ACRYLAMIDE IN POPULAR WEST AFRICAN FOODS

By

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A doctoral thesis submitted in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy in Food Science

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DECLARATION

I *Timothy Olugbenga Akinosun* hereby declare that this research work titled '*Acrylamide in West African foods'* was carried out by me under the supervision of Dr. Delia Ojinnaka (Director of Study) and Dr. Amar Aouzelleg (Second Supervisor) of the School of Applied Sciences (Division of Food Sciences), London South Bank University, London, United Kingdom.

I declare that the findings from the study has not been submitted to any University for examination or award of degree, and that all sources used or quoted have been acknowledged by means of complete references.

Timothy, Olugbenga Akinosun

CERTIFICATION

The research work titled '*Acrylamide in West African foods'* carried out by Timothy Olugbenga Akinosun (LSBU-3200452) under the supervision of Dr. Delia Ojinnaka and Dr. Amar Aouzelleg meets the regulation governing the award of the degree of Doctor of Philosophy (Ph.D.) in School of Applied Sciences, London South Bank University, London, United Kingdom. We hereby certify that it has not been submitted for the degree of Ph.D. here or elsewhere for any other award.

Dr. Delia Ojinnaka (First Supervisor)	Date
Dr. Amar Aouzelleg (Second Supervisor)	Date
Dean, School of Applied Sciences	Date
External Examiner	Date

DEDICATION

I dedicate this work first to the almighty God for giving me strength, direction and faith.
I also dedicate this work to my amiable parents for their financial, spiritual and moral support throughout the course of this work.

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My foremost acknowledgment goes to the almighty God for giving me the health, resources wisdom, knowledge and understanding to pursue the doctor of philosophy (PhD) degree in food science.I also acknowledge the quality support given to me by my parents in terms of finance, prayers and encouragements.

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ABSTRACT

Acrylamide (AA) is a toxic compound, present in a wide range of heat-processed foods prepared from materials rich in reducing sugar and asparagine. Since the time of discovery of AA in foods, no study has considered the effect of precursors, commonly used methods and temperatures of cooking on the formation of the contaminant in popular West African (WA) foods. Consequently, this study focused on the impact of these parameters on the acrylamide levels in WA foods including *akara, bread, buns, chin-chin, doughnut, fish-roll, meat-pie, plantain-chips, puff-puff,* and *yam-chips*.

The study evaluated the effect of baking and frying at 150, 180 and 210°C for 5, 10 and 15 mins on the AA levels in the selected WA foods. Potentiometric method based on the use of ammonium ion selective electrode and immobilised acrylamide amidohydrolase was used to test 150 samples of each WA food prepared using the different cooking temperatures.

In addition, recognised methods requiring glucose oxidase and asparaginase were used for measuring the amount of precursors including glucose and asparagine in the unprocessed food materials.The relationship between the contaminant and the precursors was then determined through correlation and regression analysis of the data obtained. Independent T-test of equality of sample means at $\alpha=0.05$ showed no statistical significant difference ($p > 0.05$) in the AA produced by using baking and frying at the same temperature. However, ANOVA for the AA concentrations indicated that increasing baking and frying temperatures significantly affected the amount of the process contaminants i.e. ($p < 0.05$). Highest readings for the contaminant was noted for WA foods processed for longer times at 210°C, while the lowest measurements were obtained for those processed for 5mins at 150°C. The lowest amounts of acrylamide (25±7 µg/kg) was detected in *buns* baked at 150°C, while the highest levels of the contaminant (703±27 and 706±13 µg/kg) were noticed in *yam* and *plantain chips* fried at 210°C. Overall, a significant positive relationship ($p < 0.01$) was observed between AA present in the food products and the precursors measured in the food materials. The strongest and weakest determination coefficients (r^2) of 0.88 and 0.30 were observed for WA *plantain-chips* and *bread* respectively. In conclusion, the temperature of cooking, asparagine and glucose levels in WA food materials are major determinants of the AA formed in the selected WA food products.

Keywords: acrylamide, asparagine, reducing sugars, West African foods, potentiometric analysis.

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PREFACE

The art of cooking and processing food materials with heat distinguishes human being from other animals. The heat processing of foods through methods including baking, frying, and roasting improves the microbiological safety, nutritional and organoleptic properties of foods.

In addition, antinutrients in legumes and grains such as phytate, tannins, protease inhibitors, calcium oxalate and lectins are removed by the application of heat and methods such as fermentation and soaking.

Although dry thermal treatment of food is crucial to the existence of humanity, recent studies show that several toxicants are generated as a result of it. Polycyclic aromatic hydrocarbons (PAHs), furans and nitrosamines generated after the frying and baking of certain foods exhibit carcinogenic and mutagenic properties.

About two decades ago, another process contaminant called acrylamide was found in heat-processed carbohydrate-rich foods by Swedish researchers. After the discovery, extensive and intensive scientific studies on the existence, analysis, exposure, mechanisms of formation and mitigation of the compound have been conducted.

Although many traditional foods of different part of the world such as French fries, breakfast cereals, coffee, and chocolate have been studied, no study has considered quintessential West African foods such as *akara*, *bread*, *buns*, *chin-chin*, *doughnuts*, *fish-roll*, *meat-pie, plantain-chips*, *puff-puff* and *yam-chips*.

This thesis titled **'Acrylamide in West African Foods'** focused on areas including *Awareness survey on the presence and implication of acrylamide in WA foods*; *Contribution of cooking methods and temperatures to the acrylamide in WA foods*; *Impact of essential precursors on the acrylamide levels in West African foods*. The methods described in this thesis highlights simplified and less expensive laboratory procedures for the determination of acrylamide and its precursors (asparagine and glucose) in food samples.

It is the hope of the author that science students, educators, food chemists, analysts and laboratory technicians find this thesis relevant for their class, laboratory and field works.

Timothy Olugbenga Akinosun

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ABBREVIATIONS

AA.....Acrylamide

A-ISE..... . . . . Ammonium Ion Selective Electrode

AK.....Akara (West African Bean Cake)

Asn.....Asparagine

Asp.....Aspartic acid

BR.....Bread

BN.....Buns

DRv……...…Derivatization

CN.....Chin-Chin

C-Up.....Clean up

DN.....Doughnuts

EC.....European Commission

EFSA.....European Food Safety Authority

FDE.....Food Drink Europe

FR.....Fish Roll

FSA.....Food Standard Agency

GOx.....Glucose Oxidase

JECFA.....Joint FAO/WHO Expert Committee on Food Additives

IARC.....International Agency for Research on Cancer

LOD..... Limit of Detection

LOQ.....Limit of Quantification

M.....mole per litre

Mins……Minutes

MP.....Meat-Pie

PC.....Plantain Chips

POx .....Peroxidase

ppb.....part per billion

ppm.....part per million

PF.....Puff-Puff

WA.....West Africa (n)

WHO.....World Health Organization

XTr……Extraction

YC.....Yam Chips

LIST OF PUBLICATIONS

I. List of published abstracts

(1) Timothy O. Akinosun, Delia Ojinnaka, Amar Aouzelleg (2018). An awareness survey on the presence and implications of acrylamide in West African foods and the extent of consumption of such foods. European Food Chemistry Congress Proceedings, Journal of Food Processing and Technology, Volume 9 ISSN: 2157-7110.

(2) Timothy O. Akinosun, Delia Ojinnaka, Amar Aouzelleg (2019). Contributions of different cooking methods and temperatures to the level of acrylamide in common heat-processed West African foods. Published in 26th International Conference Proceedings of Functional Food Centre, Food Science Publisher, ISBN 9781095227091.

Chapter 1

Introduction

CHAPTER 1. GENERAL INTRODUCTION

1.1 Overview

This thesis focused on the influence of important factors such as cooking temperatures and precursors on the formation of the acrylamide in popular West African (WA) foods.

For the qualitative aspect of the study, Bristol online survey (BOS) was used to assess the awareness level of WA on the subject of formation and implications of acrylamide in heat-treated foods.

In the case of the quantitative aspect of the study, analytical methods including potentiometric and spectrophotometric techniques were used for measuring the level of the contaminant and its precursors in the selected WA foods.

In addition, statistical methods such as T-tests, analysis of variance (ANOVA), correlation and regression analysis were used to present the data collected from the studies.

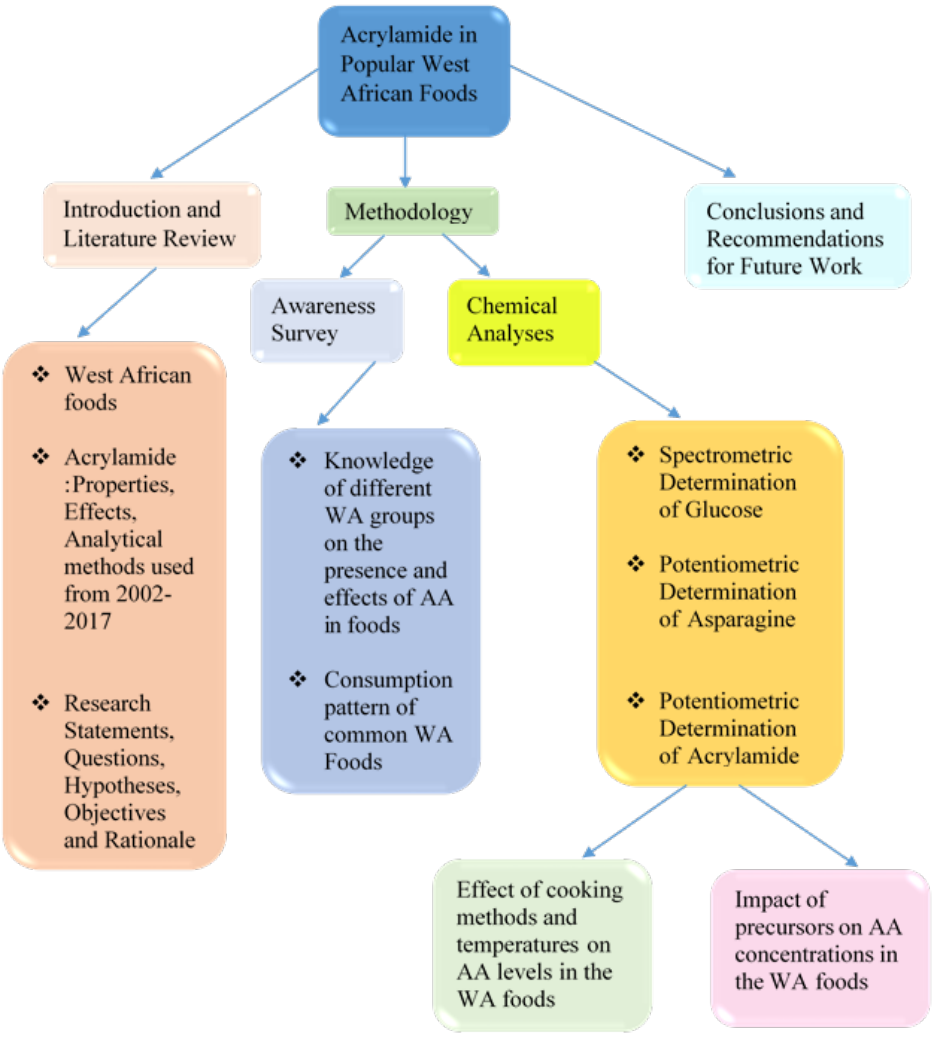

Figure 1.1. *The Research Structure.*

1.2 Introduction

The nutritional, sensory and safety aspects of food consumption are essential topics considered throughout the world by food scientists, businesses and consumers.

In many WA countries where there is poor supply of electricity, needed for the refrigeration or freezing of food items, the processing of foods by heat is the most relevant long-term food preservation technique. The heat treatment of foods destroy enzymes and microorganisms, which are important in food spoilage and poisoning. Heat processing also improves the sensory attributes and nutritional properties of the foods.

Although, heat is important in the enhancement of the sensory quality and preservation of foods, studies have shown that heat treatment of foods can also have some undesirable effects. These include destruction of antioxidants, denaturation of proteins, inactivation of vitamins, and release of processing contaminants such as N-nitrosamines, furans and polycyclic aromatic hydrocarbons (PAH) which are known to be toxic and carcinogenic.

In addition, recent researches have demonstrated that a neurotoxic and potentially carcinogenic chemical called *Acrylamide* (AA), commonly associated with plastics, cosmetics, water treatment facilities, and cigarette, is released when carbohydrate rich foods are subjected to high temperatures. The matter of acrylamide in heat-processed foods, particularly in fried and baked goods, has continued to be a topic of interest after its discovery in potato fries in 2002.

Since April 2002, when the Swedish National Food Administration (SNFA) reported the presence of acrylamide in some heat-treated foods, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have been involved in the risk assessment of acrylamide in foods. The FAO/WHO Joint Expert Committee on Food Additives (JECFA) held a special consultation to review the available data on acrylamide in 2002 and 2005.

The Centre for Science in the Public Interest (CSPI), the U.S. Food & Drug Administration (FDA) and the United Kingdom's Food Standards Agency (FSA) are other important groups that have been assessing the acrylamide content of popular heat-treated food products such as bread, potato chips, and coffee. Out of the products examined, highest levels between 400-1200 ppb were found in potato chips and French fries. This is an enormous figure when compared to the maximum limit of 0.5 μg/L (0.5 ppb) acrylamide set for drinking water by the World Health Organization (Who,1996).

Many of the popular WA heat-processed foods can be described as street foods- which are considered as ready-to- eat (RTE) commodities sold by itinerants or stationary vendors particularly on the streets and in other public places (FAO, 2009).

In various WA countries, many people depend on these ready-made convenient foods for their daily nutritional requirements. The appealing sensory attributes, availability and affordable prices of these RTE foods make them important and popular sources of energy and nutrients.

1.2.1 Some popular WA heat-processed foods and their importance

Popular heat-processed street foods of WA origin include *akara (fried bean cake) bread* , *buns*, *chin-chin (ghana chips)*, *dodo ikire*, *doughnut*, *fish-roll*, *roasted yam*, *garri*, *kokoro (corn-meal snack)*, *kuli-kuli (deep-fried groundnut snack)*, *meat-pie*, *ojojo (water yam ball)*, *pancake*, *puff-puff (bofrot)*, *roasted maize*, *roasted plantain (boli)*, *plantain chips*, *robo*, *yam chips* and *fries*.

These peculiar heat-treated foods of WA origin can be categorized as baked, fried, roasted or smoked, depending on the heating method or degree of temperature used to prepare them. In addition, some of these ready-to-eat foods have more than one method of processing. For example, *chin-chin* and *doughnuts* may be produced by methods of baking and frying.

It is worth noting that some of these foods are not only consumed in WA, but are also available in other countries e.g. Popular heat-treated foods of WA origin such as *Agege* bread, *kuli-kuli*, and *chin-chin* are available in remote countries such as United Kingdom and United State of America.

Most of these foods can serve as main meals of the day i.e. breakfast, lunch or dinner (Olumakaiye *et. al.*, 2008). These inexpensive and convenient foods can also be used as light meals at any time of the day or night. For children and adults, they serve as snacks used to assuage the hunger between meals and as momentary source of energy for the body.

A preference for street foods which are easy to prepare, convenient to carry and cheap to buy is expected for a society with a busy lifestyle. In Nigeria, They are essential components of packed lunch and takeaways.

The high rate of consumption of these street foods by children could be ascribed to factors such as time constraints and pressures of working life experienced by parents and guardians who may find it difficult to prepare breakfast and lunch at home.

The high rate of consumption of these street foods can also be attributed to the insufficiency of the common "three-meals-a-day feeding pattern" to meet the nutritional requirements of some individuals (Lobstein *et. al*., 2004). The urbanization of several region in a WA country such as Nigeria also accounts for the consumption of more street and fast food (Babatunde, 2012). Akinyele (1998) reported that the consumption of snacks contributed a considerable portion to the daily nutritional requirements of Nigerians. For instance, in 1996 two-thirds of daily meals of Nigerians were purchased from street food vendors and fast food chains (Akinyele, 1998). Between the year 1998 and 2003 there was a reported increase in the number of registered fast food vendors from 1,342 to 5,437 (Olayiwola et al., 2003).

According to Hedley *et al*., 2004, these snacks can also help adolescents concentrate at school by helping to keep their blood glucose levels high enough to prevent fatigue.

The relevance of snacks production to the small-medium enterprises (SME) and large scale businesses cannot be overemphasized. For many unemployed women in WA countries like Nigeria and Ghana, the selling of snacks is an important way to obtain income and livelihood.

Although, most of the food businesses in WA countries are operated on a small scale they provide many of the participants with a reasonable income. Hence, in WA countries with low employment rate these street foods generate sustainable income, a high volume of sales, and a high level of employment. Average profits from street food businesses have been estimated to be above earnings from alternative sources of employment (Cohen, 1986).

1.2.2 Synopsis for the preparation of popular WA heat processed foods.

Although there are several variants of each WA food, the basic ingredients for their preparation are shown in Table 1.1.

(a) Preparation of Akara

The materials for *akara* preparation are peeled beans, onions, scotch bonnet chillies, table salt, vegetable (or palm) oil, water, electrical grinder and fryer.

Table 1.1 *List of basic ingredients for the preparation of popular* WA *foods*

List of Ingredients	***Akara***	***Bread***	***Buns***	***Chin-chin***	***Doughnut***	***Fish-roll***	***Meat-pie***	***Plantain-chips***	***Puff-puff***	***Yam-chips***
Baking powder		✓	✓	✓	✓	✓	✓			
Beans	✓									
Butter		✓	✓	✓	✓	✓	✓			
Carrot							✓			
Egg					✓	✓	✓			
Fish										
Flour		✓	✓	✓	✓	✓	✓		✓	
Meat							✓			
Milk				✓	✓					
Nutmeg			✓	✓						
Oil	✓		✓	✓	✓	✓	✓	✓	✓	✓
Onions	✓									
Pepper	✓					✓	✓			
Plantain								✓		
Potatoes							✓			
Salt	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Sugar		✓	✓	✓	✓	✓	✓		✓	
Yam										✓
Yeast		✓			✓				✓	

(b) Preparation of Bread

The materials for bread preparation are plain flour, butter, sugar, fast action dried yeast, warm water, salt, stainless steel bowl and electrical oven.

(c) Preparation of Buns

The materials for *buns* preparation are all-purpose flour, butter, baking powder, sugar, table salt, water, nutmeg and electrical oven or fryer.

(d) Preparation of Chin-Chin

The materials for *chin-chin* preparation are plain flour, margarine, evaporated milk, baking powder, granulated sugar, salt, ground nutmeg, and water. Rolling pin is needed to flatten and stretch the dough. Electrical fryer or oven is required for the heat-processing of the dough.

(e) Preparation of Doughnut

The materials for *doughnut* preparation are sifted plain flour, dry yeast, butter, eggs, milk, granulated sugar, and table salt. Rolling pin and circular cutters are needed to flatten and cut the dough into ring shaped portion. Syringes may be needed to inject the ring-shaped dough with jam. Electrical oven or fryer is required for the heat-processing of the doughnut.

(f) Preparation of Meat Pie

The materials for *meat-pie* preparation are all-purpose flour, sugar, baking powder, margarine, water, yeast, and table salt. Pre-cooked minced beef, diced potatoes, onions, pepper and carrots are also needed as pie fillings. Electrical oven or fryer is needed for the heat processing of the meat-pie.

(g) Preparation of Fish Roll

The materials for *fish-roll* preparation are all-purpose flour**,** sugar**,** baking powder**,** margarine**,** water, yeast**,** and table salt. Pre-fried and mashed mackerel, onions and pepper are required for the fish roll fillings. Electrical oven or fryer is required for the heat processing of the Fish-roll.

(h) Preparation of Plantain Chips

The materials for *plantain-chips* preparation are fresh plantain and table salt. An oven or a fryer is needed for the thermal processing of WA plantain chips.

(i) Preparation of Puff-puff (Bofrot)

The materials for *puff-puff* preparation are flour, fast action yeast, sugar, table salt, chilli flakes, ground nutmeg, and water. The dough can be baked or fried using electrical oven or fryer respectively.

(j) Preparation of Yam Chips

The materials for *yam-chips* preparation are fresh yam tuber and table salt. The WA food can also be produced by baking or frying.

1.3. Background of Research

Acrylamide (AA) is a chemical substance that has beneficial applications in the paper, textile and other industries. For example, polyacrylamide, which is a derivative of AA is utilized as a paper strengthening additive in paper making, as a flocculating agent in water treatment and as electrophoresis gel in research laboratories. (FSA, 2002; USFDA, 2004).

Although, AA is useful to several industries and research agencies, it also has some hazardous properties. In 1994, the International Agency for Research on Cancer categorized acrylamide as a "Group 2A chemical", which is a tag for chemicals probably carcinogenic to humans (IARC, 1994). The scientific committee of the European Commission (EC, 2002) later classified AA as a "Category 2" carcinogenic and mutagenic substance. In 2010, the European Chemical Agency classified the chemical as a substance of "very high concern" (ECHA, 2010). The neurotoxic, carcinogenic and genotoxic effects of AA was subsequently confirmed by findings from several animal studies (Erkekoglu and Baydar, 2010; Hogervorst, 2010).

Exposure assessment of the chemical indicated that heavily exposed workers showed neurotoxic effects and their blood contained elevated levels of acrylamide bound to haemoglobin and nucleic acids (Vesper et al., 2010). Animal studies also indicated that chronic exposure to acrylamide and its reactive metabolite, glycidamide might lead to the development of tumours at several sites in rodents (Bergmark et al, 1993; Doerge et al., 2005; Manière et al., 2005)

A more recent two-year mouse and rat study by Beland et al., (2012) also supports the acclaimed potential of acrylamide as a genotoxic and carcinogenic compound (IFST, 2017)

Human exposure to AA occur by direct contact with the chemical during its industrial and laboratory handling (Bergmark et al., 1993). The smoking of cigarette (White et al, 1990; Smith et al, 2000), and the application of polyacrylamide containing cosmetics (Andersen, 2005) are also potential sources of exposure to AA.

