KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

DIETARY RISK ASSESSMENT OF ACRYLAMIDE IN FOOD COMMONLY CONSUMED AMONG CHILDREN

A THESIS SUBMITED TO THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY IN PARTIAL FULFILMENT OF REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE IN FOOD QUALITY MANAGEMENT

BY

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DECLARATION

I declare that this submission is the result of my own research and thus does not contain any previously published material except for some information which the source for each one has been stated clearly.

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KNUST

DEDICATION

This book is sincerely dedicated to the glory of God; my godfather Mr. Isaac W. Ofosu, Mary Donkor my mum and my good friend Kojo Anobel

ACKNOWLEDGEMENT

The journey to greatness in life begins with a step ordered by the Almighty God. I therefore give Him all the glory for the strength, knowledge and ideas He gives me at every moment of my life. This work is the product of the impartation by my lecturers at the Department of Food Science and Technology in general and the lecturers for the Food Quality Management programme in particular, who sacrificed their time to make me who I am now. I salute them all.

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May the Almighty God Bless You Al!!!

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ABSTRACT

A total of 300 questionnaires were administered to Primary, Junior High and Senior High school pupils (5–19 years old). Participants were made to fill out the 24 h food frequency questionnaire. A total of 100 samples of bread which was the food most frequently consumed by the participants were sampled randomly from sales points in the Oforikrom Sub-Metro and analyzed for acrylamide contents. The modal acrylamide content in the samples was 314.20 µg/kg and the modal daily intake estimated at 155.99 µg/kg/day. First order Monte Carlo simulation at 10,000 iterations estimated chronic daily intake of acrylamide as 0.014 µg /kg/day. Subsequently, the modal risk of consumption of bread within the limits of reference dose of acrylamide (0.0002) for children in the study area was estimated at 3.62×10^{-3}. This means that, the probability of children in the study area to be at a risk to cancer within a year is 4 out of every 1000 children. Hence, the need for continuous efforts to reduce dietary acrylamide exposure to children. The study recommended that baking time and temperature be well monitored by regulatory bodies and bakers.

Keywords: Acrylamide dietary intake, acrylamide in bread, risk, Monte Carlo

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CHAPTER ONE

INTRODUCTION

Acrylamide is a white odourless crystalline solid which is soluble in water, ethanol, ether and chloroform. Acrylamide decomposes in the presence of acids, bases, oxidizing agents, iron, and iron salts (Eriksson, 2005). Acrylamide is widely used in the manufacture of various polymers. The largest use of these polymers has been in water treatment. In addition, acrylamide has found many uses as binding, thickening or flocculating agents in grout, cement, sewage/wastewater treatment, pesticide formulations, cosmetics, sugar manufacturing, soil erosion prevention, ore processing, food packaging and plastic products. Polyacrylamide is also used in some potting soil (Zhang *et al*., 2005).

Notwithstanding the above beneficial uses of acrylamide in industry, it has been classified as a potential human carcinogen and an effective clastogen (IARC, 1994; FAO/ WHO, 2011). The WHO also established the neurotoxicity of acrylamide in humans in 2001 following occupational or accidental exposures to acrylamide, while experimental studies have also shown adverse reproductive effects in animals (WHO, 2011).

Hence in 2002, when the chemical acrylamide was first discovered in a variety of commonly consumed food by Sweden scientists, the news was extremely unpalatable and called for a major public health concern (WHO, 2002). This was because a known carcinogen in experimental studies and a highly reactive industrial chemical was not expected to be on the dinning plate.

Dietary acrylamide is formed when food high in carbohydrates and low in proteins are processed at high temperature or undergo thermal processing at temperatures of 120 ^{o}C or higher (JECFA, 2006). That is dietary acrylamide can be formed in a wide range of foods during frying, baking and roasting, including crisps, chips, biscuits and even coffee.

Reducing acrylamide levels in food has been a great challenge to researchers and the food industry in developed countries. This is because acrylamide forms from natural precursors in a reaction that also produces desirable colours, flavours and aromas which is known as Millard reaction. During this reaction, asparagine is decarboxylated and deaminated in the presence of reducing sugars or other carbonyl compounds during heating to form 3-aminopropionamide, a potent precursor of acrylamide or 2-propenamide (Zyzak *et al.*, 2003; JECFA, 2011).

Higher levels of acrylamide is produced at the later stages of baking, roasting, grilling or frying processes where food loses its moisture contents and surface temperature rises (JECFA, 2006). Several researches have proven that, although acrylamide concentration may vary significantly from one food item to another, the highest level of acrylamide can be found in potato chips (European potato crisps) and French fries (European potato chips). For instance, Becalski *et al.* (2003) found the concentrations of acrylamide in commercial potato chips and French fries ranging from 530 to 3700 ng/g and 200 to 1900 ng/ g, respectively.

Dybing *et al.* (2005) discovered varying concentration of acrylamide in breakfast cereals, cookies, brewed coffee and bread. In addition, the results from the research by Normandin *et al.* (2013) revealed deep fried French fries and potato chips as having the highest acrylamide concentration as 1053 ng/g and 276 ng/g respectively.

In 2002, WHO made available a document on the dietary acrylamide exposure estimates for the general adult population; they have been estimated to range from 0.3 to 0.8 l g/kg bw/d and may reach 6 l g/kg bw/d for the 98th percentile consumer; that is the highest consumer (WHO, 2002).

1.1 PROBLEM STATEMENT AND JUSTIFICATION

The report by WHO in 2002 brought to light that, on a body weight basis, children's intake of acrylamide is generally two to three times higher than that of adults. In addition to having a higher average food intake per kg body weight than adults, Dybing *et al.* (2005) concluded that, children and adolescents also consume acrylamide rich-food, such as potato chips and French fries, on a more regular basis than the rest of the population. Ghanaian's are known to process and consume carbohydrate rich foods. Little or no information is also recorded on the actual dietary intake of acrylamide among Ghanaians. From the literature, only two North American and Canadian studies have attempted to document acrylamide exposure among teenagers and adolescents respectively (Tran *et al.*, 2010; Katz *et al.*, 2012; Normandin *et al.*, 2013). It is therefore important to monitor levels of acrylamide in a food regularly consumed by children and to assess their dietary intake as well as their risk.

Literature concludes that, little information is available for exposure among teenagers (Tran *et al.*, 2010; Katz *et al.*, 2012) and adolescents respectively (Normandin *et al.*, 2013). Coupled with the fact that no assessment of the risk of exposure to acrylamide has been reported so far for developing countries. It is therefore important to monitor levels of acrylamide in food regularly consumed by children and to assess their dietary intake as well as their risk.

1.2 OBJECTIVE

The aim of this study was to estimate dietary exposures of children in a sub population in Ghana, to acrylamide and thus calculate the associated health risk.

1.3 SPECIFIC OBJECTIVES:

1. To determine the food commonly consumed by school children in the Oforikrom sub-metro.
2. To estimate dietary intake of acrylamide among school going children in the Oforikrom sub-metro.
3. To calculate the health risk of children in the Oforikrom sub-metro from exposure of acrylamide.

CHAPTER TWO

LITERATURE REVIEW

2.1. DISCOVERY OF DIETARY ACRYLAMIDE

The presence of acrylamide in food was first discovered in April 2002 by Swedish researchers; Tareke *et al.* (2002) who 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.

This raised a lot of public health concerns about the possible health risks associated with the dietary exposure to acrylamide. Since their discovery especially as a chemical, naturally generated as part of the cooking process, researchers around the world have been alarmed to research into both their toxicity and occurrence in a wide variety of foods.

2.2.0. CHEMISTRY OF ACRYLAMIDE

2.2.1. The physical parameters of acrylamide

The chemical formula for acrylamide is CH_2=CH-CO-NH_2; 2-propenamide. Acrylamide is a small hydrophilic molecule. It is an odourless white crystalline solid with molecular weight of 71.08 g/mol that is generally formed from the hydration of acrylonitrile with sulphuric acid between the temperatures; 90-100 ^oC or by catalytic hydration using copper catalyst. It is soluble in a number of polar solvents like water, acetone and acetonitrile. It is very difficult to determine the boiling point of acrylamide at ambient pressure because there is the susceptibility of acrylamide to polymerization during heating. It has a melting point of 84.5 ^{o}C, low vapour pressure of 0.007 mmHg at 25 ^{o}C and a high boiling point of 136 ^{o}C at 3.3 kPa/25 mmHg (Eriksson, 2005).

Table 1: Physical Parameters of Acrylamide

Parameter	Specification
Chemical Formula	CH2=CH-CO-NH; propenamide
Molecular Weight	71.08g/mol
Solubility	216g/100g water @30^{o}C
Boiling Point	136^{o}C at 3.3 kPa/25 mmHg
Melting Point	84.5 ^{o}C
Vapor Density	2.45 (air = 1)
Density/Specific Gravity	1.122kg/dm2 at 30 ^{o}C
Vapour Pressure	0.007 mm Hg at 25 ^{o}C

Source (Eriksson 2005).

2.2.2. Chemical structure of acrylamide molecule.

The acrylamide structure consists of two functional groups, 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 nuleophilic and Diel-Alder reactions which are very important in biological systems.

Studies have shown that, the amide group undergoes hydrolysis, alcoholysis, dehydration and condensation reactions with aldehydes. The vinylic double bond on the other hand also reacts with ammonia, aliphatic amines, bisulphite and dithiocarbonates, chlorine, phosophines, bromine and proteins (Friedman, 2003; Girma *et al.*, 2005).

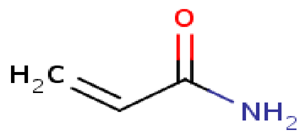

Figure 1: Structural representation of acrylamide molecule.

2.3 EFFECTS OF REACTION OF ACRYLAMIDE WITH METALS

According to Girma *et al.* (2005) acrylamide as a weak acidic and basic conjugated amide has the ability to conjugate with metals ions. The coordination occurs either at the organic group or at the amide residue. Acrylamide undergoes addition reactions to the vinylic double bound.

This makes it reactive to the nucleophiles as well as amino and thiol groups in amino acids and proteins. The formation of acrylamide adducts with the N-terminal valine residue in haemoglobin is as a result of this reaction. Such adducts are very useful biomarkers for acrylamide exposure in both animal and human studies ((Bergmark *et al.*, 1993; Rice, 2005; Tareke *et al.*, 2000).

2.4. USES OF ACRYLAMIDE

Acrylamide is an industrial biodegradable chemical that has been used since mid-1950s in the production of polyacrylamide which has a wide range of applications, mainly in the agricultural and industrial sector. For instance, it has been used over the years for the synthesis of polymers and gels, as flocculent for clarifying drinking-water, as a sealant for construction of dams and tunnels, as a binder in paper and pulp industry, and dye synthesis

(Bologna *et al.,* 1999). Acrylamide has also find uses in research laboratories for the preparation of polyacrylamides for gels for electrophoresis.

2.5. STUDIES THAT LED TO THE IDENTIFICATION OF THE ORIGIN OF DIETARY ACRYLAMIDE

Acrylamide always emerged as a factor that could be associated with considerable cancer risk in several studies which aimed at the identification of the causes of background carcinogenesis. For instance, reaction products (adducts) of acrylamide with N termini of haemoglobin are regularly observed in unexposed control persons (Bergmark, 1997; Hagmar *et al.,* 2007).

The average haemoglobin adduct level measured in Swedish adults is preliminarily estimated to correspond to a daily intake approaching 100µg of acrylamide. Because this uptake rate could be associated with a considerable cancer risk, it was a big issue to consider the origin of acrylamide.

2.6 OCCURRENCE OF ACRYLAMIDE IN FOOD

The presence of the potential human carcinogen; acrylamide was suspected in cooked food and mentioned for the first time by Tareke *et al.* (2000) who observed that, rats which were fed with fried feeds had their haemoglobin adduct highly increased. He concluded that the haemoglobin adduct he observed was N-(2-carbamoyl methyl) valine from acrylamide.

On the 24th of April 2002, a group of researchers from Sweden reported and provided data that showed high concentrations of acrylamide in a variety of baked and fried foods cooked at elevated temperatures (WHO, 2002).

Several studies from thence have provided data on the presence of high levels of acrylamide in a wide variety of heat treated starch–rich food products. Potato crisps, French fries, biscuits, crisp bread and crackers, and coffee were reported to contain acrylamide in significant levels in many countries, and their ranges of mean acrylamide levels were 399 – 1202 µg/kg, 159 – 963 µg/kg, 169 – 518 µg/kg, 87 – 459 µg/kg and 3 – 68 µg/L, respectively (JECFA, 2011).

Vegetables other than potatoes cooked in high temperature, such as baking, grilling, roasting and pan-frying, were also reported to contain acrylamide in some researches. A Turkish study revealed that the acrylamide level in grilled vegetables can reach 359 µg/kg (Şenyuva and Gökmen, 2005).

In Japan, research revealed that vegetables, such as asparagus, pumpkin, eggplant and green gram sprouts, contained acrylamide at levels of > 100 µg/kg, with the highest at 550 µg/kg (for green gram sprouts) after baking at 220 ^{o}C for 5 min (Takatsuki *et al.*, 2004). Pan-fried vegetables as reported by Japan were also found to contain acrylamide ranged from 30 to 393µg/kg in Japan (393 µg/kg in snow peas, 100 µg/kg in bean sprouts and asparagus, 30 µg/kg in broccoli, cabbage, pumpkin, eggplant, haricot beans and onion (JECFA, 2011). Among cereal products, the main contribution is from pastries and biscuits as well as processed cereal products and breads (Surdyk *et al*., 2004; Tareke *et al.*, 2002; Svensson *et al*, 2003).

