines, bisulphite and dithiocarbonates, chlorine, phosphines, bromine and proteins ($CH_2CHCONH_2$) (Friedman, 2003; Girma *et al.*, 2005).

2.2.3 Determination of Acrylamide

Intensive refinement in the determination of acrylamide has been carried out since the discovery of acrylamide in foods. Wenzl *et al.,* (2003), is of the view that gas chromatography with mass spectrometric detection (GC-MS) and high performance liquid chromatography (HPLC) with tandem mass spectrometric detection (LC-MS/MS) appeared to be the most widely used methods. According to international guidelines such as ISO 17025, few reports indicate that a rigorous and systematic determination of the characteristics of the method has been performed by European Committee for Standardization (CEN) and Eurachem (Schaller, 2003). By comparing methodologies, there were huge differences in the cleanup strategies. This was found for both GC and LC based methods (Tareke *et al.*, 2002).

Isotopically labelled acrylamide at the beginning of the sample pre-treatment is a way to take into consideration the losses of acrylamide that can occur during the whole sample pre-treatment, but does not reflect correct recoveries in the case of addition before extraction. This spiking approach is based on the establishment of equilibrium in the matrix interactions between the added internal standard native analyte. As long as equilibrium is not established, differences in the extraction procedure might have a great influence on recoveries (Thompson *et al*., 1999).

The paramount importance is if the knowledge of the exact recovery achieved for an analyte when applying a certain analytical method; this is known to affect the accuracy of the results (Eurachem, 1998). Therefore, reference materials are normally accepted for comparison.

2.3 HAZARD IDENTIFICATION

In biological targets, acrylamide and glycinamide with nucleophilic centres (i.e., unshared electrons) acts as the basis for acrylamide interactions with other compounds in a chemical reaction. This is highly correlated with the level of electron inadequacy. Electrophiles and nucleophiles during the reaction are usually identified as delicate or hard in respect to either low or high-charged densities in a spectral for reactivity (Pearson & Songstad, 1967).

Glycidamide (a genotoxic carcinogen in the liver of neonatal mice) is identified as hard electrophile which has high positive charge density, thus has the capability of reacting with hard nucleophiles for example pyrimidine and purines bases of DNA. (De Caprio and Lopachin, 2005) Also delicate-delicate bonds with marginal molecular orbital attributes suggest that the corresponding adduct target for acrylamide is the thiolate state of cysteine residues (Lopachin *et al*., 2007).

Experts from EFSA's Panel on Contaminants in the Food Chain (CONTAM) reconfirmed previous evaluations that acrylamide in food potentially increases the risk of developing cancer for consumers in all age groups (EFSA, 2015). Evidence from animal studies shows that acrylamide and its metabolite glycinamide are genotoxic and carcinogenic: they damage DNA and cause cancer. It has been shown from human studies that dietary exposure to acrylamide that causes cancer is currently limited and inconclusive.

2.3.1 Acrylamide content in foods/regulatory levels

In addition, the type of food as a source of variation, acrylamide content in foods vary according to processing factors, storage and handling of the same food (Vinci, 2012).

This is logical since food is a chemical repository with each food type, indeed each food cultivar, having a unique chemical composition, constituents of which may be differentially amenable to acrylamide formation. Foods with mainly carbohydrate content and some amount of amino acids tend to produce a high level of acrylamide compared to those with mainly protein and some carbohydrate when processed at elevated temperatures. Eight protein foods analyzed had 22–116 ppb acrylamide content whereas eight cereals had 47–266 ppb and seventeen potato chips had 117–2764 16 ppb (Burcham, 2014).

Apart from certain food category, acrylamide may vary according to cultivars or varieties of foods of the same species. Marchettini *et al*., (2013) found high amount of fructose and glucose in Rossa di Colfiorito and Kennebec cultivars of potato. The presence of free reducing sugars tends to increase the amount of acrylamide formation during food processing at elevated temperature. As a result, they found high levels of acrylamide in the former relative to the latter. To reemphasize, acrylamide content increases with increasing temperature of processing up to a certain level. This is consistent with the fact that generally the rate of a chemical reaction increases with increasing temperature. Bråthen (2005) found that a dry-processing temperature of 190 ^{o}C to 210 ^{o}C yields the highest acrylamide levels in starch foods and cereals. Another processing parameter key to acrylamide content in addition to temperature is time of processing. Below a certain temperature limit, time of heating increases the acrylamide production. Beyond that limit the, heating time reduces the level of acrylamide formed by elimination reaction, the nature of this reaction being unclear Bråthen (2005). Conditions under which foods are stored before processing were found to affect the acrylamide level during processing. For instance, acrylamide is

was found to be higher in stored potato since it is affected by the storage temperature (Marchettini *et al*., 2013)

2.4 MECHANISM OF ACRYLAMIDE FORMATION

Maillard reaction being a major mechanism for the formation of acrylamide The maillard reaction is responsible for giving colour and tasty flavour to food products. Production of foods with little or no levels of acrylamide without causing a change to the sensorial properties is a major challenge in the food industry (Zhang and Zhang, 2007).

Some of the methods used in mitigating the formation of acrylamide actually removes precursors of acrylamide from the product. Some of these precursors include glucose, fructose and asparagine. They can also be as a result of modifications in processes that are used in the inhibition or reduction in intensity of the reaction.

Acrylamide has not been found in unheated and boiled foods. It is therefore considered to be formed during heating at high temperatures. This fact has been attributed to the higher temperatures which have been reached during the maillard nonenzymatic browning reactions. This has been required for desirable colour, flavour and aroma production (Coughlin, 2003). It has been shown by Tareke et al., (2002) that acrylamide was formed by heating starch based foods above temperatures of 120^{o}C.

In addition it is believed that acrylamide is formed by a heat induced reaction between an amino acid called asparagine and a sugar (Rosen and Hellenas, 2002; Mottram *et al*., 2002; Stadler *et al*., 2002; Pedreschi *et al*., 2005). Work by Mottram *et al*., (2002) has showed that acrylamide could be formed in food components by heat treatment. This was a result of a reaction between amino acids and reducing sugars (Stadler *et al*., 2002).

The different fatty acid levels in cooking oils are likely to affect the acrylamide formation during processing. Studies have shown that high levels of the free amino acid asparagine and of reducing sugars in food processed at temperatures ranging between 120 and 230 degrees Celsius has the possibility of high acrylamide formation by milliard browning reaction (Harlfinger *et, al*., 2004).

2.4.1 Formation of acrylamide in foods during processing

The Swedish National Food Agency in 2002 after discovering high levels of acrylamide foods, it became of great interest and concern for researchers to discover acrylamide form non-industrial sources a public health concern. Foods which were fried or baked exhibited high concentrations of acrylamide. Subsequent researches revealed that carbohydrate rich foods which are processed at high temperatures greater than 120 ^{o}C (Tareke *et al*., 2002, 2000) exhibited a concern level of acrylamide. Here it was observed that a non- enzymatic browning reaction (Maillard reaction) which occurs during high heating of food during processing attributed to the high levels of acrylamide in foods (Mottram *et al*., 2002; Stadler *et al*., 2002). According to Roach *et al*. (2003), the browned crispy crusts in foods which are either baked or fried tend to have the highest levels of acrylamide. Also at temperatures between 98 and 116 degrees celcius processed foods in moist environments such as canned black olives and purine juice exhibited high levels of acrylamide. Thus, an observation that the formation of acrylamide in foods in respect to crispiness and temperatures greater than 120 $^{\circ}$C as reported by JIFSAN in 2004 indicates that there are varied pathways for its formation.

In the USA, the overall estimated daily intake of acrylamide from diet as reported by the US FDA in 2006 is 0.4 μg/kg-day with a 90^{th} percentile of 0.95 μg/kg-day. However, the estimated daily intake per the US FDA in other global population is invariably similar with the difference resulting from cultural differences in food preferences.

2.4.2 Foods acrylamide determination has been conducted on

A survey of substrates on which acrylamide studies have been conducted are presented. They have been categorized into purely potato-based, non-potato based and model systems. From this work, potato *Solanum tuberosum L*. is the most studied food crop with reference to acrylamide. Acrylamide studies has been conducted on biologically cultivated potato (Marchettini *et al.,* 2013), potato crisps (Knol*et al.,* 2009; Kita *et al.,* 2007; Senyuva *et al.,* 2006), French fries (Pedreschi *et al.,* 2007; Sanny *et al.,* 2012, fried potato strips (Zeng *et al.,* 2009), potato chips and French fries (Gökmen et al., 2007). Acrylamide studies have also been conducted on potato matrices in combination with other products. For example, potato chips and biscuits (Anese *et al.,* 2010), potato chips along with biscuits and noodles (Zhu *et al.,* 2008). Even organically produced potato powder and conventionally cultivated potato powder have also been analyzed for acrylamide after heat processing (Carillo *et al.,* 2012). Similarly, studies have also been conducted on chestnut-based foods (Karasek *et al.,* 2009), cereal-based foods (Senyuva *et al.,* 2006), roasted coffee (Bortolomeazzi *et al.,* 2012), cookies from dough (Gökmen *et al.,* 2007), Starch and cereals (Bråthen *et al.,* 2005), sweet potatoes and plantain (Quayson *et al.,* 2007).