Apart from the aforementioned sources of exposure to AA, people all over the world are exposed to varying amounts of acrylamide through the consumption of heat-processed foodstuffs (Vesper et al 2008, 2010).

The reports by Tareke et al., (2000) and the Swedish National Food Administration, SNFA (2002) created an awareness of the public to the presence of acrylamide in heat-treated foods.

These reports launched an intense investigation of important food items with the potential to contain high levels of AA. The survey of the process contaminant in different groups of

European foods including French fries, potato crisps, bread, breakfast cereals, biscuits and crackers, coffee, and baby foods led to the recommendation of appropriate benchmark levels for the various foods. For example, potato crisps, French fries and wheat based soft bread were assigned maximum limits of 750, 500 and 100 ppb respectively (EC Regulation 2017/2158**).**

For WA countries, exposure to acrylamide via diets is of great concern. In many WA countries where there is poor supply of electricity, refrigeration and freezing are not the primary method of food preservation. The processing and preservation of foods depends largely on cooking methods such as frying, grilling, roasting and smoking of foods. These different methods of cooking are used to enhance and modify the flavour, colour, texture and taste of WA foods to appealing states.

In addition, the heat treatment of these WA foods destroys or inhibit enzymes and microorganisms, which are important in food spoilage and poisoning.

In various WA countries, many people depend on these so-called "street" or convenient foods, which are sold by itinerants or stationary vendors on the streets and in other public places (FAO, 2009), for their daily nutritional requirements.

The high consumption of these street foods in and out of WA countries makes them an indispensable source of public health concern, particularly with regard to the discovery of acrylamide in heat-processed foods.

1.3.1. Problem Statements

The report by the Swedish National Food Administration (SNFA) in 2002 created public health concerns regarding the formation of the neurotoxic, genotoxic and potentially carcinogenic process contaminant in heat-treated carbohydrate-rich foods (Capuano and Fogliano, 2011). Many studies and reports have confirmed the presence of this compound in a range of baked, fried and roasted foods (Rosen and Hellenas, 2002)

Although, the research on the presence and level of acrylamide in food has been intense since 2002, there is still paucity of information on the acrylamide content of popular heat-processed WA foods.

Most of these foods are not only carbohydrate and protein rich, but also made by heat processing methods such as baking, frying, grilling, roasting and smoking. It is also worth noting that in this region of the African continent, where electric power supply is not reliable, frying, grilling, roasting and smoking are not only for food preparation, but also employed as necessary methods of food preservation.

In addition, many of these foods are commercially available throughout the world and the United States Department of Agriculture (USDA) has a devoted database for the nutritional composition of some of these foods (USDA Research Service, 2019).

Hence, the huge gap in the knowledge on the level of acrylamide in these important WA foods constitute a challenge, which requires consideration.

The problem statements for this research are summarised below.

a. There is a huge gap in knowledge of the acrylamide (AA) content in WA foods.
b. Methods of preservation for most WA foods are mainly heat-dependent.
c. No study has considered the AA content of WA heat-treated foods such as *chin-chin* and *kuli-kuli*.
d. No study has considered the AA mitigating factors for the aforementioned WA heat-treated foods.
e. No study has considered the relationship between the levels of AA precursors (such as reducing sugars) and the concentrations of AA in these WA foods.
f. Although the concentrations of AA in these foods are unknown, several WA foods are available and consumed in and outside WA countries. For example *chin-chin* is also sold at various markets in U.K and U.S.A.
g. The level of awareness of WA on the connection between heat-treatment of foods and the concentration of this processing contaminant is unknown.

1.3.2. Research Questions

Several questions arising from the identified problems are listed below.

(i) What is the level of awareness of WAs on the presence and effect of acrylamide in popular WA foods?
(ii) Based on the levels of precursors in the selected WA foods, what are their acrylamide forming potentials?
(iii) What is the level of acrylamide in popular baked, fried and roasted WA foods?
(iv) How does the use of different cooking methods (e.g. baking, frying, roasting) affect the AA content of these WA foods?
(v) What is the relationship between the concentration of precursors such as reducing sugars, asparagine and the AA levels in these foods?
(vi) What are the effects of recognized mitigating factors?
(vii) Are the levels of AA in these foods greater than recommended limits?

1.3.3. Research Hypotheses:

The objectives of this research are several thus several hypotheses are considered as single hypothesis will not be satisfactory.

(a) Null hypothesis (H_o; $\mu_{A'} = \mu_{A'}$) No difference in the level of awareness for the groups of participants; Alternative or research hypothesis (H_A; $\mu_{A'} \neq \mu_{A'}$)- Different level of awareness for the groups of participants

(b) Null hypothesis (H_o; $\mu_B = \mu_F = \mu_R$) No difference in the concentration of AA produced by baking-b, frying-f or roasting-r; Research hypothesis (H_A; $\mu_B \neq \mu_F \neq \mu_R$) Different cooking methods produce different amount of AA.

(c) Null hypothesis (H_o; $[r_{PA}] = 0$) There is no relationship between the levels of precursors and the AA concentration in the WA foods. Research hypothesis (H_A; $[r_{PA}] \neq 0$) There is a relationship between the levels of precursors and the AA concentration in the WA foods.

(d) Null hypothesis (H_o; $\mu_T = \mu$) The use of mitigating factor (T) does not affect the concentration of AA in the WA foods; Research hypothesis (H_a; $\mu_T \neq \mu$) or ($\mu_T < \mu$) –The use of mitigating factor (T) affects or reduces the concentration of AA in heat-treated WA foods.

(e) Null hypothesis (H_o; $[\mu] = [I_v]$) or or H_o; $[\mu] \leq [I_v]$ - The concentration of AA in the WA foods is not different from recommended or indicative values (I_v). Research hypothesis (H_a; $[\mu] \neq [I_v]$ or H_a; $[\mu] > [I_v]$) - The concentration of AA in the WA foods is different from I_v (or exceeds recommended values).

1.3.4. The Aims and Objectives

The primary aim of the project is to determine the concentration of acrylamide in selected heat-processed WA foods using rapid and inexpensive potentiometric method. The objectives to achieving the goals are listed below.

(1) Evaluation of the level of awareness of acrylamide in heat-processed WA foods, through an online survey.

(2) Comparison of the effects of different types of cooking methods or heat processing (frying, baking, roasting and toasting) on the concentration of acrylamide produced.

(3) Establishment of a possible correlation between the concentration of precursors such as asparagine, glucose and the concentration of acrylamide produced.

(4) Exploration of the different factors, which may mitigate or retard the production of acrylamide in heat-processed foods. For example, using raw materials with different concentrations of precursors, altering the recipe by using different flour etc.

(5) Consideration of the national/global regulatory levels of acrylamide in foods in order to establish significance of the levels in the selected WA foods.

1.3.5. Research Rationale

There justification for the project are outlined below.

(a) *The confirmation of the toxic effects of acrylamide*: Many reports from animal and human studies have established the neurotoxic, genotoxic and potential carcinogenic effects of acrylamide (IARC, 1994; IFST 2017; JECFA 2010).

(b) *The establishment of the presence of acrylamide in heat-treated foods*: The findings from the investigation of several groups of foods by the European Food Safety Authority (EFSA) and other agencies gives credence to the acclaimed presence of acrylamide in heat-treated carbohydrate-rich foods (Amrein et al, 2005; Croft et al., 2004; European Commission, 2013; EFSA, 2012; IFST, 2017; Leung et al., 2003; Lineback et al., 2005; Murkovic, 2004; Ono et al., 2003; Tareke et al., 2002; Roach et al., 2003; Rosén and Hellenäs, 2002).

(c) *The public awareness campaign by FSA*: The intense effort of the Food Standard Agency (FSA) in creating public awareness on the formation and health implications of this toxic compound in heat-treated foods. The FSA warnings on the potential cancer risk of consuming browned toast and crispy roast potatoes (The Telegraph, 2017)

(d) *The efforts of Food Drink Europe*: The Food Drink Europe (FDE), which is a representative of the European food and drink industry, continues to work in association with the European Commission to develop a "toolbox" designed to assist food business operators (FBO) in reducing the acrylamide content of their products. In May 2016, The FDE toolbox tagged "Code of Practice for Managing Acrylamide Formation" was revised (FDE, 2016).

(e) *The benchmark level for dietary acrylamide*: The European Commission (EC) adopted a "Recommendation on the monitoring of acrylamide in foods" in 2007. This recommendation led to the collection and compilation of data on the acrylamide content of some important European foods. The result of the data collection is the 2007-2010 EFSA report on dietary acrylamide. The emergence of the EC recommendation 2010/307/EU, which revised the categorisation of food products for monitoring purposes, was the consequence of the 2007-2009 data collection.

In the year 2011, there was adoption of another recommendation on the investigations into the levels of acrylamide in food by the EC. This recommendation requires Member States to carry out investigations in cases where the levels of acrylamide in a foodstuff, tested in the monitoring exercise, exceeds set indicative values. In such event, the concerned Member State(s) should report the results from the investigations to the Commission. The EC recommendation 2013/647/EU on investigations into levels of acrylamide in food replaced the previous recommendations (European Commission, 2017). The World Health Organization (WHO) has a standard of 0.5µg/L for drinking water. The previous Drinking Water Directive of the European Union (EU 98/83 Directive) amended by the Commission Directive (EU) 2015/1787 also has a permissible residual acrylamide from polymer in contact with drinking water of 0.1µgl−1 (Cavalli, Polesello, & Saccani, 2004)

(f) *The proposal for a regulation:* In February 2017, there was a consultation by the European Commission concerning a proposal to make the FDE "Code of Practice for Managing Acrylamide Formation in Foods" mandatory for food business operators. The proposal followed prolonged deliberations on the use recommendations or a more legally binding Act based on maximum concentration limits or upon a mitigation (Code of Practice) approach (IFST, 2017). Although, there is still no legally binding legislation on the management of acrylamide in food manufacture, the draft code of practice in circulation since January 2016, serves as the best practice guide for some food manufacturers (IFST, 2017)

(g) Unknown level of awareness of WA on the relationship between the heat-treatment of foods and the production of processing contaminants such as acrylamide.

(h) *The gap in knowledge of the acrylamide levels in WA foods:* Since the discovery of acrylamide in heat-treated foods, no research has focused on the popular WA heat-treated foods such as Chin-chin, Kuli-Kuli to mention a few.

An important goal of the research is to determine the concentration of acrylamide in the common WA foods. Consequently, the observed AA concentrations will be compared to national /global recommended levels. The research will also explore other factors, which may affect the production of acrylamide in heat-processed WA foods. For example, the effect of concentrations of precursors such as glucose and asparagine present in raw food materials etc.

Chapter 2

Literature Review

CHAPTER 2. LITERATURE REVIEW

2.1 Overview

This chapter presents the findings from previous works on dietary acrylamide. The physico-chemical properties, formation mechanisms, uses and health effects of the chemical compound are discussed. The methods used for the quantitation of the process contaminant from 2002 to 2017 are also described.

2.2 Introduction

Acrylamide is a chemical substance that has several applications and advantageous uses in the paper, petroleum and textile industries**.** Acrylamide is an important chemical used by industries for the production of polyacrylamide or its copolymers. The beneficial use of polyacrylamide, a derivative of acrylamide, includes its utilization for the purification of drinking water as well as sewage and process waters, where it serves as a flocculating agent. It is also utilized in the synthesis of dyes and copolymers for contact lenses. Polyacrylamide is also employed in the paper industry as papermaking additive used to enhance the resistance of paper to tearing.

Polyacrylamide gels, derived from acrylamide are used as grouting agent in enhanced oil recovery, mining and construction of dam foundations, tunnels, and sewers. It is also used as an essential agent in soil-conditioning, mining and production of cosmetics (FSA, 2002; USFDA, 2004). Acrylamide is also an important component of cigarette smoke.

In addition, electrophoresis gels from polyacrylamide are used by molecular biologists and genetic engineers in analytical and research laboratories.

According to the report by the United States National Institutes of Health, NIH (2007), there was a net increase of 18 million kg in the industrial demand of acrylamide from the year 1998 to 2003.

Human exposure to acrylamide was recognized to occur by direct contact when handling the chemical or products of the chemical e.g. during industrial production, preparation of gels (Bergmark et al., 1993), through cigarette smoke (Smith et al., 2000; White et al., 1990), and by application of polyacrylamide containing cosmetics (Andersen, 2005).

Apart from the potential exposure of industrial and laboratory users of acrylamide to the chemical, the general population is exposed to varying amounts of the chemical via the diet. Vesper et al., (2008, 2010) also noted that apart from the possibility of exposure of industrial

workers to acrylamide, people all over the world are exposed to different levels of acrylamide through the consumption of foodstuffs.

The reports by Tareke et al., (2000, 2002) and the Swedish National Food Administration, SNFA (2002) created an awareness of the public to the presence of acrylamide in heat-treated foods. These reports launched an intense analytical survey of food items and mechanistic studies to identify conditions for formation of acrylamide.

In the year 1994, the International Agency for Research on Cancer (IARC) investigated the toxic effects and exposure assessment of acrylamide. Exposure assessment of the chemical indicated that heavily exposed workers showed neurotoxic effects and their blood contained elevated levels of acrylamide bound to haemoglobin. In- vivo and in- vitro studies showed that acrylamide is metabolized to a reactive epoxide, called glycidamide which binds to proteins and DNA. The assessment also demonstrated that chronic exposure to acrylamide and glycidamide may lead to the development of tumours at several sites in rodents (Bergmark et al., 1993; Doerge et al., 2005; Manière et al., 2005). Based on these results the International Agency for Research on Cancer classified acrylamide as probably carcinogenic to humans. Another evaluation of the risk of dietary acrylamide was conducted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2005. Most of the analytical data (67.6 and 21.9%) on the occurrence of acrylamide in foods were obtained from the study of European and American foods respectively. The study of foods from Asia and the Pacific contributed 8.9 and 1.6% respectively to the total data. The surveillance exercise did not consider foods from Latin America and Africa. Consequently, part of the recommendations of the committee was the consideration of foods from developing countries in future studies (IFST, 2017).

2.3 Physical Properties of Acrylamide

Acrylamide is a polar organic chemical compound with the structure shown in Figure 2.1

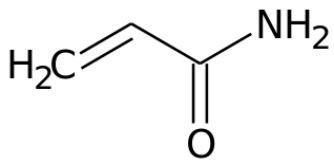

Figure 2.1. *Chemical structure of acrylamide*

At room temperature, acrylamide or 2-propenamide as it is otherwise called is a crystalline solid which is whitish in colour. It is a polar organic compound which as a melting point of

84.5º ± 0.3ºC, boiling point of 136ºC at 3.3kPa, density of 1.13 g/cm^3 and a low molecular weight of 71 Daltons(Da). Its polar nature makes it remarkably soluble in water (2155 g/L at 30ºC), in polar organic solvents and in lower alcohols. Its solubility in methanol is 1550 g/L, while its solubility in ethanol is 860 g/L. It is almost insoluble in non-polar organic solvents such carbon tetrachloride, hexane and heptane (<1g/L; Chemistry of Acrylamide, 1969; Habermann, 1991), but dissolves slightly in polar organic solvents (631 g/L in acetone, 396 g/L in acetonitrile, and 126 g/L in ethyl acetate). Its low molecular weight, low volatility and solubility behaviour in the different solvents determine the appropriate approach for its separation and determination. For example, it is difficult to employ the method of UV detection for the determination of acrylamide. This is because it does not fluoresce and lacks chromophore for UV spectrophotometric-based methods.

2.4 Chemical Properties of Acrylamide

Monomeric acrylamide rapidly polymerizes into polyacrylamide at temperatures above its melting point of about 85 °C (IARC, 1994). During the heat treatment of foods or metabolism of heat-processed foods acrylamide is oxidized to a more reactive epoxide called glycidamide. Acrylamide is naturally oxidized to glycidamide in the presence of cytochrome P450 2E1 (CYP2E1) (Besaratinia and Pfeifer, 2004; 2005). Apart from its direct reaction with the DNA, acrylamide indirectly reacts with DNA to form DNA adducts through its reactive epoxide, glycidamide. According to Beland et al., (2015) out of the several DNA adducts characterized, the main DNA adducts are N7-(2-carbamoyl-2-hydroxyethyl)-guanine (or N7-GA-Gua) and N3-(2-carbamoyl-2-hydroxyethyl) adenine (or N3-GA-Ade). The cysteine adduct from the addition of haemoglobin (Hb) to glycidamide is S-(20hydroxy-2carboxyethyl) cysteine (Von Tungeln et al 2012).

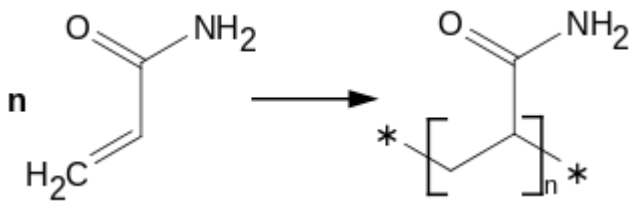

Figure 2.2. *Polymerization of acrylamide to polyacrylamide*

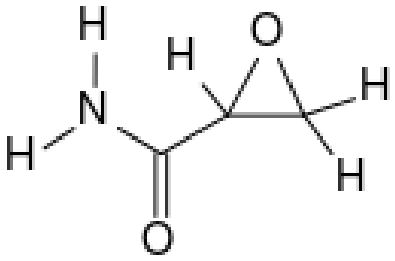

Figure 2.3. *Chemical Structure of Glycidamide*

The hydration of acrylonitrile in the presence of microbial nitrile hydratase, or the hydration of acrylonitrile in the presence of copper catalysts is used for the industrial production of acrylamide (Friedman, 2003; IARC, 1994). According to Friedman (2003), microorganisms can also produce acrylamide naturally.

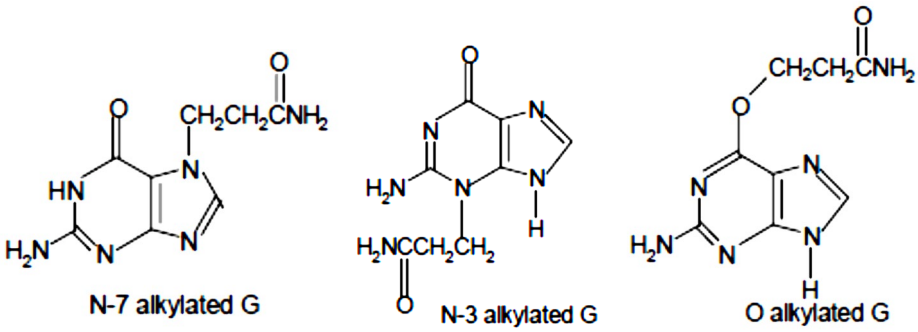

Figure 2.4. *N-7, N-3 and O Acrylamide Adducts of Guanine*

The reactive electron-deficient double bond conjugated to the amide group in acrylamide enables it to undergo Michael addition reaction with reagents having nucleophilic groups such as SH- and NH_2-groups (Friedman, 2003).

Consequently, acrylamide can react with the thiol, hydroxyl, and amino groups in DNA, which leads to DNA damage (Doroshyenko et al., 2009; Erkekoglu and Baydar, 2010; Hogervorst et al., 2010; Watzek et al., 2012; Zeiger et al., 2009). In addition, acrylamide can form adducts with ammonia, amines, phosphines, bisulphites, sulphides, mercaptans, ketones, nitroalkanes, and alcohols (Castle and Eriksson, 2005).
These addition reactions are the underlining reasons for some of the toxic effects of acrylamide. For example, its mutagenic, genotoxic and carcinogenic effects from the formation of haemoglobin and DNA adducts. Some of these addition reactions are also reversible (Besaratinia and Pfeifer, 2004; 2005).

2.5 Formation Mechanisms of Acrylamide in Foods

Extensive studies on the possible mechanisms of acrylamide formation followed few months after the announcement of the presence of the chemical in heat-processed foods (IFST, 2017; SNFA, 2002; Tareke et al., 2002)

The early studies concluded that the only reasonable pathway for the formation of acrylamide is through the reaction of reducing sugars and free amino acids, such as asparagine (Mottram et al., 2002; Stadler et al., 2002; Zyzak et al., 2003). This reaction otherwise known as *Maillard reaction* is favoured by temperatures above 120º C and low moisture condition. Asparagine and the reducing sugars required for Maillard reaction and acrylamide formation are essential components of cereals, potatoes, vegetables, cocoa, coffee and other plants or plant-derived ingredients used in food preparation (IFST, 2017; Lineback et al., 2012, Stadler and Studer, 2016; Stadler, 2006; Taeymans et al., 2004;2005).

Succeeding studies also substantiated the claim that the primary mechanism for the formation of acrylamide is via Maillard reaction (MR), which involve the reaction of glucose and/or fructose with asparagine (IFST 2017). This is also the reaction primarily responsible for the development of flavours, aromas and brown colours in foods subjected to baking, frying and roasting (Stadler and Studer, 2016)

A series of reactions and intermediate chemical compounds make up the Maillard reaction (Figure 2.5).An important intermediate chemical compound in Maillard reaction is 3-APA or 3-aminopropionamide. This biogenic amine is an essential precursor and transient intermediate, which can easily convert to acrylamide under aqueous conditions (Granvogl and

Schieberle, 2007; Schieberle et al., 2005; Zyzak et al., 2003). Experiments based on model systems and actual food samples such as cheese and cocoa (Granvogl and Schieberle, 2006; 2007) corroborated the importance of 3-APA in acrylamide formation.

According to Zyzak et al., (2003), 3- APA is formed after a carbonyl compound reacts with asparagine to furnish the Schiff base that decarboxylates under dry conditions. A subsequent de-amination of the 3-APA leads to the formation of acrylamide (Figure 2.6). Ordinary thermal treatment of 3-APA (without the addition of carbonyls) affords acrylamide in a very high yield, >60 mol %, under ideal reaction conditions (Granvogl et al., 2004).

The stable isotope-labelling experiments and extensive studies conducted by researchers including Stadler, Granvogl and Schieberle (Bagdonaite et al., 2006; Granvogl and Schieberle, 2006; 2007) provided concrete evidence for the intermediacy of 3-APA in the reaction to acrylamide.