2.7 FACTORS LEADING TO VARIABILITY IN ACRYLAMIDE (AA) CONTENT AND EXPOSURE IN DIFFERENT STUDIES

The contribution of each individual product varies between countries depending on food habits among many other factors. Coffee and green teas are among the products with high acrylamide content, as are cocoa products. Milk products, fish and seafood are examples of products that are found at the lower end of acrylamide content range. The content in the different foods shows a wide range of variation. Consequently, the average exposure rate differs not only between countries but also among age groups.

For example, in the Netherlands children and teenagers have a higher exposure rate (Konings *et al.*, 2003). Fohgelberg *et al.* (2005) estimated the acrylamide intake for Swedish infants in their first year of life to be in the range 0.04-1.2 μg/kg bodyweight/day based on analyses of breast milk and infant formulae.

In Germany, bread accounts for about 18-46 % of acrylamide intake due to the high consumption (Hilbig *et al.*, 2004). In the Netherlands, the mean acrylamide exposure is in the order of 0.48 μg/kg bodyweight /day (Konings *et al.*, 2003).

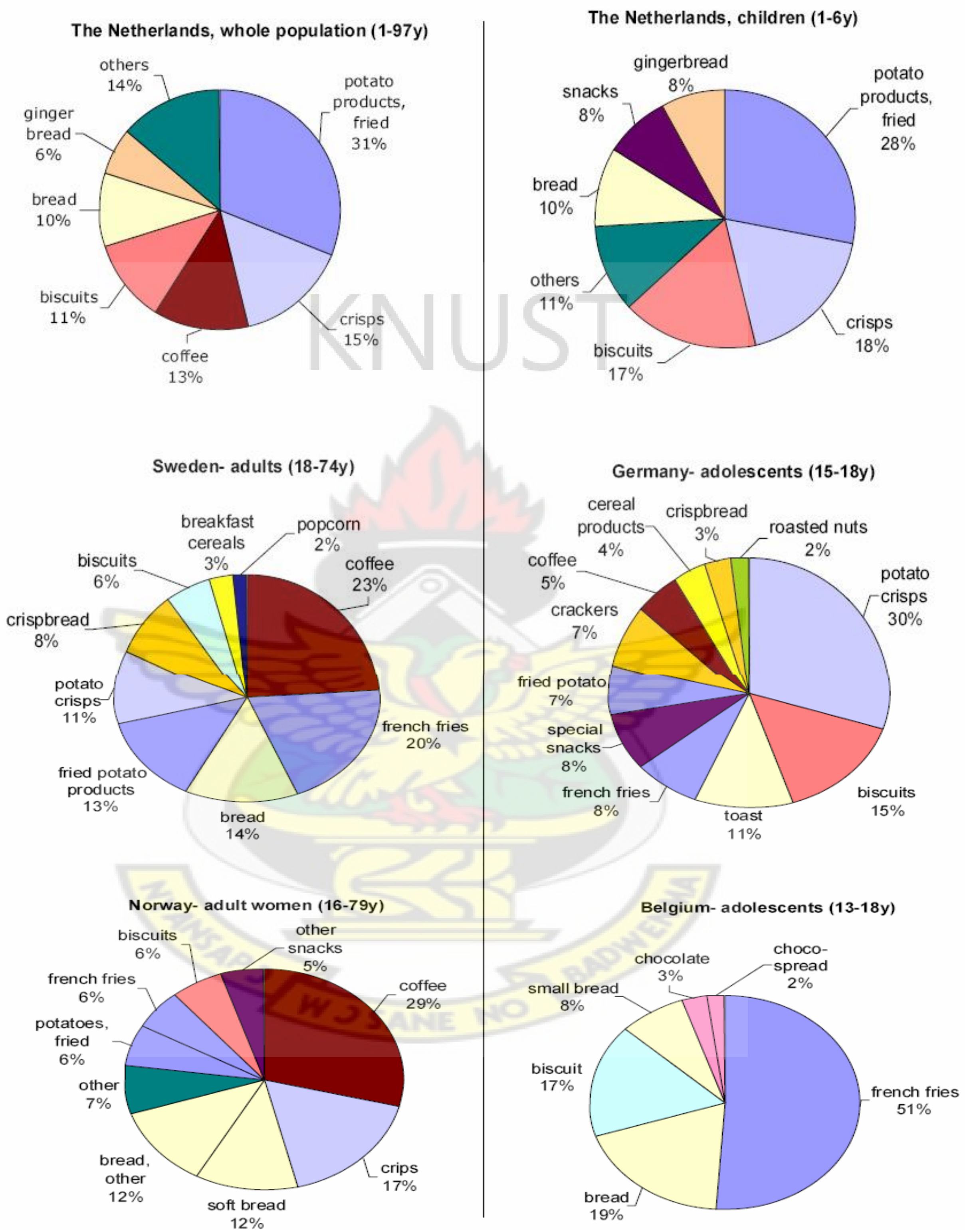

Figure 2: Contribution of food groups to acrylamide exposure in different countries for different age groups (HEATOX Project 2007)

2.8 ACRYLAMIDE'S MECHANISTIC AND MODELLING STUDIES

In the early stages of investigations into the mechanism of acrylamide formation in heated foodstuffs, two routes to the formation of acrylamide were thought possible. Since acrylamide levels were high in fatty foods such as potato crisps and French fries the fatty acid oxidation product acrolein (CH_2=CH-CHO) was noted as a possible precursor and forming acrylamide through direct reaction with ammonia followed by oxidation to acrylamide (Gertz and Klostermann, 2002).

Another possible pathway is via the reaction between reducing sugars and amino acids in the Maillard reaction. A number of recent mechanistic studies have shown that the latter route is the most likely vehicle for acrylamide formation. Mottram *et al.* (2002) illustrated that significant quantities of acrylamide were formed when the amino acid asparagine and the reducing sugar glucose were reacted at 185°C in phosphate buffer. Asparagine is the most likely amino acid precursor as it possesses an amide group attached to a chain of two carbon atoms and also occurs in significant quantities in potatoes and cereals (Brierley *et al.*, 1992, 1996, 1997).

In similar studies by Stadler *et al.* (2002, 2004), it was reported that significant quantities of acrylamide were formed when equimolar amounts of glucose and asparagine were pyrolysed at 180 °C. A conclusion was finally drawn by Biedermann *et al.* (2003) that acrylamide formation resulted from the degradation of asparagine by reaction with a carbonyl source most likely from glucose and fructose.

Becalski *et al.* (2003) showed that ^{15}N- labelled glucose and asparagine in ratios similar to those found in potatoes produced ^{15}N-labelled acrylamide. Both Stadler *et al.* (2002) and Mottram *et al.* (2002) also postulated reaction pathways to acrylamide with the sugar asparagine adduct N- glycosylasparagine being suggested as a possible direct precursor of acrylamide under pyrolytic conditions.

More recently this has been confirmed using pyrolysis gas chromatography/mass spectrometry (Py-GC/MS) and Fourier Transform Infra-Red (FTIR) spectroscopy (Yaylayan *et al.*, 2003) and model studies (Stadler *et al.*, 2004). Zyzak *et al.* (2003) using isotope substitution studies have elucidated the mechanism of acrylamide formation by confirming the presence of key intermediates such as a decarboxylated schiff base and 3-aminopropionamide.

The acrolein route to acrylamide formation has been virtually discounted as recent studies have confirmed that the addition of antioxidants did not affect acrylamide formation (Vattem and Shetty, 2003). In addition, real time monitoring of reducing sugars, asparagine and water contents in heated potato, wheat and rye systems have shown that losses are accompanied by increases in acrylamide formation and that this maximizes near the end of the heating cycle (Elmore *et al.*, 2005). At the present time most available data points to the formation of acrylamide in foods by the route as shown in Figure 3.

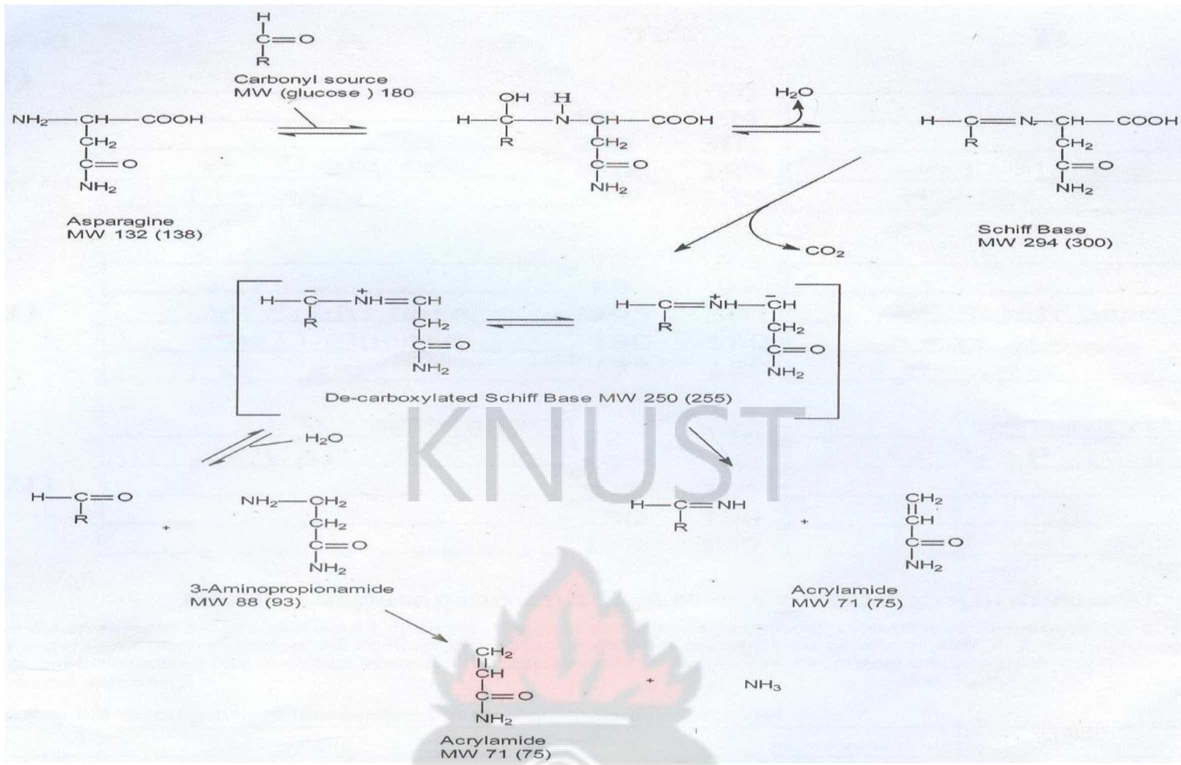

Figure 3: Probable route to the formation of acrylamide in starchy foods (Zyzak *et al.*, 2003).

2.9 FORMATION OF ACRYLAMIDE VIA MAILLARD REACTION

The Maillard reaction has been recognized for over 60 years as a major route to flavour and to brown cooked foods. The reaction between amino compounds and reducing sugars has been subject of a great amount of research done by food scientists, decided to identify the compounds responsible for certain flavour and colour characteristics in heated foods. It was recognized that acrylamide is formed in foods cooked at high temperatures when the moisture levels become low. The first scheme for the general pathway of Maillard reaction was suggested by Hodge (1953) and detailed by Martins *et al.* (2001).

In the first step a reducing sugar condenses with a specific compound possessing a free amino group, such as a ε-amino group of lysine or α-amino group of a terminal amino acid of a protein. The result is a condensation product N-substituted glycosylamine which rearranges to give an Amadori rearrangement product (ARP). Depending on the pH, the subsequent degradation could, at pH = 7, undergo 1, 2-enolization with formation of furfural when pentoses are involved, or hydroxymethyl- furfural (HMF) when hexoses are involved.

The Schiff base intermediate can either hydrolyse to form 3-aminopropionamide, a potent precursor of acrylamide (Granvogl *et al.*, 2004) or undergo 1, 2-elimination to directly form acrylamide (Claus *et al.*, 2008). At pH higher than 7 the degradation of the Amadori compound is thought to involve 2,3- enolization, where reductones, such as Hydroxymethylfurfural and a variety of fission products, including acetol, pyruvaldehide and diacetyl are formed.

Subsequently, in Strecker degradation, dicarbonyl compounds will react with amino acids to form aldehydes and α-aminoketones. Along with the enolization reactions, the Amadori product rearrangements, izomerizations and further condensations will produce brown nitrogenous polymers and co-polymers, known as melanoidins (Martins *et al.*, 2001). Formation of the advanced glycation end products (AGEs) and melanoidins, with a partially known structure, are still under study.

2.10 MONITORING OF POTENTIAL ACRYLAMIDE PRECURSORS IN FOODSTUFFS

The earlier researches by Mottram *et al.* (2002) and Stadler *et al.* (2002) strongly suggested that acrylamide formation in heated potato products resulted from the reaction of amino acids such as asparagine (and to some extent glutamine) and reducing sugars (glucose and fructose).

