KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

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RISK OF DIETARY ACRYLAMIDE EXPOSURE TO AN ADULT SUB POPULATION OF CONSUMERS OF *KOOSE*

THESIS SUBMITTED TO THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

MASTER OF SCIENCE IN FOOD QUALITY MANAGEMENT

BY:

SYLVESTER OTENG KYEI

NOVEMBER, 2014

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DECLARATION

I hereby declare that the submission of this compilation is the true findings of my own researched work presented towards an award of a second degree in Food Quality Management and that, to the best of my knowledge, it contains no material previously published by another person nor submitted to any other University or institution for the award of degree except where due acknowledgement has been made in the text. However, references from the work of others have been clearly stated.

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Student Name & ID Signature Date

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Mr. Isaac W. Ofosu …….. …….

Supervisor's Name Signature Date

Certified by:

Prof. (Mrs.) I. Oduro …….. …….

Head of Department Signature Date

DEDICATION

I dedicate this work to my late parents Opanyin Kofi Kyei and Maame Abena Gyasiwaa, for the great and solid foundation they gave me in my formative years of life.

To my uncle Mr. Philip Kwaku Gyembibi Agyapong who but for his initiative, formal education would have eluded me. To my brother, Mr. Peter Addai Kyei and my seven sisters Magarete Tuahene (Mrs), Grace Kyei, Rose Kyei, Ernestina Kyei, Debora Kyei, Hilda and Linda Kyei whose sacrifices and little helps saw me through my education.

Last but not the least, to my lovely wife Barbara Adomako whose inspiration and encouragement contributed immensely to this achievement.

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My lovely wife Barbara Adomako needs special mention. She has been my support and a source of encouragement through thick and thin since we engaged each other. I owe you a lot. Thank you so much.

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ABSTRACT

Acrylamide is proven to be carcinogenic in rodents and a probable human carcinogen with increasing evidence of positive associations with human cancers. Acrylamide forms in certain foods, particularly plant based foods that are rich in carbohydrates and protein, during processing or cooking at high temperatures. The objective of the study was to determine the acrylamide content in *koose* and to assess the average dietary acrylamide consumption and the risk posed to Ghanaian adult sub population living in Tamale. The content of acrylamide in the tested 153 samples ranged from 1.12- 74.77 µg/g. A first order Monte Carlo was run in favour of acrylamide content, mass of koose, body weight of respondents, daily consumption of *koose* and ingestion rate of acrylamide for determination of the risk. The daily dietary acrylamide exposure was computed using a probabilistic risk assessment method. Acrylamide content contributed the largest to the cancer risk associated with consumption of *koose* according to the correlation analysis. From the study, six (6) out of every ten thousand (10,000) consumers of *koose* in the Ghanaian sub population of Tamale stand the risk of getting cancer from acrylamide every year. The high risk obtained in this study calls for the creation of awareness of the presence of acrylamide in *koose* and the potential danger to the health of Ghanaians with its attendant socio-economic effects.

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LIST OF ACRONYMS

CAC	Codex Alimentarius Commission
CDI	Chronic Daily Intake
CERHR	Center for the Evaluation of Risks to Human Reproduction
CIR	Cosmetic Ingredient Review

DNA	Deoxyribonucleic acid
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization
FTIR	Fourier Transform Infra-Red
GC-MS	Gas Chromatography–Mass Spectrometry
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Cancer Research
IFST	Institute of Food Science and Technology
JECFA	Joint Expert Committee on Food Additives
LC-MS	Liquid Chromatography Mass Spectrometry
LOAEL	Lowest Observed Adverse Effect Level
LOD	Limit of Detection
MOE	Margin of Exposure
NOAEL	No Observed Adverse Effect Level
OSHA	Occupational Safety and Health Administration
PAGE	Polyacrylamide Gel
PF	Potency Factor
SCF	Scientific Committee on Food
SNFA	Swedish National Food Administration
RfD	Reference Dose
USEPA	United States Environmental Protection Agency

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WHO World Health Organization

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CHAPTER ONE

INTRODUCTION

1.1 Background

Acrylamide has been used in the chemical industry since the 1950s as a monomer in the production of polyacrylamide polymers and copolymers in order to improve adhesion and cross-link polymers. The polymerized acrylamide is widely used as flocculants in waste water treatment and paper and textile manufacturing (Hagmar *et al.*, 2001). In 1994, the International Agency for Cancer Research (IARC), taking into consideration an option of industrial exposure to acrylamide and its intake from drinking water and tobacco smoke classified acrylamide as a compound probably carcinogenic to humans (IARC, 1994).

Acrylamide is an odourless, white, crystalline organic solid with melting point of 8486^{o}C. It readily undergoes polymerization to form polyacrylamide which is highly crosslinked gel polymer with many uses in the industry (*Centre for Food Safety*, 2012).

When acrylamide was first detected in heated food, how it was formed, the precursors and the conditions involved in its formation was not clear to scientists. Initially it was thought that the fatty acid oxidation product acrolein was a possible precursor, forming acrylamide through direct reaction with ammonia followed by oxidation to acrylamide (Gertz and Klostermann, 2002). Another possible route was via the reaction between reducing sugars and amino acid in the Maillard reaction. This latter route was shown to be the most likely vehicle for acrylamide formation.

The discovery of acrylamide in fried, baked, roasted and grilled foods was alarming because these foodstuffs are widely and heavily consumed all over the world. No epidemiological data has established any relationship between dietary acrylamide and human cancer (Mucci *et al.,* 2003 and Pellucchi *et al.,* 2006.), yet based on the available animal testing data, acrylamide has been recognized as genotoxic and carcinogenic compound causing gene mutation and DNA damage both in vivo and in vitro, and its consumption may represent health hazard for human beings (FAO/WHO, 2002).

1.2 Problem Statement

Koose as it is called in Ghana is patronized by people from many African countries under different names. It is normally taken with maize or rice porridge. *Koose* is a bean paste fritter prepared by deep frying in oil at a very high temperature. It is a high carbohydrate and protein food. It therefore has the potential to contain acrylamide after thermal processing. Data on acrylamide concentration have been determined in foods such as French fries, potato chips, cookies, breakfast cereals and other foods that are processed at high temperatures such as coffee, roasted almonds and grain based coffee substitutes. All these foods are foreign foods not generally and frequently patronized by the adult population in Ghana.

There is hardly any data on acrylamide contents in foods like fried yam, fried plantain, deep fried dough and *koose* that are staple foods in Ghana but those foods are heavily consumed by adults. Besides that the foods that have been analyzed to date represent only a portion of the total diet and do not include foods representative of those consumed in developing countries. Many other foodstuffs may be found to contain acrylamide. Dietary acrylamide

exposure estimates are mainly available for the general adult population. Average intakes of acrylamide are estimated to be 0.3-0.8 microgram acrylamide per gram of body weight per day in diets of people in the developed countries like U.S, U.K, Sweden, Poland, etc. The average intake of consumers in the developing countries especially Ghana may be many folds higher than the national average (0.3-0.8µg/g BW/day) because of the way our foods are over cooked. These data available from the Western countries cannot therefore be relied on as an estimate of the dietary acrylamide exposure of Ghanaians in order to determine the risk posed to consumers.

Because *koose* is a high carbohydrate and protein food and fried in oil at a very high temperature, it is a very suitable candidate / substrate for excessive production of acrylamide. *Koose* is a food that is heavily patronized by a traditional group subpopulation in Ghana and Ghanaians in general. There is no known research conducted to determine acrylamide content in *koose* and the associated risk consumers of *koose* are exposed to.

In 2005 the Joint Expert Committee on Food Additives (JECFA) conducted a risk assessment of acrylamide in foods. Analytical data on the occurrence of acrylamide in foods from different countries was obtained from 24 countries with most of the samples from Europe (67.6%) and the US (21.9%), the remainder were from Asia (8.9%) and the Pacific (1.6%). No analytical data were obtained from Latin America and Africa (IFST Information Statement). This means that analytical data on the occurrence of acrylamide in foods are not available in the developing countries. Besides, the non- availability of data on acrylamide content in food consumed by the people in the developing and underdeveloped countries, the type of foods, the processing methods and the frequency of consumption of even common foods like bread differ markedly from those of the West.

These, among other facts, make it difficult to estimate the contribution to total dietary exposure of acrylamide and the risk to the people who consume it. The study would give a more realistic and reliable estimate of the dietary acrylamide exposure and the risk posed to adult consumers of *koose* in the adult subpopulation.

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1.3 Objective

The objective of the research was to measure the probabilistic risk associated with the consumption of *koose* in a subpopulation of Ghanaian adults living in Tamale over a one year period.

CHAPTER TWO

LITERATURE REVIEW

2.1 Discovery of Acrylamide in Food

In April 2002, the Swedish National Food Administration published for the first time data about high content of acrylamide in food (SNFA, 2002). It is now known that acrylamide is formed in food as a result of a reaction between amino acid asparagine and reducing sugar particularly glucose and fructose as part of Maillard reaction (Mottram *et al.*, 2002 and Stadler and Scholz, 2004). Acrylamide has now been identified as present in many baked, fried and grilled foods. Acrylamide is known to be formed when a high carbohydrate

and low protein food is subjected to a high temperature treatment like frying, roasting, etc during cooking.

2.2 Nutritional Information of *Koose*

Koose is a whole meal with all the required nutrients needed for the proper growth of the body. It provides the five major classes of food nutrients namely carbohydrate, protein, fat, vitamin and mineral. *Koose* has for many years served as part of breakfast meal and sometimes as a dessert for Ghanaians in general and the people of the Northern Ghana in particular. In her research into the analysis of the nutrient composition of *koose*, Nti (2005) found reasonable quantities of almost all the body required nutrients. For every 100 g of *koose*, the quantities 13.0g, 4.6g, 46.7g of proteins, fat, and carbohydrates respectively were determined. Significant quantities of minerals and vitamins namely calcium, iron, vitamin A, ascorbic acid and thiamin were also present.

2.3 Known Sources of Acrylamide before Discovery in Food

Before the findings of high levels of acrylamide in heated foods, drinking water and tobacco smoking had been regarded as the main sources of exposure for acrylamide among the general population. Acrylamide had been well studied since the 1950s as a neurotoxin owing to its extensive use for polyacrylamide production (Deafied *et al.*, 1988).

2.4 Routes of Human Exposure to Acrylamide

The discovery of acrylamide in food became a major concern because it pre-supposes that man had been exposed to the chemical from the time fire was used as a means for cooking.

There are several foodstuffs that have been cooked at high temperatures with the potential to contain acrylamide that have been patronized for many years. Humans can be exposed to acrylamide via oral or inhalational routes and also by dermal absorption (Dearfield *et al.*, 1995). Before its discovery in food however, other sources of exposure were occupational, residues from polyacrylamide and drinking water. The rest are cosmetics and smoking (European Union, 2002).

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2.5 Workplace Exposure

Occupational exposure to acrylamide occurs during the production or processing of acrylamide, during grouting and during laboratory preparation of polyacrylamide gels.

The main routes of exposure are dermal absorption of acrylamide monomer from solution and inhalation of dry monomer or aerosols of acrylamide solution (SCF, 2002). Although a maximum allowable concentration for acrylamide cannot be assigned (DFG/MAK Commission, 2007) due to the proposed non-threshold mode of action concerning carcinogenicity, a workplace exposure limit of 0.3 mg/m³ (OSHA, 2006) (resp. 0.03 mg/m³ (MSDS, 2005)) has been established. Several exposure assessments in workers employed in the acrylamide producing or processing industry were performed using Hbadducts as biomarkers of exposure. In laboratory personnel working with Polyacrylamide Gel (PAGE), the acrylamide burden was relatively low, corresponding to an uptake of acrylamide from approximately 3 cigarettes per day (Bergmark, 1997). This finding was confirmed by inhalation exposure evaluations resulting in mean workplace exposures of approximately 0.01 mg/m³ acrylamide for PAGE workers (Pantusa *et al.*, 2002). In factory workers and tunnel construction workers however, exposures were extremely high (up to

153 mg/m³), inducing neurotoxicity in a number of cases (Calleman *et al*., 1994; Hagmar *et al*., 2001). From these studies, a Lowest Observed Adverse Effect Level (LOAEL) of 6 × 10^3 pmol acrylamide-Val/g globin for development of peripheral neuropathy could be derived.