By demonstrating via stable isotope dilution assays the presence of more than 98% of ^{15}N-labelled asparagine in the generated acrylamide, Stadler et al., (2002) provided evidence for the importance of free amino acid asparagine as an essential precursor for acrylamide formation. Stadler et al (2002) also showed that *N*-glycosides (*N*-(d-glucos-1-yl)-1-asparagine and *N*-(d-fructos-2-yl)-l-asparagine) are important precursors of acrylamide, yielding >1.3 mmol acrylamide per mol of *N*-glycoside (Stadler and Studer, 2016)

Mottram et al (2002) provided an alternative explanation for the mechanism of acrylamide formation. This group showed that an optional mechanism for the formation of acrylamide is through Strecker reaction (SR), which involve the reaction of a dicarbonyl such as 2,3-butanedione with asparagine (Stadler and Studer, 2016)

Mottram et al., (2002) also proposed acrolein and acrylic acid as potential intermediates in the pathway of acrylamide formation. Acrolein and acrylic acids may be derived from the degradation and oxidation of carbohydrates, proteins, and fat/oil systems. However, for acrolein and acrylic acid to be effective precursors in the reactions leading to the formation of acrylamide, there must be availability of ammonia. For this reason, acrolein and acrylic acid are considered marginal and less important source of acrylamide (Mottram et al., 2002).

According to Stadler and Studer (2016) the major pathways for the formation of acrylamide contain reactions of dicarbonyls or hydroxycarbonyls with asparagine. Important carbonyl compounds in the pathways leading to acrylamide include aldo and keto sugars, 3-APA, and some glycoconjugates.

Independent studies have shown that both dicarbonyls and hydroxycarbonyls react rapidly with asparagine to release acrylamide. However, the studies indicated that the reactivities of the carbonyls compounds are dissimilar (Stadler et al., 2004; Yaylayan et al., 2005). For example, significantly lower amounts of acrylamide are formed when sucrose is employed as reactant, but about equal amounts of acrylamide are formed when asparagine is added to fructose or glucose. Some reports also indicated release of lower amounts of acrylamide from the reaction mixture of glucose and asparagine compared to that of fructose and asparagine.

According to Yaylayan et al., (2005) the higher yield of acrylamide from the reaction mixture of fructose and asparagine may be due to the lower melting point and higher mobility of fructose.

Other carbonyl that reacts with asparagine to produce varying amounts of acrylamide include pyruvic acid (2-oxopropionic acid), hydroxyacetone, and propanal. Pentosans and cellulose have also been reported to contribute to acrylamide formation.

2.5.1 Acrylamide Formation via Non-Enzymic Browning Pathway

The non-enzymic browning reaction popularly called Maillard reaction is initiated with the formation of the Schiff base and Amadori reaction products, formed through the reaction of reducing sugars with amino acids. Amadori rearrangement of the *N*-glycosides transforms them to dicarbonyls (1-deoxysone or 3-deoxysone). This rearrangement of the Amadori reaction products may occur via enolization, dehydration or hydrolysis reactions. Dry and low moisture conditions may favour rearrangement of the *N*-glycosyl compounds via the Schiff base.

The pre-existence of the decarboxylated Schiff base (imine 2), which is relatively stable under low-moisture conditions, and the decarboxylated Amadori compounds which serve as direct precursors of acrylamide was confirmed by some studies (Perez Locas et al., 2008; Stadler and Studer, 2016; Yaylayan et al., 2005; Zyzak et al., 2003).

The decarboxylation of the Schiff base via a cyclic intermediate (oxazolidin- 5-one) or through a dipolar Schiff base betaine leads to the formation of a more stable azomethine ylide.

Perez Locas et al., (2008) used Fourier-transform infrared spectroscopy to confirm the formation of the cyclic 5-oxazolidinone from a reaction mixture of asparagine and sugar (Stadler and Studer, 2016)

The subsequent rearrangement of the decarboxylated Schiff base leads to an Amadori product which directly generates acrylamide via a 1-2 or β-elimination. According to Stadler et al (2004), the cleavage of the carbon–nitrogen bond by β-elimination reaction is the most likely rate-limiting step in the Maillard pathway to acrylamide.

The hydrolysis of the decarboxylated Schiff base (imine 2) releases 3-APA, which in turn undergoes a deamination reaction to produce acrylamide.

The hydrolysis of the other decarboxylated Schiff base (imine 1) leads to the formation of 3-oxopropoanamide (commonly called Strecker aldehyde). The Strecker aldehyde formed can then undergo a reduction and hydrolytic reaction with a corresponding alcohol to finally generate acrylamide. The reaction steps involving imine 1 represents only a minor pathway in terms of its contribution to the overall acrylamide yield.

According to Yaylayan and Stadler (2005) the structure of the initial carbonyl compound may affect the yield of acrylamide. This may be particularly true for the functional group in the beta position to the nitrogen (Stadler and Studer, 2016; Stadler et al., 2004). For instance, a possible explanation for the higher amounts of acrylamide (>4 mmol/mol) released from a reaction involving hydroxyacetone (acetol) compared to those involving methylglyoxal (<0.2

mmol/mol), may be the rearrangement of decarboxylated Amadori compounds supported by the hydroxyl group of hydroxyacetone.

The reaction of asparagine with a dicarbonyl follows a similar route to the one described for carbonyl or hydroxycarbonyl, which involves the formation of two possible imines after the delocalization of the charge of azomethine ylide (Stadler and Studer, 2016; Stadler et al., 2004).

Although, the route leading to the formation of acrylamide from the direct fragmentation of an imine via an elimination reaction is feasible, it is considered a minor route compared to the 3-APA Maillard-driven pathway. Unlike the 3-APA Maillard-pathway which has been reported to be associated with high yields of acrylamide, no experiments have been conducted to corroborate the production of acrylamide from the fragmentation of the imine group (Stadler and Studer, 2016). Factors such as pH, temperature, type of food matrix, and the moisture content of the sample may also affect the predominant pathway to acrylamide (Stadler and Studer, 2016).

In a nutshell, acrylamide is mainly formed from the reaction of free asparagine with reactive hydroxycarbonyls (reducing sugars), α-dicarbonyl compounds, n-aldehydes, or 2-oxo acids at a temperature of about 100 °C or higher. Stable isotope-labelling experiments also confirm the source of the backbone of acrylamide molecule to be mostly a moiety from asparagine. The effective mitigating role of asparaginase in the production of acrylamide is also a pointer to the importance of asparagine as an essential precursor for acrylamide formation. Studies using food models have demonstrated that asparaginase can be very efficacious in lowering acrylamide. In certain cases, the use of the enzyme has been reported to result in reductions above 95% (Stadler and Studer, 2016).

Most studies on the route of acrylamide formation agree that the first step in the pathway is a nucleophilic attack of the α-NH_2 group of the free amino acid, asparagine (adjacent to the carbonyl group). This attack leads to the formation of Amadori compounds and N-glycosides which undergoes decarboxylation, hydrolytic and deamination reactions to produce acrylamide.

In addition, acrylamide can be formed from the amination of acrylic acid produced from the degradation and oxidation of carbohydrates, proteins or fat and oil systems. The production of acrylamide from the deamination of 3-aminopropanamide is a key reaction step in most of the likely routes that have been described.

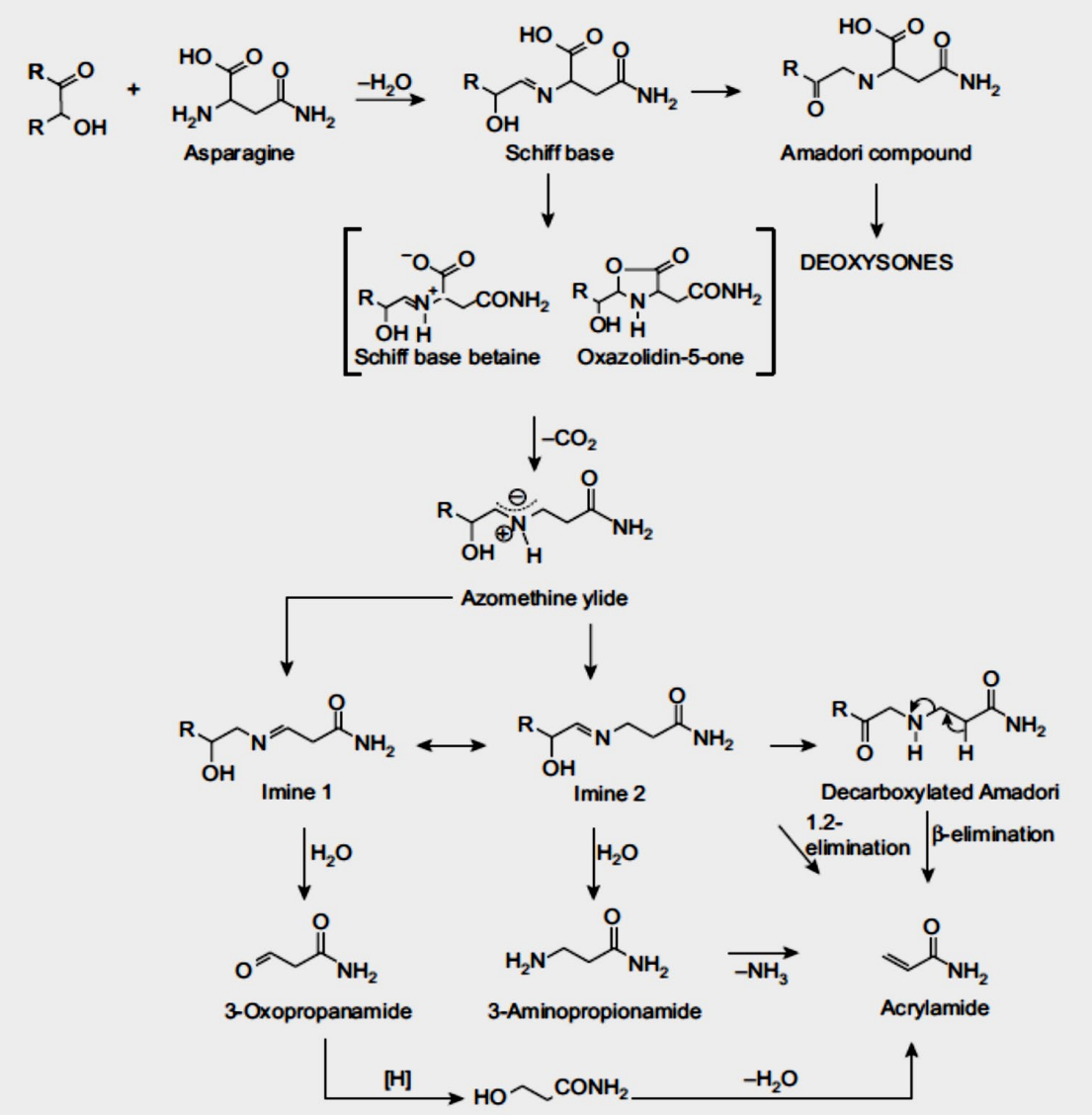

Figure 2.5. *The asparagine-hydroxycarbonyl reaction pathway to acrylamide (Stadler and Studer, 2016)*

2.5.1 **Alternative Reaction Pathways to Acrylamide**

Initial investigations into acrylamide formation focused on Maillard reaction and alternative reactions which are today considered as marginal and less important in the route leading to acrylamide formation(Stadler et al., 2003; Yaylayan et al., 2005).

Important molecules which are components of the alternative reaction pathways include acrolein (2-propenal), acrylic acid, alanine, methionine, lactamide, peptides and the ever-essential precursor, 3-APA.

Acrolein, an aldehyde with three-carbon can be formed both by the Maillard and non-Maillard reaction routes. The non-Maillard reaction routes for acrolein formation include the oxidative degradation of lipids, carbohydrates, proteins and amino acids (Stadler and Studer 2016)

Strecker degradation of the amino acid, methionine produces methional, which in turn fragments to acrolein.

Acrolein levels between 5–250 mg per kg of frying oil can be formed depending on conditions such as the frying temperature and grade of the cooking oil (Ehling et al., 2005). The use of high-temperature for frying may lead to volatilization of acrolein which has a relatively low boiling point of about 51 °C. This may lead to the loss of acrolein to other reactions in the food matrix. Nevertheless, the oxidation of acrolein can lead to the formation of acrylic acid, which can further react with ammonia through amino-dehydroxylation to produce acrylamide (Figure 2.7).

Under certain conditions, acrolein may directly react with asparagine to produce significant amounts of acrylamide. This implies that an additional amount of acrylamide may be generated from lipid systems, provided acrolein is oxidized to the intermediate acrylic acid or reacts with the free amino acid, asparagine.

In a reaction pathway analogous to that described for asparagine to acrylamide, acrylic acid can also be produced from the starting amino acid, aspartic acid (Stadler et al., 2003)

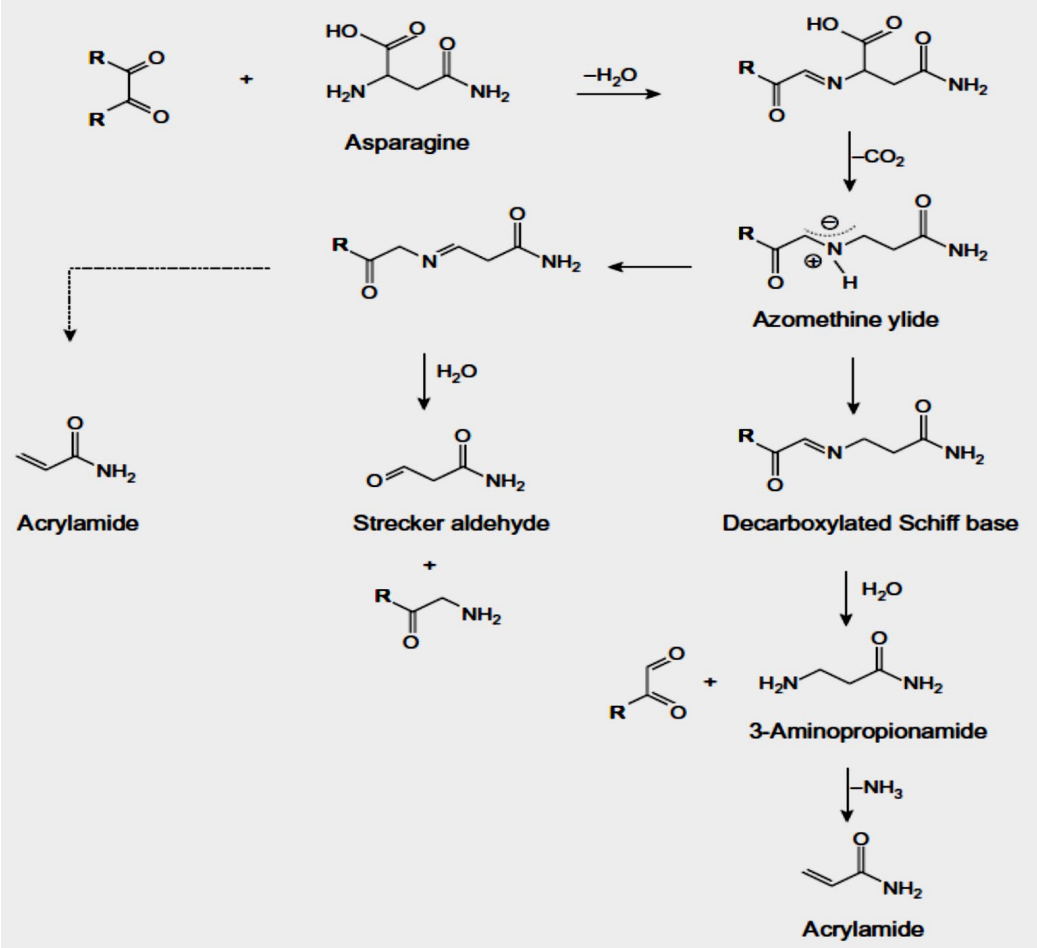

Figure 2.6. *The asparagine–dicarbonyl reaction pathway to acrylamide (Stadler and Studer, 2016).*

The volatility of ammonia makes the reaction step which includes the *amination* of acrylic acid the most likely rate-limiting step for the acrylic acid-acrylamide route.
The heating of acrylic acid with amino acids having a nitrogen atom in their side chain (asparagine, glutamine, lysine, and arginine) at 120 °C for 30 min. produced significant amounts of acrylamide ranging from 0.9–1.7 mg acrylamide per kg amino acid.
According to Ehling et al., (2005), the heating of acrolein with the same amino acids did not produce a measurable amount of acrylamide, suggesting that oxidation of acrolein may not be a major route to acrylamide or its precursor acrylic acid.

Another important amino acid which can produce acrylic acid via thermolytic deamination reaction is alanine. When heated at temperatures over 180 °C, L-arginine may also serve as an essential precursor for acrylic acid.

The fragmentation of proteins and peptides containing alanine can also be an alternative route to acrylamide. The fragmentation of wheat gluten (peptides) under baking and roasting conditions has been reported to produce varying levels of acrylamide.
According to Claus et al., (2006), there was a 20% increase in acrylamide with increasing amounts of gluten added to bread roll dough and the prerequisite for the release of acrylamide in the pyrolytic pathway is a β-proton in the amino acid adjacent to alanine. An example of a peptide chain that fragments under pyrolytic conditions to release β-alanine and consequently acrylamide is carnosine, *N*-β-alanyl-l-histidine (Yaylayan and Stadler, 2005).
The central role of 3-APA in the reaction routes leading to acrylamide has been described. A simpler pathway to the formation of 3-APA, which does not require the presence of reducing carbohydrates, hydroxycarbonyl or Maillard reaction was suggested by Granvogl et al., (2004). This alternative pathway proceeds via the decarboxylation of asparagine in the presence of histidine decarboxylase and the cofactor, pyridoxal phosphate. As previously noted, the yield of acrylamide from 3-APA by far exceeds the yield from asparagine.
This enzymatic 3-APA pathway may be the answer to the observation of a significant amount of acrylamide (>0.2 mg/kg AA) formed during the production of prune-based food products and beverages at a relatively low temperature of 95 °C (Becalski et al., 2011)

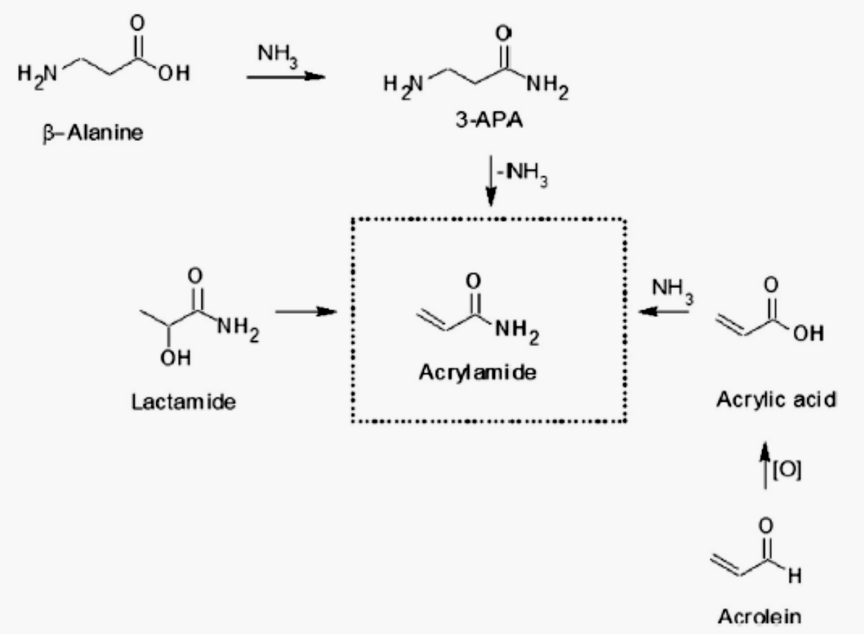

Figure 2.7. *Alternative reaction pathways to acrylamide.(Stadler and Studer, 2016)*

2.6 Adverse Health Effects of Acrylamide

The toxic effects associated with acrylamide include neurotoxicity, carcinogenicity, mutagenicity and genotoxicity. A more detailed description of these ill- effects is given below.

2.6.1. Neurotoxicity

Although, no recent study on the dietary exposure to acrylamide has indicated a severe neurotoxicological effect, ataxia, which is the inability to coordinate the voluntary muscular movements, has been shown to be a result of exposing laboratory animals and human subjects to high dose of acrylamide. Neurotoxic effects was observed in laboratory animals upon daily exposure in the range of 0.5 mg - 50 mg per kg bw (LoPachin et al., 2004).
Acrylamide has also been shown to cause impairment in the function of the sensory, motor and autonomic nervous system (LoPachin et al., 2004).

The toxic effects of chronic occupational exposures to acrylamide and accidental intoxications from contacting the chemical also lend support to this observation. Peripheral neuropathy including impairment of vibration sensation, loss of ankle reflexes, weakness in legs, tremor, numbness and tingling in the limbs was observed in human subjects upon occupational exposure (Calleman et al., 1994; Hagmar et al., 2001; He et al., 1989).

According to Hagmar et al., 2001, the neurotoxic effects were temporary and acute, if a non-severe treatment of the experimental subjects is halted.

In addition, the clinical signs were positively correlated with the haemoglobin adducts and the concentration of mercapturic acids in the urine of the subjects.

Acrylamide has been shown to degrade certain sections of the peripheral and central nervous systems e.g. the damage of the cerebellar Purkinje cell and degradation of distal axons in the central nervous system and peripheral nervous system.

This degradation of terminal nerves has been reported to be responsible for the impairment in cognitive functions and damage to the cerebral cortex, thalamus and hippocampus.

At the moment, there are two possible explanations for the mechanism of acrylamide neurotoxicity. The first hypothesis emphasizes axonopathic alterations and inhibition of fast axonal transport in the central and peripheral nervous system (Gold et al., 2000; LoPachin et al., 2002; Sickles et al., 2002). The second proposition suggests that acrylamide acts directly at nerve terminals causing presynaptic dysfunction and eventual degeneration (LoPachin et al., 2002, 2004).