Their discovery led to studies aimed at examining factors which affect the levels of these substances in raw potatoes. Noti *et al.* (2003) examined the effect of storage temperature and reconditioning at ambient temperature on sugar levels and the potential for acrylamide formation in potatoes. They recommended that, in order to avoid extensive acrylamide formation in fried or roasted foods, they must not be stored below 8 °C .

Similarly Biedermann *et al.* (2003) showed that in order to avoid excessive acrylamide formation while retaining adequate browning and flavour, potatoes with glucose and fructose contents in the range 0.2-1.0 g/kg fresh weight should be used for roasting and frying. Chuda *et al.* (2003) found that the acrylamide level of potatoes stored for 2 weeks post-harvest at 2 °C was ten times higher than those held at 10 °C and was highly correlated with both glucose and fructose levels in the tubers.

Amrein *et al.* (2003) examined the effect of variety and farming systems on glucose, fructose and asparagine content and on subsequent acrylamide levels after frying. Variety had a significant effect on acrylamide formation which was primarily related to the reducing sugar content.

In a separate study, Amrein *et al.* (2004) found that the reducing sugar content was strongly correlated with acrylamide levels in the cooked product, whereas no correlation was found between acrylamide levels and free asparagine, or the pool of free amino acids. On a molar basis the mean content of asparagine was up to 5.6 times higher than that of glucose or fructose. Olsson, (2004) examined the effect of storage temperature, storage time and variety on the levels of asparagine, glutamine and reducing sugars in eight potato clones.

These researchers then concluded that variety had a marked effect on all the components examined. In addition, storage at a low temperature (3°C) for nine months resulted in an increase in glucose and fructose levels. However, amino acid levels were not significantly affected by storage time. In a study on the effect of free amino acid and sugar levels on acrylamide formation in French fries, Becalski *et al.* (2004) found a significant correlation between acrylamide content and reducing sugar level.

In addition, they found that the presence of asparagine had a major effect on acrylamide levels although this was less significant than the effect of reducing sugars. In conclusion, the levels of acrylamide in potato products are primarily influenced by the levels of reducing sugars in raw potatoes, and this in turn is influenced by storage time, temperature and variety of potato used.

2.11. EFFECT OF PROCESSING ON ACRYLAMIDE FORMATION IN FOOD PRODUCTS

Potato products undergo a variety of processing steps prior to ingestion. The challenge for food processors and advisory/regulatory agencies is to establish processing protocols that limit the formation of acrylamide while maintaining finished product quality. A number of studies have shown that both the temperature and duration of heating have a significant influence on acrylamide levels. Rydberg *et al.* (2003) found that acrylamide levels increased in French fries as the oven temperature increased from 100-220 °C, reaching a maximum level of 5000 μg/kg.

However, with prolonged heating at the maximum temperature acrylamide concentrations decreased presumably due to thermal degradation. Taubert *et al*. (2004) also demonstrated that heating potato slices with high to intermediate surface to volume ratios to temperatures above 180°C resulted in a rapid decrease in acrylamide levels. In contrast, Pedreschi *et al*. (2004) and Matthaus *et al.* (2003) showed that increasing the frying temperature from 150-190°C resulted in a marked increase in acrylamide levels. No decrease in acrylamide formation was observed presumably because temperatures above 190 °C and prolonged heating times (max 10 min) were not employed.

Granda *et al.* (2004) and Williams *et al.* (2005) also showed that both frying time and temperature increased acrylamide levels in potato chips. The former authors also showed that the use of low-temperature vacuum frying reduced acrylamide levels. It is worth noting that a number of authors have reported an inverse relationship between acrylamide contents and the moisture content of a foodstuff (Leung *et al.*, 2003: Amrein *et al.*, 2004). With particular reference to potato products Elmore *et al.* (2005) noted that at moisture contents of 0.1-0.5 % a linear inverse relationship existed between acrylamide levels in potato cakes and their moisture contents.

The techniques applied prior to thermal processing also reduce acrylamide formation. Soaking or blanching of the raw product can reduce acrylamide content in the cooked product (Haase *et al.* 2003b; Grob *et al.*, 2003; Pedreschi *et al.*, 2004, 2005). However, Williams *et al.* (2005) found that inclusion of a water soak prior to frying had no effect on acrylamide formation. A further reduction in acrylamide levels can be achieved by immersion of the raw product in an acidic solution prior to cooking (Jung *et al.,* 2003; Kita *et al*., 2004; Pedreschi *et al*., 2004).

This method is particularly effective as it enhances the extraction of reducing sugars and amino acids from the product. Other pre- treatments shown to reduce acrylamide formation include addition of a flavanoid spice mix (Fernandez *et al.*, 2003), use of asparaginase to breakdown asparagine in the raw product (Zyzak *et al.*, 2004) and the use of genetically modified potatoes having a reduced content of soluble sugars (Soyka *et al.*, 2004).

2.12. TOXICOLOGY OF ACRYLAMIDE

Evidence to consider acrylamide a neurotoxicant was derived from observation of humans (NOAEL 0.5 mg/kg body weight/day) and studies of laboratory animals. Acrylamide is known to have caused neurotoxicity in highly exposed humans to 200 mg/kg-day via tap water and 8 mg/m^3 air (Hagmar *et al.*, 2001). These effects observed in laboratory rodents for doses of 2–3 mg/kg-day via tap water for life time, could not be associated with the ingestion of food origin acrylamide. Moreover, the peripheral neuropathy was described as slowly reversible in humans.

The degeneration of the nerve fibre axons is related to the impairment of axonal transport of proteins kinesin and dynein (JECFA, 2002). Evidence that acrylamide can cause cancer comes entirely from rat studies, for an exposure dose of 0.5–3 mg/day in drinking water, but not in humans, even among highly exposed workers (Erdreich and Friedman, 2004). In mice and experimental rats acrylamide is causing tumours at multiple organ sites in both species increasing the incidence of lung tumours, skin tumours, tyroid tumours and pituitary tumours (Klaunig, 2008).

The genotoxic metabolite of acrylamide is glycidamide. Glycidamide and acrylamide have been also positive for mutagenicity and DNA reactivity in a number of in vitro and in vivo assays. Using the relative cancer risk model, researchers (Törnqvist *et al*., 2008) arrived at a lifetime risk of 16×10-3 İμg acrylamide/kg / bw for laboratory rodents. However, validation in humans of risk estimates derived from animal experiments is not straightforward. All the compounds occurring naturally in foods represent a general exposure difficult to account due to associations of different foodstuffs related to different dietary habits.

More recently, studies by Olesen *et al.* (2008) have reported a linkage between dietary intake of acrylamide and endocrine tumours in women (endometrial, ovarian and breast). The authors concluded that a linkage between acrylamide in food and the induction of cancer was apparent.

Olesen *et al.* (2008) showed positive associations between acrylamide haemoglobin adduct levels and breast cancer incidence from examination of blood samples from 374 postmenopausal women.

2.13. ESTIMATION OF POTENTIAL SAFETY AND RISK TO THE GENERAL PUBLIC FROM DIETARY EXPOSURE TO ACRYLAMIDE

The physiologically base toxicokinetic (PBTK) model used by Sweeney *et al.* (2010) was applied to non-cancer and cancer dose-response assessments for acrylamide to derive appropriate tolerable daily intake (TDI) values to be used to assess the potential safety and risk to the general public from dietary exposure to acrylamide. To estimate safe levels of exposure, the MoEs for neurotoxicity of acrylamide (AA) exposures over life time were calculated (Tardiff *et al*., 2010) for a dose of 1 µg acrylamide/kg-day for an average consumer and a dose of 4 µg acrylamide/kg-day for an average consumer. The BMDL10 (benchmark dose lower bound) values for acrylamide (AA) and glycidamide (GA) corresponding to external doses of 0.3 and 0.5 mg/kg day (Table 2).

Table 2: Summary of the margins of exposure for acrylamide and glycidamide

ENDPOINT	CONSUMER	Margin of exposure	
		AA	GA
Neurotoxicity	Average intake	300	500
	High intake	80	130
Tumours	Average intake	200	1200
	High intake	50	300

Source: (Tardiff *et al.*, 2010)

2.14. POPULATION INTAKE, RISK ASSESSMENT AND TOXICITY STUDIES.

The toxicity of acrylamide was well known prior to the Swedish discovery and a number of excellent reviews are available regarding acrylamide toxicity (Dearfield *et al.*, 1988, 1995; Freidman, 2003; Tayemans, 2004; LoPachin, 2004; Rudēn, 2004).

Literature reviewed so far has made it clear that, risk assessment studies on potential acrylamide intake from foods are available so far on populations in Belgium (Matthys *et al.*, 2005), Sweden (Svensson *et al.*, 2003; Mucci *et al.*, 2003), Holland (Konings *et al.*, 2003), Germany (Hilbig *et al.*, 2004, Schettgen, 2002), Slovakia (Ciesarova *et al.*, 2004), Japan (Maitani, 2004), Norway (Norwegian Food Control Authority, 2002), United Kingdom (FSA, 2005ab), Australia (Croft *et al.*, 2004) and the USA (Javier, 2002, Petersen, 2002, DiNovi, 2004).

Most of these studies have concentrated on assessing acrylamide intake in food products containing low to high levels of acrylamide and do not represent a complete dietary intake for the substance.

Dietary intakes of acrylamide for the general population were estimated by FAO/WHO to be in the range of 0.3 to 0.8 µg /kg bw/day (Petersen, 2002). However, these dietary exposures are not directly comparable because of the different methods used for assessment that is different age groups, whole populations/ consumers of particular products using limited food groups rather than the whole diet.

It is still not clear whether or not acrylamide from food represents a risk to public health and a recent population-based study in Sweden failed to find a link between dietary intake of acrylamide and cancer of the bowel, kidney and bladder (Mucci *et al*., 2003). However, it is clear that the high profile nature of acrylamide in foodstuffs has raised public awareness to a level were further investigation is warranted (Gormley and Mee, 2003).

2.15. ANALYSES OF ACRYLAMIDE IN FOOD PRODUCTS

Despite acrylamide being a relatively 'new' contaminant for food analysts, intensive method development and refinement have been carried out for its identification in various food products.

An extensive review of analytical methods used for the determination of acrylamide has been published (Wenzl *et al.*, 2003) followed by a substantial number of more recent papers.

Several methods have used to determine acrylamide in foods up to the present date has made it evident that, GC-MS and LC-MS/MS are the most widely used methods for acrylamide determination. This was borne out in a recent proficiency study by the German

Federal Institute on methods used for acrylamide determination where GC- MS and LC-MS/MS represented up to 94% of the methods (Clarke *et al*., 2003; Wenzl *et al.*, 2004).

GC-MS based methods fall into two categories those which include a derivitisation step which serves to increase selectivity and improve the volatilty of the compound and those without a derivitisation step.

Bromination is the usual route to derivitisation for GC-MS with a variety of agents being used. GC-MS methods for the determination of acrylamide without derivitisation require exhaustive extraction of the compound from the food matrix with extraction times extending to ten days in some cases (Pedersen and Olsson, 2003).

LC-MS/MS methods generally do not require derivitisation. Quantification for both LC-MS/MS and GC-MS methods is generally achieved by the inclusion of either a [^{13}C3]-acrylamide or [D3]-acrylamide labelled internal standard during homogenisation of the sample. The use of MS detection as a method of quantification is probably related to the regulatory nature of the acrylamide problem.

Analysis based on liquid chromatography-diode array detection (LC-DAD) and HPLC separation is available. However it may be suitable for more routine type analyses (Gökmen *et al*., 2004).

2.16. LEVELS OF ACRYLAMIDE IN FOODS

Since the Sweden scientist reported the formation of acrylamide in food in the year 2002, many researchers have focused on the detection of acrylamide. Certain potato cultivars have been known to contain considerable amounts of acrylamide precursors such as free asparagines, glucose and fructose, which might explain their high concentration of acrylamide. For instance, Erntestolz cultivar stored at 4 °C for 15 days showed an increase in reducing sugars from 80 to 2,250 mg/kg. The impact of variety, harvest year, fertilization and storage conditions on the acrylamide content has also been extensively studied (Amrein *et al.*, 2003; EU, 2003; Jung *et al.*, 2003; Zyzak *et al.*,2003; Andrzejewski *et al.*, 2004; Taubert *et al.*, 2004).

Cereals have been less studied than potatoes, but the influence of variety gave a strong variation of the asparagine content, ranging from 319 to 791 mg/kg (Springer *et al.,* 2003). Unfavourable weather conditions can also result in sprouting and, as a consequence result in significantly higher levels of asparagines. Noti *et al.* (2003) also reported levels of 150–400 mg/kg of asparagines in wheat flour. The bakery temperature, time and presence of precursors make bread a preferred food for acrylamide formation. Gingerbread may contain up to 1,000 μg/kg of fresh weight due to presence of ammonium hydrogen carbonate, which strongly enhances acrylamide formation.

Almonds contain free asparagines in large amount, 2,000–3,000 mg/kg, but also reducing sugars such as glucose and fructose 500–1,300 mg/kg and sucrose 2,500–5,300 mg/kg. In roasted almond, the average acrylamide content is 582 μg/kg (Lukac *et al.,* 2007). Coffee beans were roasted at temperatures from 220 to 250 °C and despite the relatively high free asparagine ranging from 30 to 90 mg/100g that stimulates acrylamide formation at first, reduction in the final acrylamide content was registered due to 40 to 60 % losses in ground coffee stored at room temperature (Taeymans *et al.*, 2004). At the very beginning of the roasting step more than 7 mg/kg acrylamide is formed and then the amount declines towards the end of the roasting cycle.