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2.6 Exposure to Acrylamide Residues from Polyacrylamides

Approximately 99.9% of acrylamide produced in the EU is used in the production of polymers. Whereas the release of free monomeric acrylamide by degradation of polymers is reported to be unlikely, monomeric acrylamide may be present in polyacrylamides due to incomplete polymerization during production. The residual content of acrylamide in polymers produced in the EU is kept below 0.1% (w/w) to avoid classification as a Category 2 carcinogen under the Dangerous Preparations Directive (88/379/EEC). For polymers used in the preparation of drinking water, the maximum permitted amount of free acrylamide in the polymer is 0.025% (w/w) (European Union, 2002).

A major part of the produced polymers is used as flocculants in the treatment of municipal drinking water and waste water. Due to its excellent solubility in water, free acrylamide is readily released. In drinking water, the maximum content of acrylamide has been limited to 0.125 μg/L. Accordingly, the maximum daily intake from drinking water has been estimated to approximately 0.0036 μg/kg bw in adults (European Union, 2002).

Another possible source of exposure to residual acrylamide in polyacrylamides is cosmetic products. Acrylamide polymers are used as thickeners in soap and various other cosmetic formulations, such as pre-shave lotions and hair grooming preparations (European Union, 2002).

Polyacrylamide is used in concentrations ranging from 0.05 % to 2.8 %. Residual levels of acrylamide in the polyacrylamides used, can range from <0.01 % to 0.1% (CIR, 2005). Whereas polymers do not penetrate the skin due to their size, approximately 4.5% of the applied acrylamide is absorbed through human skin (Fennell *et al.,* 2006). As acrylamide uptake via the skin is relatively slow, acrylamide content in leave-on formulations has been limited to 0.1 ppm and to 0.5 ppm in other cosmetic products (CIR, 2005).

Acrylamide is a constituent of cigarette smoke (>1 – 2 μg/cigarette) (Schumacher *et al*., 1977, Smith *et al*., 2000) and elevated Hb adduct levels (2 – 3 times the background level) have been reported in smokers (Kutting *et al.,* 2008). It has been estimated that one cigarette raises the acrylamide Hb adduct level by 3.4 pmol/g globin (Schettgen *et al*., 2003). Smokers also excrete more acrylamide-derived metabolites in urine compared to the non-smoking population (3 – 4 times). Therefore, smoking status can be correlated directly to elevated levels of Hb-adducts as well as urinary excreted metabolites of acrylamide (Schettgen *et al.,* 2002).

2.7 Foods of Known Acrylamide Content

Cereals and potatoes contain high levels of reducing sugars and the amino acid asparagine. When roasted, baked or fried they generate acrylamide with highest level of acrylamide reported in potato products (roasted and fried) and in cereal products. Coffee and cocoa beans that are exposed to high temperatures during roasting have relatively high levels of acrylamide (Stadler and Scholz, 2004).

Data on acrylamide concentration have been determined in foods such as French fries, potato chips, cookies, breakfast cereals and other foods that are processed at high

temperatures such as coffee, roasted almonds and grain- based coffee substitutes. The foods that have been analyzed to date represents only a portion of the total diet and do not include foods representative of those consumed in developing countries. Table 2.1 shows acrylamide levels in different foods and food product groups from Norway, Sweden, Switzerland, United Kingdom and United States of America.

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Table 2.1 Acrylamide Levels in Different Foods and Food Product Groups.

Food/Product Group	Acrylamide levels (µg/kg)			
	Mean	Median	Minimum-Maximum	Number of Samples
Crisps, potato/sweet potato	1312	1343	170- 2287	38
Chips, potato	537	330	< 50 – 3500	39
Batter based products	36	36	< 30 – 42	2
Bakery products	112	< 50	< 50 – 450	19
Biscuits, crackers, toast, bread crisp	423	142	< 30 – 3200	58
Breakfast cereals	298	150	< 30 –1346	29
Crisps, corn	218	167	34 – 416	7
Bread, soft	50	30	< 30 – 162	41
Fish and seafood products, crumbed, battered	35	35	30 -39	4
Poultry or game, crumbed, battered	52	52	39 – 64	2

Instant malt drink	50	50	< 50 – 70	3
Chocolate powder	75	75	< 50 – 100	2
Coffee powder	200	200	170 – 230	3
Beer	<30	<30	<30	1

Source: FAO/WHO Consultation on the Health Implications of Acrylamide in Food, (2002)

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Acrylamide Exposure, 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.*, 1995).

Risk assessment studies on potential acrylamide intake from foods have been published by many researchers throughout the world. 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. Cooked potato products represents up to 35% of total daily intake of acrylamide (Norwegian Food Control Authority, 2002).

Several studies have been conducted on the acrylamide intake among several population groups. The average intake by adults was estimated to be 0.3 - 0.6 µg/kg body weight/day (Wilson *et al.*, 2006) and 0.5µg/kgbw in Western countries (Dybing *et al.*, 2005). However, these dietary exposures are not directly comparable because of the different methods used for assessment i.e. different age groups, whole populations/ consumers of particular products using limited food groups rather than the whole diet. It is important to stress that 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 where further investigation is warranted (Gormley and Mee, 2003).

Most studies indicate that exposure to high levels of acrylamide leads to tumor development in animals (Friedman, 2003). There has been variation in results between acrylamide and cancer formation in humans with some indicating a relationship between acrylamide and ovarian and endometrial cancer (Hogervorst *et al*., 2007). Pedersen *et al.* (2010) in their breast cancer research and treatment, suggested a possible positive association between acrylamide and breast cancer among post-menopausal non-smokers.

Serious concerns about the safety of foods have been raised in different parts of the world due to the dietary availability of acrylamide (Halford *et al.,* 2012). Acrylamide is a suspected human carcinogen and has been proven to be carcinogenic in animals as it interferes with the normal metabolism (Ghanayem *et al*., 2005). Studies in animals have shown carcinogenic, reproductive and genotoxic effects of acrylamide mediated partly by epoxide glycidamide, its main toxic metabolite (Blasiak *et al*., 2004; Baum *et al.*, 2005; Annola *et al.*, 2008). Human studies have, however, shown varied results; some indicating positive associations of cancer with acrylamide exposure (Bongers *et al.*, 2012) while it lacks in other studies (Lin *et al.*, 2011; Pelucchi *et al.*, 2011; Lipworth *et al.*, 2012). It therefore remains a probable human carcinogen due to limited and inconsistent human carcinogenicity evidence arising from epidemiological studies (JECFA, 2011).

2.9 Risk Assessment

Food risk assessment brings together all the relevant scientific information about a particular food chemical. This may include any toxicological data in the hazard characterization and information on the foods affected and widely taken by consumers.

In all human activities there is an element of risk associated with it. Routine human activities like eating a meal, taking medication, becoming pregnant and giving birth cannot be done without taking some risk. There is no doubt that food carry risks in many forms. Prehistoric man had to deal with the problem of risk when he fed on a variety of foods. In this age of nano technological advancement, many equipment have been developed with low limits of detection, new analytical protocols and improved sensitivity that can detect the presence of toxins in otherwise previously ―no contaminated‖ foods. In risk assessment, problems are defined, goals are articulated and the questions to be answered by the risk assessment are also clearly defined. Codex Alimentarius commission defines risk assessment as a scientifically based process consisting of four steps namely, Hazard identification, hazard characterization, exposure assessment and risk characterization. Risk assessment includes quantitative risk assessment which foundation based on numerical expression of risk and qualitative risk expression which foundation is based on categorical expression of risk. A cancer risk of 1 in 10^6 is generally considered to be acceptable by the Food and Drugs Administration (FDA, 1977)

2.10 Hazard Identification

The first step in risk assessment is to determine the nature of the hazard. Hazard is defined as a biological, chemical, or physical agent that is reasonably likely to cause illness or

injury in the absence of its control. Hazards exist in many forms in various human activities. Some of these hazards are identified in the environment and in outbreaks. Hazards are inherent in food and may also be produced during cooking process.

Hazard identification is defined in the codex ―Procedural Manual, Fourteenth edition‖ (CAC, 2004) as the identification of biological, chemical and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods. Published literature, scientific journals, food borne disease reports,

epidemiological studies etc. are sources used to identify hazards.

2.11 Hypothesis of Acrylamide Formation in Food

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 route 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.

Acrylamide Formation Reaction Mechanism

One of the areas of focus to scientists when acrylamide was discovered in food was the reaction mechanism of formation of acrylamide. Zyzak *et al.* (2003) approached it from the identification and profiling of asparagine/sugar intermediates and products using aqueous reaction system, experimenting with isotope- substitute asparagines to determine the source of acrylamide nitrogen and carbon atoms and the formation of acrylamide from asparagines and various carbonyl groups to determine the ability of other carbonylcontaining groups to produce acrylamide. Understanding of the reaction mechanism and knowing the reaction intermediates and factors influencing acrylamide formation could lead to the development of methods to prevent or limit its formation.

2.13 Monitoring of Reaction Intermediates

In an experiment to monitor acrylamide reaction intermediates by Zyzak *et al.* (2003), aqueous solution of a mixture of concentrations of various amino acids and sugars were prepared and subjected to a constant heating (190-200^{o}C) at varying heating times (0, 180, 210, 240, and 270s). After 180s, solution mixtures were light brown, after 210s, solutions were medium brown and after 240s, free water was gone and samples were moist and dark brown to black; after 270s, residue was dark brown to black. Solutions of these mixtures were transferred to LC auto sampler vials for analysis and determination of reaction intermediates and products. The experimental concentrations of the various amino acids and sugars are as provided in Table 2.2.

The resulting LC Chromatograms monitored the disappearance of asparagine and Dglucose and the formation of Shiff's base, 3-aminopropionamide and acrylamide. Several

techniques were used to identify the intermediate products. The molecular formulas of the asparagine, D-glucose, Shiff's base and acrylamide peaks were all confirmed by high resolution mass spectrometry. All these identifications i.e. asparagines, D-glucose, Schiff's base were confirmed in the same experiment but with uniformly isotopesubstituted asparagines. The concentrations displayed are for the same compounds as in the unsubstituted experiment (Table 2.2) but adjusted in mass to account for incorporation of the number of isotopes substitutions.

Table 2.2: Experimental Concentrations of Various Amino Acids and Sugars.

	Amino acid	Conc. mM	Sugar	Conc. mM
1	Asparagine	16.8	D- glucose	13.9
2	Asparagine	5.6	D- glucose	23.2
3	U-$^{13}C_4$, U-$^{15}N_2$-asparagine	18.2	D- glucose	13.9
4	Asparagine	16.8	2-deoxyglucose	15.6
5	Asparagine	5.6	2-deoxyglucose	25.6
6	3-aminopropionamide	20.2	None	-
7	3-aminopropionamide	13.4	D-glucose	9.3

(Source: Zyzak *et al*. (2003), Acrylamide Formation Mechanism in Heated Foods)

2.14 Amide Nitrogen as a Source of Acrylamide Nitrogen

To explain the role of asparagine in generating acrylamide, a series of stable isotope substitution experiments were conducted. In their first experiment, an isotopic substitution of the amide nitrogen of asparagine and in another experiment an isotopic substitution of the amino nitrogen of the asparagine was also examined. In the first experiment, the amide ^{15}N- substituted asparagine was heated with D-glucose to form acrylamide. If the amide

group of asparagine is the source of acrylamide nitrogen, ^{15}Nsustituted acrylamide should result at m/z 73. Integration of the acrylamide peaks of the first experiment revealed that more than 97% of the acrylamide formed is at m/z 73. This clearly indicated that the asparagine amide nitrogen was the source of acrylamide nitrogen. In the second experiment the α-amino ^{15}N-sustituted asparagine was heated with D-glucose. If the α-amino group of the asparagine was the source of the acrylamide nitrogen, ^{15}N-sustituted acrylamide should result at m/z 73. Since the α-amino group is not the source of acrylamide nitrogen, only unsubstituted acrylamide at m/z 72 resulted.