Another explanation for the acrylamide-induced synaptic dysfunction is on the bases of the binding of acrylamide to SH-groups in presynaptic proteins, which leads to a reduced release of neurotransmitter (LoPachin et al., 2004). A study by Kim (2005) also suggested that the neurotoxicity of acrylamide might be associated with alteration in the expression of an enzyme called nitric oxide synthase which in turn influences the release of neurotransmitters.

The majority of data on the NOAEL of acrylamide was obtained by the administration of very high doses of acrylamide, in the range of 10 - 50 mg per kg body weight (BW) to animal subjects (LoPachin et al., 2004).

A 90-day dose-response study which administers drinking water spiked with measured amounts of acrylamide to animal subjects showed a no-observed-adverse-effect-level, NOAEL of 200 µg/kg bw/day and a lowest-observed-adverse-effect level, LOAEL of 1000 µg/kg bw/day (Burek et al., 1980)

Another two year dose-response animal studies carried out by Johnson et al., (1986) revealed a NOAEL for microscopic degenerative nerve changes of 500 µg/kg bw/day and a LOAEL of 2000 µg/ kg bw/day.

Hitherto, no report has indicated any appreciable level of exposure of acrylamide

through diets which is up to 0.5 mg/kg BW (the no observed adverse effect level, NOAEL in animals) and enough to elicit a neurotoxic effect (Dybing et al., 2003; Madle, 2003)

Following the results from the various studies on the neurotoxicity of acrylamide, the high dose of acrylamide required to trigger neurotoxic effects in animal and human subjects is far beyond any dietary exposure reported (Tritscher, 2004)

Consequently, the neurotoxicological relevance of dietary acrylamide cannot be concluded. Nonetheless, it is important to note that the comparatively low dietary exposure might not be negligible, since the neurotoxic effect of acrylamide might be cumulative (LoPachin et al., 2004).

2.6.2 Carcinogenicity

After a thorough risk assessment by the International Agency for Research on Cancer (IARC) in 1994, acrylamide was classified in to Group 2A or "probably carcinogenic to humans" category (Besaratinia and Pfeifer, 2004; Carere , 2006; IARC, 1994; Rice, 2005; Shipp et al., 2006; WHO, 2005).

This conclusion was reached after animal studies which used rodents as subjects indicated that acrylamide is metabolized by mammalian tissues to a reactive and carcinogenic epoxide called glycidamide which causes cancerous growths in several organs (IARC, 1994).

A two years study which administers drinking water with acrylamide at doses of 0 to 2 mg/kg bw to Fischer 344 rats supported the acclaimed oncogenic potential of acrylamide.

Administration of high dose of acrylamide to the animal subjects caused significant development of tumourous growth in testes, central nervous system, oral cavity, uterus, clitoral, thyroid and mammary glands (Johnson et al, 1986). A study by Rice (2005) also showed the oncogenic effect of acrylamide on the brain, testes, lungs, thyroid, clitoral, and mammary glands of the animal subjects

Other oncogenicity studies by Thulesius et al., (2004) and Erdreich et al., (2004) confirmed the significant carcinogenic effect of high dose of acrylamide on the testes, thyroid and mammary glands of the animal subjects.

The no observable effect level (NOEL) for tumour development in the testes (scrotal mesotheliomas) was found by Friedman et al., (1995) to be as 0.5 mg/kg BW

A more recent two-year carcinogenicity study by Maronpot et al., (2015) also supported these findings.

The carcinogenic effects of acrylamide and its epoxide derivative, glycidamide on several organs of animal subjects indicates that this chemical is potentially carcinogenic to humans.

In contrast to this speculation, the findings from studies by Thulesius et al., (2004) and Erdreich et al., (2004) suggested that the probability of incurring cancer from acrylamide is overestimated and human exposure is too low and inadequate to be of concern.

In support of these findings, a previous study of 8500 occupationally exposed workers did not conclude any significant association between the exposure to acrylamide and mortality from cancerous growths (Marsh et al., 1999)

In addition, epidemiologic studies of dietary acrylamide intake have not convincingly demonstrated an increased risk of cancer for human (Lipworth et al., 2012).

The threshold required for the development of cancerous growths in humans has not been established. Consequently, the dietary exposure to acrylamide should be as low as reasonably achievable, ALARA (INFOSAN, 2005; JECFA, 2005; Madle et al., 2003; WHO/FAO, 2005).

2.6.3 Mutagenicity and Genotoxicity

A concentrated solution of acrylamide is required to form a DNA adduct, while glycidamide induces DNA adducts dose-dependently (Besaratinia et al., 2004, 2005). Acrylamide and glycidamide are loci-specific in their reaction to form the DNA adducts (Besaratinia et al., 2004).

Under in-vitro conditions, acrylamide is slightly mutagenic when combined with the DNA, but extremely mutagenic after it is metabolically activated to its epoxide derivative, glycidamide. A notable amount of DNA adducts is therefore formed from the reaction of glycidamide with nucleic acids compared to the reaction of acrylamide with the DNA (Gamboa da costa et al., 2003). The reactivity of glycidamide with the nucleic acids has been attributed its epoxide group (Besaratinia et al., 2004, 2005; Segerbäck et al., 1995).

The main adduct from the reaction of acrylamide with DNA is (N1-(2-carboxyethyl)-adenine), whereas (N7-(2-carbamoyl-2-hydroxyethyl)-guanine) is the dominant adduct from the reaction of glycidamide with DNA.

These adducts was observed in almost all the organs of the rodents treated with acrylamide. Gamboa da Costa et al., (2003) also reported the detection of these adducts in the liver, kidney and lungs of rodents treated with acrylamide.

In addition, the genotoxicity of acrylamide was also established in studies using mice and rats as experimental subjects. In the germ cells of male rats, the effect of acrylamide is almost exclusively clastogenic (Favor and Shelby, 2005). According to independent reports by Russell et al., (1991) and Sega et al., (1990), genotoxic effects were observed in the germ cell of male mice and rats treated with acrylamide in several studies. These genotoxic effects were observed after treating the animal subjects with acrylamide concentrations equal or greater than of 25 mg/kg.

No significant mutagenic effect was observed in the bacterial systems treated with acrylamide. Hagmar et al., (2005) also reported the binding of AA metabolites to human haemoglobin. According to the study, acrylamide-adducts of human haemoglobin were significantly higher in smokers than in non-smokers.

An in-vitro study of the DNA of human lymphocyte showed that 0.5–50 μM of acrylamide is required for the aberration of the chromosomes (Blasiak et al., 2004). However, these aberrations were reversed after incubating the reaction mixture for 60 min.

Acrylamide and glycidamide did not induce genetic mutations in Salmonella, but there is clear evidence that both compounds are mutagenic and clastogenic in mammalian cells: This is corroborated by the observation of gene mutations and chromosomal aberrations in the germ and somatic cells of rodents (IARC, 1994; Rice, 2005).

The mechanism for the genotoxicity of acrylamide is not yet fully understood, but there are indications that the alkylation of DNA by glycidamide is the critical step (Baum et al., 2005; Besaratinia and Pfeifer, 2004; Puppel et al., 2005; Rice, 2005).

According to Ghanayem et al., (2005) the presence of glycidamide is a critical factor in the mutagenicity of germ cell. Contrarily, several studies have reported that glycidamide is not a potent mutagen and not very effective in DNA strand breaking as compared to other mutagens (Baum et al., 2005; Puppel et al., 2005; Silvari et al., 2005). Apart from its direct reaction with the DNA, another explanation for the mutagenic effect of acrylamide is its

potential to interfere with DNA repair (Blasiak et al., 2004) or increase DNA synthesis in target sites (Lafferty et al., 2004)

From the findings of various studies, it can be concluded that acrylamide is a constituent of heat-treated foods which can be carcinogenic, genotoxic and mutagenic at high concentrations. Its carcinogenic, genotoxic and mutagenic effects can also be attributed to its reactive metabolite, glycidamide.

Presently, from the standpoint of dietary acrylamide intake, there is still insufficient information on the deleterious and detrimental effect of the chemical on human being (EFSA, 2015). Notwithstanding, there are health concerns based on the possible mutagenic or carcinogenic effects of low dose and long-term exposure (EFSA, 2015).

2.7 Reported Levels of Acrylamide in European Foods

After the report of acrylamide in heat-treated foods by the SNFA in 2002, an acrylamide-monitoring program commenced in some member states of the European Union.

The European Commission (EC) adopted a recommendation in the year 2007, which pertains to the investigation of foodstuffs with the potential to contribute appreciably to the dietary acrylamide intake. The results submitted by twenty-five European countries indicated mean acrylamide values ranging from 31 μg/kg to 1 350 μg/kg for 'other processed cereal based foods for infants and young children' and 'coffee substitutes' respectively (EFSA, 2012). According to the EFSA scientific report on the acrylamide levels in food from monitoring years 2007 to 2010, the highest 95th percentile value of 8 044 μg/kg was reported for 'instant coffee'. In addition, the EFSA report did not indicate any significant variation in the acrylamide levels from 2007 to 2010 (EFSA, 2012).

Results from the acrylamide monitoring program conducted in the Member States between 2007 and 2011 gave rise to preliminary safety limits for foodstuffs described as "indicative values". These "indicative values" are not absolute safety thresholds, but only intended as bases for controlling the formation and level of acrylamide in foodstuffs (European Commission, 2011). The acrylamide content of same foods produced in the same or different manufacturing facilities may vary widely, depending on factors such as processing conditions and formulations used (IFST, 2017)

The European Commission and the US Food and Drug Administration manage a large data base on the occurrence of acrylamide in foods (IFST, 2017). These are the European Union acrylamide monitoring database (European Commission, 2006), and the survey data on the level of acrylamide in individual food products (USFDA,
2006).

The indicative acrylamide values based on the EFSA monitoring data from 2007 to 2012 (European Commission, 2013), above which investigations should be carried out are shown in Table 2.1.

Table 2.1 *Indicative acrylamide values for different categories of foods (European Commission, 2013)*

Category of food product	**Indicative values (µg/kg)**
Potato chips (crisps)	*1000*
French fries (potato chips)	*600*
Wheat-based soft bread	*80*
Other soft bread	*150*
Breakfast cereals	*200 – 400*
Gingerbread	*1000*
Other biscuits	*500*
Roast coffee	*450*
Instant coffee	*900*
Coffee substitutes	*2000 – 4000*
Infant food (biscuits and rusks)	*200*
Infant food (containing prunes)	*80*
Other infant food	*50*

The actual acrylamide values for a food product will vary depending on the food manufacturer and the country. For instance, the data for breakfast cereals was from testing and assessing common breakfast cereals available in Europe, which does not include the toasted bran flake cereals popular in the UK (IFST, 2017). Therefore, the sole purpose of the indicative values is to serve as a guide for food business operators (FBO).

Other investigations into the acrylamide content of a broad range of food products present figures similar to the indicative values previously mentioned (Amrein et al., 2005; Croft et al., 2004; Leung et al., 2003; Lineback et al., 2005; Murkovic, 2004; Ono et al., 2003; Roach et al., 2003; Rosén and Hellenäs, 2002; Tareke et al., 2002)

Table 2.2 *Acrylamide contents of different food categories* (Amrein et al., 2005; Lineback et al., 2005;Roach et al., 2003; Rosen et al., 2002;Tareke et al., 2002)

Category of food product	**Acrylamide content (µg/kg)**
Potato chips	*100-3770*
French-fries	*50-680*
Fried meat	*15-45*
Fried noodles	*10-580*
Fried rice crackers	*17-64*
Bread	*20-40*
Crisp bread	*25-2800*
Gingerbread	*80-7800*
Fine bakery	*60-3300*
Breakfast cereals	*25-850*
Coffee (ground)	*40-480*
Roasted nuts	*10-2000*
Popcorn	*160-180*
Black olives	*1960*
Prune juice	*270*

Factors such as raw materials, formulations, and processing conditions may account for the large variation in the acrylamide contents of some categories of food products.

Figure 2.8 and 2.9 are pictorial presentations of the findings from a spectrophotometric-based determination of the acrylamide content of some WA tuber and plantain food products (Quayson and Ayernor, 2007).The AA content of the foods were determined based on the non-enzymatic browning and optical density values which were correlated with acrylamide concentrations.

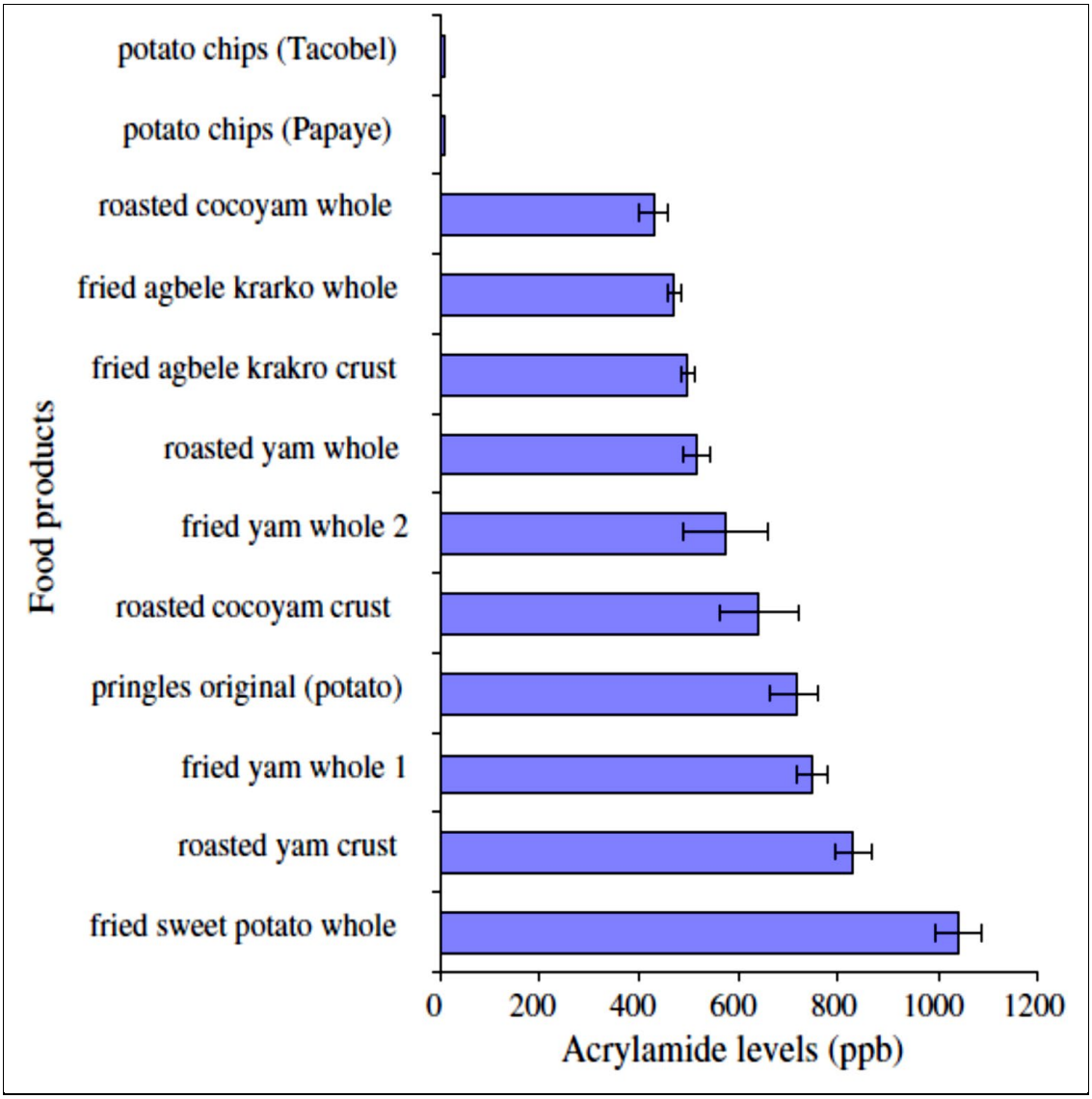

Figure 2.8. *Comparative histogram of the acrylamide content of some WA root and tuber-based products* (Quayson and Ayernor, 2007).

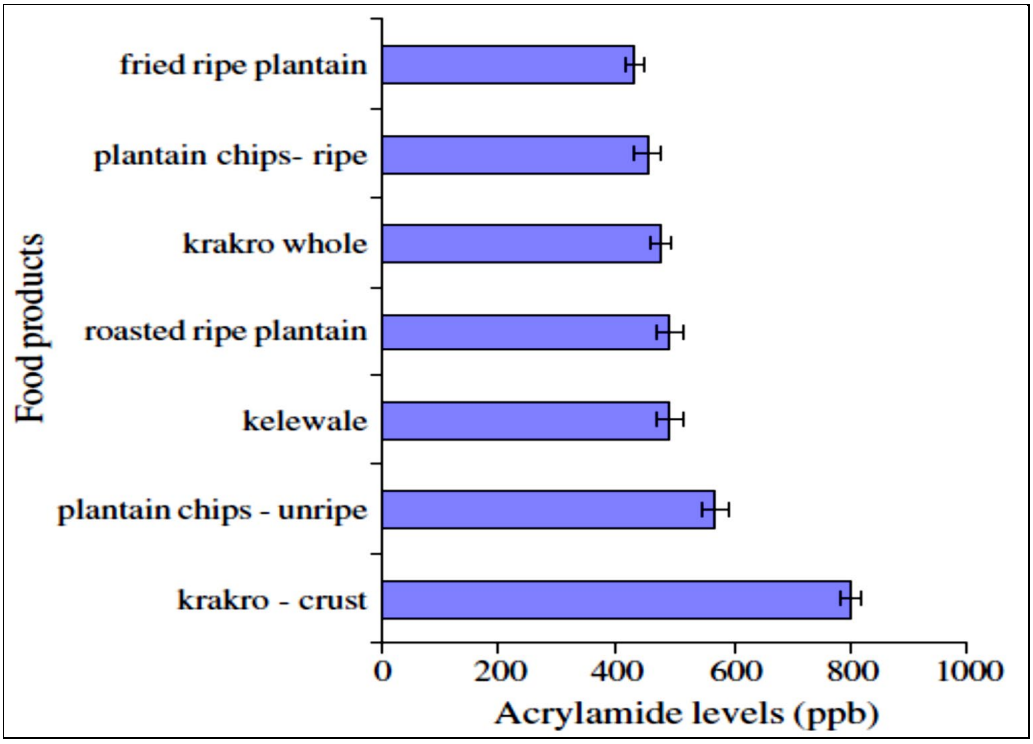

Figure 2.9. *Comparative histogram of the acrylamide content of some WA plantain-based products* (Quayson and Ayernor, 2007).

Based on dietary intake, an overview of the exposure assessment of acrylamide for various countries is presented in Table 2.3.

Table 2.3 *Overview of exposure to dietary acrylamide.*

Country	**Group of Population**	**Mean Exposure ($\mu g kg^{-1} bw d^{-1}$)**	**References**
Belgium	*Adolescents*	*0.51*	*Matthys et al., 2005*
France	*Adults*	*0.5*	*EC, 2003*
Germany	*Children*	*0.43*	*Hilbig et al., 2004*
Germany	*Adolescents*	*0.30*	*Hilbig et al., 2004*
Netherlands	*General*	*0.48*	*Konings et al., 2003*
Netherlands	*General*	*0.5*	*Boon et al., 2005*
Norway	*Children*	*0.34*	*SNT, 2002*
Norway	*Adults*	*0.34*	*EC, 2003*
Sweden	*Babies*	*1.2-1.4*	*Fohgelberg et al., 2005*
Sweden	*General*	*1.5*	*Svensson et al., 2003*
Switzerland	*Adults*	*0.28*	*Swiss FOPH, 2002*

Investigations into the importance of different categories of foods as dietary source of acrylamide indicate that fried potato products (chips, French fries), coffee, breakfast cereals, bread and crisp bread and sweet bakery (gingerbread, biscuits, and cookies) are the major source of dietary acrylamide (Boon et al., 2005; Dybing et al., 2005; Konings et al., 2003; Svensson et al., 2003)

For different countries and continents, the contribution of a particular food product to the total exposure may vary considerably, reflecting the dissimilar food consumption patterns and cooking traditions, which prevails in these regions (Dybing et al., 2005).

For instance, the exposure assessment for the US indicates 35% contribution from fried potato products and only 7% contribution from coffee (Dybing et al., 2005). On the other hand, the acrylamide exposure assessments for the Swedish (Svensson et al., 2003) and Dutch population (Konings et al., 2003) show that coffee consumption contributes 39% and 13% respectively.

Hitherto, there is paucity of information on the acrylamide content of WA foods.
In addition, no study has considered the dietary exposure of WAs to this toxic compound. An attempt to determine the acrylamide level in some important WA food products, such as roasted yam and fried plantain did not consider official and standard method of analysis, such as GC-based methods and LC-based methods. The gas-chromatographic and liquid-chromatographic methods were the methods initially developed for the determination of acrylamide in water and foods (Castle and Erickson 2005, Mastovska and Lehotay 2006). In addition, the liquid-chromatography-mass spectrometric (LC-MS) method of testing acrylamide is the most common approach presently used by most laboratories (Tekelli et al., 2012).

The method used for the determination of the acrylamide content of the tuber and plantain-based WA foods include spectrophotometric-based method and graphical extrapolations based on another study (Quayson and Ayernor, 2007). The use of graphical extrapolations based on correlated colorimetric values and acrylamide concentrations could increase the error and uncertainty in the measurement of the acrylamide content of the WA foods.

2.8 Recommended Levels for Dietary Acrylamide

The European Commission draft proposal updated in 2016 includes the responsibilities of the food business operators, which are to apply mitigation measures to reduce the presence of acrylamide in their food products below 'benchmark levels' and to verify through sampling and analysis that the levels of acrylamide are below established 'benchmark levels'.

According to the draft, the Commission shall review the set benchmark levels of acrylamide every three years.
As previously mentioned, the European Commission (EC) adopted a “Recommendation on the monitoring of acrylamide in foods” in 2007. This recommendation led to the collection and compilation of data on the acrylamide content of some important European foods.
The result of the data collection is the 2007-2010 EFSA report on dietary acrylamide.
The emergence of the EC recommendation 2010/307/EU, which revised the categorisation of food products for monitoring purposes, was the consequence of the 2007-2009 data collection.
In the year 2011, there was adoption of another recommendation on the investigations into the levels of acrylamide in food by the EC. This recommendation requires Member States to carry out investigations in cases where the levels of acrylamide in a foodstuff, tested in the

monitoring exercise, exceeds set indicative values. In such event, the concerned Member State(s) should report the results from the investigations to the Commission.