2.16.1. Comparism between acrylamide levels of food samples at different year periods

In 2009, the European Food Safety Authority published a report on results of the monitoring of acrylamide levels in food. The report compares the 2007 results with the results collected by the European Commission Joint Research Center's Institute for Reference Materials and Measurements in the years 2003 to 2006.

There was variability between the results for 2007 and the ones from 2003–2006 resulting in a p-value lower than 0.05 that suggest a statistically significant difference between the two compared periods. The report published by the European food safety Authority showed that acrylamide content was reduced in 2007 for a half of the products including potato crisps, jarred baby food, coffee, bread and others analyzed, while for the other five (home cook products, French fries, breakfast cereals and biscuits) a slight increase was noticed. Potato crisps, French fries, coffee and biscuits recorded the highest levels of acrylamide of all their researches.

2.16.2 Comparism between acrylamide levels of food studied in different countries.

A recent study done in Turkey (Ölmez *et al.*, 2008) reported the level of acrylamide in potato crisps of 834 µg/kg, higher than the level registered in EU of 628 µg/kg. However, the Turkish coffee had an acrylamide content of 266 µg/kg, similar with the one reported for the EU in 2007, 253 µg/kg. Traditional Turkish foods, desserts cooked at high temperatures, oven baking such as baklava and tulumba also contained higher levels of acrylamide (Ölmez *et al.*, 2008).

Studies coming from China showed much higher values of acrylamide content in potato crisps (3,016 µg/kg), but also notable contents in nuts, such as roasted hazel (357 µg/kg), roasted walnut (208 µg/kg) and stir-fried chestnuts (196 µg/kg). Dried mushrooms and spices also contain high amount of acrylamide (Chen *et al.*, 2008).

When the exposure of Polish population to dietary acrylamide was evaluated, researchers (Mojska *et al*., 2010) found that potato chips and French fries were very close to the average values reported for EU. Crisp bread and salty sticks were also found to have high acrylamide content.

2.17. DIETARY ACRYLAMIDE EXPOSURE AMONG DIFFERENT AGE GROUPS.

Assessment of the Polish population exposure was made considering 3 categories: children between 1–6 years old, children and adolescents between 7–18 years old and adults between 19–96 years old and different dietary habits for each category. A disturbing conclusion that resulted from this study was that decisively much higher acrylamide intake per kg of bodyweight was found in the group of children and adolescents (Mojska *et al*., 2010).

2.18.0 METHODS APPLIED TO MITIGATE THE PRESENCE OF ACRYLAMIDE IN FOOD

Understanding the consequences of acrylamide presence in food and the importance of its precursors, the Confederation of the European Food and Drink Industry (CIAA, 2006) has developed the Toolbox approach.

Both asparagine and sugars are not only important and desirable nutrients, naturally present in many foods, they are also important for plant growth and development. In most foods, they cannot be considered in isolation, since they are part of the highly complex chemical composition and metabolism of food plants.

The Maillard reaction depends on the presence of a mixture of these common food components to provide the characteristic flavour, colour and texture of a given product. Thus, most of the Maillard reaction products are highly desirable.

2.18.1. Agronomical factors

Effects of nutrient availability during cultivation can influence free asparagines accumulation in many plant species by the multiplicity of stresses, including exposure to toxic metals such as cadmium, pathogen attack, and drought or salt stress. It can also be induced in many plant species as response to nutrient availability, with different nutrients having contrasting elects (Muttucumaru *et al*., 2008). Free asparagines can accumulate when there is a plentiful supply of reduced nitrogen and low rates of protein synthesis.

Sulphur deficiency leads to asparagines accumulation in cereal grains. For wheat cultivation there are recommendations that producers should follow in order to mitigate acrylamide presence, such as: – avoiding sulphur, phosphate and potassium deficiency; – soil sulphur content should be at least 15 kg per hectare; – nitrogen feeding increases acrylamide risks as the excess is stored as free asparagines in plant. In potatoes the relationship between free asparagines, sugars and acrylamide is more complicated, but some recommendations for cultivators are: – control sulphur and nitrogen feeding that can increase acrylamide risks; – changes in amino acid profile in response to feeding is highly variety-depending; – storage at 4°C must be avoided because it causes rapid accumulation of sugars; – advice to growers should be related to the soil used and selected variety.

2.18.2. Biotechnological factors

The use of biotechnological processes to determine precursor consumption is seen as a powerful method to limit acrylamide presence in food. Such tools are represented mainly by fermentation and asparaginase pre-treatments (Anese *et al*., 2009).

Fermentation of dough or potatoes favours kinetic control of the rate of acrylamide formation, controlling also precursors consumption, as well as pH. Fermentation time has a strong impact on acrylamide levels (Claus *et al*., 2008) and fermenting yeasts consumed high amounts of free asparagine, leading to a decay of 60 % and 90% in the precursors of acrylamide in cereal products. Therefore, prolongation of the fermentation time to at least one hour was found to be appropriate for acrylamide reduction in bread making (Fredriksson *et al.*, 2006). Lacto-fermentation used for preparation of sourdough has been assessed and it could be noticed that a decrease in acrylamide by 95% was obtained when a fermentation step using lactic acid bacteria NCIMB 40450 was applied (Baardseth *et al*., 2006).

However, lactic acid bacteria seems to have a negative impact on yeast fermentation, which might lead to elevated acrylamide levels in bread produced with sourdough, as compared with ones with yeast (Claus *et al*., 2008). Lactic acid fermentation of potatoes before deep- frying lowered the acrylamide content from 48 % to 78 % in the end product (Anese *et al*., 2009; Baardseth *et al*., 2006). Combination of lactic fermentation with blanching would lead to even higher reduction of acrylamide levels (79–94 % less acrylamide) or the addition of 0.05 M glycine to the incubation medium (80% less acrylamide) (Anese *et al*., 2009; Baardseth *et al*., 2006).

2.18.3. Additives

The use of asparaginase to attain asparagines consumption was suggested to control acrylamide levels. Asparagine is hydrolyzed to aspartic acid, thus inhibiting acrylamide generation in the Maillard reaction.

When enzyme was added to crackers, acrylamide levels decreased by at least 70 %, and no changes in colour or flavour of the products could be noticed (Vass *et al*., 2004). In a series of experiments at industrial level made by Dobrogea Group Corporation of Romania, acrylamide level in biscuits proved to decrease from 400 µg/kg to 170 µg/kg for the trials with asparaginase, while for breakfast cereals, the registered detection limit and the low values of acrylamide did not allow to see the same reduction.

This very promising way of reducing acrylamide levels has as a main disadvantage the high price for asparaginase, that prevent it to be used in low price foods such as bread. Asparaginase based on cloning of Aspergillus oryzae has been very recently developed and was recognised as GRAS (Ciesarová *et al*., 2006). The process of using asparaginase requires optimization of the processing parameters, thus more studies are necessary to model the influence of factors such as time, temperature and water content in industrial processes.

The addition of consumable acids is a very simple but efficient method to reduce acrylamide in bakery products. When increasing amounts of citric acid (0.1 % or 0.2 %) were added to bake corn chips, acrylamide decreased almost linearly without any impairment on taste of flavour (Jung *et al*., 2003). Similar effects were reported for lactic, tartaric and hydrochloric acids added to biscuits (Graf *et al*., 2006).

The mitigation of acrylamide by adding amino acids or protein-based ingredients was recently investigated. Brathen *et al*. (2005) added glycine to dough prior fermentation and a high rate of (80 %) reduction was achieved in flat bread.

In potatoes, derivates depending on the applied processing conditions and the amount of added glycine (0.3–1 % w/w) registered a reduction ranging from 30 to 90 % (Rydberg *et al*., 2003; Kim *et al*., 2005). Cookies formulations added with 2.5 to 5 % protein-based ingredient had 65–75% less acryl- amide than the control sample (Anese *et al*., 2009). However, higher dairy protein content (10 %) was less effective in lowering acrylamide formation, maybe due to asparagines residues from caseinates (Lingnert *et al*., 2002).

A combination of soy protein and glycine had a synergistic effect on acrylamide reduction (Cook and Taylor, 2005). Adding divalent cations such as Ca^{2+} or Mg^{2+} to dough had a strong effect on acrylamide content. Elder *et al*. (2004) reported an almost 20% reduction in acrylamide when these ions were added and a decrease of 50 % when slightly acidic conditions (pH = 5.5) were present. Gokmen and Senyuva (2007) reported a negative correlation between acrylamide formation and added Ca^{2+} concentration in potato products.

Studies on the effect of NaCl in crackers and wheat bread have shown that relatively low concentrations of Na^{+} (1–2 % w/w) decreased acrylamide formation, whereas at higher level of salt the concentration was increased (Levine and Smith, 2005).

2.18.4. The use of antioxidants

Contradictory results were published on the impact of different types of antioxidants on acrylamide formation. Tareke *et al*. (2003) found that the addition of Butylated hydroxytoluene, sesmol and Vitamin E to meat prior heating enhanced the formation of acrylamide, by protecting acrylamide. Enhanced formation of acrylamide was observed in potato slices soaked in 1% w/v cranberry and Oregano before frying (Vattem and Shetty, 2003), but decreased acrylamide formation was obtained when there were added rosemary extracts to oil (Taeymans *et al*., 2004).

Zhang *et al.* (2005, 2008) demonstrated that the addition of bamboo leaves antioxidant (AOB) and green tea extract could effectively reduce acrylamide presence in different heated foods. AOB proved to be effective in different combinations, with other plant extracts such as ginkgo biloba, grape seeds extracts, etc.

2.18.5 Conventional process parameters

The important factors that influence the process of acrylamide formation are: heating temperature and time, relative humidity and heat transfer. Many invitations were directed towards clarifying the temperature-time influence on acrylamide formation and researchers' contributions (Vass *et al*., 2004; Lukac *et al*., 2007) revealed that it was obvious that proper choice of temperature and time could act actively to prevent acrylamide formation.

In addition, the water activity influences acrylamide formation. Keeping the relative humidity high during baking proved to be effective in reducing acrylamide levels in bakery products (Ahrné *et al*., 2007; Vleeschouwer *et al*., 2007). This can be achieved not only by reducing the temperature, but also by injecting steam during baking. Heat transfer based on conduction and radiation was more effective in acrylamide reduction than convection ovens.

2.18.6 Non-conventional processes to reduce acrylamide

Few non-conventional processes have been investigated to minimize acrylamide formations in potato and cereal derivates (Granda *et al*. 2004; Erdoğdu and Sahmurat, 2007; Anese *et al*., 2009). The application of a microwave precooking step resulted in a very effective reduction of acrylamide formation (Erdoğdu and Sahmurat, 2007) Low temperature vacuum frying was described by Granda *et al*. (2004) in order to reduce concentration of acrylamide up to 95% without significant changes of colour and texture attributes. Combination of conventional and dielectric heating led to a reduction of acrylamide formation in bakery products (Anese *et al*., 2009). The radiofrequency-assisted baking process can be used for improving heat transfer and control moisture, which would also impact acrylamide reduction (Anese *et al*., 2010).

2.18.7 Reduction of acrylamide levels in food

In order to control acrylamide presence in foods, different strategies could be adopted. Following the CIAA "toolbox" recommendations, producers can identify the basic methods to reduce acrylamide in their processes. However, the fast scientific advancements of the methods to mitigate acrylamide presence impose constant re-evaluation of the level of knowledge and decide if new ways of acrylamide reduction should be applied. Moreover, pilot and plant scale researches are needed for the most promising methods to be confirmed in different food matrixes or processing conditions.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

The bread samples *(brown bread, tea bread, sugar bread, butter bread*) were obtained from bread vendors in the O*forikrom sub-metro*, Kumasi and stored in plastic bags and then kept in a refrigerator prior to analysis. Carrez I and II solutions, n-hexane and Methanol used for extraction and analyses were purchased from Sigma Aldrich Chemicals (UK).

3.2 METHODS

3.2.1 Study population

A total of 300 school going children of both genders, aged 5 to 19 years old were recruited in the general population of the Oforikrom Sub-metro, Kumasi-Ghana. This study was conducted between 20th December, 2013 and 30th March, 2014.

3.2.2 Study sampling and participation

A list of Primary, Junior High School and Senior High School in the Oforikrom sub-metro totaling 245 schools was obtained from the Ghana Education Service. Seventy (70) schools representing 33.33 % of the total number of schools in the *Oforikrom* sub-metro were randomly selected for this study. Rationale for the study was explained to the head teachers. After giving their consent, participants were selected to undertake the study. Those who were spontaneously interested in participating in the study were made to fill a self-administered questionnaire designed to obtain socio-demographic information as well as food consumption data. A minimum of three children were recruited per school. A total of 300 children were randomly recruited from the 70 selected schools in the *Oforikrom* sub-metro, Kumasi.

3.2.3 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 questionnaire required that, participants describe the types and amounts of selected carbohydrate rich foods processed at high temperatures and suspected to contain acrylamide which is usually seen to be consumed among children especially, those in school. The list of foods provided in the questionnaire for participants to choose from were; *fried yam, plantain chips, potato chips, Indomie, cocoyam chips, pop corn, fried rice, roasted plantain, roasted cookies, roasted yam, cheese balls, digestive biscuit, soda biscuit, chocolate coated biscuit, bread, corn flakes, snappy snack, wafer (jack n jill), groundnut, chocolate drink, malt and tom brown*).