The only measurable peak in this experiment was the unsubstituted acrylamide peak at m/z 72.

2.15 Source of Nitrogen and Carbon of Acrylamide

In their investigation into the source of nitrogen and carbon, all the nitrogen and carbons of the asparagine were isotope-substituted and heated with D-glucose. If the α-amino group and the carboxyl group of asparagine are not the sources of the nitrogen and carbon, then acrylamide with four isotope-substitutions at m/z 76 should result. The chromatograms from the experiment revealed that the only measurable peak was the m/z 76 peak, corresponding to incorporation of four isotope substitutions showing that all the three acrylamide carbon atoms as well as the one nitrogen atom came from asparagine. Another experiment with $^{13}C_6$ D-glucose and unsubstituted asparagine resulted in only unsubstituted acrylamide. These observations conclusively demonstrated that acrylamide is formed from the amide side chain of asparagine.

2.16 Acrylamide Formation with other Carbonyl Compounds

Zyzak *et al.* (2003) investigated the formation of acrylamide from asparagine and various carbonyl compounds. Carbonyl sources from D-glucose, 2-deoxyglucose, ribose glyceraldehyde and glyoxal were independently reacted with asparagine and acrylamide formation in each measured. The results obtained are as outlined in Table 2.3 below. In all the cases above, it was found out that a variety of carbonyl sources could generate acrylamide from asparagine under heat as illustrated by Table 2.3. As the sugar chain gets shorter, it was revealed, the molecule is strained to form a cyclic hemiacetal structure and subsequently the carbonyl becomes more readily available for nucleophilic attack from the α- amine of asparagine.

Table 2.3: Formation of Acrylamide from Asparagine and Various Carbonyl Compounds

Carbonyl Source	Acrylamide / **µg**
D-glucose + asparagines	1454
2-deoxyglucose+ asparagines	1036
Ribose + asparagines	2425
Glyceraldehyde + asparagines	2669
Glycoxal + asparagines	3936

Source: Zyzak *et al*. (2003)

Thus, the shorter chain sugars become more reactive and more acrylamide is produced. Compare acrylamide production between D-glucose and asparagine and that between glycoxal and asparagine. From the study, it was concluded that the first step in acrylamide production is the Schiff's base formation between the carbonyl and the amino group of asparagine. On the bases of the isotope-substitution results and the carbonyl studies

described, mechanism similar to the Maillard reaction (Figure 2.1) was proposed as the mechanism of acrylamide formation in heated foods.

Mottram *et al.* (2002) and Stadler *et al*. (2002) showed again that acrylamide may be formed through the Maillard reaction from amino acid (asparagine) and reducing sugars (glucose). Beclaski *et al*. (2003) showed that ^{15}N-labelled asparagine and glucose 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.

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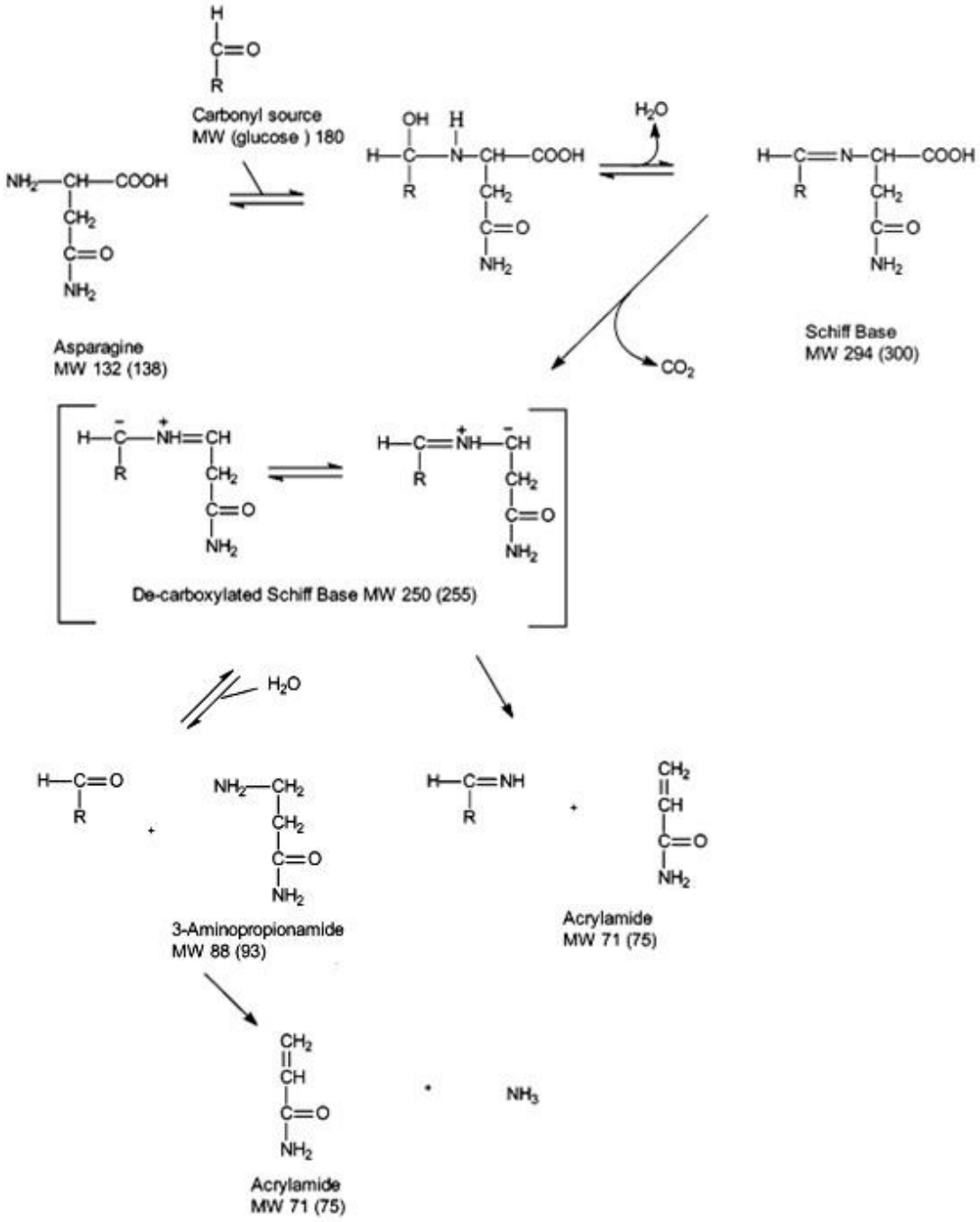

Figure 2.1: Mechanism of acrylamide formation in heated foods. Numbers in parentheses are the molecular weights observed when uniformly isotope-substituted asparagine was used. Source: Zyzak *et al.* (2003), Probable route to the formation of acrylamide in starchy foods

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). 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 maximises near the end of the heating cycle (Elmore *et al*., 2005).

2.17 Factors Influencing Acrylamide Formation in Foods

Influence of acrylamide formation by various parameters and ingredients also attracted other researchers. These researches were aimed at identifying the presence of ingredients in foodstuffs and processing conditions/parameters such as temperature and time that could have an effect on the amount of acrylamide formed

2.17.1 Effects of Initial Concentration of Amino Acid and Sugars

According to Becalski *et al.* (2004) who conducted a research on how the initial concentration of free amino acids and sugar in raw potatoes influences acrylamide formation in French fries, asparagine had a considerable positive effect on acrylamide production but the effect was not as significant as sugars. Raw samples of potatoes were selected and the concentrations of sugar and amino acid were determined before subjecting them to cooking. French fries were produced in a laboratory-scale simulation of an

industrial process followed by a finish fry at 150 ºC for 3.5 min using a restaurant fryer. Acrylamide was detected after treatment of the potato samples this way but its concentration significantly varied from 50- 1500 μg /g.

Statistical analysis of the data obtained indicated that the effect of sugar and asparagine on the concentration of acrylamide in French fries is positive and significant. Levels of sugar correlated well with levels of acrylamide, whereas asparagine levels were not strong predictors of potential levels of acrylamide in French fries. The research concluded that by selecting potato materials low in sugar (and to a lesser extent low in asparagine) acrylamide content in the finished product (French fries) can be substantially reduced when using an industrial standard procedure and a frying temperature of 180 ºC.

One of the preferred approaches the industry should focus on is selective reduction of acrylamide through the control of these plausible precursors, particularly asparagine if feasible, while allowing the Maillard reaction to proceed, at least to a certain extent, because that reaction is largely responsible for the desirable aroma and taste of food and also produces beneficial compounds.

2.17.2 Effects of Selected Food Ingredients

In the experiment to investigate the formation of acrylamide in shortbread, Marconi *et al.* (2010) varied parameters such as the amount of baking agent (ammonium and sodium bicarbonate) and baking time and temperature. Their aims were to set up an analytical method to determine acrylamide concentration in shortbread biscuits, and study the technological parameters influencing the acrylamide formation during shortbread processing.

A number of recipes were prepared which had varied amounts of baking agents namely sodium and ammonium bicarbonates and all the recipes consisted of the same measured amount of flour, water, powdered sugar (sucrose), vegetable fat, skimmed milk, a starch wheat preparation, monohydrate dextrose , eggs, mineral salt, orange and vanilla aroma. For some samples, the baking parameters were fixed at 240ºC for 7 min whereas the amount of baking agent varied. In some samples too, the amount of baking agent were fixed whereas temperature and time varied. The results showed that the ammonium bicarbonate concentration in shortbread and the high temperature influenced the acrylamide formation during the processing, highlighting that the final acrylamide concentration is influenced by the processing parameters.

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*., 1997). Similarly, Stadler *et al*. (2002, 2004) reported that significant quantities of acrylamide were formed when equimolar amounts of glucose and asparagine were pyrolysed at 180°C. Biedermann and Grob. (2003) also concluded that acrylamide formation resulted from the degradation of asparagine by reaction with a carbonyl source most likely from glucose and fructose.

2.18 Hazard Characterization

Chemical and other contaminants are not equal in their capacity to cause adverse effect.

Hazard characterization is defined in the Codex ―Procedural Manual, Fourteenth Edition‖ (CAC, 2004) as the qualitative and or quantitative evaluation of the nature of adverse health effect associated with biological, chemical and physical agent which may be present in food. To determine the capacity of agents to cause harm, we need quantitative toxicity data. For chemical agents a dose-response assessment should be performed. Animal subjects (rats and mice) are used in dose-response assessment to characterize hazards. The data are then extrapolated from the high doses actually administered to laboratory test subjects to low doses humans are likely to receive in the course of everyday living.

2.19 Reference Dose

Acrylamide damages DNA and causes mutations in human cells. Cancer is seen in animals exposed over their lifetime to acrylamide orally. After eating food containing acrylamide, the chemical is taken up by the body and distributed to tissues in the body. For these reasons, dietary exposures are considered to pose a cancer risk to human. However human cancer studies conducted to date are insufficient to determine the level of cancer risk from acrylamide.

The US Environmental Protection Agency (EPA) defines reference dose (RfD) as an estimate of a daily exposure to human population that is likely to be without an appreciable risk of deleterious effect during a lifetime and has set a chronic reference dose for acrylamide to be 2µg/kg/day (EPA, 2010). The US EPA (1991) has established a reference dose (RfD) for acrylamide of $2x10^{-4}$mg/kg/day based on neurotoxic effects.