The EC recommendation 2013/647/EU on investigations into levels of acrylamide in food replaced the previous recommendations.

The Food Drink Europe, which is a representative of the European food and drink industry, continues to work in association with the European Commission to develop a "toolbox" designed to assist food business operators (FBO) in reducing the acrylamide content of their product. There was a revision of the FDE toolbox tagged "Code of Practice for Managing Acrylamide Formation" in May 2016 (FDE, 2016).

In February 2017, there was a consultation by the European Commission concerning a proposal to make the FDE "Code of Practice for Managing Acrylamide Formation in Foods" mandatory for food business operators.

The proposal follows prolonged deliberations on the use recommendations or a more legally binding Act based on maximum concentration limits or upon a mitigation (Code of Practice) approach (IFST, 2017).

Although, there is still no legally binding legislation on the management of acrylamide in food manufacture, the draft code of practice in circulation since January 2016, serves as the best practice guide for some food manufacturers (IFST, 2017)

The Food Drink Europe, a representative of the food industry in Europe, and the Member States firmly support the proposal for a legal control of dietary acrylamide and legislation is expected before the end of 2017(IFST, 2017).

The Food Standards Agency (FSA), a governmental agency of the UK responsible for protecting the public health in relation to food, has also been actively involved in advising consumers and food business operators on minimising acrylamide formation in heat-processed foods (IFST, 2017; UK FSA, 2017).

Although, there are currently no enforceable law on the allowable amount of acrylamide in foods, there is an international regulation concerning the amount of acrylamide that is permissible in drinking water. The World Health Organization (WHO) has a standard of 0.5µg/L for drinking water (WHO, 1996). The previous Drinking Water Directive of the European Union (EU 98/83 Directive) amended by the Commission Directive (EU) 2015/1787 also has a permissible residual acrylamide from polymer in contact with drinking water of 0.1µgl−1 (Cavalli et al., 2004).

For several African countries, there is still need for development of regulations, which addresses issues bordering on water and food safety.

2.9 Analytical Methods for Acrylamide Determination (2002-2017)

The level of acrylamide in several foods has been determined by chromatographic techniques, including gas chromatography (GC), liquid chromatography (LC) or ion chromatography (IC). These chromatographic techniques are used in conjunction with suitable selective and specific detectors, such as electron capture detector (ECD), flame-ionisation detector (FID), nitrogen-phosphorus detector (NPD), UV detector and mass spectrometer (MS)

Alternative sensitive and selective techniques for acrylamide determination are capillary electrophoresis (CE), spectrometric techniques, bioanalytical techniques including enzyme-linked immunosorbent assay (ELISA) and electrochemical biosensors, EB (Oracz et al., 2011; *Zhu et al., 2017*). In addition, the usefulness of colorimetric methods such as an image processing technique called computer vision (CV) and quantum dots based fluorescent sensing technique has been described (Gökmen et al., 2007, 2008; Hu et al., 2015; Liu et al., 2014).

2.9.1 Common analytical techniques for acrylamide detection in foods *(Liquid and gas chromatographic (LC/GC) Techniques)*

Among all the methods that have been reported for the quantification of acrylamide, the liquid (LC) and gas chromatographic (GC) techniques stand out as well-established methods.

The liquid and gas chromatographs are mostly used in a hyphenated state with the mass spectrometer i.e. LC-MS/MS or GC-MS.

For most food matrixes, the separation and quantification of acrylamide is achieved using HPLC coupled with MS. The LC-MS method is mostly preferred due to its sensitivity, selectivity, and versatility.

The Food Analysis Performance Assessment Scheme (FAPAS) showed through proficiency tests that LC-QqQ-MS/MS is a reliable method with broad applications (Tekkeli et al., 2012).

A modified form of the conventional HPLC called ultra-performance liquid chromatography (UPLC), which uses higher pressures and lower flow rates, allows better separation of mixture components in a shorter time and with high sensitivity. The effective separation of acrylamide by the UPLC is achieved through the use of reversed-phase columns with much thinner film and particle size than in typical HPLC columns. Compared to traditional liquid chromatography techniques, the UPLC also minimizes solvent consumption, enhances separation of polar compounds such as acrylamide and improves the analyte peak symmetry (Oracz et al., 2011).

The challenges associated with the use of the conventional reversed-phase chromatography in retaining and separating polar compounds, similar to acrylamide made chromatography experts use alternative approaches such as ion-exchange chromatography and hydrophilic interaction liquid chromatography (HILIC).

Gas chromatography coupled with mass spectrometry has also been used in the quantitative assays of acrylamide and its derivatives.

For the gas chromatography-mass spectrometry (GC-MS) technique, derivatization of the analyte is necessary in order to increase volatility, selectivity, sensitivity and retention time.

Although, alternative approaches such as silylation (Oracz et al., 2011), have been reported for the derivatization of acrylamide, the most popular method of acrylamide derivatization is its bromination prior to the analysis (Zhang et al., 2005).

The high selectivity and precision of this GC-MS technique effectively compensates for the difficult and time-consuming derivatization process.

The two GC based methods including and excluding a derivatization step show satisfactory agreement with the LC-MS approach for the detection of acrylamide in various foods (Becalski, 2005; Soares et al., 2006, Wenzl et al., 2006). However, the GC-MS method

including a derivatization step is recommended by most reports for the detection of trace levels (<50 μg kg^{-1}) of acrylamide in cereal-based foods due to its high sensitivity (LOD 2 μg kg^{-1}) and great recoveries (93-104%) **(**Pittet and Perisset, 2004).

An important drawback of the GC-MS technique excluding a derivatization step is the lack of characteristic ions in the mass spectrum of the underivatized acrylamide and the interference caused by matrix decomposition. The high background noise associated with the GC-MS technique excluding a derivatization step therefore prevents achievement of a low limit of detection for acrylamide.

The utilization of gas chromatography coupled to tandem mass spectrometry (GC– MS/MS) reduces the effect of interference, which in turn results in a larger acrylamide peak area.

The application of GC– MS/MS method for baby foods resulted in a LOQ and LOD below 5 μg kg^{-1} and 1.5 μg kg^{-1}, respectively (Oracz et al., 2011).

According to Lee et al., (2007) the solid-phase micro-extraction (SPME) coupled with gas chromatography-positive chemical ionization tandem mass spectrometry is a highly sensitive technique for the determination of acrylamide in aqueous matrices, which is useful for the detection of acrylamide concentrations as low as 0.1 μg kg^{-1} (Lee et al., 2007).

In addition to the mass spectrometer (MS), other detectors that can be combined with the gas chromatographs to quantify acrylamide are flame-ion detectors (FID), nitrogen-phosphorus detector (NPD), and electron capture detectors (ECD).

For acrylamide detection and quantitation, ECD are exceptionally selective and sensitive.

The application of a GC– ECD method (including a prior derivatization step by KBrO3 and KBr) for fried foods such as potato crisps, potato chips, and fried chicken wings resulted in LOD and LOQ of 0.1 μg kg^{-1} and 3 μg kg^{-1}, respectively.

The good precision of the GC-ECD approach therefore compares with the GC–MS/MS method, and also includes an additional benefit of a lower cost of instrumentation (Oracz et al., 2011).

2.9.2 Alternative analytical methods for acrylamide detection in foods

(a) *Capillary Electrophoresis*

Alternative analytical techniques based on charge-to-mass ratio differences, and efficient separation of compounds through electrophoresis are used in acrylamide quantification.

Capillary electrophoresis (CE) is a relatively recent, emerging and rapid analytical technique which allows the resolution of polar and nonpolar compounds (Font et al., 2008).

For CE method, high voltage is used to separate or resolve components in a mixture (Geiser and Veuthey, 2009; Ravelo-Perez et al., 2009).

The application of CE allows charged compounds which are dissolved in an electrolyte to migrate to electrodes at different rates when the high voltage is applied (Anastos et al, 2005; Kataoka et al., 2009; Zhou et al., 2007)

Micellar electro-kinetic chromatography (MEKC) is a relative of CE technique which enables separation of charge and uncharged compounds that can undergo ionization due to a change in pH (Geiser and Veuthey, 2009; Ravelo-Perez et al., 2009).

The technique can be used for the separation and quantitative assay of virtually all chemical compounds soluble in water. The migration of acrylamide, which is a polar uncharged compound is influenced by its coefficient of separation between the micelles and water buffer solution.

For the quantitative determination of acrylamide, suitable detectors which can be used with the CE technique include UV spectrophotometers equipped with a diode matrix (diode-array detector, DAD) and the MS (Bermudo et al., 2004).

Recently developed MECK–UV–vis technique is characterized by low detection limit and can be used to measure trace amounts of acrylamide in complex matrices. The efficiency and selectivity of the MEKC is also comparable to the HPLC technique (Anastos et al, 2005; Bermudo et al., 2006; Zhou et al., 2007)

To further enhance the sensitivity and effectiveness of the capillary zone electrophoresis (CZE) method, the field amplified sample injection (FASI) technique including the derivatization of acrylamide by the application of 2-mercaptobenzoic acid is required.

Two capillary zone electrophoresis in-line pre-concentration technique for the analysis of acrylamide in foodstuffs (field amplified sample injection, and stacking with sample matrix removal) which require derivatization step with 2-mercaptobenzoic acid have been reported to show similar sensitivity and precision compared to the chromatography-based methods (Bermudo et al., 2006, 2007).

The CZE–FASI–UV method including a derivatization step was used by Bermudo et al., (2006) to determine the content of acrylamide in foods such as biscuits, crisp bread, cereal flakes, potato crisps and coffee. For samples tested, the LOD for this method was as low as 3 ng g^{-1}. (Bermudo et al., 2006, Tezcan and Erim, 2008).

The method was later modified and improved by the inclusion of tandem mass spectrometry i.e. capillary electrophoresis coupled to tandem mass spectrometry, CE-FASI-MS/MS (Bermudo et al., 2007)

The integration of the FASI technique with capillary electrophoresis and tandem mass spectrometry (FASI–CE–MS/MS) resulted in a more reproducible, sensitive and selective methodology of acrylamide quantification (Monton et al., 2007; Ravelo-Perez et al., 2009). The application of the FASI–CE–MS/MS for the determination of acrylamide in bread yielded values of 8 and 20 ng g^{-1} for the limit of detection and limit of quantification respectively (Bermudo et al., 2007).
These are values consistent with those obtained by the utilization of chromatographic techniques such as LC–MS/MS (Huhn et al., 2010)

The replacement of aqueous background electrolyte (BGE) solvents with organic solvents in capillary electrophoresis can also improve the sensitivity and selectivity of the CE technique (Xu et al., 2004).

The non-aqueous capillary electrophoresis (NACE) technique as it is called was used in conjunction with UV spectrophotometric detection at 200 nm by Baskan and Erim for the quantification of acrylamide in foods (Baskan and Erim 2007, Oracz et al., 2011).

The migration of acrylamide in an electric field is restricted because of its polar-uncharged property in aqueous medium. The NACE technique is based on the modification of the behaviour of acrylamide resulting from its uncharged nature.

This method uses a solvent such as acetonitrile, with low pH to protonate, charge and enhance the electrophoretic mobility of acrylamide (Oracz et al., 2011).

The NACE-UV method of detection has been shown to be more sensitive than the CZE-UV method of detection. This method has a low detection limit of 4.4 ng ml^{-1} and has been used for the determination of acrylamide in potato chips (Tezcan and Erim, 2008).
In addition, the introduction of the ESI-MS/MS technique can further enhance the accuracy and sensitivity of the NACE methodology (Monton et al., 2007)
The merits of using these electrophoretic techniques include use of minimal sample volumes for analysis, high resolution, omission of sample clean-up step required for HPLC techniques, short time of analysis, and relatively simple equipment (Oracz et al., 2011).

(b) *Enzyme-linked immunosorbent assay (ELISA):*

The Enzyme-linked immunosorbent assay (ELISA) is attracting an increasing attention for the quantitation of acrylamide in foods. ELISA is a quicker and easier method based on a highly specific interaction between an antigen and antibody (Oracz et al., 2011).This analytical technique utilizes antibodies conjugated to an appropriate enzyme for the optical detection of the antigen of interest.
The high specificity of the antibody- antigen (Ab-Ag) interaction makes ELISA technique an efficient method with other advantages including good sensitivity, selectivity, and high throughput.
The analytical detection of acrylamide through the use of ELISA technique is faced with the two important challenges which are the development of appropriate antigens to obtain high affinity antibodies, and signal amplification.
Initially, the low molecular weight of acrylamide made the synthesis of specific antibodies and the application of ELISA technique a difficult task. Usually, substances with molecular mass below 1000 Da do not trigger immunogenic response or lead to production of antibodies (Oracz et al., 2011; Zhang et al., 2009).

To overcome this challenge, the coupling of polyclonal antibodies with high molecular weight and epitope group to acrylamide was proposed by Preston et al., (2008). The use of immuno-stimulating carrier protein was found to be an effective method for the production of antibodies specific for acrylamide. Consequently, the isolation of acrylamide binding antibodies enabled quantitative determination of acrylamide in foods (Hu et al., 2015)

The chemiluminescent effect from the interaction of enzyme-Ab complex with the Ag also makes ELISA technique functional with analytical methods such as spectrophotometry.
The integration of ELISA with enhanced chemiluminescence (ECL) instead of conventional spectrophotometric techniques considerably increased the sensitivity and selectivity of the immunological tests and eliminated interferences caused by matrix components which are encountered in the analyses of potato chips and biscuits (Oracz et al., 2011; Zhang et al., 2011).

The ELISA –ECL technique was used by Quan et al. to quantify acrylamide in food products such as potato crisps, instant noodles, biscuits and cakes (Oracz et al., 2011). For this analytical determination, polyclonal antibodies obtained through the immunization of rabbits with N-acryloxysuccinimide (NAS) and keyhole limpet haemocyanin (KLH) were used. The limits of detection obtained for potato crisps, instant noodles, biscuits and cakes were 126, 41, 137 and 69 ng ml^{-1}, respectively (Zhang et al., 2011).The ELISA-ECL technique which has the potential of rapid detection of acrylamide in 1-2 minutes also uses luminol, ρ-iodophenol, and hydrogen peroxide (H_2O_2), but has lower sensitivity compared to conventional methods (Hu et al., 2015, Quan et al.,2011,)
Compared to chromatography and electrophoretic techniques, ELISA is a simpler and faster method with sufficient precision, high sensitivity, lower cost, and shorter detection time. In addition, it can be used in routine analyses of multiple samples and does not require expensive equipment or tedious sample preparation**.** Also, the results of acrylamide quantification by this immuno-enzymatic technique are consistent with those obtained from the application of HPLC (Oracz et al., 2011).
ELISA kits for acrylamide quantitation with LOD of 5 μg kg^{-1}, a linear range of 10-10000 μg kg^{-1} and good recovery of 92-96 % are now commercially available (Franek et al., 2014; Hu et al., 2015; Oracz et al., 2011).

The ELISA Kits produced by Morinaga Institute of Biological Science, Inc. Japan has sensitivity of 0.7-90ng/ml (42 -5,400 ppb) and can be used for up to 24 samples per assay.

Although, ELISA is a promising technique one important challenge facing its use remains the difficulty of producing long-lasting and stable antibodies with high affinity for acrylamide.

(c) *Quartz Crystal Microbalance (QCM) Sensing Technique*

This is based on the piezoelectric effect of supramolecular assembly which generate electric charge in response to applied mechanical stress. A nano-gram mass change in quartz crystal microbalances (QCM) produce changes in the oscillations of the crystal which can be used to detect different analytes, including biological materials. A microscopic acrylamide recognition site in specific supramolecules binds to available acrylamide in tested samples creating signals that help in the detection of the analyte (Steed & Atwood, 2009). The intermolecular interaction between the "host" supramolecule and the "guest" molecule accounts for the high selectivity and stability of the supramolecular recognition based method.

The recent molecular imprinting technique (MIT) based on the chemistry of supramolecules or "chemical antibody" has been applied in liquid chromatography, solid phase extraction, and other techniques used in food analysis (Chen et al., 2011).

Kleefisch et al., (2004) studied the supramolecule-based detection and quantitation technique. This research group assembled a supramolecule consisting of tetralactam macrocycles with binding sites having high affinity for AA.

The binding of acrylamide to the recognition cavity of the supramolecule causes changes in resonant frequency of the oscillating quartz chip, which can be used for the quantitation of the analyte.

These highly sensitive superior host compounds also identified as quartz microbalance sensors have been used for the detection of small amounts of acrylamide, and can give a detection limit of 10 μg/kg. Other interfering compounds like acrylic acid, propionamide or propionic acid showed no or significantly lower affinities to the macrocycles (Kleefisch, *et al*., 2004).

(d) *Electrochemical and biosensing methods*

This category of analytical techniques uses highly selective bio-receptors with strong affinity for acrylamide to quantify the level of the analyte in food samples. Apart from the rapidity and simplicity of these sensor-based quantitative techniques, they are also sensitive, selective

and less expensive analytical techniques with broad applications in food safety (Hu et al., 2015).

Biological entities employed for the detection of acrylamide include *Brevibacterium sp*, *Pseudomonas aeruginosa*, and haemoglobin (Hb)

Initial attempts for the determination of AA in wastewater were based on effect of the analyte on the rate of oxygen consumption of *Brevibacterium sp*. The difference between the rates of oxygen consumption of *Brevibacterium sp.* before and after the introduction of acrylamide, defined as specific respiratory activity (SRA) by Ignatov et al., (1997) was used for the determination of the analyte.

Changes in electrical signals resulting from the reduction in the concentration of oxygen and the metabolism of AA is a promising approach for the detection of AA.

An electrochemical biosensor based on the electron transfer between amidase-producing *Pseudomonas aeruginosa* and acrylamide was fabricated by Silva et al., in 2009 (Hu et al., 2015, Silva et al., 2011).

The amidase produced by this bacteria acts as a catalyst for the hydrolysis of acrylamide to acrylic acid and ammonium ion. The concentration of acrylamide in the tested sample can then be indirectly quantified by the use of ion- selective-electrodes (ISE) designed for ammonium (NH_4^+) ions.

Hence, this potentiometric biosensor-based technique can be used for online and real-time detection of acrylamide in food samples (Hu et al., 2015).

The use of amidase-producing *P. aeruginosa* and NH_4^+ ISE for acrylamide determination resulted in a linear response in the range of 0.1- 4.0 $x10^{-3}$M of acrylamide, a detection limit of 4.48 $x10^{-5}$M acrylamide, a response time of 55 s, a sensitivity of 58.99 mV mM^{-1} of acrylamide, substrate recovery of 93.3% and a maximum half-life $t_{1/2}$ of 54 days (Silva et al., 2009).

Compared to standard methods such as HPLC-MS, the biosensor is relatively cheap to produce, since whole cells of *P. aeruginosa* is used as the source of amidase activity (Silva et al., 2009).

Demerits of the use of this biosensor include its cross-reaction with acetamide and formamide.

Other concerns are the purity of the mutant strain of *P. aeruginosa* used, and the effect of side reactions catalyzed by other enzymes. The use of a living bacteria can also affect the response time of the biosensor.

The changes in electric signal following the reduction of acrylamide in the presence of electrolytes, such as LiCl and cobalt(II) ions has also been used for the detection of the analyte (Niaz et al., 2008; Zargar et al., 2009).
The merits of this method are high sensitivity (LOD of 3.52 μg kg^{-1}) and rapid responses. However, the detection of acrylamide in complex food matrixes require efficient removal of interfering substances.
An alternative biosensing technique is based on the redox reaction involving the combination of acrylamide and haemoglobin (Hb) which results in the transition of the Fe^{3+} in Hb molecule to Fe^{2+} and changes in electric current signals (Friedman, 2003; Lineback et al., 2012).
The effective interfusion of AA and Hb, together with the associated redox and electrochemical reactions endow this method with high sensitivity, high selectivity, rapid response, and a wide linear range (Stobiecka et al., 2007)

Two main concerns with the use of this biosensing technique are the efficiency of Hb immobilization and electron transfer on the surface of the working electrode (Sun et al., 2013). Hence, the use of novel materials including carbon nanomaterials and metal nanoparticles (MNP, such as Au, Ag, Pt, etc.) for the enhancement of the immobilization and electron transfer of Hb is highly recommended.
The use of materials like carbon nanotubes with abundant functional groups (such as the carboxyl and carbonyl groups) and high surface-to-volume ratio facilitates efficient immobilization of Hb to the electrode resulting in higher signal response.
The application of nanomaterials with excellent electrical conductivity for the Hb-based biosensor can enhance the transfer of electrons, causing a better signal response (Dreyer et al., 2010; Lee et al., 2010; Liu et al., 2012; Mai et al., 2011; Sun et al., 2013; Yang et al., 2010).
The application of an electrode modified with haemoglobin-gold nanoparticles (Au-NPs) by Garabagiu and Mihailescu (2011) to determine trace levels of acrylamide generated sensitivity and linearity range of 2.84 μg kg^{-1} and 0.71-710 μg kg^{-1} respectively.
The utilization of a single walled carbon nanotube (SWCNT)-Hb based biosensing technique by Krajewska et al., (2008) for potato crisps extracts produced results with a wider range of linearity (7.1 x 10^{-4} to 7.1 x 10^{-2} μg kg^{-1}) and lower detection limit (0.071 μg kg^{-1})

According to results from preliminary studies, the irreversible interaction between AA and Hb adversely affects the reusability and efficiency of the working electrodes (Garabagiu & Mihailescu, 2011; Krajewska et al., 2008; Stobiecka et al., 2007).
Later studies utilized mixture of multi-walled carbon nanotubes (MWCNTs) and copper nanoparticles (CuNPs) to construct reusable electrodes. This modified Hb- based biosensors can be used for 120 times over 100 days with good reproductively, and consistency (Batra et al., 2013).
(e) *Fluorescence-based sensing technique*

This remarkable method, which relies on the polymerization of acrylamide and the luminescent characteristics of quantum dots (QDs), was recently recommended for the detection of acrylamide (Hu et al., 2014).
A study showed that the modification and polymerization of QDs containing carbon-carbon double bonds by N-acryloxysuccinimide (NAS) and UV radiation caused a decrease in the distance between QDs and the magnitude of emitted light (Liu et al., 2011; Noh et al., 2010; Tansakul et al., 2010). The presence and involvement of AA in the polymerization reaction accounts for an increase in the fluorescence intensity caused by the increase in distance between QDs.
Consequently, there was an establishment of the correlation between the concentrations of AA and changes in the magnitude of fluorescence following UV irradiation.
For the QD-fluorescent technique, the range of linearity and LOD are 35-350000 μg kg^{-1} and 35 μg kg^{-1}, respectively.
In comparison to conventional methods and electrochemical biosensing techniques, the lower sensitivity of this method limits its applicability to the detection of acrylamide in several food samples.