In all these studies, the trend could be observed that these studies were conducted in countries where the food products were commercially important, patronage and consumption was high and processing was essentially heat-based.

Laboratory models systems have also been simulated and acrylamide investigations done on them. For instance, fructose and asparagine models (Knol *et al.,* 2010) as well as asparagine cum glucose models (De Vleeschouwer *et al.,* 2008) have been studied. Although not limited to it, these enquiries were done largely to elucidate the mechanism of acrylamide chemistry in foods and food processing.

Note would have to be made here that, additionally (EFSA 2012) has been monitoring acrylamide in ten main food groups including French fries, potato chips, pre-cooked potato chips, soft bread, breakfast cereals, biscuits and cookies, coffee and its substitutes, baby foods and other products since 2007. This is a more comprehensive effort in terms of matrices of acrylamide exposure study. There are limited studies on such African staples as cassava, groundnut, fish, cocoyam, yam and plantain thermally processed in the African context.

2.5 ABSORPTION OF ACRYLAMIDE

2.5.1 Haemoglobin Abducts as Acrylamide biomarker

Investigations by Fennell *et al*., (2005), using 24 volunteer adult males, a toxicokinetic study of the level of haemoglobin adduct levels were estimated over 120 days of continuous exposure to acrylamide based on assumptions of 1.26 μg/ kg per day exposure to acrylamide. Haemoglobin adducts levels of acrylamide observed in workers who were exposed through inhalation and demal exposure exhibited qualitative evidence of these routes of absorption. Acording to Hagmar *et al*., (2001)

and Bergmark *et al*., (1993), dermal exposure was the predominate route of absorption of the workers used in the study. Bergmark *et al*., (1993) again observed exposure to acrylamide in some chines workers who were involved in acrylamide and poly acrylamide production for a period of 0.1-8 years. Here the N-terminal valine residues in haemoglobin adduct levels were measured at an exposed level of 0.3 to 34 nmol acrylamide/g haemoglobin. An average level of 7.3 nano-mole per gram haemoglobin (n value of 12 polymerization) and 14.7 nmol/g haemoglobin (n = 14) were found in workers involved in an air space of 1.52 and 0.73 mg/m3 for work places complicated in the polymerization and amalgamation reactions respectively for acrylamide.

The authors observed the inconsistency levels of haemoglobin adduct levels during inhalation and hypothesised dermal exposure was the predominant exposure route in the workers used for the study (Bergmark *et al*., 1993). In a group of 210 tunnel construction works who were being exposed to chemical grouting agent containing acrylamide and N-methyl-acrylamide, the haemoglobin adduct levels were measured over a period of 2 months without personnel protective devices were determined (Hagmar *et al*., 2001).

At the first month of exposure, blood samples were drawn after the day's constructional activity and analysed for N-terminal valine adducts levels. From previous assumptions by researches workers were expected to have varying extent of both dermal exposure and inhalation. At a limited quantitative exposure data of two personal air samples concentrations of 0.27 and 0.34 mg/m^3 for the sum of acrylamide and N- methyl-acrylamide was obtained. Also at a mixture of equal proportions of the reactants, haemoglobin adduct concentration varied at about 18non smoking control

subject varying between 0.02-0.07 nanomole per gram globin. Out of the 210 passage works, 47, 89, 36 and 38 had their adduct levels as <0.08 nmol/g globin, 0.08–0.29 nmol/g globin 0.3–1.0 nmol/g globin, and1.0–17.7 nmol/g globin individually. Blood tests gathered at interims up to 5 months after suspension of presentation from five laborers with starting levels running from 2.2 to 4.4 nanomole per gram. The levels diminished inside 120 days to the foundation level.

2.5.2 Human oral / dermal exposure

Fennell *et al.* (2005) in respect to the Code of Federal Regulations guiding the safety of humans, 24 Caucasian aspemic male adult and had not used tobacco for a period of 6 months with weights between 71 and 101 kg and ranging between the ages of 26 and 68 years had their haemoglobin adduct formation levels evaluated following oral and dermal administration of acrylamide.

The study used radio labelled acrylamide. A low dose study protocol of 3mg/kg radio labelled acrylamide by gavage was administered in rats before human exposure in the study. A continuous monitoring of acrylamide delivered via oral routes in water solution of 0.5, 1.0, and 3.0 at 3 mg per kg body weight under controlled conditions at 3 times daily. Within 24 h of administering about thirty four percent delivered dosages was recouped in the urine. This is a representation a lower range on aggregate assimilation from the oral path. The results observed by Fennell *et al*., (2005) for dermal exposure indicated lower levels of acrylamide and glycinamide –haemoglobin terminal adduct levels. This comparably had 6.6 percent of doses delivered via the dermal route to a 100 percent oral absorption.

2.6 METABOLISM OF ACRYLAMIDE

2.6.1 Human metabolism

The majority of urinary metabolites observed from the 24 adult male volunteers in a study, the excreted N-acetyl-S-(3-amino – 3-oxopropyl) cysteine S-oxide from glutathione conjugation. Fennell *et al*., (2005). In addition whilst oral administration showed a linear dose response for acrylamide and glycinamide – haemoglobin terminal adduct levels, low levels of N-acetyl-S-(3-amino-2-hydroxy-3-oxopropyl) cysteine were additionally recognized in urine of participants. Acrylamide is metabolized is through epoxidation using cytochrome P450 (CYP450) pathway to form three possible pathways with glycinamide preceding: formation glycinamide formation is a phase 1 metabolite which is catalyzed by epoxide hydrolase, formation N-acetyl-S-(2-hydroxy-2-carbamoylethyl) cysteine or GAMA and/or with glycinamide left metabolized. Thus, the un-metabolized glycinamide, The latter is assumed to be the cause of acrylamide's genotoxicity (Shibamoto and Bjeldanes, 2009) even as both glycinamide and acrylamide can and do form adducts with DNA and as such prime suspects of mutagenicity and possible carcinogenicity. It must be qualified that latter research indicates this rate is low. On ingestion acrylamide trips to the stomach and sent into liver where hepatocytes metabolize acrylamide through either the dermal or oral routes.

Another work by Sumner *et al*., (2003) concludes that the main metabolic pathway for urinary metabolites in humans was through a conjugation of glutathione forming N-acetyl-S-(3-amino-3-oxopropyl) cysteine thus the similarities and differences in the pathways of rat and mice which occurs through conjugation of glutathione and its S-oxide respectively

2.7 TOXICITY AND HEALTH EFFECT OF ACRYLAMIDE IN FOODS.

Processing methods and its factors such as cooking time, processing temperature and variety affect acrylamide formation in foods. In the developed countries reach institution have put many efforts into determination of the toxicant acrylamide in products such as potatoes and coffee whist same cannot be said for our Ghanaian staples. As result, not much is known between the food toxicant such as acrylamide and the rising levels of non-communicable disease such as cancer in our parts of the world. However quiet a number of researches on the effect of acrylamide consumption in our foods is ongoing.

Analytical and mechanistic studies have revealed a clear mechanism by which acrylamide formation occurs in carbohydrate rich foods processed under high temperatures by non-enzymatic browning (Maillard Reaction) this browning reaction mostly affect the organoleptic properties of the food. According to Hitherto acrylamide studies generally used deterministic models which limit the application of results to real populations, as such their evident need of stochastic methods that would enhance the level of accuracy of acrylamide studies to real populations. Whereas respondent sampling methods were clearly reported especially for cohort studies, acrylamide studies generally did not report matrix sampling method, this may have a telling effect on the reproducibility and accuracy of acrylamide studies and so a clear method of matrix sampling needs research into especially for national and regional monitoring of acrylamide. Acrylamide analytical quantitation methods including HPLC–MS/MS, GC-MS and spectrophotometry are standard, although other validated methods like HPLC-DAD are used.

2.7.1 Food exposure and Risk of Acrylamide to Health

The direct exposure of acrylamide to consumers may result from the ingestion of foods high in carbohydrates. Other indirect means of exposure can be as a result of residual traces of the monomer which is present in food packages. In most of these products polyacrylamide is used as binding agent. (Samuelson 2003; Zhang et al., 2005).

Assessing the presence of acrylamide is a major concern in many countries. Some of the results obtained have shown that products such as potato account for 50% of the exposure to acrylamide. Baking products and bread also account for about 20% of human exposure to acrylamide. Acrylamide formation has been through heating or drying in the oven or frying (Eriksson, 2005; Torqvist, 2005).

Acrylamide is known to be genotoxic. It increases the incidence of cancer in rats. These have been related to doses of 1 – 2 mg/kg body weight per day. It has been reported that the maximum intake of acrylamide in foods is 0.05 mg/kg body weight per day. This level has however been challenged. There has been urgent outcry to reduce this level in foods. The heatox project in 2003 was started to investigate the risk of acrylamide to human health. The following risks were identified by the project. The evidence of acrylamide posing as a cancer risk has been strengthened. With the reduction of acrylamide levels in bread and potato in the laboratory, there is the potential of reducing human exposure. Acrylamide was found to be not the only genotoxic compound formed when food is heated. In total, close to 50 compounds have been highlighted as potential carcinogens (HEATOX project, 2003).