This reference dose was based on nerve damage observed in drinking water study in rats

(Burek *et al*., 1980). In a draft report by the expert panel on acrylamide of the National Toxicology Programs centre for the eradication of risks to human reproduction, the lowest observed effect level for both developmental and male reproductive toxicity was about 5 mg/kg-day (CERHR, 2000). Dividing this level by a standard uncertainty factor of ten (for LOEL to NOAEL extrapolation) results in a NOAEL estimate of 0.5 mg/kgday.

US EPA (1991) established a NOAEL of 0.2 mg/kg /day (200 µg/kg/ day) and a corresponding reference dose of 2µg/kg/day for non-cancer effects based on experimental neurotoxicity data. The daily dose level posing a 10^{-5} lifetime risk of cancer is 0.014 µg/kg/day. This is interpreted to mean that one out of every one hundred thousand people exposed to the consumption of acrylamide at the rate of 0.014 µg/kg/day in their lifetime is at a risk of developing cancer. According to WHO, a lifelong (70years) intake of 1 µg/kg/day of acrylamide per day would be associated with a lifetime cancer risk of $1x\ 10^{-5}$.

Exposure Assessment

Exposure assessment is defined in the Codex Procedural Manual, Fourteenth Edition (CAC, 2004) as the quantitative and or qualitative evaluation of the likely intake of biological, chemical and physical agent via food as well as exposures for other sources if relevant. Exposure assessment involves the determination of the concentration of a contaminating agent in the environment and estimating its rate of intake in the target population. An exposure assessment estimates qualitatively and quantitatively the magnitude, frequency, duration and the route of exposure for each potential or actual population to be evaluated in the risk assessment (DOE, 1991).

2.21 Acrylamide Identification and Determination

Despite acrylamide being a relatively new contaminant for food analysts, intensive method development and refinement has been carried out since its discovery in food products in 2002. 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 (Wenzl *et al.,* 2003). Only a limited number of methods have been published so far on the determination of acrylamide in foods despite the matrices where acrylamide can occur.

There are only a few reports that indicate that a rigorous and systematic determination of the characteristics of the method has performed according to international guidelines such as ISO 17025, CEN and Eurachem (Schaller, 2003). Rosen and Hellenas (2002) reported results from an in-house validation study, but admitted that these would not be applicable for all matrices and that there would still be some problems with different sample types. Most significant for the comparability of results of analysis is the harmonization of the sample preparation protocols. There were large differences in the extraction procedure, e.g. variations in composition of extractant, in swelling conditions, in the definition of extraction temperature and time, and in the mechanical treatment. By comparing methodologies, there were huge differences in the clean-up strategies. This was found for both GC and LC based methods. Especially, in LC sample preparation; there is a large spread of different procedures used, from lavish multistage solid-phase extractions to very simple, solid phase extraction-omitting protocols. Comparability of the GC methods is difficult due to differences in the sample matrices for which they were developed.

In some articles, recoveries are reported, but often there is lack of information about how they were determined. Mostly recoveries were reported in the range 90 to about 100 %. However, 60 % has also been reported (Tareke *et al.,* 2002). Adding 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 pretreatment, but invariably 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 and native analyte. As long as the equilibrium is not established, differences in the extraction procedure might have a great influence on recoveries (Thompson *et al.,* 1999).

Knowledge of the exact recovery achieved for an analyte when applying a certain analytical method is of paramount importance as it will strongly affect the accuracy of the results (Eurachem, 1998). Therefore, reference materials are normally accepted for comparison. In an experiment, the initial method in which the DB-1701 column was used, a Limit of detection (LOD) of 20 µg/kg was obtained. The LOD was lowered to 4 µg/kg when the column was replaced with the DB-VRX column which emphasized the importance of using a column which provides good resolution. Since there is no allowable limit yet established for acrylamide in foods, it is crucial that if acrylamide is present within a sample it should be quantified (Tareke *et al.,* 2002).

2.22 Exposure Studies

The intensive and extensive intake of foods with potential acrylamide and the high levels of acrylamide determined in some food items prompted the scientific community to initiate

exposure studies among the general population. In two separate exposure studies conducted in Canada and Poland, an idea of the extent of exposure was determined.

Normandin *et al.* (2013) did a research on dietary exposure to acrylamide in adolescents from a Canadian urban centre. Fourteen (14) food categories ranging from deep-fried French fries through oven baked French fries, potato chips, corn chips, pop-corn, crackers, roasted almonds, breakfast cereals, toasted bread to brewed coffee were selected for the analysis. These foods were considered to be frequently consumed by the adolescents and a potential dietary source of acrylamide. Two sets of consumption data were obtained separately from a 2- day food diary and Food Frequency Questionnaire (FFQ). Estimates from the 2-day food diary gave median total daily intake of acrylamide as 0.29 µg/kg bw/d and upper 97.5th percentile value to be 2.85 µg/kg bw/d (high consumers) while the estimates from the food frequency questionnaire gave median daily intake of acrylamide as 0.71 µg/kg bw/day and 97.5th percentile of 0.48 µg/kg bw/d.

Deep fried French fries had the highest contribution of about 50 % to the daily dietary acrylamide intake, while potato chips, oven baked French fries and breakfast cereals accounted for 10 %, 8 % and 5 % respectively.

In a similar dietary exposure studies, an estimation of the dietary acrylamide exposure to the Polish population was done. Twelve (12) food categories that were considered to be frequently consumed by the Polish were sampled for analysis of acrylamide. The food types sampled for analysis by the Polish researchers were similar to those of the Canadian researchers but in addition to the following; oak flakes, corn flakes, corn crisp pastries and salty sticks. Food consumption data was taken from the household food consumption and anthropometric survey in Poland. Estimated acrylamide mean exposure was 0.43 µg/kg

bw/d. Bread supplied 45 % of total dietary acrylamide intake, French fries and potato crisp 23 % and roasted coffee 19 %.

Acrylamide exposure was estimated to be in the range of 0.3- 0.8 μg/kg bw/day based on the exposure calculations done by FAO/WHO in 2002. Exposure to acrylamide was estimated to an average of 1 μg/kg bw/day with the highest exposure for adults close to 0.5 μg/kg bw/day, with 95th percentile values of about 1 μg/kg bw/day. The young people in Germany aged between 15-18 years are the highest exposed since the 95th percentile was 3.4 μg/kg bw/day (Dybing, *et al.*, 2005).

2.23 Food Intake and Exposure to Acrylamide

While researchers aimed at detection and quantification, acrylamide formation mechanism, influencing factors and method development for determination of acrylamide, others attention was drawn to dietary exposure and risk assessment resulting from acrylamide intake. A number of research studies have been done in the area of dietary exposure and risk assessment.

2.24 Dietary Exposure to Acrylamide

Exposure to acrylamide through the diet is the most prevalent source of acrylamide in the general population. Acrylamide is ubiquitous in the human diet. About 38% of our caloric uptake is provided from food (Petersen and Tran, 2005). Foods with the highest concentrations of acrylamide are potato products such as French Fries and chips (Kopp and Dekant 2009). Although in coffee, acrylamide concentrations are relatively low, it is a

major contributor to acrylamide exposure in adults, due to the high amounts consumed (Dybing *et al*., 2005).

Children and adolescents tend to eat more acrylamide on a per body weight basis. This may be due to a combination of factors including children's higher caloric intake relative to body weight as well as their preferred choice for consumption of certain acrylamiderich foods, such as French fries and potato crisps (Kopp and Dekant 2009). Accordingly, intakes 1.5 to 2 times the daily exposure in adults have been estimated for children and adolescents with possible worst-case scenarios of up to 6.9 μg/kg b.w. per day (Mosbach *et al.*, 2003).

2.25 Risk Characterization

Risk characterization involves the estimation of the potential impact of a hazard based on the severity of its effect and the amount of exposure. Risk characterization is defined in the Codex Procedural Manual, Fourteenth Edition (CAC, 2004) as the qualitative and or quantitative estimation, including attendant uncertainties of the probability of the occurrence and severity of known or potential adverse health effect in a given population based on hazard identification, hazard characterization and exposure assessment. Risk characterization brings together all the qualitative and quantitative information of the previous steps to provide a soundly based estimate of risk for a given population. Once the risks are characterized, various regulatory options are evaluated in the process of risk management, which includes consideration of social, political and economic issues.

2.26 Risk Estimate

Joint FAO/WHO Expert Committee on Food Additives (JECFA) used the Margin of Exposure (MOE) approach to estimate the human risk to acrylamide. MOE is a quotient of the lower limit at which no adverse effects where observed (NOAEL), over the estimated exposure from food. The lower the value, the greater is the possible effect on public health. With mean acrylamide exposure, 0.001mg/kg BW/day and high exposure of 0.004mg/kg/BW/day (based on the national intake data), the JECFA had established MOEs for acrylamide as 200 and 50 for mean dietary exposure and high dietary exposure respectively. The No Observed Adverse Effect Level (NOAEL) was taken to be 0.2 (JECFA, 2011). JECFA concluded that while adverse neurological effects are unlikely at the estimated average exposure, morphological changes in nerves could not be excluded for individuals with a high dietary exposure to acrylamide.

2.27 Cancer Studies in Experimental Animals

The potency of acrylamide as a carcinogen has been studied using animals as subjects. Acrylamide is a suspected human carcinogen and has been proven to be carcinogenic in animals as it interferes with normal metabolism (Ghanayem *et al*., 2005). Most studies on animals indicate that exposure to high levels of acrylamide leads to tumor development (Friedman, 2003; Spivey, 2010; Tardiff *et al.*, 2010). Rats and mice have largely been used as test subjects. In mice studies in which only the lungs and skin were examined, acrylamide induced lungs and or skin tumors (Bull *et al.*, 1984). In female rats, acrylamide induced tumors of the mammary glands, thyroid, central nervous system, oral cavity, uterus

and clitoral gland. Tumors of the thyroid, testis and central nervous system were also observed in male rats (Johnson *et al.,* 1986).

2.28 Cancer Studies in Human

Most of the available epidemiological studies of cancer and exposure to acrylamide have been published since acrylamide was listed in the Sixth Animal Report on Carcinogens. In a study of multi-plant cohort consisting mostly of male workers, the incidence of pancreatic cancer was significantly higher among workers with the highest cumulative exposure to acrylamide than in the US population. Among the exposed workers, the incidence of pancreatic cancer was significantly associated with duration of exposure and time since first exposure (Marsh *et al.,* 1999, Schulz *et al.,* 2001).

Several population-based studies that investigated the association between dietary intake of acrylamide and specific cancer outcomes were reviewed by Hogervorst *et al.* (2010). Several prospective cohort studies used case-cohort or nested case-control analysis to evaluate dietary exposure to acrylamide (based on a food-frequency questionnaire) and the risk of cancer of specific tissue states. These include the Swedish women's lifestyle and health cohort, the Swedish mammography cohort, the Netherland study on diet cancer, a cohort of Swedish men, the US nurses' health study and the Danish diet, cancer and health study.

There has been variation in results between acrylamide and cancer formation in humans with some indicating a relationship between acrylamide and ovarian/endometrial cancers (Hogervorst *et al.,* 2007) though no study has indicated a perfect relationship between breast cancer and dietary acrylamide (Wilson *et al*., 2009,Hogervorst *et al*.,2007). Pedersen

et al. (2010) suggested a possible positive association between acrylamide and breast cancer among post-menopausal non-smokers. Serious concerns about the safety of foods have been raised in different parts of the world due to the dietary availability of acrylamide (Halford *et al.,* 2012). Human studies have, however, shown varied results; some indicating positive associations of cancer with acrylamide exposure (Bongers *et al*., 2012) while it lacks in other studies (Pelucchi *et al.,* 2011; Lipworth *et al*., 2012). It therefore remains a probable human carcinogen due to limited and inconsistent human carcinogenicity evidence arising from epidemiological studies (JECFA, 2011).