In addition, for online detection of AA in foods, this method is limited by the high temperature associated with the polymerization reaction (Hu et al., 2015)
However, the advantages of this fluorescence-based sensing technique include the visibility of signals, easiness of operation, unneeded support for large-scale instruments (Hu et al., 2015), and the expected lower cost of analysis compared to conventional methods.

2.9.3 Sample treatment for common acrylamide determination techniques

The general sample pre-treatment procedures for conventional detection methods such as LC-MS/MS and GC-MS, include homogenization, spiking with an internal standard, extraction, defatting, de-proteinization, and purification.

Internal standards (IS) are added to the samples to enhance the accuracy, precision, and repeatability of measurements (JECFA, 2005) through efficient recoveries and monitoring of the likely loss of the analyte during the whole sample preparation (Hu et al., 2015).

Solvents such as acetonitrile have been used to prepare surrogate standards such as N,N dimethylacrylamide, $^{13}C_3$- labelled acrylamide, $^{13}C_1$- labelled acrylamide, d_3-labelled acrylamide, propionamide, methacrylamide, and butyramide. Although, most studies use $^{13}C_3$- labelled acrylamide as internal standard (Hu et al., 2015), a different internal standard called zidovudine was used for the determination of acrylamide by Veni et al., (2014).

The polar property of acrylamide makes it highly soluble in water (2155 g/L at 30ºC), in polar organic solvents and in lower alcohols. Its solubility in methanol is 1550 g/L, while its solubility in ethanol is 860 g/L. It is almost insoluble in non-polar organic solvents such carbon tetrachloride, hexane and heptane (<1g/L; Castle and Eriksson, 2005; Habermann, 1991). The solubility of acrylamide in acetone, acetonitrile and ethyl acetate are 631 g/L, 396 g/L, and 126 g/L respectively. Consequently, the solubility property of acrylamide and the method of determination determines the suitable solvent for sample extraction. Generally, polar media such as water and salt solutions (formic acid) are used to extract acrylamide from food matrixes. Polar organic solvents such as acetone and acetonitrile are also used for sample extraction. (Chen et al., 2008; Chu & Metcalfe, 2007; Gökmen et al. 2009; Klaffke et al., 2005).

The use of water as extraction solvent can reduce interferences with hydrophobic nature, but does not minimize the presence of other hydrophilic interferences (Zhang et al., 2005). Additional merits of utilizing organic solvents for the extraction of acrylamide include the convenience of evaporation and subtraction of a centrifugation stage.

Some studies made use of a combination of aqueous salt solutions and organic solvents to enhance and improve the extraction of acrylamide (Yamazaki et al., 2012; Zhang et al., 2007).

Wang et al., (2008) made use of solvents such as hexane and cyclohexane to defat the food samples while Bagdonaite et al., (2008) utilized solvents such as acetone, ethanol, methanol and Carrez I-II reagents (potassium ferricyanide and zinc sulfate) for sample de-proteination.

Ghiasvand and Hajipour, (2016) utilized a solid phase micro-extraction (SPME) fibre made from carboxen- divinylbenzene-polydimethylsiloxane (CAR/DVB/PDMS) to extract acrylamide from the headspace above the food sample for 30 min at 60 °C. This method does not require complicated sample treatment and allows direct injection of the withdrawn sample extract into the GC-FID system.

Derivatisation techniques are used to obtain an analyte that is more convenient to detect than acrylamide itself. Most studies made use of bromination as the derivatisation technique to obtain a modified analyte called 2, 3 - dibromopropionamide which can be readily detected at trace levels. Although, derivatisation is not compulsory, it can lead to achievement of lower detection limits, particularly for GC-based techniques.
Most liquid chromatographic techniques and some GC based techniques do not require derivatisation (Dunovska et al.,2006; Lee et al., 2007).
Reagents such as bromine water, potassium bromate ($KBrO_3$), potassium bromide (KBr), hydrobromic and sulphuric acid are used for the bromination step (Nemoto et al.,2002; Zhang et al., 2006).
The simplicity, stability, automation, accuracy, precision, and repeatability of solid phase extraction (SPE) technique makes it a suitable method for the cleaning of food sample extracts (JECFA, 2011).

The appropriate approach for sample extract purification is dependent on the available SPE cartridge. For SPE cartridges such as Oasis HLB, Oasis MCX, Isolute Multi-Mode, ENVI-Carb, and Isolute ENVþ (Zhang et al., 2005) the analyte is absorbed from the complex sample extract through hydrogen bonding, p-p interaction, and cation exchange. The analyte is consequently eluted using polar solvents.

The second clean-up technique retain interferences in the SPE cartridge after the elution and collection of the extract containing the analyte (Bortolomeazzi et al., 2012). Oasis HLB, Oasis HLB coupled with Bond Elut-Accucat, Isolute Multimode and a homemade SPE column filled with the mixture of C18, SCX, and SAX have been used for sample clean-up.

Recently, improvements in the purification of sample extracts was achieved through the application of novel filling materials such as carbon nanotubes, magnetic chitosan (Xu et al., 2012), and molecule imprinted polymer (Xu et al., 2013).

2.9.4 Sample treatment for alternative acrylamide determination techniques

The sample pre-treatments for alternative analytical methods of acrylamide detection such as capillary electrophoresis, electrochemical biosensing, ELISA and colorimetric techniques are similar to those described for conventional and established methods. This is particularly true for the protocols and solvents required for extraction.
However, for these optional analytical methods the SPE clean-up step may be omitted or replaced by other purification procedures.

In addition, for colorimetric techniques such as computer vision, no extraction and purification procedures are required (Hu et al., 2015)

The extraction solvent used for bioanalytical techniques such as ELISA, enhanced chemiluminescent (ECL) ELISA, and indirect competitive enzyme-linked immunosorbent (ic-ELISA) is predominantly de-ionised water (Preston et al., 2008; Quan et al., 2011; Wu et al., 2014; Zhu et al., 2017)
For some of these immunoassays, phosphate buffered saline (PBS) is also used for the purpose of analyte extraction and removal of interference (Quan et al. 2011: Hu et al., 2015)

For most ELISA based techniques, the reagents used for the derivatisation of analyte include alkaline solution of 3-mercaptobenzoic acid (3-MBA), mercaptobenzoic acid, and 4-mercaptophenylacetic acid, 4-MPA (Preston et al., 2008; Quan et al., 2011; Wu et al., 2014; Zhu et al., 2017). Some ELISA based technique also used SPE clean-up technique for extract purification (Preston et al., 2008).
Most immunoassays require a simpler pre-treatment, without complicated treatments such as spiking of sample with an internal standard (Hu et al., 2015). This is a direct consequence of the specific recognition of antibodies and the derivatised analyte.

The capillary zone electrophoresis technique used by some studies also used alkaline solution of 2-mercaptobenzoic acid for derivatisation of the analyte (Bermudo et al., 2006).

The microchip electrophoresis technique used by Wu Chen et al., (2016) utilized sulfoindocyanine succinimidyl ester (Cy5) dissolved in acetonitrile and borate solution for derivatisation.

For electrophoretic method of determination, purification of sample extract can be achieved by liquid–liquid extraction (LLE) with dichloromethane and SPE with Strata-X-C and ENV+ cartridges (Bermudo et al., 2006). Defatting of sample is via the use of solvents such as hexane, while de-proteination is through the use of Carrez I-II reagents (potassium ferricyanide and zinc sulfate). Thus, the pre-treatment of electrochemical biosensing methods is more complicated compared with immunoassays, but still simpler than standard methods (Hu et al., 2015).Ionic strength and pH adjustment of the extract by the addition of acetic acid and NaCl solutions is also required for some electrophoretic techniques (Krajewska et al., 2008; Stobiecka et al., 2007). Extraction and clean-up can also be enhanced by the use of ultrasonic treatment and filtration (Zargar et al., 2009).

Unlike the sample pre-treatments for conventional analytical techniques such as LC and GC based methods, the sample treatments for the alternative methods of detecting acrylamide are timesaving, and lower cost. Some of these optional methods may also be used without SPE clean-up step, which allows a rapid, high-throughput, and on-line detection of AA (Hu et al., 2015). Thus, the subtraction of the SPE clean-up phase and the simplicity of the sample pre-treatments used for some of the alternative techniques reduces the time and cost of the whole detection process.

2.9.5 Comparison of the common and alternative methods of acrylamide determination

(a) Versatility: Established methods which are LC and GC based techniques have been used to determine the level of acrylamide in several foods including potato chips, cereal-based foods, coffee, tea, and instant noodles. The practical application of most alternative methods for a wide-range of foods have not been confirmed, as most studies only evaluated their use for potato chips (Hu et al., 2015). Hence, most of the alternative methods still require further validation.

(b) Preference: studies have indicated that most private and official laboratories in Europe prefer the established methods to the alternative methods (Wenzl et al., 2003). According to Castle and Eriksson, (2005), the use of methods such as bromination and GC based

technique for acrylamide detection was relatively advanced even before the discovery of the toxic compound in heat-treated foods.

(c) SPE clean-up: For most established methods, the purification of sample extracts with SPE cartridges is necessary to guarantee high selectivity. Several alternative methods do not require SPE clean-up step (Hu et al., 2015)

(d) Cost of determination: The instruments and accessories needed for the established methods of acrylamide determination are quite expensive. GC and HPLC analytical systems can cost as much as £25000-£50000, while ELISA kits and accessories cost about £700-£2500. In addition, the SPE clean-up stage for most established methods raises the cost and complexity of operations. In contrast, the simpler sample pre-treatment of some bioanalytical methods reduces the cost of acrylamide determination. Considering sample pre-treatment, depreciation of equipment, labor charges, and material cost, bioanalytical technique such as ELISA can saves at least 50% in costs when compared to conventional methods (Hu et al., 2015).

(e) Duration of analysis: The sample pre-treatment is the rate determining step for the established methods. In contrast, optional methods such as electrochemical biosensors and computer vision, require simpler or no sample pre-treatment reducing the detection time by 40% or more (Hu et al., 2015). Bioanalytical methods such as ELISA enable analysis of several samples in one assay i.e. Up to 24 samples can be detected by one assay (Hu et al., 2015). In addition, the use of commercial ELISA kits reduces the time required for the determination to approximately 6 hrs. Alternative methods such as electrochemical biosensors can enable on-line and real-time detection of acrylamide.

(f) Skill and expertise: The operation of conventional methods such as LC and GC require very high level of skill and expertise. In contrast, optional methods of detecting acrylamide such as ELISA require minimal training.

Table 2.4 *Gas Chromatography (GC)-based techniques for the detection of acrylamide (2002-2016)*

Matrices/ LOD/LOQ (References)	**Sample Extraction (XTr) , Treatment, Analyte Derivatization (DRv) and Clean-up (C-Up)**
▪ Fried foods rich in protein or carbohydrates ❖ LOD of 5 µg/kg (*Tareke et al., 2000*).	XTr: Water as extraction solvent (ES) e.g.10 g sample mixed with 100 ml water. IS: N,N dimethylacrylamide or [$^{13}C_3$]-acrylamide (1 µg) as Internal Standard (IS) Derivatization (DRv) by bromination using 7.5 g KBr, HBr, bromine water and conditions of pH 1–3, 4ºC 18hr Clean-up (C-Up) by filtration of extract through glass-fibre filter, purification on carbograph column (1000mg carbon), addition of IS, bromination, titration with sodium thiosulfate solution (1 M), 15 g Na_2SO_4 added, extraction with 2x10 ml ethyl acetate (EtAc)/hexane=1/4, filtration of combined extract through 0.45 µm PTFE filter, Bio-Beads S-X3 gel (400x25mm i.d. column), eluent: ethyl acetate/ cyclohexane=1/1, fraction from 80 to 200 ml collected and evaporated to 100 µl
▪ *Carbohydrate-rich foods.* ❖ LOD of 10–30 µg/kg (*Swiss Quality Testing Service, 2003*).	XTr : using 10 g homogenized sample, α-amylase, 80 ml water, 30 s mixing, equilibration for 10 min at room temperature, 30 min. ultrasonic bath at 60ºC. IS: Propionamide as internal standard (IS) C-Up: by cooling sample at -18ºC for 15 min, addition of 20ml hexane, mixing for 30 s, centrifugation (10 min at 2000 rpm), 20ml aqueous phase pipetted onto column (Merck), elution with 300 ml EtAc, evaporation to <10ml, further evaporation to <1ml, reconstitution with EtAc to exactly 1mL, filtration through 0.45 µm syringe filter
▪ *All types of foods.* ❖ LOD of 10–20 µg/kg (*Biedermann et al., 2002*).	Water and 1-propanol as extraction solvent (ES) Methacrylamide, d_3-acrylamide (500mg/l), and butyramide as Internal Standard (IS) Clean up by addition of 10 ml supernatant, evaporation of solvent at 50 Torr and 60–70ºC, addition of 3 ml acetonitrile (AcN) and 20 ml hexane to the residue, second extraction of AcN with another 5ml hexane, transference of about 1.5ml AcN-phase into autosampler vial, addition of butyramide standard (IS2)
▪ *Various food products.* ❖ LOD of 9 ng/g and LOQ of 30 ng/g (*Nemoto et al., 2002*).	XTr: **u**sing water and defatting of sample with hexane. Centrifugation of homogenized mixture for 10 min at 2600 rpm. 20µl of 100 µg/ml $^{13}C_1$-acrylamide solution. as internal standard (IS) DRv: using bromination of sample with 10 g KBr, 6ml 0.1M $KBrO_3$ solution and 5M H_2SO_4, at 4–10ºC for a reaction time of 90min. C-Up: using 1M sodium thiosulfate to decompose excess bromine. Two-stage extraction of solution with 10 ml EtAc, drying and evaporation of the extract using Na_2SO_4 and rotary evaporator respectively, dissolution of residue using 2ml of 10% acetone/hexane solution, rinsing of the sample tube with 1ml 10% acetone/hexane onto a Florisil cartridge conditioned with 10 ml hexane. Removal of the hexane fraction, and collection of the second fraction containing the analyte in test tubes, followed by its concentration in a centrifugal concentrator. Removal of the residual solvent under a nitrogen gas stream and reconstitution in 0.5ml acetone. Addition of 20 µl triethylamine to the sample.
▪ *Baby foods.* ❖ LOD: 1–2 µg/kg (*Wiertz-Eggert-Jo¨rissen GmbH, 2003*).	XTr: Homogenization of 10g sample using 50 ml water and ultrasonic bath for 30 min at 60ºC. IS: D_3-acrylamide (0.1 µg/g sample)as IS C-Up: by addition of Carrez I, Carrez II and 30 ml n-hexane to the aqueous solution; followed by centrifugation at 45 000g for 10min, saturation of the aqueous phase with NaCl and two-stage extraction with 50 ml EtAc, the organic phases are combined, dried over Na_2SO_4 and evaporated to 1ml.

Table 2.4 *Contd- GC-based techniques for the detection of acrylamide (2002-2016)*

Matrices/ LOD/LOQ (References)	**Sample Extraction (XTr) , Treatment, Analyte Derivatization (DRv) and Clean-up (C-Up)**
▪ *Various foods including potato chips, French fries, breakfast cereals, bakery products, confectioneries, nuts, and roasted coffee.* ❖ LOD of 5 µg/kg (*Hoenicke et al., 2004*).	XTr1: Filtration of homogenized sample, followed by defatting using iso-hexane and water extraction in ultrasonic bath at 60 ºC for 30 min. XTr2: water extraction of homogenized sample in ultrasonic bath at 60 ºC for 30 min. Addition of iso -hexane and 5 ml of Carrez I and II solutions. Two-stage extraction of aqueous phase of centrifuged sample extract using sodium chloride and 50 ml ethyl acetate IS: deuterium-labelled [$^{2}H_3$]acrylamide (d_3 -acrylamide) C-Up: treatment of sample extract with acetonitrile and Carrez solutions. Alternatively, iso-hexane and Carrez solutions may be used.
▪ *Various samples like potato chips, corn snacks, biscuits, instant noodles, coffee, soy sauce.* ❖ LOD of 0.5-5 µg/kg *and* LOQ of 5–20 µg/kg *(Yamazaki et al., 2012).*	XTr: using water, and hexane for defatting. IS: d_3-acrylamide DRv: using xanthydrol in acidic media, LLE with EtAc. C-Up: Sample extract purified using Sep-Pak™ C18 and Sep-Pak™ AC-2 columns. SPE with EXtrelut™ column for clean-up of amino acid-rich samples
▪ *Coffee.* ❖ LOD of 5 µg/kg *and* LOQ of 10 µg/kg *(Soares et al., 2010).*	XTr: matrix solid-phase dispersion (MSPD) sample preparation IS: $^{13}C_3$ acrylamide dissolved in acetonitrile DRv: using bromination method C-Up: Sample extracts purified using SPE.
▪ *Asparagine-glucose model system.* ❖ LOD of 2 µg/kg *and* LOQ of 36 µg/kg (*De-Vleeschouwer et al., 2007*).	Asparagine-glucose solutions prepared in citrate buffer IS: acrylamide, methacrylamide and butyramide. C-Up : See Biedermann et al., 2002
▪ *Cereal-based foods, French fries, and potato chips.* ❖ LOD of 1 ng/kg *and* LOQ of 25 ng/kg (*Notardonato et al., 2013*).	XTr: Iso-octane and acetonitrile for homogenization and centrifugation of sample, followed by stirring in ultrasonic bath for 5 min. DRv: using trifluoroacetic anhydride C-Up: not required
▪ *Various heat-processed foodstuffs*. ❖ LOQ of 15 and 40 µg/kg (*Dunovska et al., 2006*).	XTr: using n-propanol followed by solvent exchange to acetonitrile (MeCN). IS: d_3-acrylamide DRv: Not required C-Up: using dispersive solid phase extraction, based on addition of primary-secondary amine (PSA) sorbent into defatted extract.
▪ *Fried foods, such as potato crisps, potato chips, and fried chicken wings*. ❖ LOD of 0.1 µg/kg (*Zhang et al., 2006*).	XTr: using n-hexane, and aqueous solution of sodium chloride, using liquid–liquid extraction with ethyl acetate IS: $^{13}C_3$-acrylamide DRv: using potassium bromate ($KBrO_3$) and potassium bromide (KBr) C-Up: clean up not required, but can be done using Carrez I and II solutions
▪ *Potato crisps and French fries*. ❖ LOD of 0.1 µg/L (*Lee et al., 2007*).	XTr: Carbowax/ divinylbenzene (CW/DVB)-coated fibre for solid-phase microextraction (SPME) of acrylamide in water solutions at pH 7, and extraction time of 20 min and analyte desorption at 210 ºC for 3 min IS: not required DRv: not required C-Up: no further SPE clean-up and concentration steps required

Table 2.4 *Contd-GC-based techniques for the detection of acrylamide (2002-2016)*

Matrices/LOD/LOQ (References)	Sample Extraction (XTr) , Treatment, Analyte Derivatization (DRv) and Clean-up (C-Up)
▪ *Coffee and coffee products*. ❖ LOD of 5 µg/kg and LOQ of 10 µg/kg (*Soares et al., 2006*).	XTr: using water and ethanol IS: $^{13}C_3$ acrylamide dissolved in acetonitrile DRv: using bromination method C-Up: Two purification steps. Initial stage with ethanol and Carrez solutions; and the second stage with a layered solid-phase extraction (SPE) column.
▪ *Espresso coffees*. ❖ LOD of 1.16±0.25 µg/30 ml for pure Arabica samples, and 2.31±0.43 µg/30 ml for Robusta Samples (*Alves et al., 2010*).	XTr: matrix solid-phase dispersion (MSPD) sample preparation IS: $^{13}C_3$ acrylamide dissolved in acetonitrile DRv: using bromination method C-Up: Sample extracts purified using SPE.
▪ *Potato chips*. ❖ LOD of 12.8 µg/kg and LOQ of 38.8 µg/kg (*Fernandes and Soares 2007*)	XTr: using C18 as dispersive agent and water as extraction solvent. Matrix solid-phase dispersion (MSPD) sample preparation. Hexane for defatting sample extract IS: $^{13}C_3$ acrylamide dissolved in acetonitrile DRv: using bromination method C-Up: further cleaning not necessary after the solid phase dispersion sample treatment
▪ *Processed cereal products (bread, toasts, breakfast cereals, snacks, cookies, and biscuits), chocolates, and baby food*. ❖ LOD of 5.2 µg/kg (*Soares and Fernandes 2009*)	XTr: using C18 as dispersive agent and water as extraction solvent. Sample packed in an empty SPE column, Hexane for defatting sample extract IS: $^{13}C_3$ acrylamide dissolved in acetonitrile DRv: using bromination method C-Up: further sample cleaning not necessary
▪ *Crispbreads, biscuits, chips*. ❖ LOD of 16 µg/kg (*Wenzl et al., 2006*)	XTr: using a mixture of ethyl acetate and cyclohexane (3 mL, 1:1, v/v) IS: $^{13}C_3$ acrylamide DRv: using potassium bromate ($KBrO_3$) and potassium bromide (KBr). C-Up: liquid–liquid extraction (LLE) with ethyl acetate. SPE clean-up is used for samples tested by HPLC-MS/MS technique.
▪ *Carbohydrate-rich foods*. ❖ LOD of 0.10 –0.15 µg/kg (*Zhang et al., 2007*)	XTr: homogenization and defatting of sample with petroleum ether. Extraction using aqueous solution of sodium chloride IS: $^{13}C_3$-acrylamide DRv: using potassium bromate ($KBrO_3$) and potassium bromide (KBr) C-Up: LLE with ethyl acetate and SPE technique
▪ *Heat-processed foods*. ❖ LOD of 0.60 µg/kg(*Zhu et al., 2008*)	XTr: Water as extraction solvent and n-hexane for defatting sample. IS: acrylamide standard solution for sample preparation DRv: Hydrobromic acid and saturated bromine-water for bromination C-Up: LLE with ethyl acetate (no SPE clean-up and concentration steps required
▪ *Potato chips.* ❖ *LOD of 0.22* µg/g *and LOQ of 0.77* µg/g (*Ghiasvand and Hajipour, 2016*)	To extract acrylamide, position a needle with SPME fibre made from carboxen- divinylbenzene-polydimethylsiloxane (CAR/DVB/PDMS) into the headspace above the sample for 30 min at 60 °C. Withdraw the fibre into the needle and immediately inject the sample into GC-FID system.