2.8 STOCHASTIC AND DETERMINISTIC ACRYLAMIDE RISK STUDIES

Murniece *et al.,* (2013) assessed the risk of acrylamide toxicity among consumers of fried potato in Latvia. Like many other studies in this area, the assessment fell short of stochastic standard, citing the mean as a representative point data along with the confidence interval and/or standard deviation. This deterministic approach has serious shortfalls, including limits to it extrapolability using only a handful of discontinuous data, data that is severely limited relative to the actual total population, limits to its representativeness of the distribution of acrylamide in the population it not capturing the probability of acrylamide toxicity, limits to it accuracy in it curtailing the possibility of acrylamide toxicity of every member of the population to a single data point and so ignoring variability, including vulnerable and resistant sections, and limits to its certainty in not accounting for the glaringly high level of uncertainty inherent. In short deterministic approaches to risk analysis although beneficial provide nothing more than a point data within a range of spread or error margin. Popular of deterministic methods among food scientists include hazard quotient (HQ) which is an index of the exposure denominated by the reference dose, hazard index (HI) which is an aggregate of the HQ of individual chemo-hazards.

Correlational risk studies have also been done using odds ratio (Wilson *et al.,* 2009; Larsson *et al.,* 2009) and hazard ratio (Garcia *et al.,* 2015; Burley *et al.,* 2010; Schouten *et al.,* 2009; Obon-Santacana *et al.,* 2013) as standard methods in clinical trials or epidemiological cohort studies. These methods having almost gain standard status in medical research are all point data, that is they each essentially consist of an aggregate value representing the sample, and although they have unique advantages including being easy to calculate, flexible, highly used and appreciated by the medical profession (Spruance *et al.,* 2004), they are nonetheless deterministic, they are froth

with the limitations that comes with deterministic approaches to risk estimates itemized previously. As a result, stochastic methods are being used to enhance conventional toxico-medical models dietary hazard exposure (Huybrechts *et al.*, 2011). In addition acrylamide spectral analyses is also being used but to a very limited extent, despite its immense advantages including utility and versatility (Wang *et al.*, 2010).

A stochastic approach using Monte Carlo simulation for example works by iteratively generating output distributions and their corresponding probabilities from a corresponding distribution of the sample, and in this manner create a more realistic representation of the model, capture the uncertainties across the sample population. In addition to the likelihood of each outcome, a Monte Carlo simulation spawns out a probabilistic outcome graphs. Also Monte Carlo outputs include probabilistic correlational, sensitivity, optimization and scenario analyses. Furthermore, the built-in iterations enable a more realistic extrapolation of results of studies to the general population.

2.9 ACRYLAMIDE RESEARCH IN DEVELOPING COUNTRIES

Despite the current enormous research output on acrylamide in the world, there have been little inquiries on acrylamide in Ghana and Africa generally. Quayson and Ayernor (2007) contend that the major research related to acrylamide was focused on cocoa, a cash crop and a major foreign exchange earner, despite the fact that Ghanaian staples are starch-based and prepared under high temperatures in dry cooking, such foods being highly susceptible to high acrylamide content. In the light of the obvious high exposure rate among the citizenry, the risk of acrylamide toxicity may be an issue worth investigating.

Acrylamide concentration is high in potato food products because potato is rich in acrylamide precursors: mainly carbohydrate and naturally-occurring asparagine. Also the principal use of potatoes requires processing at elevated temperatures. Thus, creating a chemical system in which acrylamide is produced principally by the Maillard reaction. French fries and potato chips being main food products from potatoes are categorized as ‘junk’ among the convenience foods served by food joints. As a result, due to their generally high patronage, potato-based products have high exposure and consumption rate occasioning high level of research focus, inquiry and activity regarding acrylamide risk.

In some countries, however coffee with a similar acrylamide profile as potato but relatively less acrylamide concentration, is ranked highest as the most important in terms of acrylamide dietary exposure, principally because it is having a high exposure frequency, being consumed more often in a day than potato.

CHAPTER THREE

MATERIAL AND METHODS

3.0 MATERIALS

The freshly refined oil (crude palm oil, groundnut oil and refined palm oil) samples was obtained from four selected Markets (Ayigya Market, Kumasi Central Market, Bantama Market and Ayeduase Market) in Kumasi and stored in plastic bags. They were stored in a refrigerator prior to analysis.

All the chemicals were purchased from Sigma Aldrich Chemicals (Germany). Homogenizer, shaker, vortex, centrifuge was purchased from Gen Lab, UK, IKA®, Germany, Gallenkemp Orbital Shaker, UK, VortexGene, Holland, Eppendoff, USA respectively.

3.1 METHODS

3.1.1 Study population and selection

A total number of 100 oils samples were purchased from retailers of cooking oil within the Kumasi metropolis over a period of three weeks. A total of 50 samples of used oils were also obtained from identified food vendors within the study area. The Retailers were randomly selected. A total of three hundred questionnaires were distributed for the study. Rational for the study was explained to the participants, after giving their consent, participants were selected to answer the questionnaire set for the study.

3.1.2 Dietary and lifestyle questionnaires

Participants were made to complete a 24-h self-administered food frequency questionnaire. First, the content of the questionnaire was explained to the participants. The consumers were made to give information on the type of cooking oils used in the

preparation of dishes. They were again asked as to whether it was used for commercial or domestic food preparation. The various types of oils were purchased from retailers in the various markets. 330 ml of each oil costing GH₵ 1.50 were bought. The participants were required to identify how much oil and the type of oil used in their food preparation in a week and how often they consume the foods prepared with oil. The questionnaire also required participants' weight to be taken. The questionnaire was pre-tested before the study to ensure all parameters needed to be addressed was made available.

3.2 DETERMINATION OF ACRYLAMIDE

3.2.1 Extraction and Clean-up

Extraction and clean-up was based on QuEChERS as proposed by UCT, (2009) with modifications. The sample (1g) was weighed into a 50 ml centrifuge tube, 5 ml of hexane added and vortexed for 1min. Subsequently 10 ml each of distilled water and acetonitrile was added and shaken vigorously for 1 min. The resulting solution was centrifuged for 5 min at 3000 rpm. To 1 ml of acetonitrile layer, 150 mg $MgSO_4$ (Sigma Aldrich, Germany) was added and mixed for 30 s. The tube was shaken at 400 rpm for 5 min and supernatant used for spectrophotometric reading.

3.2.2 HPLC

Standard of acrylamide (0.0, 0.1, 0.2 and 0.3 mg/ml) were prepared from a stock solution of 10mg/ml. The absorbance of calibration standards was measured using a Thermo Scientific Nandrop spectrophotometer at 220 nm. A calibration curve was established by plotting absorbance against the corresponding concentration. The concentration of samples was calculated by plugging the corresponding absorbance into the equation of the line. Determinations were done in triplicates.

3.3 STATISTICAL METHOD

The data was tabulated in Microsoft Excel and analyzed using Palisade @Risk software to simulate acrylamide risk in the oils using the Monte Carlo simulation. The data was entered in excel spreadsheet columns as acrylamide content, Number of times of consumption of oil per day, volume of oil used per day (M), Ingestion rate (IR), exposure frequency and exposure duration (EF and ED) and body weight (BW). Each column of data was fitted to an appropriate or best fit distribution using the @Risk distribution fitting function specifying whether it is discrete or continuous and writing the aggregate output to a designated cell.

Each value so generated was added to a unit excel cell using the "add output" tool and simulated at first order and ten thousand iterations. A construct of the empirical model was built using Excel formulas into which the model parameters were referenced to determine the risk using the potency factor value. The risk value so generated to cell was added to output and simulated at first order and ten thousand iterations. Excel reports, including graphs, of the outputs were then extracted as results for discussion.

3.4 RISK CALCULATION

Chronic Daily Intake (CDI) was computed using the equation 1 (Hans *et al*, 2003).

$$\mathbf{CDI = \frac{C \times CR \times EFD}{BW \times AT}} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots.. (\mathbf{1})$$

Where; C = *concentration of Acrylamide (mg/g),* CR = *contact rate (mL/day),* *EF = exposure frequency* (days/year), ED= *exposure duration (years), BW = body weight (kg), AT = average time of exposure* (days).

A reference dose (RfD) of 0.002 mg/kg (USEPA, 2000b) was used for this work. Thus, it is assumed that 0.002 mg/kg body weight of acrylamide may be consumed in a lifetime without any adverse health effects. The risk was finally calculated using the formula;

$$\textbf{Hazard Quotient (HQ)} = \frac{CDI}{RfD} \quad \text{.....(2)}$$

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 POPULATION DESCRIPTION

4.1.1 Gender and oil preference

The sample population was asked to identify the oil type with which they consume. In all 192 respondents participated. Results from the study show that majority of the respondents consumed "refined palm oil". They accounted for about 64% of the sample. A little over 35% of the respondents consumed crude palm oil while 0.005% of the respondents were associated with the consumption of groundnut oil. On a gender basis females were seen to consume mainly refined palm oil followed by palm oil. The same trend is also seen by the male counterparts. None of the females however was linked to the consumption of groundnut oil. Out of the 192 participants sampled for the consumption of oils, 14% were males whiles females accounted for 86%. The ratio of consumption of oil from female to male is 6:1. This is also an indication that females have a higher preference for oil when compared to males (Table 4.1).