The children were made to give information on specific foods among the list of foods provided in the questionnaire which they have consumed within 24 h from the time the questionnaire was administered to them. They were also supposed to provide the number of portions of specific foods they choose as well as the number of times they consumed per day. Real food models of standardized portions were used to help participants to better describe amounts weight. The questionnaire also required participants to provide sociodemographic data on level of education, household number, body weight and height and their ages. The actual weights and heights of the school children were measured. The questionnaire was pre-tested before the study.

3.2.4 Selection of most frequently consumed food based on data provided by the children

The database of the data obtained from the 300 participants were imported into Epi-info 7 (2013) and the food consumption was analyzed using frequencies and percentages. The food with the highest percentages was taken to be the most consumed food by respondents.

3.2.5 Food sampling and preparation

Sampling was done for the food identified to be the most often consumed by the participants, on the basis of response to the questionnaires. Since levels of acrylamide can vary considerably in the same product, the food sampling plan took into consideration both the types of consumed foods and variability within a batch and between batches. Thus, a total of 100 samples of different types of selected most commonly consumed food (*brown bread, tea bread, sugar bread, butter bread*) were randomly purchased irrespective of the type at supermarkets, minimarkets, shops, school canteens and cafeterias in the *Oforikrom* sub-metro, Kumasi. The samples were coded 1-100 irrespective of the type. Two grams of each coded sample was transferred into 50 mL Falcon tubes. Subsequently, 20 mL of extraction solution (18 mL of 1:1 distilled water: methanol + 1mL Carrez I Solution + 1mL Carrez II Solution) was added to the test samples. Additional 15 mL of hexane was added and the resulting mixture vortex for 15 s. The mixture was agitated at 250 rpm for 30 min and further centrifuged at 4000 rpm for 15 min. The aqueous phase was further filtered via micro-filters.

3.2.6 Determination of acrylamide

Standard calibration curve was used to determine the concentration of acrylamide in the samples. Standard solutions of 0.02, 0.04, 0.08 and 0.32 µg/ml of acrylamide was prepared from a stock solution of 2 µg/ml and measured using Shimadzu UV-Vis (Model =UVmini-1240). The spectrophotometer was "zero-ed" using the blank before standards and samples were measured. A calibration curve was established by plotting absorbance against the corresponding concentration. Using the equation of the calibration curve, the acrylamide in test samples were determined. Determinations were done in triplicates.

3.2.7 Dietary acrylamide intake and risk Assessment

Modal food was obtained from data collected based on the 24 h food frequency questionnaire using Epi-info 7 (2013) designed by the Center for Disease Control (CDC), USA.

3.3 DATA ANALYSIS

Acrylamide content (CW)

Values of the acrylamide content obtained from the chemical analysis of the 100 test samples were loaded into the decision tool by Palisade Corporation (2013) (@ Risk *version 6.2)* and fitted to a *Log logistic* distribution with fitting parameters -0.024911, 0.39142, and 3.5652. The distribution was then defined with the said parameters and a first order Monte Carlo simulation was run.

Number of times of consumption of bread per day: From the general pattern of consumption of bread by participants used in this study, it was obtained from the 24 h food frequency questionnaire that, consumption rate ranged between a minimum of 0 (number of consumption of bread per day) and a maximum of 3 times per day. Therefore, a histogram distribution was defined in favour of number of times of consumption of bread per day with distribution parameters 0 for minimum and 3 for maximum with p table values 0.72, 85.51, 13.04, 0.72 and subsequently simulated using a first order Monte Carlo for 10,000 iterations.

Mass of bread per day (M): According to data obtained from food frequency questionnaire, mass of bread taken per day by participants used in this study was in the range of 111-176 g. Therefore, a uniform distribution was defined using a minimum distribution value of 111 g and a maximum value of 176 g of bread which was subsequently simulated using a first order Monte Carlo for 10,000 iterations.

Ingestion rate (IR): The ingestion rate was calculated as the product of the simulated values obtained for the number of times of consumption of bread and the mass of bread taken per day.

Exposure frequency and exposure duration (EF and ED): The exposure frequency is the number of times individuals are exposed to the bread. This was obtained by fairly assuming that children in the Oforikrom sub-metro consumed bread daily all year round (365 days). Therefore, the exposure duration was for one year risk determination.

Body weight (BW): Data obtained from the survey showed that, weights of participants used in the study was in the range of 20-60 kg. A histogram distribution was defined with a minimum parameter of 20 kg and a maximum parameter of 60 kg with p-value parameters 4.67, 14.33, 18.33, 30.67. The distribution was then simulated using a first order Monte Carlo simulation at 10, 000 iterations.

Chronic daily intake (CDI*):* Chronic daily intake (CDI) is computed using the equation 1 (Hans *et al*, 2003).

$$CDI = \frac{CW \times IR \times EF \times ED}{BW \times AT} \qquad (1)$$

where; CW = *concentration of acrylamide, IR = ingestion rate, EF = exposure frequency*, ED= *exposure duration, BW = body weight, AT = average time of exposure*

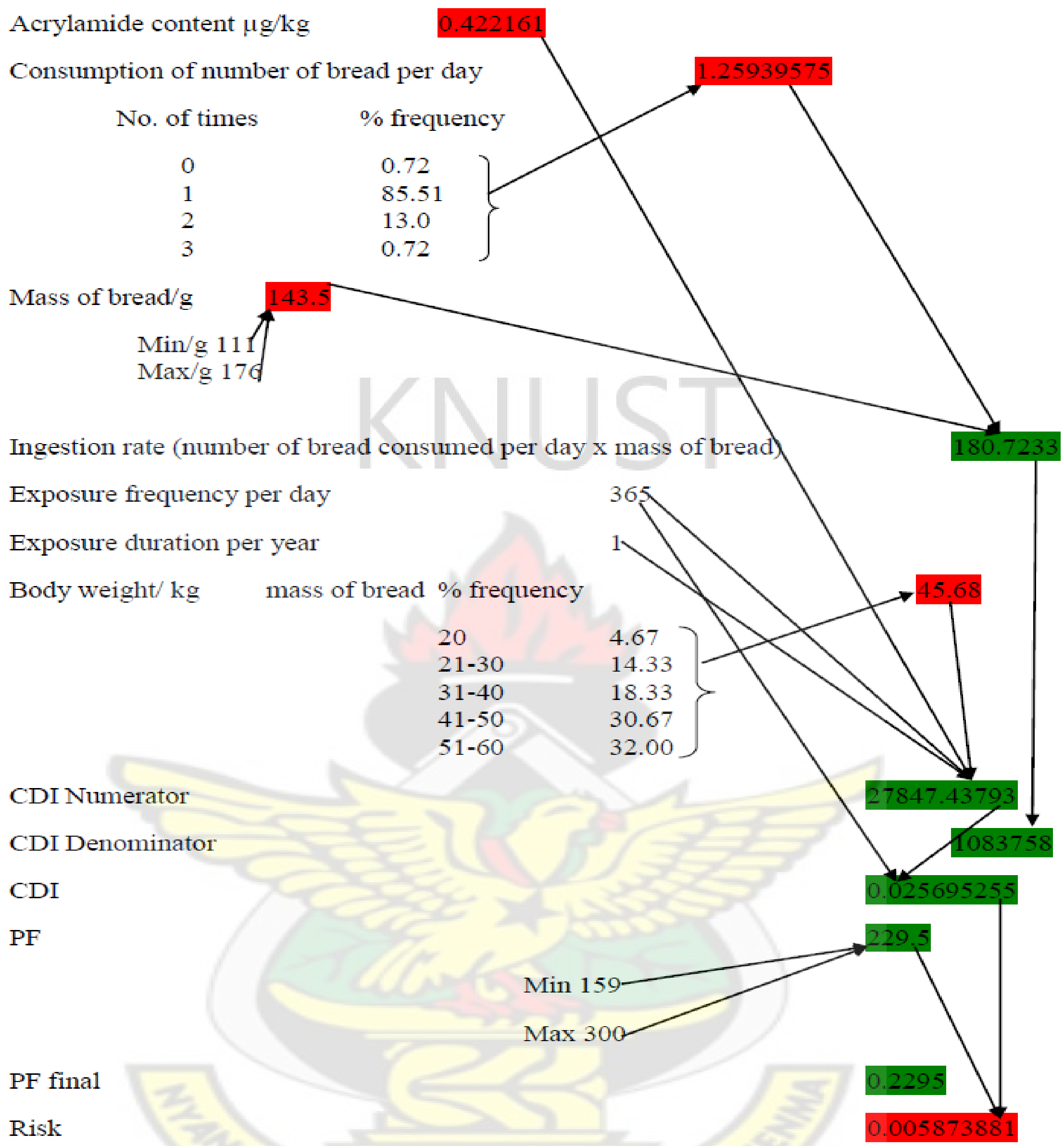

Figure 3.1: Detailed representation of various lifetime risk calculation parameters using the decision tool by Palisade Corporation (2013) (@ Risk *version 6.2)*

Potency factor (PF*)*: The potency factor (PF) is usually the slope of the dose-response curve of acrylamide in a primary collection of the dose response data. The PF of acrylamide as was used in this research was derived from the Integrated Risk Information System Database of the United States Environmental Protection Agency (USEPA, 2001).

According to the database, a range of 159-300 was quoted as PF for rodents. Therefore, a uniform distribution was defined using a minimum of 159 and maximum of 300. It was then simulated with a Monte Carlo first order simulation for 10, 000 iterations.

Final potency factor (PF Final): The final PF was derived when a safety factor of 10^3 was used to convert a rodent based PF of acrylamide to a human based system. It is explained as 10 from conversion from rodents to humans, 10 from differences in behavior and another 10 due to the highly risked chemical being analyzed (10 x 10 x 10). A Monte Carlo simulation was then run in favour of the PF final obtained.

Risk calculation: A reference dose of 2.0×10^{-4} was used for this work (USEPA, 2001). Thus, it is assumed that 2.0×10^{-4} 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;

$$\text{Risk} = \text{PF} \times [\text{CDI}-(2.0 \times 10^{-4})] \qquad (2)$$

A Monte Carlo simulation was also run in favour of the risk calculated for 10, 000 iterations.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1. POPULATION DESCRIPTION

A summary of the sociodemographic characteristics of the participants is shown in Appendix 4. The highest number of participants was from Junior High School representing approximately 61% whilst the least number of participants were from Senior High School representing approximately 7% of the total number of participants. The number of participants at the various levels of education was not the same since participants were randomly recruited for the study. That is, there was not a specific pattern for their selection.

The highest body weight among participants was 28.18 kg and the highest body weight among participants was 58.75 kg.

4.2. FREQUENTLY CONSUMED FOOD

It can be observed from the yes(Y) column of table 3 (Appendix5) and figure 4.2 that, from descending order, the participants representing approximately 44 % ate bread, 30 % ate fried yam, 13% ate groundnut, 10 % each for fried rice and chocolate drink, 9 % each ate soda biscuit, digestive biscuit and roasted cookies, 8 % ate *Indomie*, 7 % each ate tom brown, chocolate coated biscuit, popcorn and roasted plantain, 6 % each ate roasted yam and malt, 4 % ate cornflakes, 3 % ate snappy snack and 3 % ate snappy snack. Since the highest percentage of the participants representing 44 % of the responded that they ate bread than any of the food items in the questionnaire, the most common food which is eaten among the participants was chosen as bread.

In addition, 1 % responded that, they ate cocoyam chips, meaning that cocoyam chips were not commonly eaten among the children. They responded that they have consumed more bread within 24 h from the time the questionnaire was administered to them.