Table 2.5 *Liquid Chromatography (LC)-based techniques for the detection of acrylamide (2002-2016)*

Matrices//LOD/LOQ (References)	**Sample Extraction (XTr) , Treatment, Analyte Derivatization (DRv) and Clean-up (C-Up)**
▪ *Potato chips.* ❖ LOD of 1 µg/kg and LOQ of 3 µg/kg (*Zhang et al., 2007*).	XTr: Defatting of grounded sample using petroleum ether. Aqueous extraction with 2 mol/L solution of sodium chloride. Further liquid–liquid extracted with ethyl acetate IS: d_3-labelled acrylamide solution (1 µg/mL) DRv: not required C-Up: solid-phase extraction (SPE) clean-up using OASIS HLB cartridges
▪ *Coffee*. ❖ LOD of 5 µg/kg *and* LOQ of 16 µg/kg (*Bortolomeazzi et al., 2012*).	XTr: using water as extraction solvent IS: d_3-labelled acrylamide DRv: not required C-Up: SPE clean-up
▪ *Cereal-based foods, potato chips, fries, crisps, breads, biscuits and cookies.* ❖ LOD of 6 µg/kg *and* LOQ of 18 µg/kg (*Senyuva & Gökmen, 2006*).	XTr: using 0.01 mM acetic acid in a vortex mixer IS: $^{13}C_3$-labelled acrylamide DRv: not required C-Up: SPE clean-up
▪ *Tea*. ❖ LOD of 1 µg/kg *and* LOQ of 5 µg/kg (*Liu et al., 2008*).	XTr: at 25 ºC for 20 min, using water followed by acetonitrile. Thorough stirring of mixture with 4 g magnesium sulphate and 0.5 g of sodium chloride. IS: $^{13}C_3$-labelled acrylamide DRv: not required C-Up: Solid-phase extraction (SPE) clean-up using an Oasis MCX cartridge
▪ *French and roasted fries, cookies, cocoa, and coffee*. ❖ LOD of 10 µg/L (*Paleologos and Kontominas 2005*).	XTr: using water and hexane IS: $^{13}C_3$-labelled acrylamide, Methacrylamide DRv: not required C-Up: Solid-phase extraction (SPE) clean-up
▪ *Coffee, cocoa, and high-salt foods*. ❖ LOQ of 10 µg/kg (*Eberhart et al., 2005*).	XTr: Ethyl acetate extraction IS: $^{13}C_3$-labelled acrylamide DRv: not required C-Up: SPE clean-up technique
▪ *Potato-based foods.* ❖ LOQ of 4 µg/kg (*Gökmen et al., 2005*).	XTr: extraction of acrylamide with methanol IS: $^{13}C_3$-labelled acrylamide DRv: not needed C-Up: sample purification with Carrez I-II solutions and Oasis HLB solid-phase extraction (SPE) cartridge.
▪ *Deep-fried flour-based leaven dough foods*. ❖ LOD of 6 µg/kg (*Wang et al., 2008*).	XTr: using water and low temperature centrifugation at 14,500 ×g and 0 °C for 15 min. Filtration of extract through a 0.45-µm PVDF syringe filter to a vial. IS: using acrylamide as standard DRv: not needed C-Up: SPE method using Oasis HLB and Bond Elut-Accucat cartridges.
▪ *Starch-based foods* ❖ LOD of 30 µg/kg (*Geng et al., 2008*).	XTr: Extraction of acrylamide with 75% (v/v) methanol in water IS: $^{13}C_3$-labelled acrylamide DRv: not needed C-Up: SPE clean-up technique

Table 2.5 *Contd-LC-based analytical techniques for the detection of acrylamide (2002-2016)*

Matrices/ LOD/LOQ (References)	**Sample Extraction (XTr) , Treatment, Analyte Derivatization (DRv) and Clean-up (C-Up)**
▪ *Traditional Turkish foods*. LOD of 0.93 ng/ml and LOQ of 2.82 ng/ml (*Can and Arli,* 2014).	XTr: Mixing and homogenization of 50-200 mg sample using water and vortex mixer. Centrifugation of mixture at 3000g for 10 min. Removal and filtration of 2–3 mL of resulting supernatant through a 0.2µm polyvinylidene fluoride filter. IS: Methacrylamide and acrylamide C-Up: SPE clean-up technique
▪ *Breakfast cereals.* ❖ LOD of 23.2 µg/kg (Rufián-Henares, and Morales 2006).	XTr: extraction using water IS: $^{13}C_3$-labelled acrylamide DRv: not required C-Up: cleaning with Carrez solution, methanol and SPE.
▪ *Coffee*. LOD of 2 µg/L(*Granby and Fagt 2004*).	XTr: ground coffee boiled in water and centrifuged for 10 min at 3500 rpm (after spiking with IS) IS: d_3 -acrylamide DRv: not required C-Up: clean-up using multimode SPE by automatic SPE equipment
▪ *Potato chips, French fries, breakfast cereals, bakery products, confectioneries, nuts, and roasted coffee*. ❖ LOD of 30 µg/kg (*Hoenicke et al., 2004*).	XTr1: Filtration of homogenized sample, followed by defatting using iso-hexane and water extraction in ultrasonic bath at 60 ºC for 30 min. XTr2: water extraction of homogenized sample in ultrasonic bath at 60 ºC for 30 min. Addition of iso -hexane and 5 ml of Carrez I and II solutions. Two-stage extraction of aqueous phase of centrifuged sample extract using sodium chloride and 50 ml ethyl acetate IS: d_3 -acrylamide C-Up: Using SPE techniques and Carrez precipitation
▪ *Carbohydrate-based foods*. ❖ LOQ of 25 µg/kg (*Croft et al., 2004*)	XTr: Water extraction of food samples IS: d_3 –acrylamide as surrogate standard DRv: not required C-Up: SPE technique using a mixed-bed anion and cation exchange cartridge in series with a C18 extraction disk
▪ *Various processed foods*. ❖ LOQ of 2 ng/mL (*Tsutsumiuchi et al., 2004*).	XTr: Water extraction and centrifugation IS: [$^2H_3$]Acrylamide DRv: not required C-Up: SPE clean-up technique
▪ *Cooked food samples.* ❖ LOD <15 µg/kg (*Calbiani et al., 2004*).	XTr: Extraction step with acidified water without clean-up IS: $^{13}C_3$-labelled acrylamide DRv: not required C-Up: not required
▪ *Baby food products.* ❖ LOQ of 0.5 µg/kg for liquid samples and 2 µg/kg for other samples (*Fohgelberg et al., 2005*)	XTr: using aqueous solution of deuterium-labelled acrylamide. Placing of the solution in a water bath (37 ºC, 5 min) and addition of $KAl(SO4)_2$ (s) (0.23 g). Centrifugation of sample at 4500 x g and 10 ºC for 25 min. IS: d_3 –acrylamide C-Up: SPE clean-up
▪ *Infant powdered milk and baby foods in jars*. ❖ LOQ of 1 µg/kg (*Jiao et al., 2005*)	XTr: Defatting with petroleum ether, and extraction with aqueous solution of sodium chloride. Further liquid–liquid extraction with ethyl acetate. IS: $^{13}C_3$-labelled acrylamide C-Up: SPE clean-up with HLB 200 mg cartridges

Table 2.5 *Contd-LC-based analytical techniques for the detection of acrylamide (2002-2016)*

Matrices/ LOD/LOQ (References)	Sample Extraction (XTr) , Treatment, Analyte Derivatization (DRv) and Clean-up (C-Up)
▪ *Chocolate matrices.* ❖ LOD of 0.30 µg/kg and LOQ of 1 µg/kg (*Ren et al., 2006*)	XTr: Defatting with petroleum ether, extracting with aqueous solution of 2 mol L^{-1} sodium chloride IS: $^{13}C_3$-labelled acrylamide C-Up: SPE clean up with OASIS HLB cartridges.
▪ *Potato chips and biscuit, grilled meat, and chicken*. ❖ LOD of 0.5 µg/L and LOQ of 5.0 µg/kg (*Kaplan et al., 2009*)	XTr: defatting of sample with hexane, followed by treatment with Carrez solutions and acetic acid extraction. Centrifuged at 5000 rpm for 10 min at −5 °C, followed by filtration through 0.45 µm syringe filter IS: standard solutions of acrylamide C-Up: no need for further cleaning.
▪ *Snacks, potato chips, biscuits, crisp bread, and breakfast cereals*. ❖ LOQ of 5 µg/kg (*Yusa et al., 2006*)	XTr: pressurized fluid extraction (PFE) of samples with acetonitrile and precipitation with Carrez reagents. IS: $^{13}C_3$-labelled acrylamide DRv: not required C-Up: SPE clean-up technique
▪ *Potato and cereal products*. ❖ LOD of 10 µg/kg (*Govaert et al., 2006*)	XTr: water extraction followed by centrifugation of extract at 5 ºC with a speed of 4000 rpm IS: d_3 –acrylamide DRv: not required C-Up: SPE clean-up technique
▪ *Bread*. ❖ LOD < 10 µg/kg; LOQ of 13 µg/kg (*Koh 2008; Nielsen et al., 2006*)	XTr: Using de-ionized water IS: d_3-acrylamide and $^{15}N_2$-asparagine as internal standards for acrylamide and asparagine, respectively. DRv: not required C-Up: SPE clean-up technique
▪ *Potato chips, breakfast cereal, cookies, bread.* ❖ LOD of 2 ng/g (Gökmen and Şenyuva 2006)	XTr: extraction with methanol, centrifugation of extract at 10,000 rpm for 10 min and extract purification with Carrez I and II solutions. IS: 13C3-labelled acrylamide DRv: not required C-Up: SPE clean up using Oasis HLB cartridge preconditioned with 1ml of methanol and 1ml of water.
▪ *Potato crisps and chips, biscuits, crisp breads,pastry, dried fruits, chocolates, and coffee.* ❖ LOD of 2-15 ng/g and LOQ of 6-45 ng/kg (LOD of 15 ng/g for ion trap and 2 ng/g for triple quadrupole- Bermudo et al.,2008)	XTr: using water spiked with IS. Agitation of sample for 1 hr followed by centrifugation at 4000rpm for 30min IS: d_3-acrylamide DRv: not required C-Up: SPE clean up followed by filtration of extract into vials via 0.45µm nylon filter.
▪ *Processed foods*. ❖ LOD of 0.1 µg/kg and LOQ of 2 µg/kg (*Kim et al., 2007*)	XTr: using water spiked with IS. IS: $^{13}C_3$-labelled acrylamide DRv: not required C-Up: Using C18 SPE cartridge activated with 5 ml of methanol and 5 ml of water. Filtration of extract into vials via 0.45µm nylon filter.
▪ *Cocoa and coffee.* ❖ LOD of 5.5 µg/kg (*Arisseto et al., 2008*)	XTr: Extraction with water, deproteination with Carrez I and II solutions, LLE with EtAc. Addition of water followed by evaporation with N_2 IS: d_3 –acrylamide DRv: not required C-Up: SPE clean up with Isolute Multimode cartridges

Table 2.5 *Contd-LC-based analytical techniques for the detection of acrylamide (2002-2016)*

Matrices/ LOD/LOQ (References)	**Sample Extraction (XTr) , Treatment, Analyte Derivatization (DRv) and Clean-up (C-Up)**
▪ *Tea*. ❖ LOD of 1 ng/mL (*Liu et al., 2008*)	XTr: Extraction with water, LLE extraction with ACN, $MgSO_4$ and NaCl, evaporation with N_2. IS: $^{13}C_3$-labelled acrylamide DRv: not required C-Up: SPE clean up with Oasis MCX SPE cartridge
▪ Fresh, roasted, flour, cooked, glazed chestnut. ❖ LOQ of 9 µg/kg and 4 µg/kg for roasted chestnuts and chestnut puree respectively (Karasek et al., 2009)	XTr: Extraction with water IS: d_3 –acrylamide DRv: not required C-Up: SPE with Isolute Multimode cartridge and then with Isolute ENV+ cartridge
▪ Deep-fried cassava, French fries, toasted cassava flour, water and salt biscuit, cream cracker biscuit, corn flakes, crispbread, French bread, hotdog bread, Italian bread, "bisnaguinha" bread. ❖ LOD of 10 µg/kg and LOQ of 20 µg/kg (Arisseto et al., 2009)	XTr: homogenization and extraction of sample using water spiked with IS. Centrifugation at 2500 x g and 5ºC for 10 min. Filtration of 3 ml aliquot of the supernatant through a 0.45 mm nylon filter IS: d_3 –acrylamide DRv: not required C-Up: SPE clean-up of 2ml extract using Oasis HLB cartridges preconditioned with 5 ml methanol and 5 ml water.
▪ Bakery products. ❖ LOQ of 10 µg/kg (Claus et al., 2005)	XTr: using water spiked with methanolic solution of deuterium-labelled acrylamide (20 µg/mL). Sample extraction in ultrasonic bath at 40ºC for 10 min. Liquid–liquid extraction with ethyl acetate IS: d_3 –acrylamide DRv: not required C-Up: SPE clean up technique
▪ *Potato chips*. ❖ LOD of 20 ng/ml and LOQ of 40 ng/ml (*Veni et al., 2014*)	XTr: Homogenization of 1± 0.1 g sample, followed by extraction with water spiked with IS. Centrifugation of sample at 4000 rpm for 10 min. IS: Zidovudine C-Up: SPE clean-up of 1 ml of filtered supernatant by using Phenomenex cartridges conditioned with 2 ml of methanol and 2 ml of water
▪ *Water samples*. ❖ LOD of 0.20 ppb (*Cavalli et al., 2004*)	Filtration of water samples using 0.45 µm filters. Direct injection of the samples into a microbore ICE-AS1 column and separation using acetonitrile–formic acid eluent.
▪ *French fries, potato chips, tortilla chips, wheat snacks with bacon flavour and crisp bread*. ❖ LOQ of 50 µg/kg (*Cavalli et al. 2003*)	XTr: Using accelerated solvent extraction (ASE) system pure water or water with 10 mM formic acid at extraction temperature of 80 °C C-Up: Filtration of extracts using a 0.22 µm nylon filter. Alternatively use solid phase extraction or liquid extraction

Table 2.6 *Alternative analytical techniques for the detection of acrylamide (2002-2017)*

Matrices/ Electrophoretic Techniques / LOD/LOQ (References)	**Sample Extraction (XTr) , Treatment, Analyte Derivatization (DRv) and Clean-up (C-Up)**
▪ *Home-made French Fries*. Micro-emulsion electrokinetic Chromatography with UV detection at 200 nm LOD of 0.70 µg/mL (*Bermudo et al., 2004*)	Sample treatment using 0.8% m/v n-amyl alcohol, 3.3% m/v SDS, 6.6% m/v n-1-butanol, and 89.3% m/v 40 mM phosphate buffer at pH 6.5
▪ *Potato chips*. MEEKC–UV–vis LOD of 0.1 *µg/mL* LOQ 0.33 *µg/mL* (*Bermudo et al., 2006*)	Extraction with methanol, defatting with hexane, and dilution with electrolyte $Na_2B_4O_7$-SDS.
▪ *Potato chip and French fries*. Non-aqueous capillary electrophoresis method (NACE) with UV detection at 200 nm ❖ LOD of 0.041 mg/mL (*Başkan and Erim 2007*)	Sample treatment using 30 mmol/L $HClO_4$ in ACN
▪ *Crisp bread, biscuits and snacks*. CZE–FASI–UV/ For standards LOD of 1 μgL^{-1} using FASI and 7 μgL^{-1} using LVSS. For samples LOD of 3 ng g^{-1} using FASI and 20 ng g^{-1} using LVSS (*Bermudo et al., 2006*)	XTr: Extraction with water and defatting using hexane. IS: not necessary DRv: derivatization with 0.01 M NaOH and 2-mercaptobenzoic acid C-Up: Liquid–liquid extraction with dichloromethane. SPE with Strata-X-C and ENV+ cartridges
▪ *Potato chips and Almond extracts*. NACE with Diode array detection at 210 nm/ LOD of 2.6 ng/mL with electrokinetic Injection and LOD of 4.4 ng/mL with hydrodynamic injection (*Tezcan and Erim 2008*)	Sample treatment using 30 mmol/L $HClO_4$, 20 mmol/L $NaClO_4$, 218 mmol/L CH_3COOH in ACN.
▪ *Potato crisps, crispbread, biscuits, breakfast cereals and coffee* . CE with MS/MS/ LOD of 8 ng/g (*Bermudo et al., 2007*)	Sample treatment using 35 mM ammonium formate/ ammonia solution at pH 10
▪ *Potato chips*. Micellar electrokinetic capillary chromatography with UV detection at 198 nm/ LOD of 0.1 µg/mL (*Zhang et al., 2007*)	Sample treatment using 50 mmol/L $Na_2B_4O_7$ and 40 mmol/L SDS at pH 10.0
▪ *Bread*. Analyte Focusing by Ionic Liquid Micelle Collapse (AFILMC) Capillary Electrophoresis/ LOD of 0.71 ng g^{-1} for acrylamide (AA), LOD of 1.06 ng g^{-1} for asparagine (AS), and LOD of 27.02 ng g^{-1} for glucose (GL). LOQ of 2.49, 3.55 and 93.53 ng g^{-1} for AA, AS and GL respectively (*Abd El-Hady and Albishri, 2015*)	Simultaneous extraction of AA, AS and GL from homogenised bread samples by ionic liquid based ultrasonic assisted extraction (ILUAE). Extraction of 4.0 g of the homogenate in a 1-butyl-3 methylimidazolium bromide (BMIMBr) micellar matrix.
▪ *Potato chips and French fries*. Microchip electrophoresis with on-line multiple-preconcentration techniques / LOD of 1 ng/mL (*Wu Chen et al., 2016*)	XTr: Water extraction of sample. Defatting with hexane. Centrifugation at 16,000 rpm for 40 min. Filtration of 2 mL of the supernatant through filters of pore size 0.45 µm) DRv: using sulfoindocyanine succinimidyl ester (Cy5) dissolved in acetonitrile and derivatization buffer (20 mM borate solution at pH 8.5)

Table 2.6 *Contd- Alternative analytical techniques for the detection of acrylamide (2002-2017)*

Matrices/ Electrochemical, Biosensor, Imaging and Fluorescence Based Techniques / LOD/LOQ (References)	**Sample Extraction (XTr) , Treatment, Analyte Derivatization (DRv) and Clean-up (C-Up)**
▪ *Acrylamide standard solutions*. Quartz Crystal Microbalance (QCM) or Superior Host Compounds/ LOD of 10 ppb (*Kleefisch et al.,2004*)	High voltage and Hamilton syringe are used to transfer the analyte or sample dissolved in a polar solvent to Quartz sensor made of quartz plate and gold electrodes coated with sensor-active material (tetralactam macrocycle)
▪ *Potato crisps*. Voltammetric biosensor based on carbon-paste electrode modified with haemoglobin (Hb)/ LOD of 1.2×10^{-10} M (*Stobiecka et al., 2007*)	Extraction with water, defatting with hexane, precipitation with Carrez I and II solutions, addition of 0.2 M CH_3COOH and 0.05 M NaBr, pH adjustment to 4.8 with 0.1 M NaOH
▪ *Potato crisps.* Amperometric determination using Hb/cMWCNT-Fe3O4NP/CHIT/Au electrode/ LOD of 0.02 nM (*Batra et al., 2013*)	Extraction using deionised water (DW) and powdered potato crisps. Stirring of mixture after 20 min, at 60 ºC for 1 hr. Centrifugation at 4,500 rpm for 20 min. Collection of supernatant, defatting by extraction in hexane. Purification of aqueous extract by the addition of Carrez I and Carrez II solutions. Subsequent filtration of supernatant.
▪ *All types of foods*. Amperometric detection of AA using haemoglobin-gold nanoparticles (Hb-AuNP) modified electrode/ LOD of 4 x 10^{-8} M. (*Garabagiu & Mihailescu, 2011*)	-
▪ *Potato chip.* Computer Imaging (CI)/*** 1000 µg/kg** (*Gökmen et al., 2007* and *Hu et al., 2015*)	Sample extraction, treatment and clean-up are similar to those used for LC-MS. Method also include capturing and processing of the images of samples using special discriminatory features such as the red, green and blue characteristics of the image.
▪ *Cookies*. CI/***150 µg/kg** (*Gökmen et al., 2008* and *Hu et al., 2015*)	Sample extraction by solvent such as methanol; sample treatment and clean-up are similar to those used for LC-MS.
▪ *Potato chips*. Fluorescence based methods/ LOD of 35 µg/kg (*Hu et al., 2014*)	XTr: Extraction by using a vortex to mix 1 ± 0.1g blended samples and 10mL of 0.3 % formic acid for 1min. Centrifugation of sample at 4 ºC and 10,000 rpm for 10min. Removal of the top fat layer, and separation of the supernatant for clean-up. C-Up: SPE clean up technique
▪ *French fries, fried puffs, fried chicken roll, bread, biscuits*. Fluorescence based methods/ LOD of 15 µg/kg (*Liu et al., 2014*)	XTr: Homogenization of 1.0 g portion of sample (that has been blended and dried at 60 ºC) with 10 mL hexane. Centrifugation of sample at 10,000 rpm for 10 min. Ultrasonic treatment for another 10 min. Removal of the top fat hexane layer. Repetition of defatting step. Extraction of acrylamide using 10ml water, followed by another 10min ultrasonic mixing and 10 min centrifugation at 10,000 rpm. Filtration of sample through 50µ m membranes. DRv: using NaOH, NaClO, and fluorescamine in Hofmann degradation.
▪ *Industrial Effluent:* *ISE based biosensor/* LOD of 6.31×10-4 M and 9.12×10-5 M (Silva et al., 2009)	Analysis using industrial effluent, as well as 20 mM Tris buffer pH 7.2 containing 1 mM b-mercaptoethanol and 1 mM EDTA, both of which were spiked with known amounts of $^{13}C_3$-labelled acrylamide. Reaction of sample with immobilized Pseudomonas aeruginosa with amidase activity.