Table 4.1: Gender and Oil Preference

	TYPE OF OIL			
Gender	Crude Palm oil	Ground nut oil	Refined palm oil	Total
Male	11	1	15	**27 (14%)**
Female	57	0	108	**165 (86%)**
Total	**68 (35%)**	**1 (0.005%)**	**123 (64%)**	**92**

4.1.2 Age and oil preference

The age of respondents in relation to the type of oil consumed was also analysed. The study looked at the ages between 18 and 60 years. Consumption of refined palm oil was found to be highest (65.7%) in all age groups. This was followed by crude palm oil (33.7) and groundnut oil (0.5%) (Table 4.2).

Table 4.2 Age and Oil preference

	TYPE OF OIL			
Age	Crude Palm oil	Groundnut oil	Refined palm oil	**Total**
18-28	9	0	45	**54 (30.3%)**
29-39	22	0	41	**63 (35.4%)**
40-60	29	1	31	**61 (34.3%)**
Total	**60 (33.7%)**	**1 (0.005%)**	**117 (65.7%)**	**178**

Generally, 25.2% of the respondents aged between 18 – 28 years were found to consume refined palm oil. In the same age group only 5% consumed palm oil. This confirms that respondents in the age group of 18 – 28 years have high preference for refined palm oil when compared to palm oil. None of the respondents in this age group however consumed groundnut oil. The same trend follows for respondents between the ages of 29 – 39 years. For age groups between 40 to 60 years however some of the respondents consumed groundnut oil. The study concludes that 17% of the total respondents preferred refined palm oil, 16% preferred crude palm oil while 0.5% preferred groundnut oil. That is groundnut oil consumption is seen in the age group between 40 to 60 years.

4.1.3 Occupation and oil preference

The occupation of the respondents was also identified. Out of the sampled participants 37.6% were students, 58.0% were works and 3.4 % were found to be unemployed. In all three occupations results from table 5 indicates that about 59% of workers consume all three types of oil. Students who consume all three types of oils also accounted for 37.5% of the respondents. The unemployed made up 3.4% for the consumption of oil.

Table 4.3: Occupation and Oil Preference

	TYPE OF OIL			
Occupation	Crude palm oil	Groundnut oil	Refined palm oil	**Total**
Student	11	0	54	65 (37.6%)
Worker	34	1	68	102 (58.9%)
Unemployed	2	0	4	6 (3.4%)
Total	**60 (34.7%)**	**1 (0.005%)**	**91 (52.6%)**	**173**

For crude palm oil consumption, workers were found to consume higher proportions. They accounted for 56.6% of the crude palm oil population. This was followed by students with a percentage of 18.3. The least respondents to consume crude palm oil were found to be unemployed; they made up 3.3% of the crude palm oil consumption population. Workers again dominated the consumption of refined palm oil. They accounted for about 74.7% of the respondents. Students also made up 59.3% of the refined palm oil consumers while the unemployed accounted for 4.3% of the respondents. Generally, only 0.5% of the entire population consumed groundnut oil were found in the worker's category.

4.1.5 Distribution of Data Gathered

Data gathered from the field of study were characterized using statistical probabilistic distributions. As shown on Table 4.4. Acrylamide concentration of Groundnut oil follows the triangular distribution whilst that of Crude palm oil and refined palm oil had exponential distribution using the dietary recall food survey. Both Exposure frequency and Exposure duration followed a uniform distribution. Body weight and Consumption Rate follows Poisson and Log logistic distribution respectively.

Table 4.4. Model parameters and primary and secondary data sources used for the estimation of neurotoxicity risk

Risk parameters	Data sources and values	Fitted Distribution/references
Hazard concentration(HC) Groundnut oil Crude Palm oil Refined palm oil	Dietary Recall Food Survey	Triangular (0.27996, 1.12738) Exponential (0.15072,0.22421) Exponential (0.1725, -0.0000541)
Consumption rate(CR)	Dietary Recall Food Survey	Log logistic (-113.30, 121.161,12868)
Exposure frequency	365 days per year	Uniform (1,365)
Exposure duration	5 years	Uniform (1,5)
Body weight	Dietary Recall Food Survey	Poisson (61.667)
Averaging time(AT) for carcinogenicity	365 x Exposure duration/days	
Reference dose(RfD)	0.00002 mg/g-day oral acrylamide slope factor for water)	

4.2 ACRYLAMIDE CONCENTRATION

The mean concentration of exposure to acrylamide contaminant in the oils (groundnut, crude palm oil and refined palm oils) decreases respectively (0.1720, 0.2319 and 0.084 mg/g). The concentrations of acrylamide in all oils decrease at 5%, 50% and 95% groundnut, crude palm oil and “refined palm oils” Table 4.5.

The least recorded acrylamide concentration in “refined palm oil” was found to be (0.0083 at the 5th percentile. The value is found to be higher than the 0.001mg/g acrylamide levels in foods as recoded by (EFSA 2012). And again, the acrylamide concentration was all exceeding maximum value of 0.004533 mg/g recommended by EFSA (2012). Groundnut oil recorded 0.4693 and Crude palm oil recording 0.2319 at the fifth percentile.

Recent studies have shown that acrylamide is formed in foods with a high content of the free amino acid and reducing sugar of asparagine. Taubert *et al*., 2003 and Harlfinger *et al.,* 2007 in addition discovered that when these conditions are met under high temperature. It is also known that asparagine is readily found in some plant based foods such as legumes, nuts and seeds. Longer cooking or processing times at elevated temperature affect acrylamide formation in foods. Thus the high concentration levels of acrylamide in groundnut oil and least in refined palm oil.

Table 4.5: Sample size (N), mean, mode, 5 %, 50 % and 95 % percentile values of acrylamide in mg/g of foods sampled in the study and iterated 10000 in a Monte Carlo simulation.

Matrix	N	Mean	Mode	5% Percentile	50% Percentile	95% Percentile
Groundnut oil	28	0.8449	1.1252	0.4693	0.8791	1.1231
Palm oil	15	0.2319	0.2250	0.2319	0.3623	0.6755
Refined palm oil	19	0.1720	0.0003	0.0083	0.1191	0.5161

4.3 CHRONIC DAILY INTAKE (CDI)

The mean daily intake of acrylamide of acrylamide per body mass per day (mg/kg/day) is recorded as 0.8449, 0.0906 and 0.0431 for groundnut oil, crude palm oil and "refined palm oil" respectively. In Table 4.6 the chronic daily intake at the 5th percentile of exposure to acrylamide recorded 0.003, 0.0006 and 7.21005E-05 respectively for groundnut, palm and refined palm oils. In addition at the 95th percentile were 0.4844 for groundnut oil, and 0.208 for palm oil. (Table 4.6)

The dietary intake of acrylamide recorded for groundnut oil in this study is far higher than that estimated by FAO/WHO (0.3-0.8 mg /kg/day) for the general population (Petersen, 2002). Acrylamide, a genotoxic (mutagenic) contaminant increases the incidence of cancer in rats at doses of 1–2 mg/kg bw/day. The maximum acrylamide intake in the oils is recoded as (0.4844mg/kg/day) for groundnut oil is far higher than the maximum acrylamide intake from EU foods (0.05 mg/kg/day) which even called for urgent action to minimize the level of acrylamide in foods.

Table 4.6: Chronic daily intake

CDI (mg/kg/day)	Mean	Mode	5% Percentile	50% Percentile	95% Percentile
Groundnut oil	0.8449	0.0007	0.003	0.0272	0.4844
Crude palm oil	0.0906	0.001	0.0006	0.011	0.208
Refined palm oil	0.0431	1.64736E-05	7.21005E-05	0.0034	0.0885

4.4 RISK OF CONSUMPTION OF GROUNDNUT OIL, CRUDE PALM OIL AND "REFINED PALM OIL"

Hazard Quotient (HQ) is the ratio of the potential exposure (Chronic Daily Intake) to a substance and the level at which no adverse effects are expected (Reference Dose). From the study the mean recorded HQ values were 0.0538, 0.0144 and 0.0142 respectively for groundnut, palm and refined palm oils (Table 4.7). At the 50^{th} percentile exposure to acrylamide, groundnut oil recoded the highest value of 0.0.00195. The least value recorded was for "refined palm oil" which is 0.00063. Crude palm oil recorded 0.00167. From the results, it can be observed that the HQ values for all the oils were less than 1. A ratio larger than unity suggests that the concentration of the chemical is high enough to cause adverse health effect

Table 4.7: Sample size (N), mean, mode, 5 %, 50 % and 95 % percentile values of acrylamide in mg/g of foods sampled in the study and iterated 10000 in a Monte Carlo simulation.

Matrix (HQ)	Mean Hazard Quotient	5% Percentile	50% Percentile	95% Percentile
Groundnut oil	0.0538	0.00011	0.00195	0.0338
Palm oil	0.0144	9.769E-005	0.00167	0.0294
Refined palm oil	0.005	9.902E-006	0.00063	0.0142

.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.0 CONCLUSION

The risk associated however varies with the different type of oil used in the study. The study concluded that the acrylamide concentration in the sampled oils ranges from 0.172 to 0.844 mg/g. Groundnut oil had the highest concentration of acrylamide, this was followed by crude palm oil and refined palm oil. Although the hazard quotient for all the oils was found to be less than one, consumption of groundnut oil presents the highest risk compared to crude palm oil and then refined palm oil. The population is therefore at risk of ingesting acrylamide.