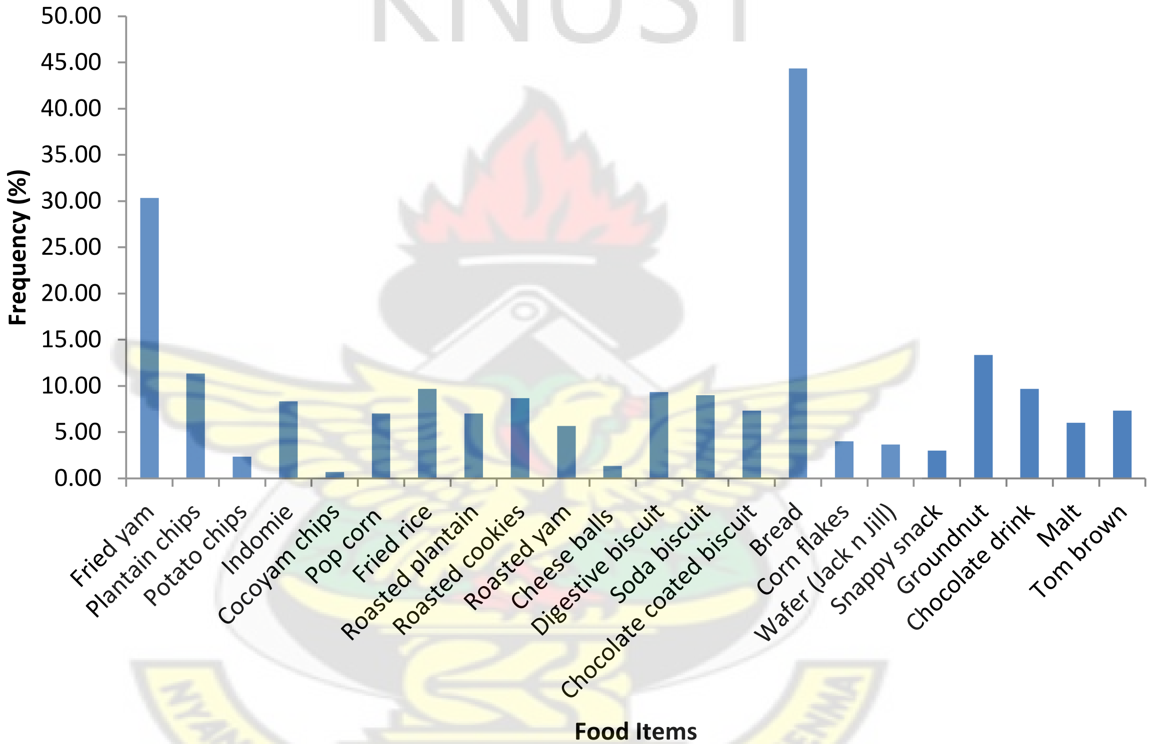

Figure 4.1: Distribution of participants' preference to food items suspected to contain acrylamide in the 24 h recall questionnaire

The selection of bread by the participants as the most common food they eat may be due to the fact that, most school going children eat bread as part of their breakfast at home or their lunch boxes are packed with either a slice or more of bread. Even on the school premises, children who didn't eat breakfast at home happens to get their breakfast complimented with bread from the school's cafeteria.

Finally, bread happens to be among the cheapest, readily available and ready to eat commodities one can lay hands on in almost every Ghanaian community.

4.3 STATISTICAL RESULTS

4.3.1 Acrylamide levels in bread

At a significance level of 0.05, the mean acrylamide content in the bread is 0.423 µg/g (423 µg/kg). We can conclude that bread consumed by these school going children contains an average acrylamide content of 423 µg/kg. From Appendix 7, it can be observed that at most times, bread from the study area has; minimum acrylamide content (5^{th} percentile) of 0.15 µg/g (150 µg/kg) and maximum acrylamide content (95^{th} percentile) of 0.87 µg/g (870 µg/kg). The median acrylamide content was 366.5 µg/kg and a modal acrylamide content of 314.26 µg/kg.

The high levels of acrylamide in the bread confirms the results from JECFA'S studies in the year 2011 on different food groups from 24 countries which named bread and its products among the major foods that contribute to high levels of acrylamide (87- 459 µg/kg).

However the mean acrylamide content (423 µg/kg) in the bread used in this study is lower than that reported by the JECFA, 2011 studies for other baked foods like biscuits (159 – 963 µg/kg). The variation between the acrylamide content of the bread consumed in the study area and that of the Western countries could be due to several factors such as variability in agronomy of the wheat used, the baking time, baking temperature, formulation, the packaging storage, type of additives used just to mention a few.

In addition, the high level of acrylamide could also be due to the fact that all the bread samples were not obtained from the same vendor and bakery within the study area resulting in variability in their acrylamide concentrations. That is, even within the Oforikrom sub-metro, different bakeries may apply different formulation methods, baking temperature and time. High temperature and long baking time have been proven to cause a high concentration of acrylamide in bread (JECFA, 2006).

Finally, the high acrylamide distribution in the samples could be attributed to the fact that the current study did not consider individual bread types but focused on bread in general therefore samples were coded randomly irrespective of the type of bread prior to extraction. That is, the probability of variability in acrylamide levels between the bread types.

4.3.2. Daily dietary acrylamide intake (ingestion rate per day)

The mean daily acrylamide intake among the children was 180.83 µg/kg/day. That is, it can be said that, at a significant level of 5%, the children consume an average of 180.83 ug/kg bw/day of acrylamide per body mass per day. Appendix 7 gives the minimum ingestion rate per day (5^{th} percentile) as 83 ug/kg bw/day and the maximum ingestion rate (95^{th} percentile) as 347 ug//kg/day at most times. The median ingestion rate (50^{th}

percentile) was 166.00 ug/kg bw/day whilst the mode was 155.99 ug/kg bw/day. The median ingestion rate of 166.00 ug/kg bw/day indicates that, the acrylamide intake among 50% of the children is 166.00 ug/kg bw/day.

The dietary intake of acrylamide recorded in this study is far higher than that estimated by FAO/WHO (0.3 - 0.8 µg /kg bw/day) for the general population (Petersen, 2002).

Although other studies in other countries have recorded higher acrylamide exposure rates among children, infants and teenagers, the dietary intake (83-347 µg/kg bodyweight/day) in this study still remains higher. For example, in the Netherlands children and teenagers have a higher exposure rate (Konings *et al.*, 2003). Fohgelberg *et al.* (2005) estimated the acrylamide intake for Swedish infants in their first year of life to be in the range 0.04-1.2 µg/kg bodyweight/day based on analyses of breast milk and infant formulae. In Germany, bread accounts for about 18-46 % of acrylamide intake due to the high consumption (Hilbig *et al.*, 2004). In the Netherlands, the mean acrylamide exposure to children and teenagers is in the order of 0.48 µg/kg bw/day (Konings *et al.*, 2003).

Acrylamide is genotoxic (mutagenic), which increases the incidence of cancer in rats at doses of 1–2 mg/kg bw/day. In this study, the maximum acrylamide intake (0.52 mg/kg/day) 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 when it was reported by Tareke *et al.* (2002) who isolated and characterized acrylamide in heated foodstuffs.

The high acrylamide intake recorded in this study may be attributed to the baking temperature and time. In addition, the differences in the agronomy (sugars and asparagines), processing, final preparation and handling of bread may be the other factors for the higher level of acrylamide intake.

4.3.3 Mass of bread

Appendix 7 explains that, at a 90 % confidence interval, the mass of medium sized bread range between 114.2 g and 172.7 g. The mean mass (g) of medium sized bread per day is 144 g. That is on the average, a child consumes 143.5 g of bread per day. The average consumer consumes approximately 143.5 g of bread per day, whiles at most times, the minimum mass (5^{th} percentile) of a medium sized bread is 114.2 g and the maximum mass (95^{th} percentile) of a medium sized bread is 172.7 g. The median and modal acrylamide recorded were 143.51 g and 145.13 g respectively.

4.3.4 Body weight of the children

The minimum body weight of the children was approximately 28.1 kg whilst the maximum body weight was approximately 58.75 kg (Appendix 7.). The most likely weight among the participants was 52.13 kg, mean body weight was 45.68 kg and median weight was 47.30 kg. A body weight of 28.18 kg was recorded for the participant with the least body weight (5^{th} percentile) while the highest body weight (95^{th} percentile) among the children was 58.75 kg.

4.3.5 Number of bread per day

From Appendix 7, at a 90 % confidence interval, it can be said that, the number of bread consumed by a child per day range between 0.588 and 2.411. The mean number of medium sized bread consumed by a child per day is 1.2594 which is approximately, 1. That is, we are 90 % confident that at most times, a child consumes a minimum of approximately 1 portions of bread per day and a maximum of 2 portions of bread per day. On the average, a child consumed 1 portions of 143.5 g sized bread per day.

4.3.6 Chronic daily acrylamide intake (CDI)

The mean CDI of acrylamide per child is 0.0272 μg/kg/day, median CDI is 0.0212 μg/kg/day and modal CDI of 0.0141 μg/kg/day (Appendix 7). That is, within the children population the mean CDI of acrylamide is 0.0272 mg/kg/day at most times.

However, the CDI for the 95th percentile consumer is 0.0680 μg/kg/day and the 5th percentile consumer is 0.0055 μg/kg/day. It can further be explained that, the concentration of acrylamide that a child must ingest per day in order to stand a probable risk of getting cancer within 1 year is 0.0272 μg/kg/day for the average consumer, 0.0055 μg/kg/day for the 5th percentile consumer and 0.0680 μg/kg/day for the 95th percentile consumer.

4.3.7 Final potency factor (PF final)

The mean, modal and median PF final obtained from the distribution shown in Appendix 7 were 0.2295, 0.1907 and 0.2295 respectively. The PF final obtained for the lower consumer of acrylamide (5th percentile) was 0.1660 and that for the higher consumer (95th percentile) was 0.2929. That is at most times the PF final was between 0.1660 and 0.2929.

4.3.8. Dietary acrylamide risk

The study resulted in a mean, median, and modal acrylamide risk of 6,200, 4,718 and 3,624 respectively (Figure 4.2). At 90 % confidence interval, the probability of a child to the risk of carcinogenicity due to the eating of bread is between 1,169 and 15,766. That is, at most times, approximately 1 out of every 1000 children who eat bread stand a minimum probable risk of carcinogenicity in a year whiles 15 out of 10000 children who ingest bread stands a maximum risk of carcinogenicity in year.

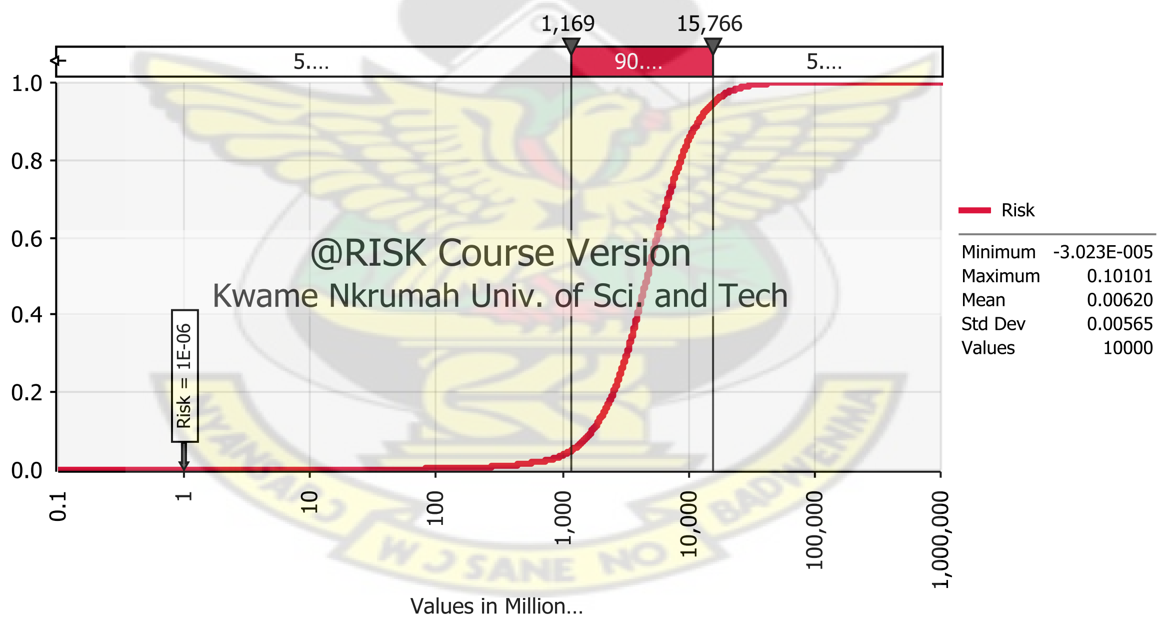

Figure 4.2: Daily consumption of bread and its acrylamide risk after Monte Carlo simulation over 10,000 iterations

From figure 4.2, it is clear that this study recorded a risk which is far beyond the benchmark dose (10^{-6}). These results are different from that reported by Dybing *et al.* (2003) which concluded that a lifetime risk to cancer from the consumption of acrylamide was 6 out of 10,000. The difference in the results may be due to differences in assessment methods as well as variability in acrylamide contents of bread samples used in this study.

This result is also higher than the internationally accepted risk for cancer which is 1 out of 1,000,000 persons as reported in 2003 by Hans *et al*. and WHO (2011).