Though the daily dietary acrylamide intakes vary with age and consumption, the world wide estimation by World Health Organization is in the range of 0.3-2.0 μg/kg body weight for the total population. The intake may, however, reach 5.1 μg/kg bw when the 99th percentile is considered (JECFA, 2011). The average daily acrylamide intake by children has been reported to be 0.54 μg/kg bw/day (Heudorf *et al.,* 2009).

2.29 Present Research Focus on Acrylamide

Many research studies have focused on different aspects of acrylamide since it was first discovered to be present in foods processed or cooked at high temperature. Among the areas of studies were the identification and quantification of acrylamide in food matrices, mechanism of formation of acrylamide in heated foods and methods of determining acrylamide in various food matrices. The rest are dietary exposure, risk assessment of acrylamide intake as well as the influence of other ingredients on the formation of acrylamide. There have been extensive studies devoted to the reduction of acrylamide in foods. Acrylamide formation mitigation effort have been approached through removing of

reactants, that is; fructose, glucose, asparagine before the heating process, changing reaction conditions and removing acrylamide after its formation during heat processing.

In spite of its recent discovery, intensive and extensive studies have been done by the academia, the food industry and regulatory authorities worldwide. The stakeholders showed much interest because of both economic and health implications resulting from the presence of acrylamide in food. The areas of current research include method of identification and determination of acrylamide in a number of food matrices, the reaction mechanism involved in the formation of acrylamide and monitoring of factors like time, temperature, food ingredients and food additives. Other areas in acrylamide formation in food may include estimated acrylamide exposure to the population of a number of countries, risk assessment and acrylamide content in a limited number of foodstuff mostly foreign foods.

2.30 Potential Areas for Further Research

There are identifiable areas in which research work on acrylamide has been limited or non-existent. A lot of research has gone into determination of acrylamide content in many foodstuff yet the number of foodstuffs whose acrylamide content have been determined so far are very few compare to the number of food with the potential to contain acrylamide available worldwide. *koose* is one of these foods whose acrylamide content cannot be cited in any literature. There is therefore the need for researchers to widen the scope of research to cover many food items.

Acrylamide is formed in both food prepared by consumers in the home and in the industry. To date most of the research work on acrylamide content has been focused on products

manufactured by the food industry. The last but not the least, investigation of acrylamide in air surrounding food production plants like bakeries, potato chips factories, restaurant kitchen and other places where larger amount of materials which contain components which are known to be able to give rise to acrylamide when heated have received little attention.

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CHAPTER THREE

MATERIALS AND METHODS

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3.1 MATERIALS

3.1.1 Source of *koose*

koose was purchased from *koose* sales points in four geographical divisions of Tamale metropolis.

3.2 METHODS

3.2.1 Sampling of *koose* for Analysis

Tamale metropolis was divided into four geographical zones, Tamale North, South, East and West. *koose* sales points were identified in each zone. In Tamale North, twelve (12) sales points were identified. Eleven (11) sales points were identified in Tamale south, ten (10) in Tamale east and eight (8) in Tamale west.

The *koose* for acrylamide contamination were sampled from all these sales points which numbered forty one (41). For a continuous six (6) days, two pieces of *koose* was picked from each sales point in a small plastic bag and stored in a deep freezer. On each day forty-one samples (sample made up of 2 pieces) was picked from across the four geographical zones. Two hundred and forty six (246) samples were picked for the analysis of acrylamide content.

3.2.2 *Koose* Consumption Data

Consumption data were collected from *koose* preparation and retail outlets in Tamale. Tamale was purposively selected because *koose* happens to be a traditional food of the people and hence there is a high concentration of koose consumers. The survey was carried out between 4th and 14th November, 2013 and it involved a random survey applying quantitative data collection methods. Adults between the ages (18-96) were randomly invited to provide answers to a set of questionnaires. Before asking them to provide answers to the questionnaires, the details of the study were explained to the participant and a verbal consent obtained. The directions as to how the self-administered questionnaire be filled was explained.

In all 354 respondents made up of 207 males and 147 females in the four geographical zones were available for interview in the compilation of the consumption data. Data were collected on gender of consumer, frequency of consumption level, estimated weight and mass of *koose*. The daily *koose* consumption was calculated by dividing the weekly intake per person by a factor of 7 and by the estimated body weight of an individual or an average weight of 60.3kg (JECFA).

3.2.3 Extraction of Acrylamide

A method by Lubomir *et al*. (2009), Zhiming *et al*. (2011) and Daniali *et al*. (2010) was used for the extraction and determination of acrylamide. Details are outlined as follows. Two grams (2g) of homogenized sample were weighed into 50ml Falcon tubes. 20ml of extraction solution (18ml of 1:1 distilled water: methanol + 1ml Carrez I Solution + 1ml Carrez II Solution) to the sample. Additional 15ml of hexane was added and the resulting

mixture was vortex for 15 – 30 s. The mixture was agitated at 250 rpm for 30 min and further centrifuged at 4000rpm for 15 min. The aqueous phase was further filtered via Micro-spin centrifuge filter tube, 0.45 micron (Alltech Associates, Deerfield, IL).

3.2.4 Acrylamide Concentration Determination

Standard solution of 0.02, 0.04, 0.08 and 0.32 ug/ml of acrylamide was prepared from a stock solution of 2ug/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 (appendix E), the acrylamide in test samples were determined. Determinations were done in triplicates.

3.2.5 Statistical Methods

Acrylamide content: The acrylamide content values obtained from the chemical analysis of the *koose* were loaded into the Palisade @Risk software 6.2 (2014) and fitted to a *RiskLognorm* distribution with fitting parameters 15.949, 20.247. The distribution was then defined with the said parameters and a first order Monte Carlo simulation was run in favour of acrylamide content at 10, 000 iterations.

Number of times of consumption of koose per day: From the general pattern of consumption of *koose* in Ghana, the consumption rate ranged between a minimum of 1.00 and a maximum of 3.00 times per day. Therefore, a uniform distribution was defined in favour of the consumption of number of times per day with distribution parameters 1 for

min and 3 for max subsequently simulated using a first order Monte Carlo for 10,000 iterations.

Mass of koose: According to data obtained from Infocomm Commodity Profile, (2014), per capita range of 30-50 g of *koose* was the mass of *koose* taken per day by individuals across the Tamale Metropolis. Therefore, a uniform distribution was defined using a min distribution value of 30 g and a max value of 50 g of *koose* after which a first order Monte Carlo simulation was run again for 10,000 iterations.

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

Exposure frequency and exposure duration: The exposure frequency is the number of times individuals are exposed to the acrylamide in the consumed *koose*. Generally, it was fairly assumed that consumers in the study area consumed *koose* daily for all around the year (365 days) and the exposure duration was for a period of one year for this research.

Body weight: A data of body weight per kg for the consumers of *koose* presented a histogram which were grouped as 40-45, 46-52, 53-60, 61-70, and >70, with percentage frequencies respectively as 0.0227, 0.0823, 0.202, 0.372 and 0.321. Subsequently, a histogram was defined using these percentage frequencies over the population who eat *koose* in the Tamale metropolis. The distribution was also simulated at a first order Monte Carlo at 10, 000 iterations.

Chronic daily intake (CDI): Chronic Daily Intake is computed using the equation 1

(appendix F) (Hans *et al*, 2003). In essence, it is defined as the product of chemical concentration of acrylamide, ingestion rate, exposure frequency and exposure duration per the product of body weight and average time of exposure.

Potency factor: The potency factor (PF) is usually the slope of the dose-response curve of acrylamide for 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). According to the database, a range of 0.5-4.5 was quoted as the PF of a rodent. Therefore, a uniform distribution was defined using a minimum of 0.5 and maximum of 4.5. It was then simulated with a Monte Carlo first order simulation for 10,000 iterations. However, the final PF was derived when a safety factor of 10^3 was used to convert the rodent based PF of acrylamide to a human based system. It is explained as a factor of 10 from conversion from rodents to humans, another factor of 10 for differences in human behaviour and yet another 10 due to the highly risked chemical being analyzed. A Monte Carlo simulation was then run in favour of the PF final obtained from database at 10,000 iterations.

Risk calculation: A reference dose of 2 μg/kg/day (0.0002 mg/kg/day) (EPA, 2010) was used for this work. Thus, it is assumed that 2 μg/kg/day of acrylamide may be consumed in a lifetime without any adverse health effects. The risk was finally calculated using equation (2);

$$\text{Risk} = \text{PF} \times (\text{CDI} - 0.0002) \quad (2).$$

where PF is the Potency factor and CDI is the chronic daily intake. A Monte Carlo simulation was also run in favour of the risk calculated for 10, 000 iterations.

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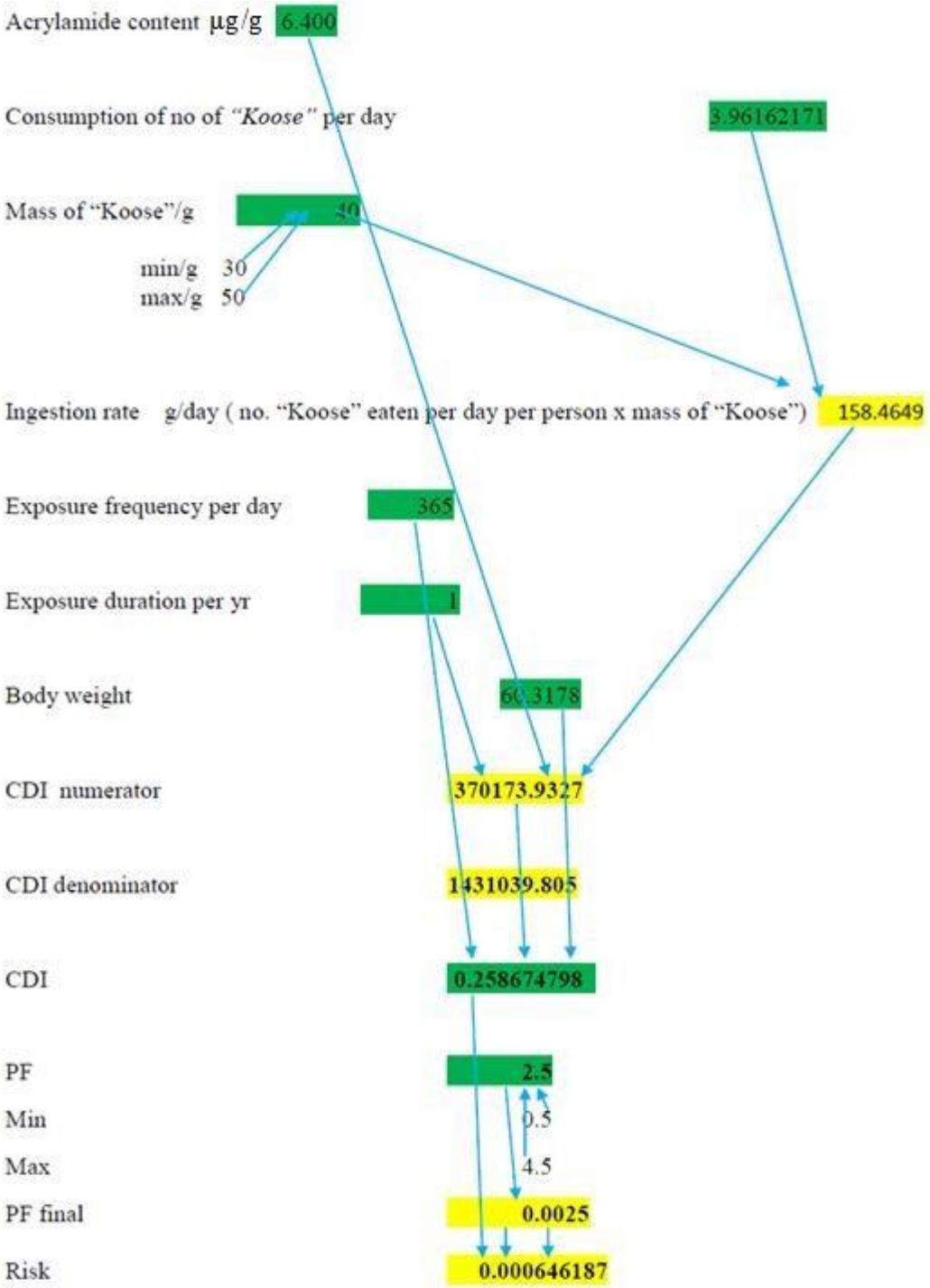

Fig 3.1: Flowchart of risk assessment of acrylamide from *koose* consumption.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Acrylamide content

The acrylamide content in the 153 samples of pieces of *koose* collected from the Tamale metropolis ranged from 1.12µg/g to 74.77µg/g. The mean acrylamide content after a first order Monte Carlo stimulation was run in favour of acrylamide content at 10,000 iterations was 6.395µg/g and the modal concentration was 0.377µg/g.