5.1 RECOMMENDATION

It is recommended that further work be done to identify the processing methods in which our local manufactures go through during processing of our local oils. It also recommended that regulatory bodies in the country educate manufactures on the need to adhere to Good Manufacturing Practices during processing to prevent contamination during processing.

Finally, the risk results of this study call for the concern of government and public health officials to put in measures on how to reduce the risk of consumers of cooking locally produced oils.

REFERENCES

Adjei-Nsiah.S, Zu, A.K.S. and Nimoh F (2012) Technological and Financial Assessment of small scale palm oil production in Kwaebibrem District, Ghana. *Journal of Agricultural Science*, 4, (7) 111-119.

Agyei-Mensah.**S** and de-Graft Aikins.,A (2010) Epidemiological transition and the double burden of disease in Accra, Ghana. *Journal of Urban Health*: Bulletin of the New York Academy of Medicine, 87, (5) 11-13.

American Institute for Cancer Research (2007). *Food, Nutrition, Physical Activity and the Prevention of Cancer*: *a Global Perspective*: 4,119-140.

Barber D.S; Hunt, J.R. ; Ehrich, M.F; (2001) Metabolism, toxicokinetics and haemoglobin adduct formation in rats following subacute and subchronic acrylamide dosing. Neuro Toxicology 22: 341–353.

Bergmark, E**.** (1997) Haemoglobin adducts of acrylamide and acrylonitrile in laboratory workers, smokers and non-smokers. Chemical Research Toxicity l10(1):78–84.

Bergmark, E; Calleman .C.J.; Costa L.G. (1991) Formation of haemoglobin adducts of acrylamide and its epoxide metabolite glycidamide in the rat. Toxicology and Applied Pharmacology 111:352–363.

Bergmark, E; Calleman, C.J; He, F. (1993) Determination of haemoglobin adducts in humans occupationally exposed to acrylamide. Toxicology and Applied Pharmacology 120:45–54

Bråthen, E., and Knutsen, S. H. (2005). Effect of temperature and time on the formation of acrylamide in starch-based and cereal model systems, flat breads and bread. *Food Chemistry, 92*(4), 693–700.

Bjellaas, T; Stolen, L.H; Haugen.,M . (2007) Urinary acrylamide metabolites as biomarkers for short-term dietary exposure to acrylamide. Food Chemical Toxicology. 45(6):1020-6.

Boettcher, M.I; Bolt, H.M; Angerer, J. (2006) Acrylamide exposure via the diet: influence of fasting on urinary mercapturic acid metabolite excretion in humans. *Archives of Toxicology.* 80(12):817-819

Boettcher, M.I; Schettgen, T; Kutting, B (2005) Mercapturic acids of acrylamide and glycidamide as biomarkers of the internal exposure to acrylamide in the general population. Mutation Research 580(1–2):167–76.

Burley, V. J., Greenwood, D. C., Hepworth, S. J., Fraser, L. K., de Kok, T. M., van Breda, S. G., …Cade, J. E. (2010). Dietary acrylamide intake and risk of breast cancer in the UK women's cohort. *British Journal of Cancer*, *103*(11), 1749–1754. http://doi.org/10.1038/sj.bjc.6605956

Calleman C.J. 1996. The metabolism and pharmacokinetics of acrylamide: Implications for mechanisms of toxicity and human risk estimation. Drug Metabolism Reviews 28:527-590

Calleman C.J; Wu, Y; Tian G. (1994) Relationships between biomarkers of Exposure and neurological effects in a group of workers exposed to acrylamide. Toxicology Applied Pharmacology 126:361–371.

Crofton K.M; Padilla,.S; Tilson, HA; et al. (1996) The impact of dose rate on the neurotoxicity of acrylamide: the interaction of administered dose, target tissue concentrations, tissue damage, and functional effects. Toxicology Applied Pharmacology 139:163–176.

Dai Q, Shu X, Jin F, Goo Y, Ruan Z and Zhang W (2001) Consumption of Animal Foods, Cooking Methods and Risk of Breast Cancer. Cancer epidemiology,Biomarkers and Prevention. American Association for Cancer Research http://m.cebp.aacrjournal.org/content/11/9/801. Accessed on 12th March, 2015.

Dearfield K.L; Douglas, G.R; Ehling,.U.H. (1995) Acrylamide: a review of its genotoxicity and an assessment of heritable genetic risk. Mutation Research 330:71–99.

Doerge D.R; Twaddle, N.C; Boettcher, M.I (2007) Urinary excretion of acrylamide and metabolites in Fischer 344 rats and B6C3F(1) mice administered a single dose of acrylamide. Toxicology Letters. 169(1):34-42.

Doerge D.R; Young, J.F; McDaniel, L.P. (2005) Toxicokinetics of acrylamide

and glycinamide in Fischer 344 rats. Toxicology Applied Pharmacology. 208, 199-209.

Donovan J.W; Pearson,T. (1987) Ingestion of acrylamide with severe encephalopathy, neurotoxicity and hepatotoxicity. Veterinary Human Toxicology 29:462.

Dourson M., Becker, R. a, Haber, L. T., Pottenger, L. H., Bredfeldt, T., and Fenner-Crisp, P. a. (2013). Advancing human health risk assessment: integrating recent advisory committee recommendations. *Critical Reviews in Toxicology*, *43*(6), 467–92. http://doi.org/10.3109/10408444.2013.807223

Dung C.H, Wu S.C. and Yen G.C. (2006) Genotoxicity and oxidative stress of the mutagenic compounds formed in fumes of heated soybean oil, sunflower oil and lard. *Toxicology In-Vitro,* 20: 439-447

Dybing E; Sanner, T. (2003). Risk assessment of acrylamide in foods. Toxicological Sciences 75:7–15.

EFSA. (2012). Scientific Report of EFSA, Update on acrylamide levels in food

from monitoring years 2007 to 2010. *EFSA Journal*, *10*(10), 1–38.

Elijah I.O, Cletus IE, Sylvester CI, Dorcas A.E (2014). Small-Scale Palm Oil Processing Business in Nigeria; A Feasibility Study: Greener Journal of Business and Management Studies, 4 (3): 070-082.

Eriksson. E., Acrylamide in food products: Identification, formation and analytical methodology. *Doctoral Thesis,* Stockholm University, 2005

Eurachem. (1998).Guide: The Fitness for Purpose of Analytical Methods — A Laboratory Guide to Method Validation and Related Topics, English edn 1.0 (EURACHEM, LGC (Teddington) Ltd: UK).

Fennell T. R**.**; Sumner S.C; Snyder R.W. (2005) Metabolism and haemoglobin adduct formation of acrylamide in humans. Toxicol Sci. 85(1):447–59.

Friedman, M. (2003): Chemistry, biochemistry and safety of acrylamide. A review Journal of Agriculture & Food Chemistry, 51: 4504-4526.

Fuhr, U; Boettcher**,** M.I; Kinzig-Schippers, M. (2006). Toxicokinetics of acrylamide in humans after ingestion of a defined dose in a test meal to improve risk assessment for acrylamide carcinogenicity. Cancer Epidemiol Biomarkers Prev. 15(2):266-71.

Garland, T.O; Patterson, MW. (1967) Six cases of acrylamide poisoning. Brit Med J (4):134–138.

Garcia, E., Hurley, S., Nelson, D. O., Hertz, A., and Reynolds, P. (2015). Hazardous air pollutants and breast cancer risk in California teachers : a cohort study, 1–14.

Gjerløff, T**;** Elsborg, H; Bonde, J.P. (2001) Svaerkroniskacrylamid-intoksikation

[Severe chronic acrylamide intoxication].UgeskrLaeg 163:4204–4205.

Gökmen, V., Açar, Ö. Ç., Köksel, H., and Acar, J. (2007). Effects of dough formula and baking conditions on acrylamide and hydroxymethylfurfural formation in cookies. *FoodChemistry*,*104*(3),1136–1142. http://doi.org/10.1016/j.foodchem.2007.01.008

Gunstone F.D**.** (2002) *Vegetable oils in food technology*. Composition, properties and uses. Blackwell publishing, CRC Press, LLC Boca Raton, FL. Ch. 1: 28.

Hagmar L; Törnqvist, M; Nordander, C; et al. (2001) Health effects of occupational exposure to acrylamide using haemoglobin adducts as biomarkers of internal dose. Scand J Work Environ Health 27(4):219–226.

Hagmar L; Wirfalt, E; Paulsson, B; et al. (2005) Differences in haemoglobin adduct levels of acrylamide in the general population with respect to dietary intake, smoking habits and gender. Mutation Research 580(1–2):157–65.

IARC (2010) International Agency for Research on Cancer, Monographs, High Temperature Frying, 95:392- 393.

Igisu, H; Matsuoka, M. (2002) Acrylamide encephalopathy. J Occup Health 44:63–68.

JIFSAN. (2002) Acrylamide in Food Workshop: Scientific Issues Uncertainties, And Research Strategies. Available from: http://www.jifsan.umd.edu/acrylamide2002.htm.

JIFSAN. (2004). Proceedings and Reports from the Joint Institute for Food Safety and Applied Nutrition (JIFSAN) 2004 Acrylamide in Food Workshop: Update - Scientific Issues, Uncertainties, and Research Strategies; April 13– 15, 2004; Chicago, Illinois. Available from: http://www.jifsan.umd.edu/acrylamide2004.htm.