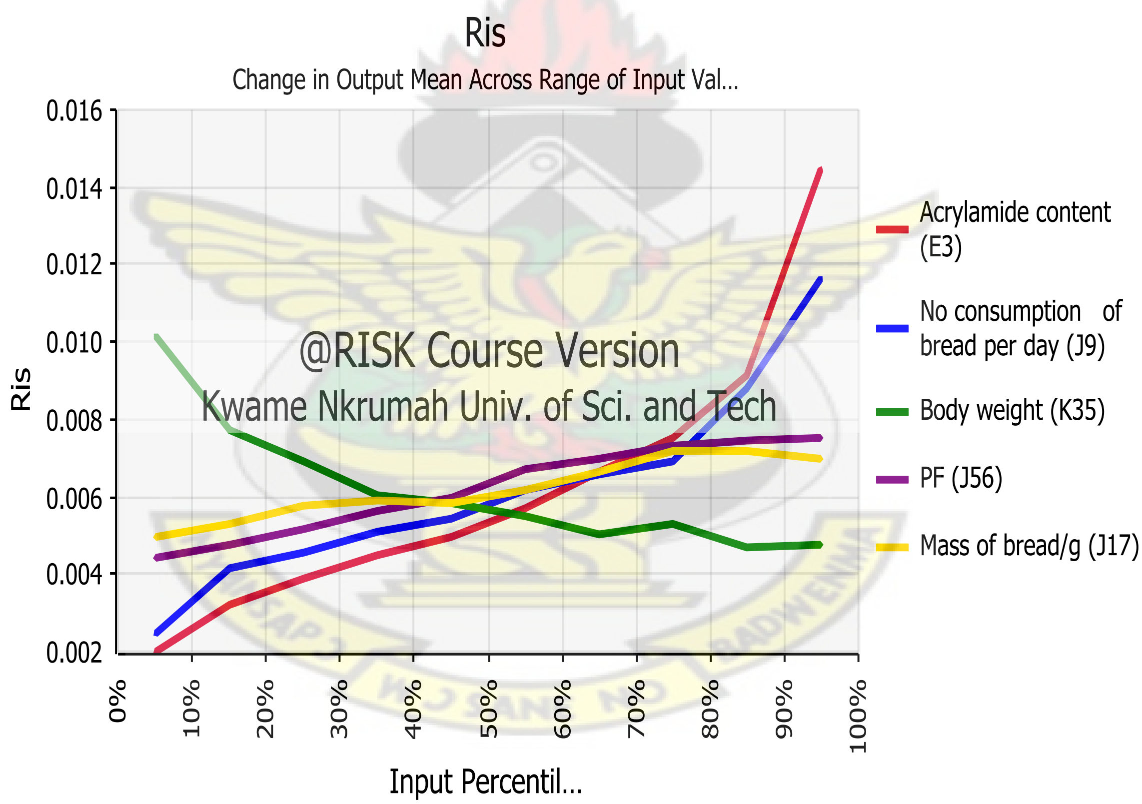

Figure 4.3: Changes in children's risk to cancer across range of input percentiles; exposure to acrylamide, body weight, number of consumption of bread per day and mass of bread consumed per day.

4.3.9. Correlation between risk and input parameters

Correlation between acrylamide and risk: Figure 4.3 gives a summary of the correlation between acrylamide content and its risk. It can be observed that before the 90th percentile, the risk is gradual and even becomes constant right after the 60th percentile and before the 90th percentile. There is a great increase in risk just before and after the 90th percentile. From this result, it can be said that, as the acrylamide content in bread increases, a consumers risk to cancer also increases. That is the higher consumer will tend to consume more acrylamide and have a higher probable risk to cancer.

Correlation between number of bread consumed per day and risk: From Figure 4.3, there is gradual increase in risk for consumers of bread before the 90th percentile. The risk tends to increase steadily right before the 90th percentile as the number of bread consumed by a child increases the risk also increases. That is an increase in risk of persons who consume large number of bread per day than people who consume fewer amounts per day as well as those who consume in moderation. This confirms the saying that "every substance is poisonous but its effect can be reduced if taken in moderation".

Correlation between body weight and risk: As the body weight of children increases, risk decreases (Figure 4.3). This means that, children with larger body weight who consume bread from the study area have a lesser risk of getting probable cancer than those with small body weight. This assessment differs from the report by Konings *et al.* (2003) that, Netherlands children and infants have high acrylamide exposure rates.

Correlation between number of bread consumption per day and risk: There is gradual increase in risk for consumers of bread before the 90th percentile (Figure 4.3). The risk tends to increase steadily right before the 90th percentile as the number of bread consumed by a child increases the risk also increases. That is here fast increase in carcinogenic risk of persons who consume large number of bread per day than people who consume fewer amounts per day as well as those who consume in moderation.

Correlation between potency factor and risk: As the potency factor increases a child's probable risk of carcinogenicity increases (Figure 4.3). This means that, the potency factor of lower and average consumers of bread is low and hence such consumers stands less risk of getting cancer from the consumption of bread than the 95th percentile.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

In conclusion, this study showed that bread, a food commonly consumed among Ghanaian children in the Oforikrom sub-metro contained a modal acrylamide content 314.30 µg/kg. This high level of acrylamide indicates that bread contributes to the acrylamide exposure of children.

In addition, the estimated mean total dietary intake of acrylamide in children, from the consumption of bread based on the food frequency questionnaire is higher than other studies.

The estimated risk value shows that consumers of bread from the Oforikrom sub-metro are at a greater health risk of carcinogenicity. Thus, the probability of 50% of children who consume bread obtained from the Oforikrom Sub-metro to the risk of carcinogenicity within a year is 5 out of every 1000 children. This is a serious issue which calls for great and immediate public health concern since this value (4×10^{-3}) is far higher than the recommended WHO cancer risk of 10^{-6}.

5.2 RECOMMENDATIONS

It is recommended that further work be done in the same study area which will focus on the effects of varying temperature and time on the acrylamide content of bread from various bakeries which supply bread to the *Oforikrom* Sub-Metro.

On the basis of the current results, efforts should be pursued by parents, children and bakers to reduce exposure to acrylamide by both changing food habits and lowering contents of bread consumed especially by children.

The regulatory bodies like the Food and Drug Authority must educate the public on dietary acrylamide as a food toxicant even in bread and train the bakery industry on the formulation and processing methods that will reduce the level of acrylamide in bread.

Finally, the risk results of this study calls for the concern of government and public health officials to put in measures on how to reduce the risk of consumers of bread to cancer.

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APPENDIX

APPENDIX 1: The schematic representation sample extraction is shown in the figure below;

2.0g of homogenized sample in 50ml of Falcon tubes

↓

Add 18ml of DI Water+ 1ml Carrez I Solution + 1mlCarrez II Solution

Carrez I solution = 15 g of potassiumhexacyanoferrate in 100 mL of water

Carrez II solution = 30 g of zinc sulphate in 100 mL of water.

↓

Add 15ml of Hexane

↓

Vortex for 15 – 30sec

↓

Shaker for 30mins agitation @ 2500rpm etc)

↓

Centrifuge

(4000rpm for 15mins)

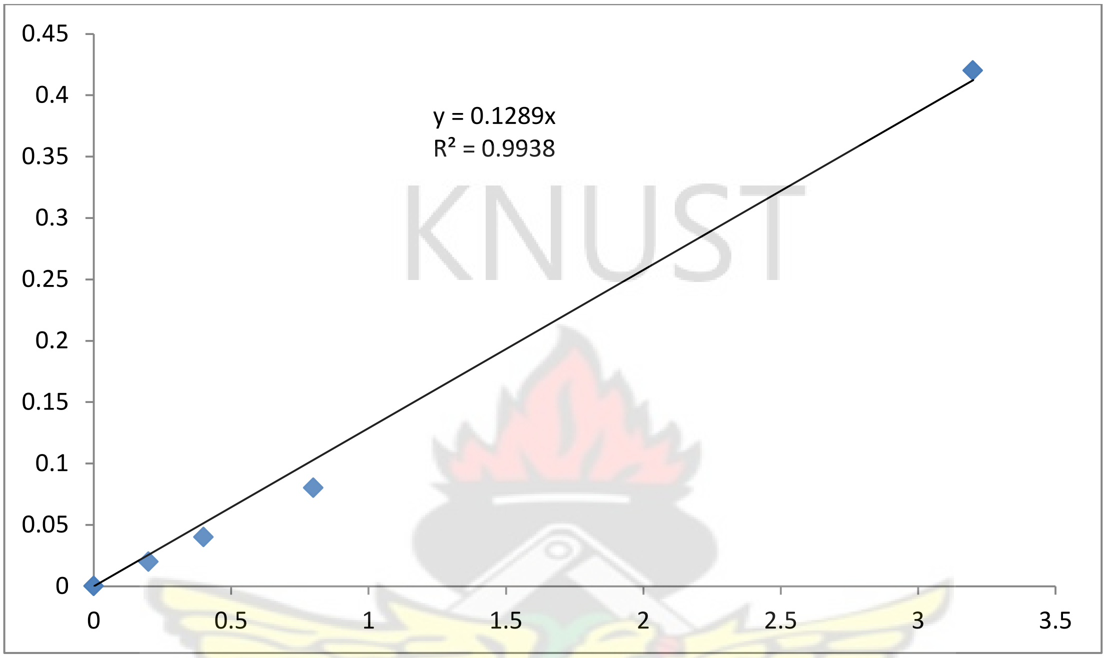
↓

Filter aqueous phase via micro-filters

↓

Read using Scanning UV-Vis Spec (@ 285nm)

APPENDIX 2. ACRYLAMIDE STANDARD CURVE FOR DETERMINING THE CONCENTRATION OF ACRYLAMIDE CONCENTRATION IN SAMPLED FOOD



APPENDIX 3. CONCENTRATION OF ACRYLAMIDE IN SAMPLED FOOD

Table 2 : The Results of Acrylamide concentration(mg/g) in the bread Samples

Sample ID	Conc of acrylamide (µg/g)	Sample ID	Conc of acrylamide (µg/g)	Sample ID	Conc of acrylamide (µg/g)	Sample ID	Conc of acrylamide (µg/g)
1	0.54	26	0.58	51	0.25	76	0.35
2	0.34	27	0.51	52	0.21	77	0.52
3	0.49	28	0.62	53	0.59	78	0.27
4	0.61	29	0.71	54	0.30	79	0.21
5	0.39	30	1.54	55	0.30	80	0.25
6	0.39	31	0.64	56	0.16	81	0.28
7	0.44	32	0.72	57	0.30	82	0.49
8	0.39	33	0.54	58	0.26	83	0.42
9	0.38	34	0.13	59	0.39	84	0.22
10	0.43	35	0.34	60	0.63	85	0.12
11	0.43	36	0.21	61	0.33	86	0.17
12	0.46	37	0.23	62	0.30	87	0.33
13	0.35	38	0.51	63	1.02	88	0.34
14	0.44	39	0.37	64	0.18	89	0.25
15	0.57	40	0.20	65	0.42	90	0.34
16	0.34	41	0.25	66	0.63	91	0.29
17	0.55	42	1.43	67	0.36	92	0.17
18	0.46	43	0.03	68	0.30	93	0.25
19	0.51	44	0.47	69	0.34	94	0.16
20	0.62	45	0.50	70	2.12	95	0.36
21	0.48	46	0.50	71	0.30	96	0.38
22	1.01	47	0.27	72	0.31	97	0.15
23	0.46	48	0.37	73	0.23	98	0.49
24	0.27	49	0.11	74	1.69	99	0.41
25	0.39	50	0.40	75	0.27	100	0.21

Mean:0.423854326
Stdev:0.351490212
Variance:0.123545369
Median: 0.36646617

APPENDIX 4. SOCIODEMOGRAPHIC CHARACTERISTCS

Table 3 Sociodemographic characteristcs of participants (n=300)

Characteristics	Number (% Frequency)
Age	
<9	29 (9.67)
9-11	51 (17.00)
12-14	123 (41.00)
15-17	81 (27.00)
18-19	16 (5.33)
Education level	
lower primary	43 (14.33)
upper primary	64 (21.33)
JHS	182 (60.67)
SSS	11(3.67)
Body weight	
<20	14 (4.67)
21-30	43 (14.33)
31-40	55 (18.33)
40-50	92 (30.67)
51-60	96 (32.00)
Height	
< 1.0	5 (1.67)
1.1-1.4	115 (38.33)
1.5-1.8	178 (59.33)
1.9-2.2	2 (0.67)

APPENDIX 5. PARTICIPANTS PREFERENCE OF FOOD

Table 4: Participants Preference Of Food

Food items	Y (% Frequency)	N (% Frequency)
Fried yam	30.33333	69.66667
Plantain chips	11.33333	88.66667
Potato chips	2.333333	97.66667
Indomie	8.333333	91.66667
Cocoyam chips	0.666667	99.33333
Pop corn	7	93
Fried rice	9.666667	90.33333
Roasted plantain	7	93
Roasted cookies	8.666667	91.33333
Roasted yam	5.666667	94.33333
Cheese balls	1.333333	98.66667
Digestive biscuit	9.333333	90.66667
Soda biscuit	9	91
Chocolate coated biscuit	7.333333	92.66667
Bread	44.33333	55.66667
Corn flakes	4	96
Wafer (Jack n Jill)	3.666667	96.33333
Snappy snack	3	97
Groundnut	13.33333	86.66667
Chocolate drink	9.666667	90.33333
Malt	6	94
Tom brown	7.333333	92.66667

APPENDIX 6

Department of Food Science and Technology
College of Science, KNUST

Demographic, Anthropometric and Consumption Survey of Foods Commonly Consumed By School Children

DEMOGRAPHY

1	***What is your religion?***	*None*	*Traditional*	*Muslim*	*Christian*	*Others: specify*			
	Responses →								
2	***What is your level of education?***	*Lower primary*	*Upper primary*	*JHS*	*SSS*				
	Responses →								
3	***How many people do you live with in your house?***	*Alone*	1	2	3	4	5	6	>6
	Responses →								
4	***How many people do you eat with?***	*Alone*	1	2	3	4	5	6	>6
	Responses →								

ANTHROPOMETRY

1	***How old are you?***	*< 9*	*9-11*	*12-14*	15-17	18-19
	Responses →					
2	****What is your height/m?***	*< 1.0*	*1.1-14*	*1.5-1.8*	*1.9-2.2*	*>2.2*
	Responses →					
3	****What is your weight?***	*<20*	*21-30*	*31-40*	*40-50*	*51-60*
	Responses →					

***** *Please measurement would be taken on the scales provided.*

CONSUMPTION DATA

1 ***Since yesterday, which of these food categories did you often eat? Thick appropriate categories.***