The 95th percentile of acrylamide concentration was found to be 24µg/g indicating that 95% of the *koose* sampled had concentrations 24µg/g or less and the remaining 5% of the samples had concentrations 0.26 µg/g or more. The asymptotic nature of the graph indicates that acrylamide concentration has no limit and can increase to infinite levels if no efforts are taken to mitigate its formation in *koose*. Acrylamide contents have been determined in a number of food products.

There have been wide ranges of acrylamide concentration within the same food product and across food categories. In a study to estimate dietary acrylamide exposure of the Polish population, acrylamide content of various food groups with the potential to contain acrylamide were determined, in potato crisp alone, acrylamide content in the individual samples varied between 113µg/kg to 3647µg/kg. Acrylamide concentration as low as 5µg/kg has been determined for roasted coffee and as high as 3647µg/kg in potato crisp. The monitoring studies carried out in Europe from 2007 showed that acrylamide content in food ranges from less than 30µg/kg-4700µg/kg depending on product type (EFSA,2009).

In this study the mean value of acrylamide content after the simulation was 6396µg/kg. The modal value was 377µg/kg and the piece of *koose* with the least concentration was 253µg/kg. The result of this study like many other studies shows a wide range between the mean, modal and least values. The wide difference between the mean and the modal acrylamide may be attributed to the effect of some of these *koose* pieces being fried at a high temperature and for a longer time than others.

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4.2 Daily Consumption of *koose*

When the pieces of *koose* that were taken from the four geographical zones were run in @Risk (2014), using first order Monte Carlo simulation at 10,000 iterations the distribution of the daily consumption of *koose* in the sub population was as follows. The 95th percentile of the respondents consumed 5.71 pieces of *koose* per day or less and 5% consumed 2.24 pieces or more. Maximum consumption per day was 6.00 pieces and the minimum was 1.00 piece. Three hundred and fifty four well-structured food frequency questionnaires were administered to 354 subjects in the Tamale metropolis in order to estimate their daily intake of *koose*. The mean, modal and least intake of 3.96, 4.20 and 2.21 respectively were determined using Monte Carlo simulation and fitting a distribution.

4.3 Masses of pieces of *koose* sampled

The masses of pieces of *koose* sampled were between 30 g and 50 g. The mean of the sampled *koose* was 40 g. The 95th percentile of weights (masses) of *koose* sampled was 49.00 g. in other words, 95 % of the pieces of *koose* sampled had masses 49.00 g or less while the remaining 5 % had masses 31.00 g or more.

4.4 Acrylamide Ingestion rate

The three common routes of acrylamide exposure reported by EPA are contaminated water, contaminated food and dermal exposure while working in polyacrylamide production industry. Ingestion of acrylamide through contaminated food is however the most common. The acrylamide ingestion per day of the first 5% low consumers (5^{th} percentile) was 86.4µg/day while the acrylamide ingestion rate per day of the upper 95% high consumers (95^{th} percentile) was 243.8µg/day. The minimum ingestion rate was 32.02µg/day and the maximum was 297.27µg/day. The mean and least ingestion rate were respectively 158.50µg/day and 32.02µg/day. The ingestion rate depended on the number and masses of pieces of *koose* consumed per day. The acrylamide ingestion depended more on the number of pieces of *koose* consumed per day than the mass of *koose* consumed (Appendix I).

4.5 Body weight of respondents

The weights of three hundred and fifty-two respondents were measured in the survey. Thirty-two percent (32 %) of respondents had weights above 72 kg and 2.2 % had weight between 40 kg and 45 kg. The remaining 65.73 had weights between 46 and 70 kg. The 95^{th} percentile of the weight was 69.06 kg. This means that 95% of the adult population of the Ghanaian subpopulation in Tamale weighs 69.06 kg or less. The remaining 5% had weights of 47.99 kg or more. The mean, median, mode and the least weights were 60.3 kg, 61.1 kg, 58.3 kg and 47.5 kg

4.6 Chronic Daily Intake (Exposure)

Chronic daily intake of acrylamide is the mean amount of acrylamide ingested everyday by a community of people over a long time, usually in their lifetime which is taken as seventy (70) years. Estimates of chronic daily intake (exposure) of acrylamide may be determined in different ways and dietary habits also differ among countries, a mean chronic daily intake can be considered to be about 0.4µg/kg/bw/day. The mean chronic intake for high level consumers is taken to be about 1.0µg/kg/bw/day (Mills *et al*., 2009).

Average U.S.A daily intake of acrylamide for all individuals over the age of two was estimated at 0.43µg/kg of body weight, however the estimated exposure of children aged two to five years was 1.06µg/kg (Manson *et al.*, 2005). For France, Germany, the Netherlands, Norway, Sweden, the U.K and the U.S.A acrylamide exposures ranged from 0.3-3.2µg/kgbw/day with considerable variation in the estimations.

The 95th percentile of exposure (CDI) was estimated to be 1.00µg/kgbw/day. In other words 95% of adult population of the Ghanaian subpopulation in Tamale may be exposed to acrylamide at a dose of 1.00µg/kgbw/day. The 5^{th} percentile of consumers is also exposed to acrylamide at a dose 0.01µg/kgbw/day. The mean exposure was found to be 0.262µg/kgbw/day.

The CDI obtained in this study seemingly compares favourably with the national exposure value range of 0.3-0.8µg/kgbw/day. While the national exposure value is quoted for exposure from a number of foodstuffs with potential to contain acrylamide and over a lifetime period (70 years), the CDI obtained from this study is for one particular food product and over an exposure duration of just one year. This means that

the CDI obtained in this study is very high. The large variability in acrylamide levels within a given type of food, the high percentage of foods in the diet containing acrylamide, and the variability in food preparation methods and food consumption rates among the population suggest that surveys of dietary recall do not provide accurate measure of acrylamide intake.

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4.7: Risk Determination

The risk of acrylamide consumption depends on the amount of acrylamide containing foods and the frequency of their consumption among others. Apart from *koose*, there are other food categories, cosmetics and acrylamide polluted air that are sources of acrylamide exposure to the Tamale people. The risk obtained in this study was determined from exposure to acrylamide from only one food product (*koose*).

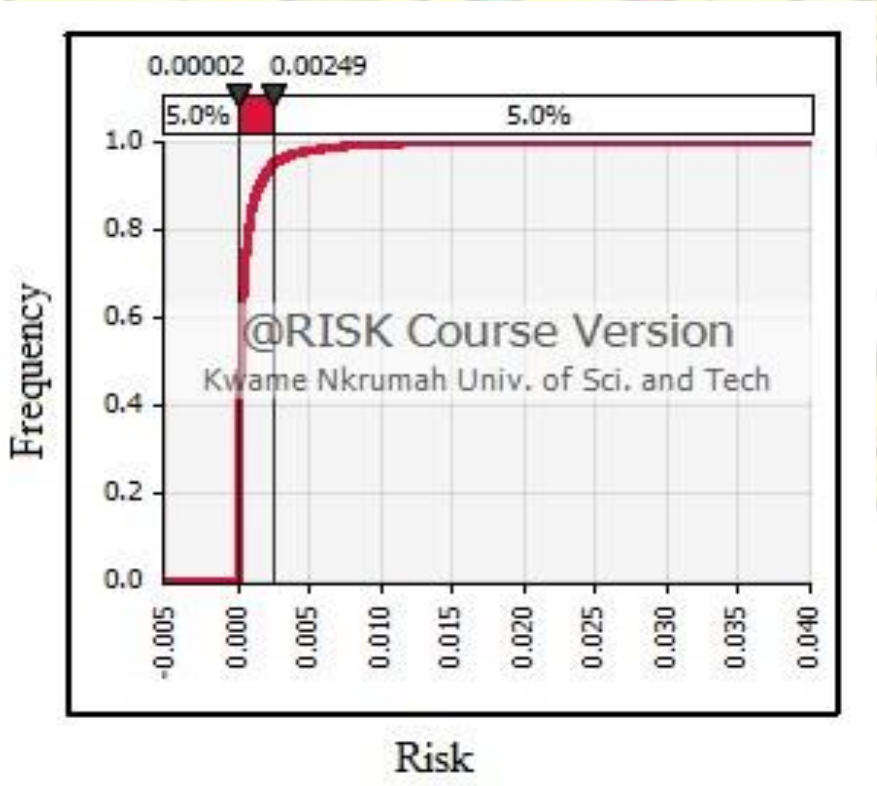

Fig 4.1: Risk distribution of acrylamide from *koose* consumption

Using the potency factor of 4.5 and an assumed exposure of 1μg/kg/day (ie. 0.001mg/kg/day) U.S EPA has calculated a lifetime risk of 4.5 excess cancers per 1000 population for acrylamide (US EPA, 1991). Using models but the same exposure factor, other calculations of risk for cancer offer probabilities of 0.7 per 1000 and 10 per 1000 (ECSCE, 2002). The result of these calculations should not be interpreted to mean that 10 people will actually get cancer. Rather these risk represent the probability that x number of people in the population of 1000 will develop cancer if exposed to acrylamide over a lifetime. Using the modal concentration and the potency factor of 4.5, the risk obtained after calculation was 0.00064.

From the distribution above (Fig 4.1), the 95th percentile, 95% of all respondents who are considered as high level consumers are at a risk of 0.0025 and the 5% (5th percentile) of all the respondents who are considered as low level consumers are at risk of 0.0000171. This means that for high level consumers of *koose* 3 out of every one thousand are at the risk of getting cancer from acrylamide every year, for low level consumers, 2 out of every hundred thousand stand the risk of getting cancer from acrylamide through the consumption of *koose* every year. The mean risk was determined to be 0.000646, implying that six (6) out of every ten thousand consumers of *koose* are exposed to the risk of getting cancer from acrylamide. These risk values were influenced by acrylamide content, the potency factor, the number of pieces of *koose* consumed, the mass of *koose* in grams consumed and the body weight of the respondents in kilograms (Appendix H). Acrylamide content had the biggest influence to the risk than the other parameters. According to the FAO/WHO (2002), one out of every hundred thousand (100,000) who take 1μg of acrylamide per day in their

lifetime is likely to get cancer.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

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5.1 Conclusion

The study has shown that *koose* contains a very high level of acrylamide. The content of acrylamide ranged from 1.12 – 74.77 µg /kg with the mean and the modal concentration being 15.89mg/g and 6.40mg/g respectively. Acrylamide was present in all the samples analyzed. The mean Chronic Daily Intake of 0.262 µg/Kg/bw/d for one year duration determined in this study is associated with a probable cancer risk of 0.000646. This implies that six (6) out of every ten thousand (10000) consumers of *koose* in the Ghanaian sub population of Tamale stand the risk of getting cancer from acrylamide every year. The level of acrylamide present in *koose* contributed the highest to the risk followed by potency factor, number of pieces of *koose* consumed per day, mass of *koose*, in that order. The body weights of the respondents, that is, whether one is a heavy weight or light weight had very little significance to the risk.