Kadry, AM; Friedman, MA; Abdel-Rahman, MS. (1999) Pharmacokinetics of acrylamide after oral administration in male rats. Environmental Toxicological Pharmacology 7:127–133.

Larsson, S. C., Akesson, A., and Wolk, A. (2009). Long-term dietary acrylamide intake and risk of epithelial ovarian cancer in a prospective cohort of Swedish women. *Cancer Epidemiology, Biomarkers and Prevention : A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology*, *18*(3), 994–997. http://doi.org/10.1158/1055-9965.EPI-08-0868

LoPachin, R.M and De Caprio, AP. (2005) Protein adduct formation as a molecular mechanism in neurotoxicity. Tox.Sci. 86(2): 214-225.

LoPachin, R.M; Gavin, T; Geohagen, B.C; Das, S. (2007) Neurotoxic mechanisms of electrophilic type-2 alkenes: soft interactions described by quantum mechanical parameters. Toxicology Science. 98(2):561-70.

Mahan L.K, Escott-Stump S and Raymond J.L (2012) *Krause's Food and Nutrition Care Process*, Elsevier,St.Louis Missouri 13th edition, Chp.3:.40,Chp.37:835-393.

Michels, K.B; Rosner, B.A; Chumlea, W.C. (2006) Preschool diet and adult risk of breast cancer.Int J Cancer 118(3):749–54.

Miller, M.J.; Carter, D.E; Sipes, IG.(1982) Pharmacokinetics of acrylamide in Fischer-334 rats.ToxicolApplPharmacol 63:36–44.

Mottram D.S; Wedzicha, B.L; Dodson, AT. (2002) Acrylamide is formed in the Maillard reaction. Nature 19:448–449.

Mulloy, K.B. (1996) Two case reports of neurological disease in coal mine preparation plant workers. Am J Ind Med 30:56–61.

Murniece, I., Karklina, D., Galoburda, R., and Santare, D. (2013). Risk Assessment of Acrylamide Intake from Deep-Fat Fried Potatoes in Latvia. International Journal of Bioscience, Biochemistry and Bioinformatics, 3(4), 318–321. http://doi.org/10.7763/IJBBB.2013.V3.222

Nelson W.G, DeMarzo AM and Issacs WB (2003) Prostate Cancer: Mechanisms of Disease.*The New England journal of Medicine*; 349:366-381.

NTP/CERHR (2004) National Toxicology Program / Center for the Evaluation of Risks to Human Reproduction Expert Panel Report on Reproductive and Developmental Toxicity of Acrylamide. http://cerhr.niehs.nih.gov/.

Obón-Santacana, M., Slimani, N., Lujan-Barroso, L., Travier, N., Hallmans, G., Freisling, H., and Duell, E. J. (2013). Dietary intake of acrylamide and pancreatic cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. *Annals of Oncology*, *24*(10), 2645–2651. http://doi.org/10.1093/annonc/mdt255

OECD-FAO (2011) *Agricultural Outlook 2011-2020*, Chapter 5, Oil seeds and Oil seed products: 108.

P., Lalljie, S., Lingnert, H., Lindblom, M. and Matissek, R. (2004). A review of acrylamide: An industry perspective on research, analysis, formation, and control. *Critical Reviews in Food Science and Nutrition*, 44, 323–347.

Poku, K. (2002). Small-Scale Palm Oil Processing in Africa.Rome, Italy: Agriculture Services Bulletin 148.Food and Agricultural Organization of the United Nations.

Roach, J.A; Andrzejewski, D; Gay, M.L (2003). Rugged LC–MS/MS survey analysis for acrylamide in foods. J Agricultural Food Chemistry 51:7547–7554.

Rosen J, Hellenas K.E. (2002). Analysis of acrylamide in cooked foods by liquid chromatography tandem mass spectrometry analyst, 127: 880-882.

Rosillo-Calle F, Pelkmans L and Walter A (2009) *A global overview of vegetable oils with reference to Biodiesel.* IEA Bioenergy Task 40 report, 3-19.

Schaller, U. (2003). Experiences with acrylamide determination review from a retailer's laboratory. Presentation at the workshop Analytical Methods for Acrylamide Determination in Food, Oud-Turnhout, Belgium

Schouten, L. J., Hogervorst, J. G. F., Konings, E. J. M., Goldbohm, R. A., and Van Den Brandt, P. a. (2009). Dietary acrylamide intake and the risk of head-neck and thyroid cancers: results from the Netherlands cohort study. *American Journal of Epidemiology*, *170*(7), 873–884. http://doi.org/10.1093/aje/kwp213

Shibamoto, T., and Bjeldanes, L. (2009). *Introduction to food toxicology*. Amsterdam: Academic Press.

Spruance, S. L., Reid, J. E., Grace, M., and Samore, M. (2004). Hazard Ratio in Clinical Trials MINIREVIEW Hazard Ratio in Clinical Trials. *Antimicrobial Agents and Chemotherapy*, *48*(8), 2787–2792. http://doi.org/10.1128/AAC.48.8.2787

Stadler, R. H; Blank, I; Varga, N. (2002) Acrylamide from Maillard reaction products. Nature 419:449.

Sumner, S.C; Fennell, T.R; Moore, TA. (1999) Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. Chemical Research Toxicology 12:1110–1116.

Sumner, S.C; MacNeela, JP; Fennell, TR. (1992) Characterization and quantitation of urinary metabolites of 1,2,3- [13C]-acrylamide in rats and mice using 13C nuclear magnetic resonance spectroscopy. Chemical Research Toxicology 5:81–89.

Sumner, S.C; Williams, CC; Snyder, RW; et al. (2003) Acrylamide: a comparison of metabolism and haemoglobin adducts in rodents following dermal, i.p., oral, or inhalation exposure. Toxicology Science 75(2):260–70.

Swaen, G.M; Haidar, S; Burns, CJ; et al. (2007) Mortality study update of acrylamide workers. Occupational Environmental Medicine. 64(6):396-401.

Taeymans, D., Wood, J., Ashby, P., Blank, I., Studer, A., Stadler, R., Gondé, P.,

Van Eijck, Taiwo KA, Owolarefe OK, Sanni LA, Jeje JO, Adeloye K and Ajibola OO (2000) Technological assessment of palm oil production in Osun and Ondo states in Nigeria. *Technovation*, 20:215-223.

Tareke E, Rydberg P, Karlsson P, Eriksson S, Torenquit M (2002). Analysis of acrylamide
a carcinogen formed in heated foodstuffs. Journal of Agriculture & Food Chemistry, 50: 4998-5006.

Tareke, E; Rydberg, P; Karlsson, P. (2000) Acrylamide: A cooking carcinogen? Chemical Research Toxicolgy 13:517–522.

Twaddle, N.C; McDaniel, LP; Gamboa da Costa, G; et al. (2004)
Determination of acrylamide and glycidamide serum toxicokinetics in B6C3F1 mice using LC-ES/MS/MS. Cancer Letters 207(1):9–17.

U.S. FDA (2006) The US FDA / Center for Food Safety and Applied Nutrition (CFSAN) site for information on acrylamide and FDA research. Available from http://www.cfsan.fda.gov/~lrd/pestadd.html#acrylamide.

Vissoh P, Adjei-Nsiah S, van Huis A and Röling N (2010) Opportunities for oil palm development in Benin and Ghana: Institutional conditions for technological change. In *Aspects of Applied Biology*, Special Issue, Agriculture: Africa's Engine for Growth- Plant Sciences and Biotechnology hold the key, 96: 207-214.

Weibhaar, R. (2004). Acrylamide in heated potato products analytics and formation routes.*European Journal of Lipid Science and Technology*, 106, 786–792.

Wenzl T, Beatriz De La Calle M, Anklam E (2003). Analytical methods for the determination of acrylamide in food products: a review, Food Additives and Contaminants: Part A, 20: 885-902.