Fried foods (such as fried yam, plantain chips, potato chips, fried rice, fried fish/chicken/sausage or others)

Baked foods (such as cookies, meat pie, cake, yam, bread or others)

Roasted foods (roasted plantain, cookies, plantain, yam, malt, coffee, groundnut, tom brown or such others)

Cooked (such as boiled yam, rice(plain rice), boiled potatoes, banku, konkonte or such others)

		Responses				
		Never	1 time	2 times	3 times	>3 times
2	***How often did you eat fried foods?***					
3	***How often did you eat baked foods?***					
4	***How often did you eat roasted foods***					

5. When you ate roasted, baked or fried foods which of these foods did you eat? Thick as many as you have eaten.

Fried yam		Pop corn		Roasted yam		Sandwich		Chocolate drink	
Plantain chips		Fried rice		Cheese balls		Corn flakes		Malt	
Potato chips		Roasted plantain		Digestive biscuit		Wafer (Jack n Jill)		Tom brown	
Indomie		Roasted cookies		Soda biscuit		Snappy snack		Others: specify	
Cocoyam chips		Roasted plantain		Chocolate coated biscuit		Groundnut			

6. Since yesterday, when you ate roasted, baked or fried foods how often did you eat? Thick appropriately.

	Responses				
	Never	1 time	2 times	3 times	>3 times
Fried yam					
Plantain chips					
Potato chips					
Indomie					
Cocoyam chips					
Pop corn					
Fried rice					
Roasted plantain					
Roasted cookies					
Roasted plantain					
Roasted yam					
Cheese balls					
Digestive biscuit					
Soda biscuit					
Chocolate coated biscuit					
Sandwich					
Corn flakes					
Wafer (Jack n Jill)					
Snappy snack					
Groundnut (pea nut)					
Chocolate drink					
Malt					
Tom brown					
Others: specify					

7, When you ate roasted, baked or fried foods below;

a) How much did you eat? *How did you like the colour of the food?*

	Responses							
	Quantity of food			*Colour of food*				
	Small	*Medium*	*Large*	*Yellowish*	*Light brown*	*Brownish*	*Slightly burnt*	*Very burnt*
	Thick appropriately			*Thick appropriately*				
Fried yam								
Plantain chips								
Potato chips								
Indomie								
Cocoyam chips								
Pop corn								
Fried rice								
Roasted plantain								
Roasted cookies								
Roasted plantain								
Roasted yam								
Cheese balls								
Digestive biscuit								
Soda biscuit								
Chocolate coated biscuit								
Sandwich								
Corn flakes								
Wafer (Jack n Jill)								
Snappy snack								
Groundnut (pea nut)								
Chocolate drink								
Malt								
Tom brown								
Others: specify ➡								

8. ***When you ate fried, baked or roasted food, how was the feel in your mouth like the hardness? Thick appropriately.***

	Responses
a. Did it feel like you were eaten banana (very Soft)?	
b. Did it feel like you were chewing jack n jill (soft)?	
c. Did it feel like you were chewing toffee (Crispy)?	
d. Did it feel like you were chewing crackers (hard)	
e. Did it feel like you were chewing bones (Very hard)	

APPENDIX 7: RESULT GRAPHS AFTER MONTE CARLO SIMULATION OVER 10,000 ITERATION

7.1 Acrylamide content

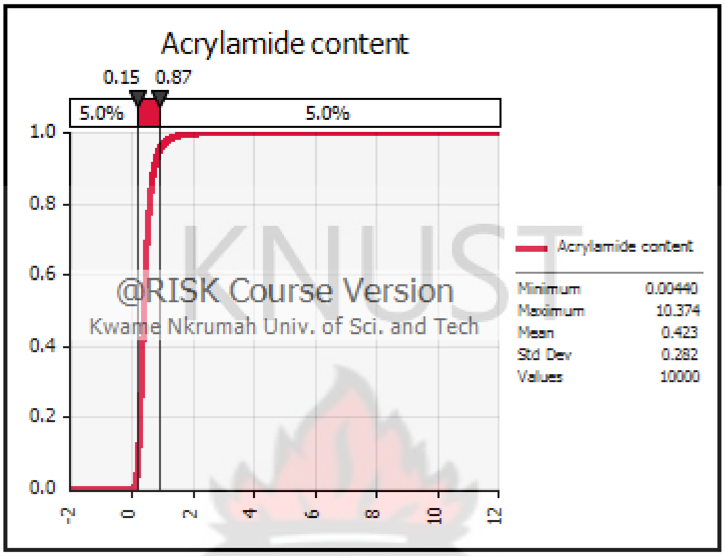


Figure 7.1: log logistic distribution of acrylamide content in bread samples obtained from the oforikrom sub-metro based on a first order Monte Carlo simulation.

7.2 Ingestion rate per day

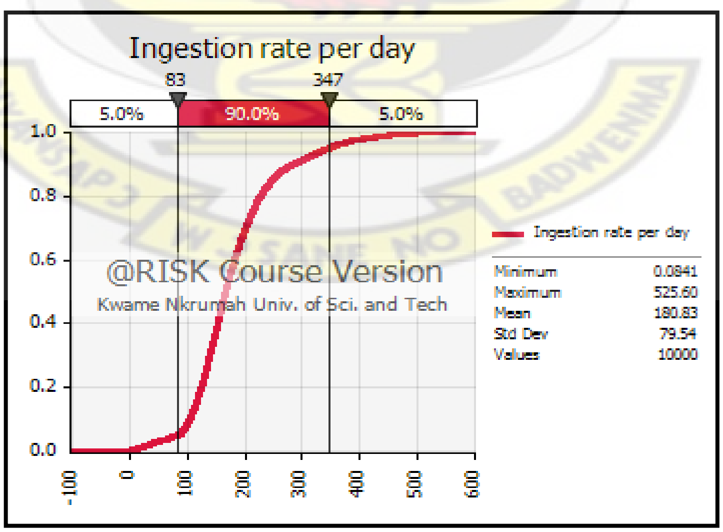


Figure 7.2: Distribution of the daily dietary acrylamide intake of bread among children in the Oforikrom sub-metro.

7.3 Mass of bread

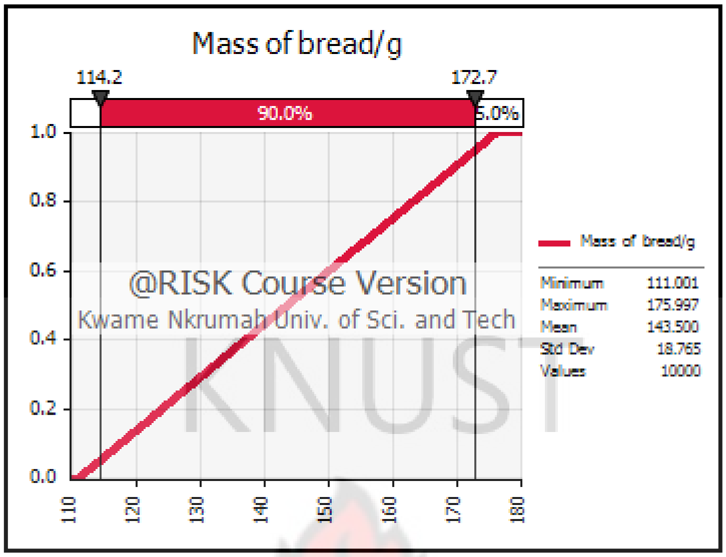


Figure 7.3: Uniform distribution representing the first order Monte Carlo simulation of the mass of bread eaten among the children group in the Oforikrom sub-metro.

7.4 Body weight of the children

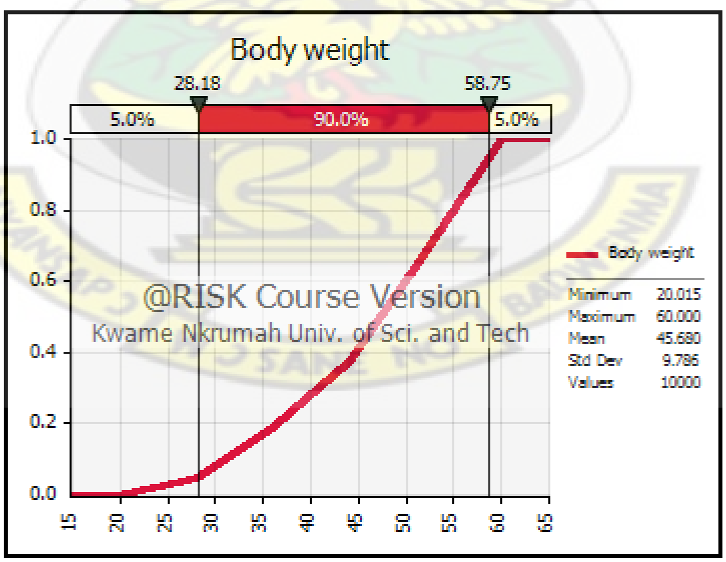


Figure 7.4: Histogram distribution representing the Monte Carlo simulation of the body weight of children in the Oforikrom populace

7.5 Number of bread per day

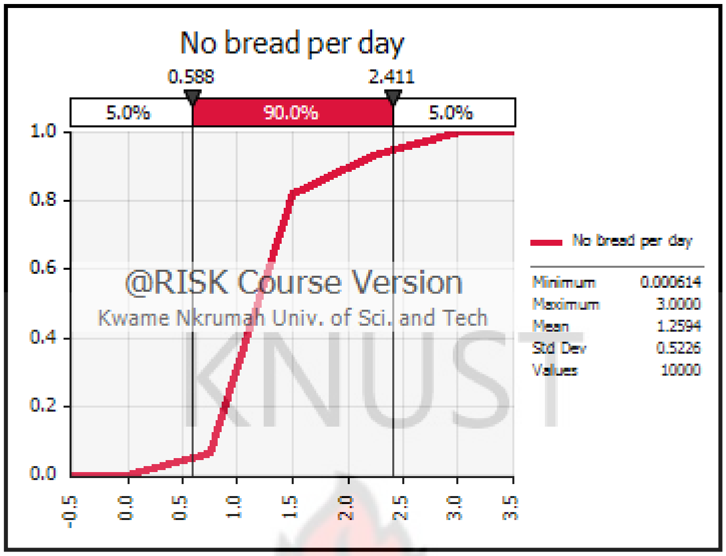


Figure 7.5: Histogram distribution for the number of bread consumed per day by children from the Oforikrom populace after Monte Carlo simulation over 10,000 iterations

7.6 Chronic daily acrylamide intake (CDI)

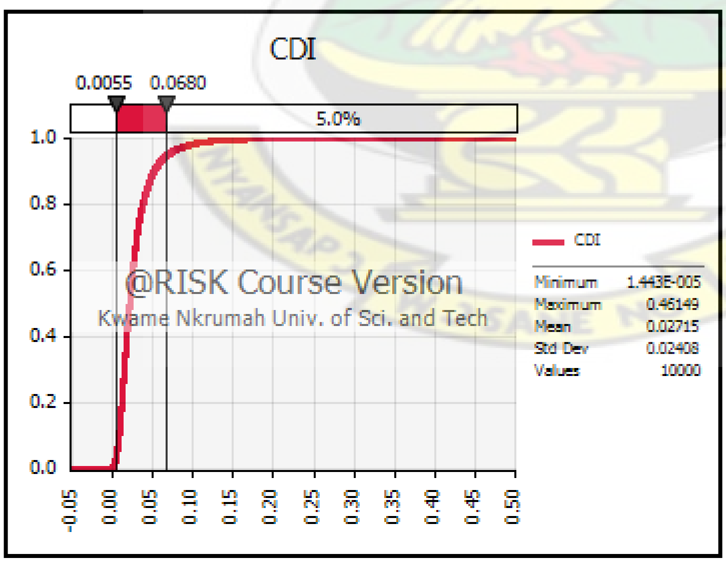


Figure 7.6: Chronic daily intake of acrylamide after Monte Carlo simulation over 10,000 iterations

7.7. Final potency factor (PF final)

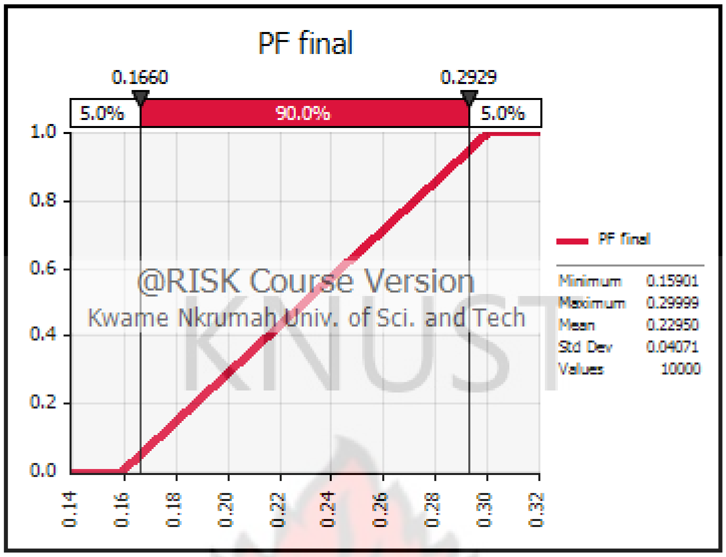


Figure 7.7: Distribution of potency factor final after Monte Carlo simulation over 10,000 iterations