5.2 Recommendation

The presence of acrylamide in cooked foods and the consequences of intake of acrylamide contaminated food is not known to most Ghanaians. There are so many Ghanaian foods with the potential to contain acrylamide. Research must go into investigation of such foods to establish the presence or otherwise of acrylamide in order to properly educate the public. Meanwhile, effort should be made to reduce exposure

to acrylamide by avoiding burnt foods and reducing consumption of high heat treated carbohydrate rich foods, including deep frying and baking.

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APPENDICES

APPENDIX A

Food Frequency Questionnaire

Demographic, Anthropometric and Consumption Survey on koose

KNUST

A. DEMOGRAPHY

1. What is your Religion?	None	Traditional		Muslim		Others: Specify		
Responses								
2. What is your level of education?	Primary	JHS		SHS		Tertiary		Others: Specify
Responses								
3. What is your Marital status?	Single	In a Relationship		Married		Widowed		
Responses								
4. How many people do you live with in your house?	Alone	1	2	3	4	5	6	>6
Responses								
5. How many People do you eat with?	Alone	1	2	3	4	5	6	>6
Responses								

B. ANTHROPOMETRY

1. How old are you?	18- 25	26- 35	36-45	46-55	56-75	76-96
Responses						
2. *What is your weight/kg?	<40	40-45	46-52	53-60	61-70	>70
Responses						

***** *Please measurement would be taken on the scale provided*

C. koose CONSUMPTION DATA

This section of the survey is on the koose you have ate during the last month

1 Do you take koose as part of your meal? Yes No

Responses						
2. How often do you take koose	Never	Once per year	Once per month	once per week	Once per day	Others, Specify
Responses						
3. What meal times do you eat koose?	Breakfast	Lunch	Supper	In between Breakfast and Lunch	In Between Lunch and Supper	Others, Specify
Responses						
4. How many pieces of koose do you take per meal?	1 Piece	2 Pieces of koose	3 Pieces of koose	4 Pieces of koose	5 Pieces of koose	Others, Specify
Responses						
5.How many pieces of koose do you take a day						
Responses						

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APPENDIX B

The Results of Acrylamide Content in the Samples

SAMPLE CODE	ACRYLAMIDE CONTENT (µg/g).
K1	12.75
K2	4.35
K3	5.87
K4	8.60
K5	6.17
K6	5.37
K7	10.47
K8	5.52
K9	14.70
K10	6.87
K11	5.30
K12	6.42
K13	10.42
K14	6.90
K15	2.97
K16	3.77
K17	1.42
K18	5.67
K19	9.32
K20	4.65
K21	20.92
K22	4.85
K23	1.45
K24	4.37
K25	11.77
K26	7.97
K27	4.62
K28	11.30
K29	11.65
K30	9.05
K31	17.30
K32	3.30
K33	6.20

SAMPLE CODE	ACRYLAMIDE CONTENT (µg/g).
K34	3.65
K35	8.40
K36	5.85
K37	17.82
K38	4.20
K39	3.32
K40	48.07
K41	30.45
K42	29.85
K43	33.87
K44	44.75
K45	23.82
K46	28.55
K47	31.60
K48	33.22
K49	33.37
K50	32.52
K51	27.87
K52	39.82
K53	34.37
K54	32.67
K55	26.67
K56	40.92
K57	29.55
K58	39.92
K59	27.72
K60	12.30
K61	7.40
K62	15.30
K63	11.15
K64	22.15
K65	16.57
K66	8.70

SAMPLE CODE	ACI C
K67	
K68	
K69	
K70	
K71	
K72	
K73	
K74	
K75	
K76	
K77	
K78	
K79	
K80	
K81	
K82	
K83	
K84	
K85	
K86	
K87	
K88	
K89	
K90	
K91	
K92	
K93	
K94	
K95	
K96	
K97	
K98	
K99	

SAMPLE CODE	ACRYLAMIDE CONTENT (µg/g).
K100	12.07
K101	5.62
K102	5.50
K103	5.77
K104	9.20
K105	6.40
K106	2.22
K107	5.55
K108	11.57
K109	6.40
K110	5.20
K111	3.95
K112	7.85
K113	3.72
K114	11.55
K115	4.05
K116	16.60
K117	5.00
K118	3.72
K119	13.70
K120	12.00
K121	10.00
K122	7.90
K123	21.60
K124	16.80
K125	15.67
K126	6.05
K127	7.14
K128	13.33
K129	6.79
K130	10.32
K131	6.91
K132	4.91

KNUST

SAMPLE CODE	ACRYLAMIDE CONTENT (µg/g).
K133	18.40
K134	14.47
K135	13.62
K136	11.62
K137	2.77
K138	2.77
K139	15.00
K140	3.50
K141	1.12
K142	1.60
K143	4.05
K144	7.45
K145	3.35
K146	3.65
K147	3.02
K148	2.37
K149	2.20
K150	5.87
K151	7.77
K152	3.10
K153	3.05

KNUST

APPENDIX C

Results of Analysis of Food Frequency Questionnaire Data

KNUST

C1 Weights of Respondents

Weight/Kg	Frequency	Percent
>70	113	32.10%
40-45	8	2.27%
46-52	29	8.24%
53-60	71	20.17%
61-70	131	37.22%
TOTAL	352	100.00%

C2 Frequency of Consumption of ***koose*** by Respondents

Frequency Of Consumption	Frequency	Percent
NEVER	8	2.76%
ONCE PER DAY	102	35.17%
ONCE PER MONTH	55	18.97%
ONCE PER WEEK	73	25.17%
ONCE PER YEAR	18	6.21%
OTHERS	34	11.72%
TOTAL	290	100.00%

C3 Number of ***koose*** Pieces Eaten Per Day

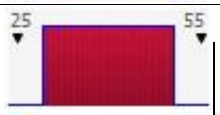
Number of ***koose*** Pieces	Frequency	Percent
1 PIECE	2	0.81%
2 PIECES	21	8.50%
3 PIECES	63	25.51%
4 PIECES	73	29.55%
5 PIECES	52	21.05%
OTHERS	36	14.57%
TOTAL	247	100.00%

KNUST

KNUST

APPENDIX
D

Summary of the Analysis of Acrylamide Content in the ***koose*** Sampled

Name	Cell	Graph	Min	Mean	Max	5%	95%	Errors
Acrylamide content	E3		0.0147343	6.4393	974.9818	0.2592928	23.86487	0
No of koose per day	J9		1.004182	3.961623	5.999527	2.244939	5.713967	0
Mass of koose/g	J17		30.00106	40	49.99875	30.9998	48.99997	0
PF	J56		0.5002823	2.500001	4.49984	0.6997687	4.2999	0
Body weight	K35		40.02163	60.3178	69.9988	47.98441	69.06359	0

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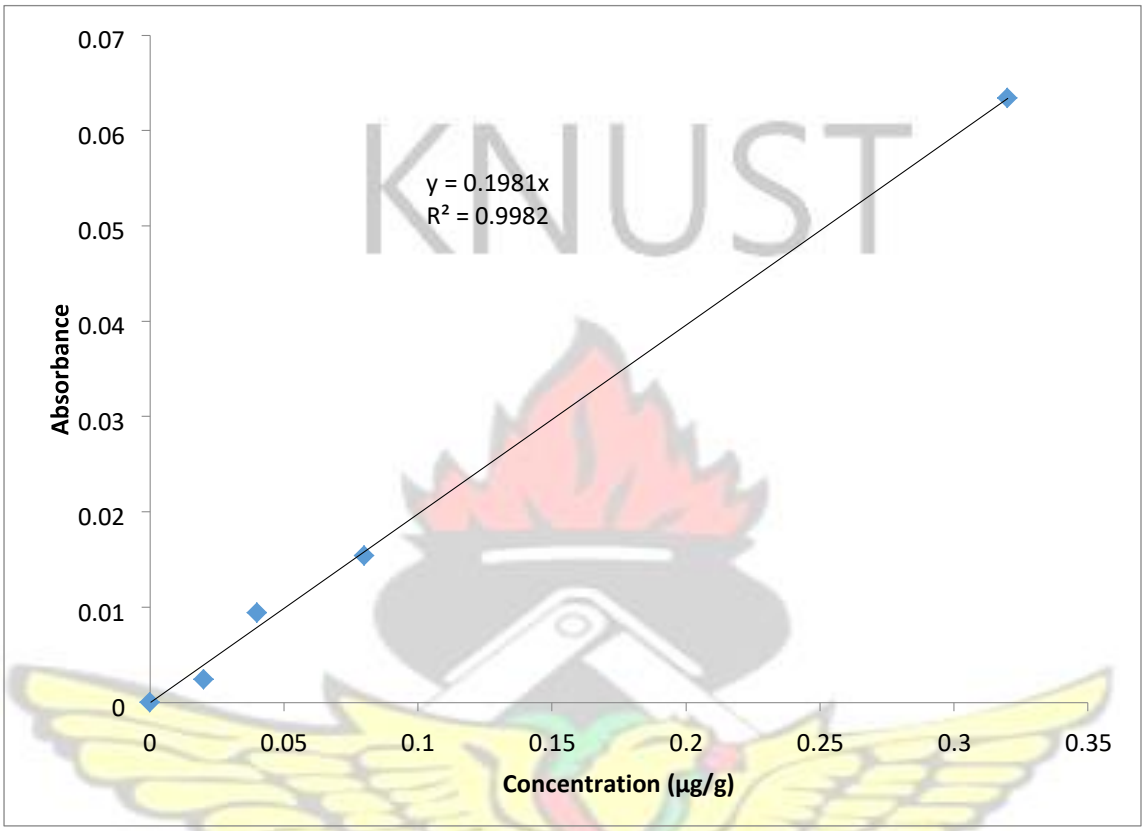
APPENDIX E

Standard Acrylamide Calibration Curve



F

APPENDIX
Formular for the Calculation of Chronic Daily Intake (Exposure) and Risk of Chemical Toxins.

The formular for calculating Chronic Daily Intake (CDI) or exposure is given as:

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$$CDI = \frac{C \times CR \times EF \times ED}{BW \times AT}$$ Where:

C = The average exposure concentration

CR = Contact rate, the amount of contaminated medium.