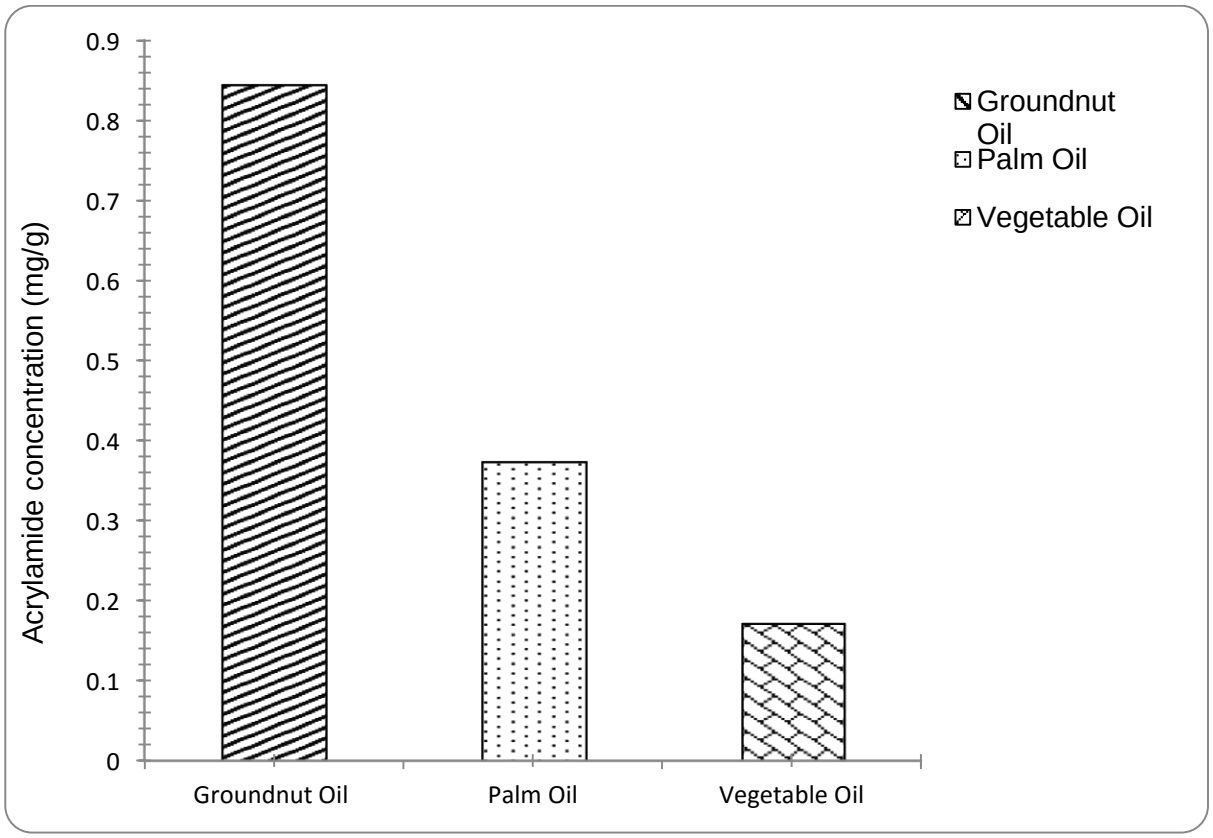
Wilson, K. M., Bälter, K., Adami, H. O., Grönberg, H., Vikström, A. C., Paulsson, B.,
… Mucci, L. A. (2009). Acrylamide exposure measured by food frequency questionnaire and hemoglobin adduct levels and prostate cancer risk in the Cancer of the Prostate in Sweden Study. *International Journal of Cancer*, *124*(10), 2384–2390. http://doi.org/10.1002/ijc.24175

Wu S.C and Yen GC (2004) Effects of cooking oil fumes on the genotoxicity and Oxidative stress in human lung carcinoma (A-54a) cells. *Toxicology In vitro,* 18(5):571-580.

Zu K.S.A., Adjei-Nsiah S and Bani R.J (2012) Effect of processing equipment and duration of storage of palm fruits on palm oil yield and quality in the Kwaebibrem District, Ghana.*Agricultural Research and Reviews*, 1 (1):18-25.

APPENDIX

APPENDIX 1: ACRYLAMIDE CONCENTRATION OF OILS USED



APPENDIX 2: PARAMETER ESTIMATES OF ACRYLAMIDE RELATED MATRICES IN GROUNDNUT OIL.

Parameter	Mean	Mode	5 % Percentile	50 % Percentile	95 % Percentile
Acrylamide concentration/mg/g	0.844	1.1252	0.413	0.879	1.116
No. of times of consumed/per day	0.763	0.7596	0.200	0.763	1.327
Mass of medium/g	430.369	3.5850	3.841	41.734	626.413
Ingestion rate/g/day	289.538	2.2186	1.752	28.650	480.053
Weight of respondent/kg	61.6	61.0	49	61	75
Cancer CDI (mg/kg-day)	0.32	0.0007	0.0031	0.0272	0.4844
Hazard Quotient	131.31	0.895	0.824	13.54	227.96

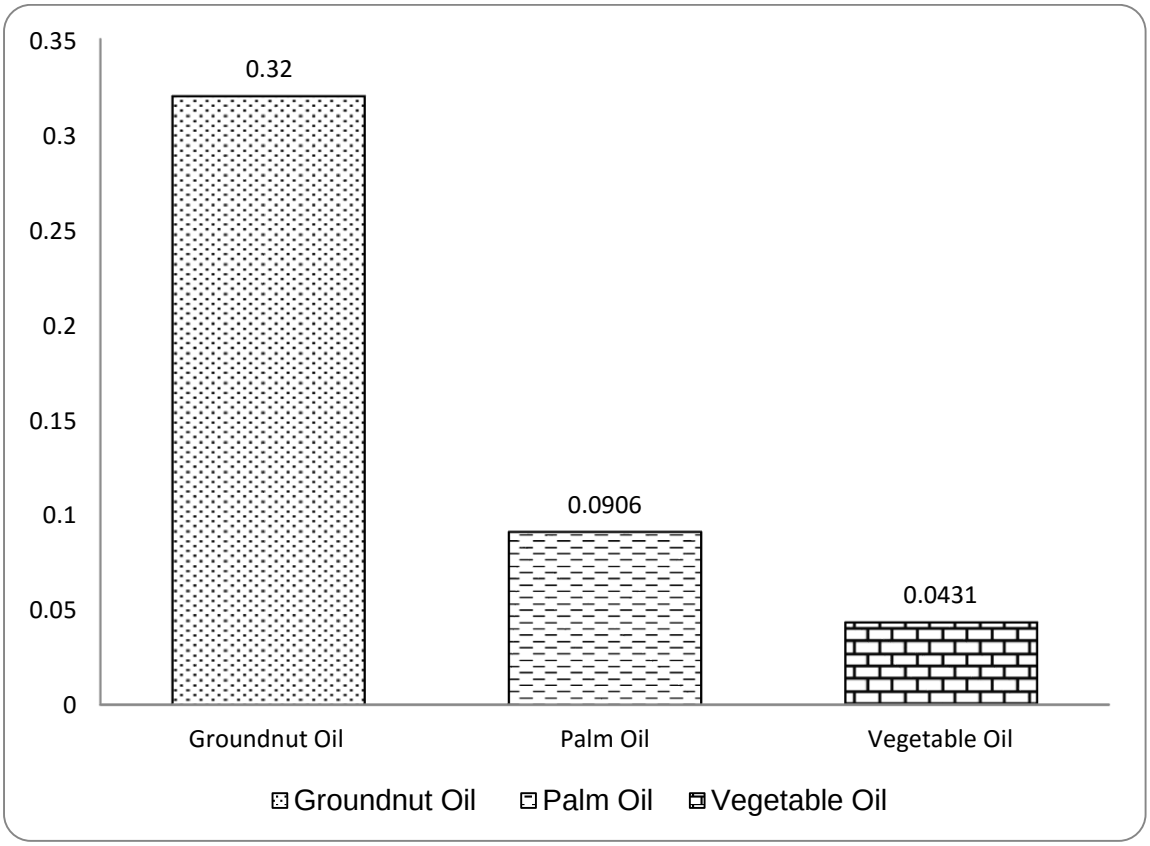
APPENDIX 3: **PARAMETER ESTIMATES OF ACRYLAMIDE RELATED MATRICES IN CRUDE PALM OIL**

Parameter	Mean	Mode	5 % Percentile	50 % Percentile	95 % Percentile
Acrylamide concentration/mg/g	0.374	0.224	0.231	0.328	0.675
No. of times of consumed/per day	0.763	0.742	0.199	0.763	1.327
Mass of medium/g	269.515	2.561	3.836	41.740	625.884
Ingestion rate/g/day	204.639	4.360	1.733	28.524	470.875
Weight of respondent/kg	61.6	61	49	61	75
Cancer CDI (mg/kg-day)	0.0906	0.001	0.0006	0.011	0.208
Hazard Quotient	48.61	0.501	0.353	5.88	98.76