EF = Exposure frequency (days/years)

ED = Exposure duration (years)

AT = Average time, thus period over which the exposure is average (days)

The formular for calculating Risk of chemical toxin is

Risk = PF (CDI - RfD)

Where;

PF = Potency factor of toxins

RfD = Reference dose

However in the absence of the reference dose of toxins (thus if a little of such reference toxins availability is assumed to have a risk, then there will be no level of which it is assume as safe for consumption, therefore RfD = 0)

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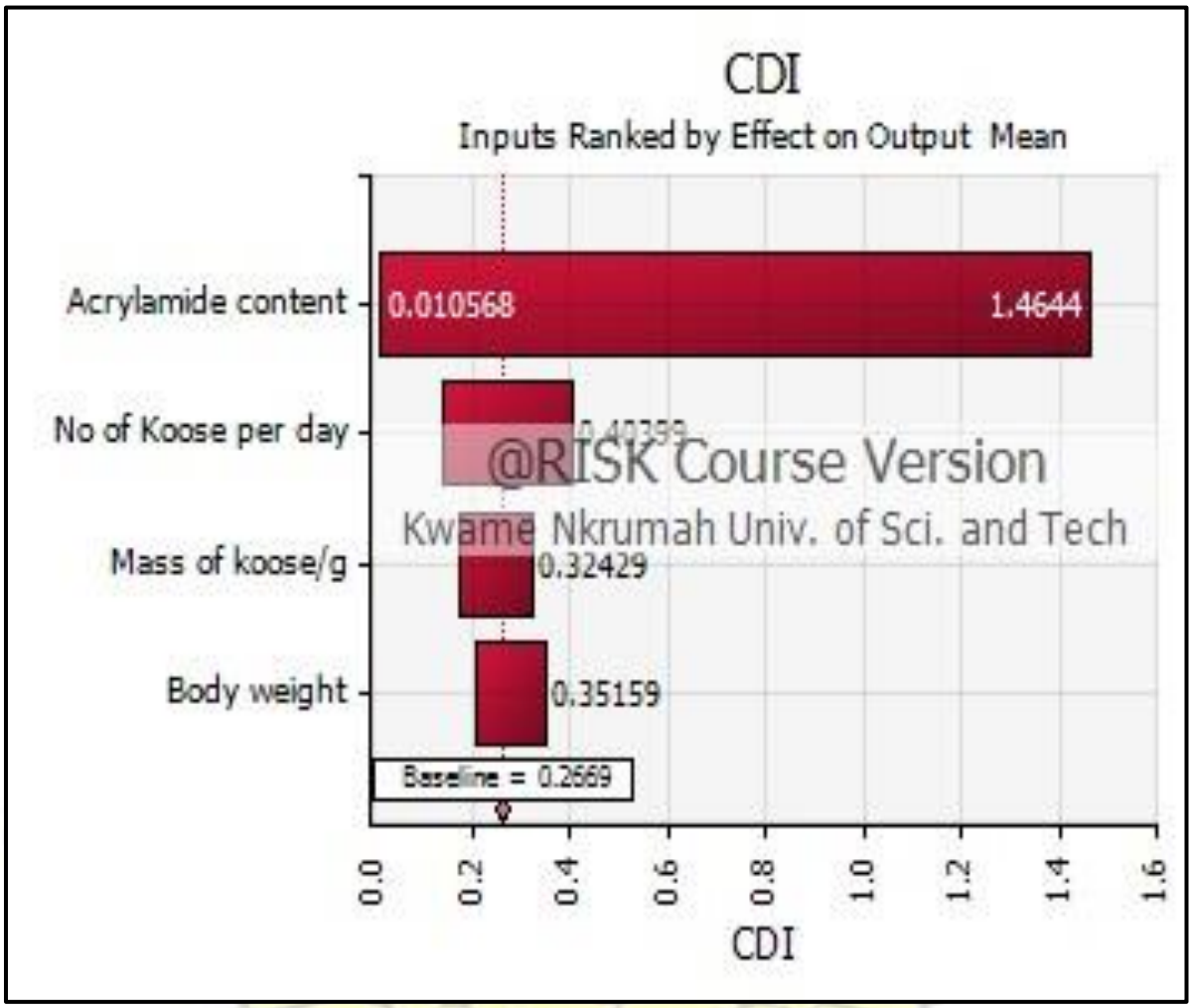
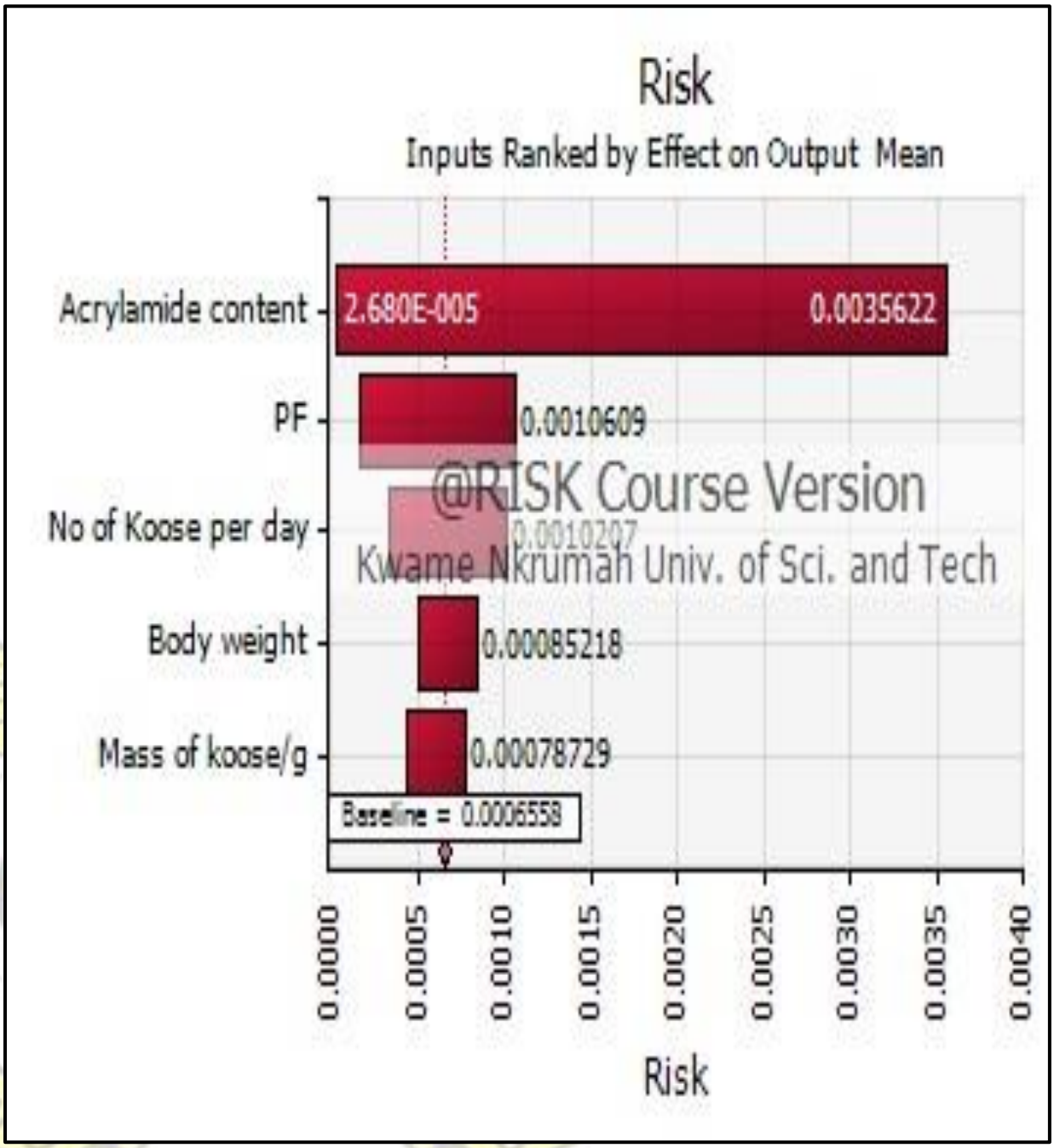
G

APPENDIX

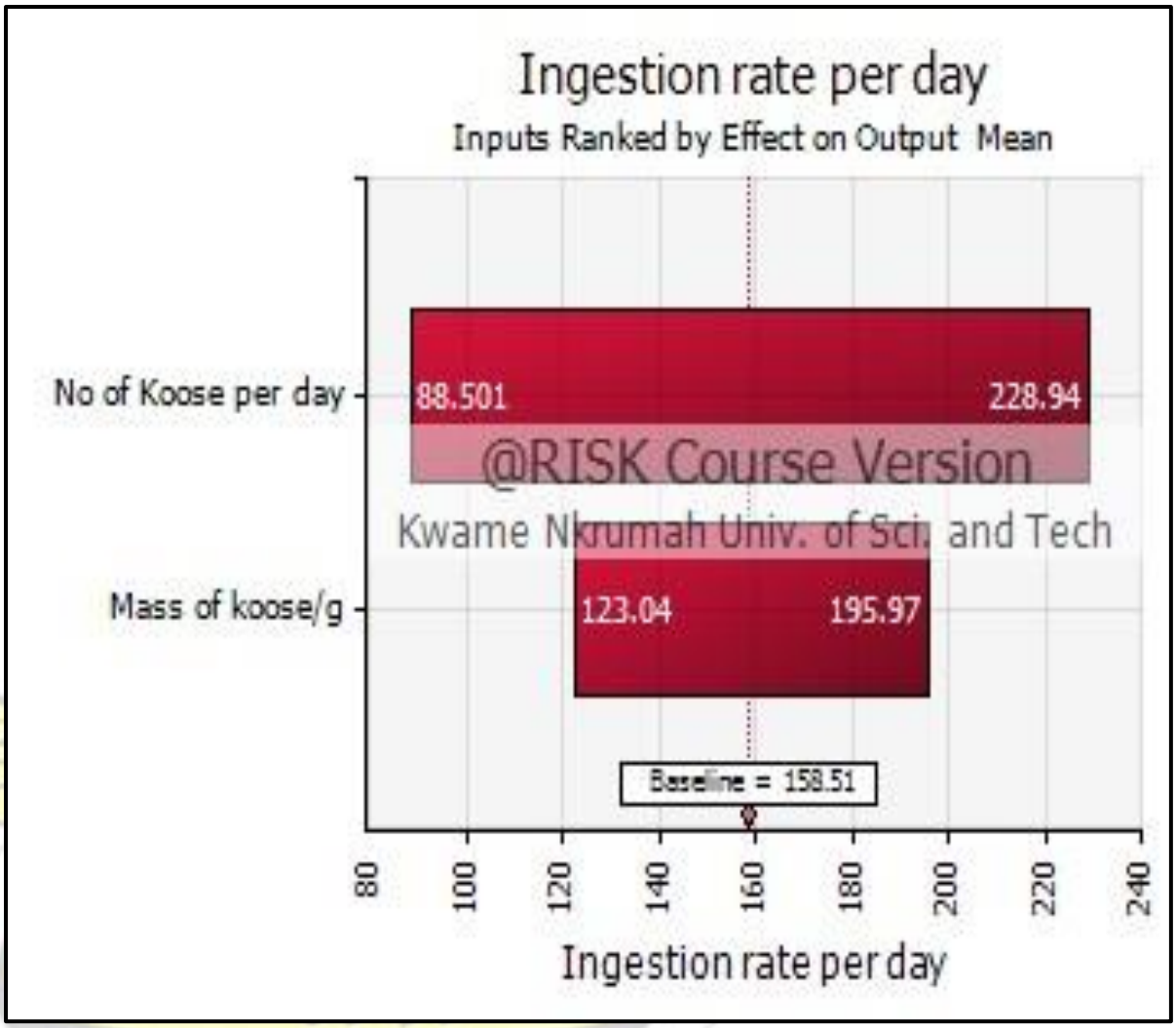

CDI
Inputs Ranked by Effect on Output Mean
Acrylamide content
0.010568
1.4644
No of Koose per day
@RISK Course Version
Kwame Nkrumah Univ. of Sci. and Tech
Mass of koose/g
0.32429
Body weight
0.35159
Baseline = 0.2669
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
CDI

APPENDIX H


Risk
Inputs Ranked by Effect on Output Mean
Acrylamide content
2.680E-005
0.0035622
PF
0.0010609
No of Koose per day
0.0010207
@RISK Course Version
Kwame Nkrumah Univ. of Sci. and Tech
Body weight
0.00085218
Mass of koose/g
0.00078729
Baseline = 0.0006558
0.0000
0.0005
0.0010
0.0015
0.0020
0.0025
0.0030
0.0035
0.0040
Risk

APPENDIX

I


Ingestion rate per day
Inputs Ranked by Effect on Output Mean
No of Koose per day
88.501
228.94
@RISK Course Version
Kwame Nkrumah Univ. of Sci. and Tech
Mass of koose/g
123.04
195.97
Baseline = 158.51
80
100
120
140
160
180
200
220
240
Ingestion rate per day