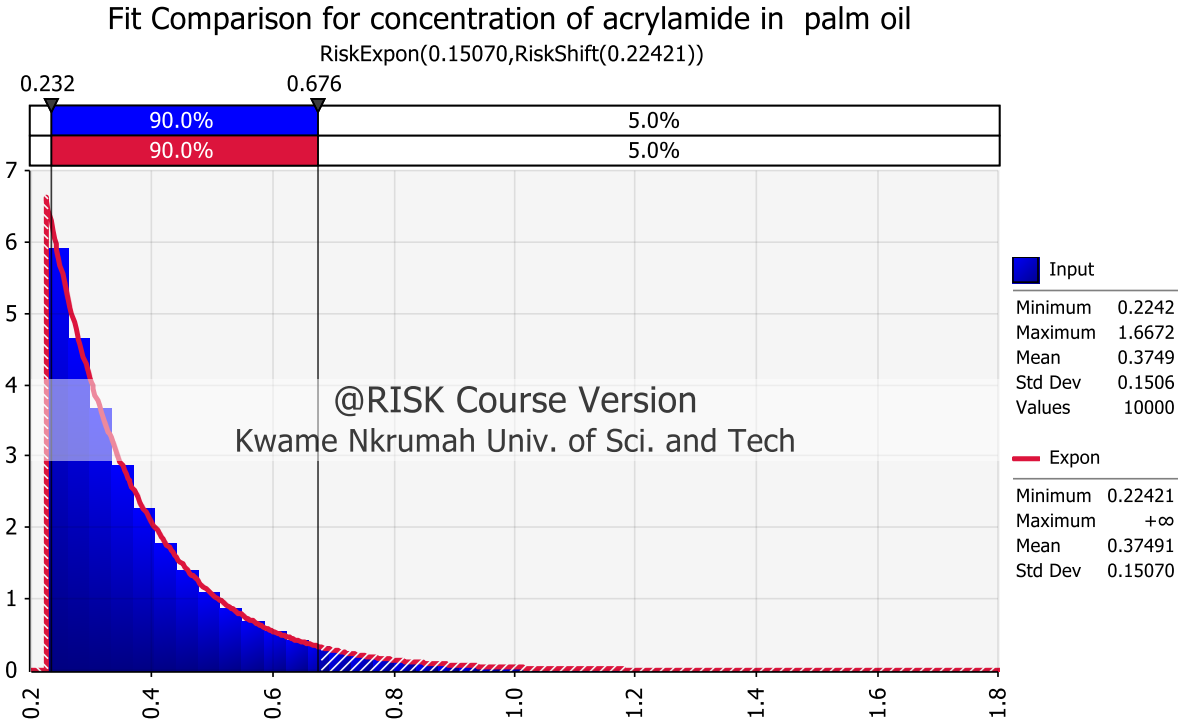
APPENDIX 4: PARAMETER ESTIMATES OF ACRYLAMIDE RELATED MATRICES IN REFINED PALM OIL

Parameter	Mean	Mode	5 % Percentile	50 % Percentile	95 % Percentile
Acrylamide concentration/mg/g	0.172	0.0003	0.008	0.119	0.516
No. of times of consumed/per day	0.763	0.7596	0.199	0.763	1.327
Mass of medium/g	413.20	3.07	3.84	41.74	626.19
Ingestion rate/g/day	303.66	1.92	1.78	28.81	485.09
Weight of respondent/kg	61.665	62.34	47.48	61.664	75.832
Cancer CDI (mg/kg-day)	6.224E-005	1.616E-008	7.1495E-008	3.496E-006	9.570E-005
Hazard Quotient	31.12	0.00808	0.0365	1.75	47.85

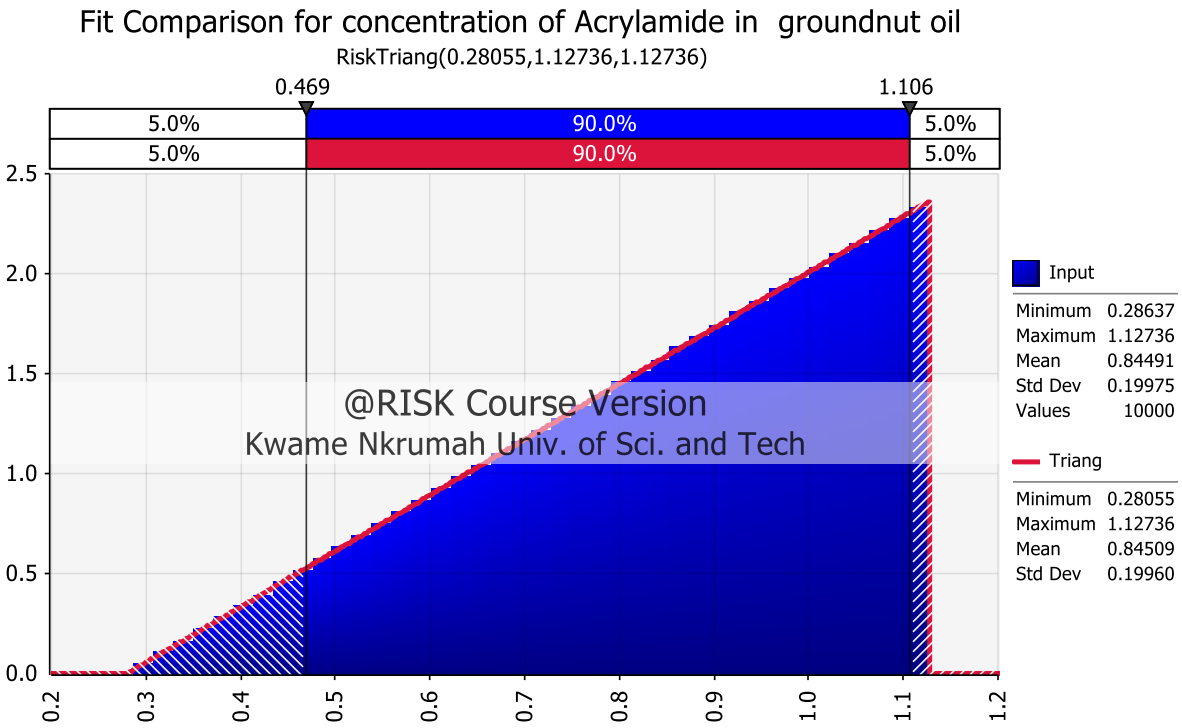
APPENDIX 5: MEAN OF CDI FOR OILS USED.



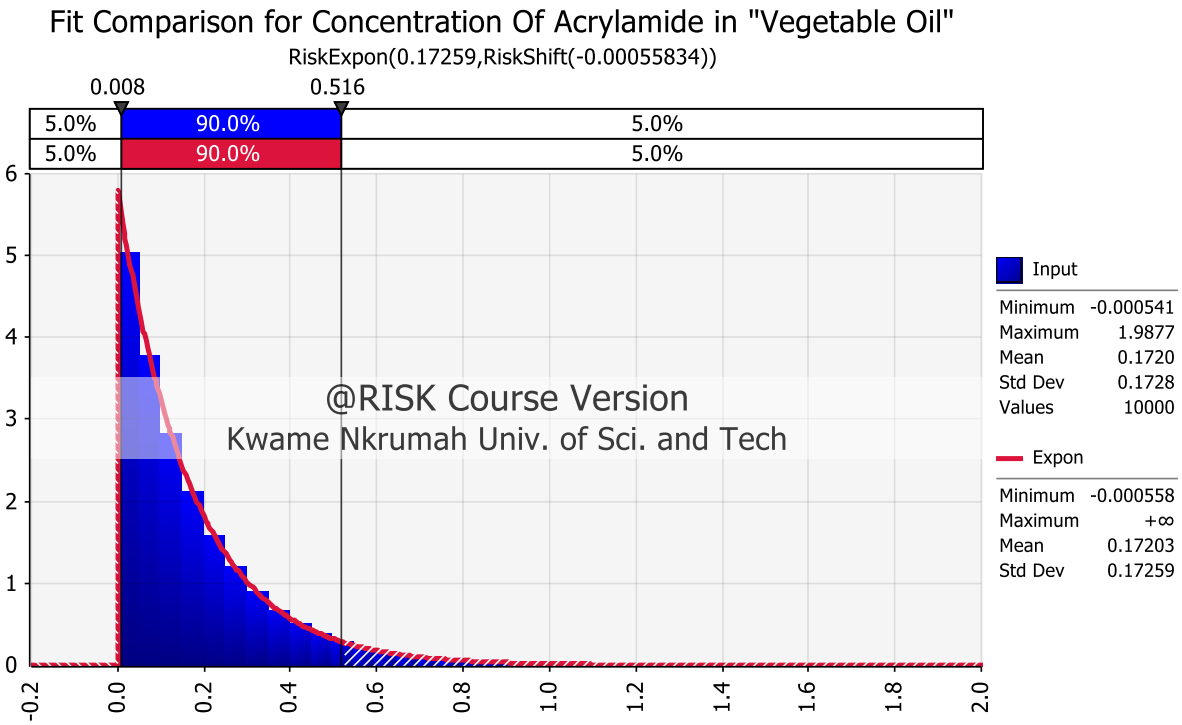
APPENDIX 6 A GRAPH SHOWING RISK EXPONENTIAL REPRESENTATION OF ACRYLAMIDE CONCENTRATION IN PALM OIL.



APPENDIX 7 A GRAPH SHOWING RISK TRIANGULAR REPRESENTATION OF ACRYLAMIDE CONCENTRATION IN PALM OIL.



APPENDIX 8 A GRAPH SHOWING RISK EXPONENTIAL REPRESENTATION OF ACRYLAMIDE CONCENTRATION IN "REFINED PALM OIL".



APPENDIX 9: QUESTIONNAIRES

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

Please tick where applicable

1. Sex Male ( ) Female ( )

2. Age …….

3. Educational/Professional background? Student ( ) Worker ( ) Unemployed ( )

4. Do you use cooking oil in your food preparation?

Yes ( ) No ( )

5. If Yes is it for… Domestic ( ) Commercial ( )

*Commercial

6. What type of Oil do you use in your preparation?

Crude palm oil() Refined palm oil ( ) Others …….

7. How often do you change the oil used for commercial frying?

Daily …… Others ……...

*Domestic

8. What type of Oil do you use in your preparation?

Crude palm oil( ) Refined palm oil ( )Others ……

9. How often do you change the oil used for domestic frying?

Daily …… Others ……...

10. How much oil do you use in a week? …….

11. How often do you consume oil foods in a week? ……

12. Do you cook for a family? Yes ( ) No ( )

13. If Yes How many in a family? …….

14. Body weight of participant……..…