Table 2.6 *Contd- Alternative analytical techniques for the detection of acrylamide (2002-2017)*

Matrices/ Enzymatic and Spectrophotometric Techniques / LOD/LOQ (References)	**Sample Extraction (XTr) , Treatment, Analyte Derivatization (DRv) and Clean-up (C-Up)**
▪ *Water samples, Pringles crisps*. Enzyme-Linked Immunosorbent Assay Methods (ELISA)/ LOD of 65.7 µg/kg (*Preston et al., 2008*)	XTr: Homogenization of 2.0g samples in 20mL of deionised water. DRv: 3-mercaptobenzoic acid (3-MBA); Derivatization using 7.5mg of 3-MBA and 100 L of 1M NaOH at a temperature of 50◦ C and incubation period of 1 hr C-Up: SPE clean up technique
▪ *Potato crisps, instant noodles, biscuits, and cakes*. Enhanced chemiluminescent ECL-ELISA/ LOD of 18.6 µg/kg and LOQ of 60.6 µg/kg (*Quan et al., 2011*)	XTr: Water extraction DRv: using mercaptobenzoic acid
▪ *Potato chips, cookies, and coffee*. ELISA/ LOD of 0.036 µg/kg (*Wu et al., 2014*)	XTr: Water extraction DRv: 4-mercaptophenylacetic acid (4-MPA)
▪ *Various foods including biscuits and cakes.* Indirect competitive enzyme-linked immunosorbent assay (ic-ELISA)/ LOD of 8.87 ng/ml (*Zhu et al., 2017*)	XTr: Homogenization of 1.0g of sample using 20 ml deionized water. Centrifugation at 4000× g for 20 min. Spiking of supernatant with standard solutions of AA. DRv: 4-mercaptobenzoic acid (4-MBA) for derivatization.
▪ *Potato chips.* Portable and Handheld Infrared Spectrometers / LOD of 40 µg/kg and LOQ of 119 µg/kg for regular potato chips (*Ayvaz and Rodriguez-Saona, 2015*)	Using hydraulic press to remove oil from 10 g blended potato chip. Collection of the NIR spectra of the resulting ‘‘cakes’’ using a dispersive handheld NIR (Thermo Fisher Scientific Inc., Wilmington, MA, USA) equipped with a single Indium Gallium Arsenide (InGaAs) detector.
▪ *French fries*. Near-Infra Red Spectroscopy (NIRS)/ LOD of 50 µg/kg (*Adedipe et al., 2016*)	Extraction of acrylamide from 1g sample using a combination of hexane, deionized water, and acetonitrile
▪ *Potato chips, corn chips crackers and cereal-based baby foods*. Flame Atomic Absorption Spectrometry (FAAS)/ LOD of 0.08 µg/kg and LOQ of 0.28 µg/kg (*Altunay et al., 2016*)	Homogenisation of 3g food sample in blender. Treatment of homogenate with 5mL n-hexane ,4.0g anhydrous $MgSO_4$, 0.5g NaCl,10mL ultra-pure water and 10mL acetonitrile to remove interferences such as fat and other water soluble compounds. Centrifugation of treated homogenate for 5min at 4000rpm is required for effective separation. For further extraction of acrylamide, transfer 2mL acetonitrile phase to a centrifuge tube containing 50mg primary secondary amine (PSA) and 100 mg anhydrous $MgSO_4$. For preconcentration prior to analysis of the sample by FAAS, ultrasonic-assisted cloud point extraction (UA-CPE) based on ion–pairing of AA with fluorescein (F^{2-}) in the presence of Ni(II) ions at pH 9.0, and subsequent extraction of the formed ternary complex into micellar phase of poly(ethylene glycol-mono-p-nonylphenylether –PONPE 7.5) is required.

Chapter 3

An awareness survey on the presence and implications of acrylamide in West African foods and the extent of consumption of such foods

CHAPTER 3. An awareness survey on the presence and implications of acrylamide in West African foods and the extent of consumption of such foods

3.1 Overview

This chapter discusses the findings from an awareness survey of different WA groups. The study investigated the consumption pattern of popular WA foods amongst different groups of participants. The survey also evaluated the knowledgeability of the respondents on dietary acrylamide and its toxic effects.

3.2 Introduction

The nutritional composition of a diet is an essential factor, which determines the health status of an individual. In addition to the nutrients available in a food, other chemical components of the food may also be important in the onset of neurodegenerative diseases and cancers. Such non- nutrient compounds include the so-called process contaminants; acrylamide in heat-processed foods, nitrosamines in cured fish and meats, furans in baked, fried and caramelized foods, and polycyclic aromatic hydrocarbons (PAH) in smoked and roasted meats. Recent studies have indicated that the correct application of food processing methods may also determine a healthy diet and aid in disease prevention.

The presence of acrylamide in heat-processed carbohydrate rich foods has been a subject of interest since 2002, when the toxic compound was incidentally found in potato fries. Studies conducted before and after 2002 confirmed the adverse health effects of the compound which include carcinogenicity, neurotoxicity, mutagenicity and genotoxicity (Beland et al., 2012; ECHA, 2010; IARC, 1994; Lyon, 1994; Maronpot et al., 2015). Several surveys on the presence and level of the compound in different groups of heat-treated foods were conducted between the year 2002 and 2017. Indigenous carbohydrate-rich foods of regions of the world including Austria, Caribbean, China, Colombia, India, Iran, Italy, Saudi-Arabia, Turkey, United States, Poland and several other European countries have been tested through survey exercises (Bent, et al., 2012; Boroushaki et al., 2010; Can & Arli, 2014; El-Ziney et al., 2009; Murkovic, 2004; Pacetti et al., 2015; Shamla & Nisha, 2014; Vesper et al., 2010; Vivanti et al., 2006; Wang et al., 2013; Wang et al., 2008; Wyka et al., 2015; Zhang et al., 2007; Ziney, 2008).

In 2007, the European Commission (EC) adopted a recommendation which pertains to the official survey of foodstuffs with the potential to contribute appreciably to the dietary

acrylamide intake. The United States Food and Drug Administration (USFDA) also published a guidance document in 2016, which contains non-binding recommendations for food industries (FDA, 2016). In addition, the European Commission (EC) and the USFDA manage a large database on the occurrence of acrylamide in foods (European Commission, 2006; IFST, 2017; USFDA, 2006).

The varying cooking methods and dissimilar food consumption patterns prevalent in different countries and continents are important determinants of the contribution of a particular food product to the total amount of acrylamide consumed (Dybing et al., 2005). For instance, the exposure assessment for the US indicates 35% contribution from fried potato products and only 7% contribution from coffee (Dybing et al., 2005). On the other hand, the acrylamide exposure assessments for the Swedish (Svensson et al., 2003) and Dutch population (Konings et al., 2003) show that coffee consumption contributes 39% and 13% respectively.

Although, the level of acrylamide in traditional foods of several countries have been determined, there is still paucity of information on the acrylamide content of popular WA heat-processed foods.

In addition, there is a gap to be filled with regards to the knowledge of the level of awareness of WAs on the presence and health implications of process contaminants including acrylamide. For WAs, an awareness of the existence of these process contaminants and the mitigating strategies required to reduce them would lead to making of informed decisions in the preparation and consumption of foods with the propensity to contain acrylamide.

For United Kingdom (UK) and Europe, the Food Standards Agency (FSA) and Food Drink Europe (FDE) are two important organizations that have undertaken the role of creating awareness and advising individuals and food business operators on best ways to reduce the amount of acrylamide in heat-processed foods. Such advice include avoidance of excessive browning during home or industrial cooking of foods and selection of ingredients and materials with low asparagine content for cooking (FDE, 2016; FSA, 2014). However, in the case of WA countries, no organization is yet responsible for creating awareness on the occurrence and ill effects of process contaminants such as acrylamide. This study focuses on the assessment of the current awareness level of different groups of WAs on the presence and health implications of food processing contaminants including acrylamide and the consumption pattern of heat-processed foods of West African origin.

The WA foods selected are bread, chin-chin, kokoro, doughnut, pancake, fish roll and meat pie. These foods are heat processed, carbohydrate -rich and may contain asparagine, thus there

is the propensity for development of acrylamide. Asparagine and reducing sugars such as fructose and glucose are important precursors for acrylamide formation (Amrein et al., 2004; Bråthen and Knutsen 2005; Surdyk et al., 2004, Vass et al., 2004), Figure 2.5.

Several studies on baked products such as gingerbreads, crackers and biscuits indicated that the ingredients; flours, baking agents, honey, inverted sugar and sucrose could affect the acrylamide concentration. Similar ingredients are also used in the production of the WA foods, Table 1.1.

3.2.1 Problem Statements

Although most of these foods are commercially available throughout the world and have sufficient amounts of carbohydrate and protein (USDA, 2019), which are required for the formation of high acrylamide, there is still absence of any report or data on the risk of consuming them. In addition, this suggests that most people from this part of the world are uninformed on the presence and toxic effects of acrylamide.

Hence, the problem statements for this study are:

a. The unknown state of awareness of the different groups of WA on the formation of acrylamide in thermally processed foods.
b. The unknown consumption pattern of popular heat-processed WA foods among this group of Africans.
c. The unknown exposure of WA to dietary acrylamide based on the consumption frequency of each food.

3.2.2 Research Rationale

The justifications for the study, which are partly discussed in Chapter 1, include the following:

(a) *Absence of acrylamide monitoring studies for West African foods*: No WA country has a database for the nutritional and acrylamide composition of popular WA foods. Existing information on dietary acrylamide are mainly those from the monitoring exercise funded by the European Food Safety Authority (European Commission, 2013; EFSA, 2012; IFST, 2017)

(b) *Public awareness campaign*: Unlike the United Kingdom and other European countries, no WA country has a group such as Food Standard Agency (FSA) and Food Drink Europe (FDE), with the responsibility to make the public aware of the formation and undesirable implications of dietary acrylamide (FDE, 2016; The Telegraph, 2017).

(c) *Regulations and recommendations for acrylamide:* No WA country has any legal document on the monitoring of acrylamide in popular WA foods. This is unlike European countries with EC regulations and recommendations on methods to monitor, manage and mitigate acrylamide in foods.

3.2.3 Research Questions

Based on the identified problems, some of the questions worth answering are listed below.

(i) How knowledgeable are WA on the presence and effect of acrylamide in popular WA foods?
(ii) What is the pattern of consumption of the popular WA foods among this group of Africans?
(iii) Based on the consumption pattern, which foods are the most important source of dietary acrylamide for this group of people?

3.2.4 Research Hypotheses:

Several hypotheses arising from the questions for the study are listed below.

(a) Null hypothesis (H_o; $\mu_{A'} = \mu_{A'}$) There is no difference in the knowledgeability of the different groups of WA; Alternative or research hypothesis (H_A; $\mu_{A'} \neq \mu_{A'}$)- There is a difference in the knowledgeability of the different groups of WA.

(b) Null hypothesis (H_o; $\mu_{cp} = \mu$) - There is no difference in the pattern of consumption of the WA foods for the different groups of participants; Research hypothesis (H_A; $\mu_C \neq \mu$) There is a difference in the pattern of consumption of the WA foods for the different groups of WA.

(c) Null hypothesis (H_o; $\mu_{aa} = \mu$) – Based on the consumption pattern, the different WA foods are alike in their importance as source of acrylamide; Research hypothesis (H_A; $\mu_{aa} \neq \mu$) - Based on the consumption pattern, the different WA foods are unequal in their importance as source of acrylamide.

3.2.5 Research objectives

See Chapter 1 for the objectives of the study.

3.3 MATERIALS AND METHOD

3.3.1 Background

The investigation was conducted using Bristol Online Survey website. The focus was on the awareness of the presence and health implications of dietary acrylamide. The survey covered aspects such as the participant's country of residence, knowledge of the occurrence and health implications of dietary acrylamide and other process contaminants such as nitrosamines, furans, and PAHs.

Figure 3.1. *Map of West Africa (Encyclopaedia Britannica, Inc.)*

The WA countries covered are shown (Figure 3.1). The United Kingdom (UK) was also included in the survey as there are many British nationals with WA background. The survey included questions addressing the awareness of WA groups on the presence and health implications of process contaminants such as nitrosamines, furans, and polycyclic aromatic hydrocarbons (PAHs). Particular attention was given to the assessment of the knowledge of the WA participants on the occurrence and possible ill-effects of acrylamide in heat-processed foods.

3.3.2 Design of the survey

The survey was designed with easy-to-complete multiple-choice questions (MCQs) and polar (yes-no) questions. The number of answer alternatives for each multiple-choice question (MCQ) also varied depending on the purpose of the question. For instance, the MCQ for the country of residence allowed up to 21 answer options. Polar questions were used for aspects such as the respondent's knowledge of the existence and health effects of process contaminants. In addition, the MCQ on the pattern of consumption of baked WA foods contained scaled answer options such as 'weekly' and 'once in a while'. A web link for the survey was created and sent to invitees via social platforms such as Facebook, WhatsApp and email. The account was protected by password to ensure the confidentiality and protection of the collected responses. Further protection of the data subjects (respondents) was provided by complying with the institution's ethics and data protection policies. The results were analysed and validated using appropriate statistical methods.

3.4 Results

3.4.1 Country of residence.

WA respondents from eight major national groups completed the survey (Figure 3.2). Out of the 1103 total survey responses, 116 (10.5%) responses came from participants residing in UK. The 346 responses from participants dwelling in Nigeria represent the highest percentage of the total responses (31.4%), while the 235 responses from Ghanaian respondents represent the second highest percentage of the total responses (21.3%).The responses collected from respondents dwelling in Ivory Coast represent the third highest percentage of the total responses (15.7% of total responses). Benin, Gambia, Liberia and Sierra Leone contributed 7.9, 0.6, 11.1, and 1.5% respectively to the total responses collected (Figure 3.2).

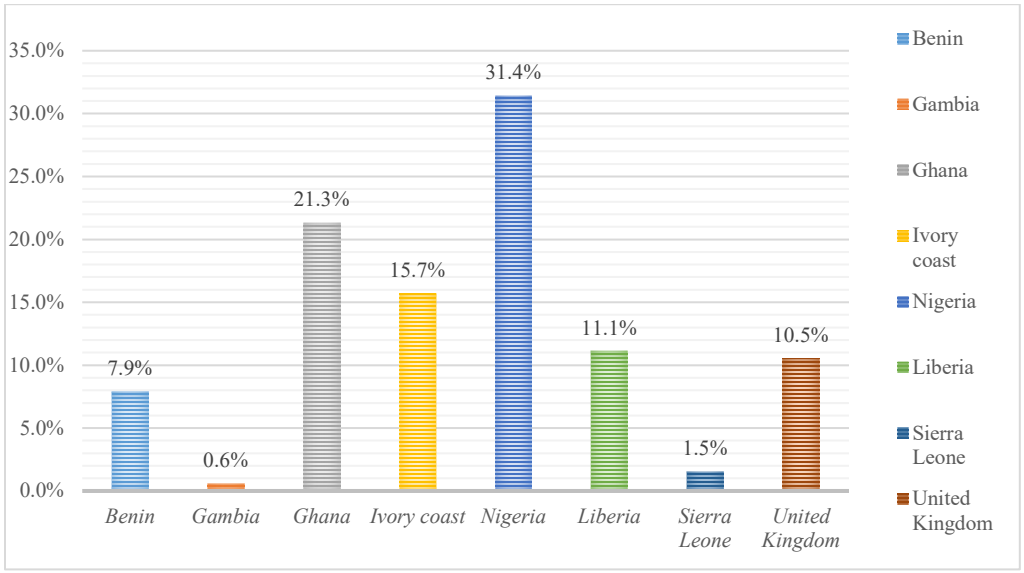

Figure 3.2. *Country of residence, and response percentage*

3.4.2 Knowledge of participants on the presence and health implications of acrylamide and other process contaminants in heat-treated WA foods

As depicted in Figure 3.3 only 5.1% of the 1103 participants were cognisant of the association between heat-processing of foods and release of toxic chemicals.

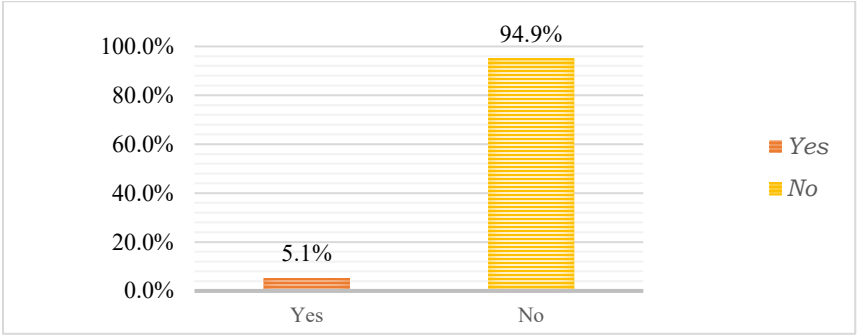

Figure 3.3. *Knowledge of chemical hazards associated with heat-processed foods*

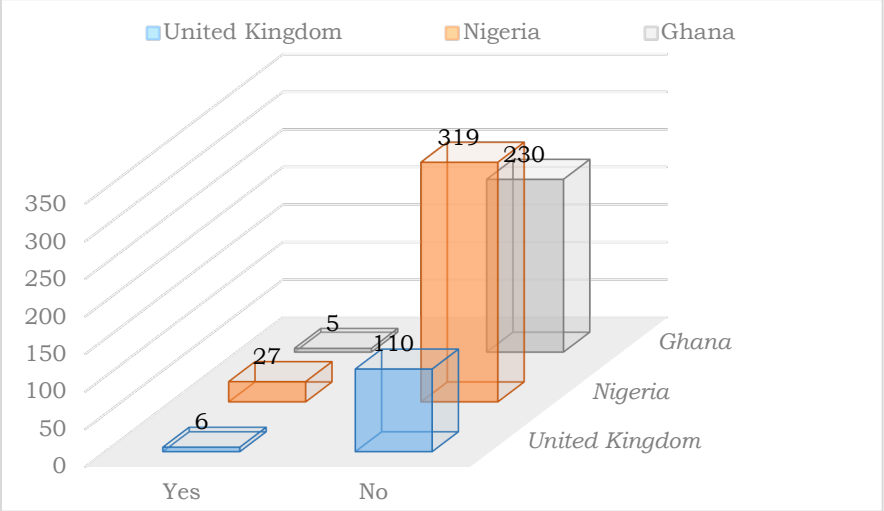

Figure 3.4. *Comparison of the knowledge of GHN, NGR and UK respondents on the chemical hazards associated with heat-processed foods*

Responses received from GHN, NGR and UK participants for the question on the knowledge of the link between chemical hazards and heat processing of food showed 2.1, 7.8, and 5.2 % respectively were informed. Altogether, 2% of all the participants had knowledge of the presence and ill effects of these process contaminants (Table 3.1).

Table 3.1. *Knowledge of Furans, Nitrosamines, and PAHs*

Knowledge of Process Contaminants e.g. Nitrosamines, Furans, PAHs	Response Percent	Response Count
Yes	2.0%	22
No	98.0%	1081
Sample size (N)		1103

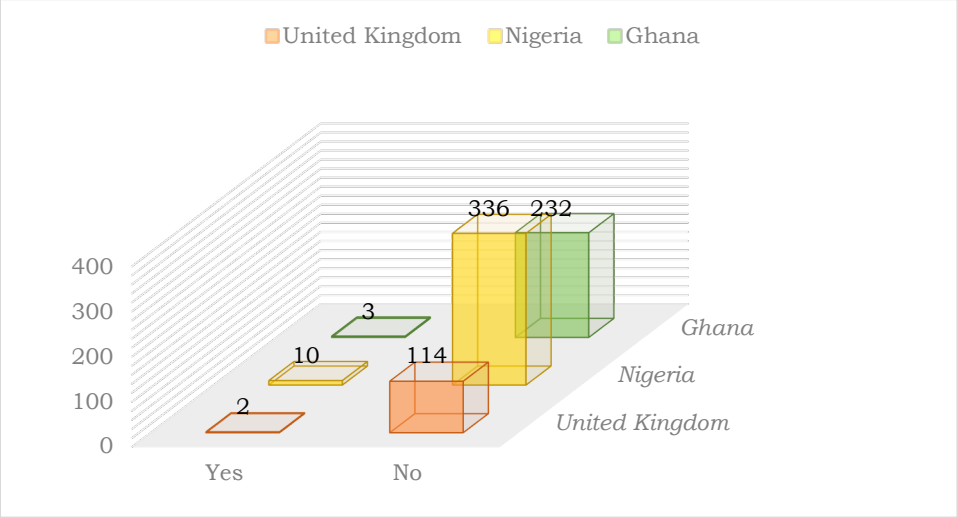

Figure 3.5. *Comparison of the knowledge of GHN, NGR and UK respondents on the presence and health implications of food-borne furans, N-nitrosamines, and PAHs.*

The percentage of participants informed on the presence and deleterious effects of N-nitrosamines, furans and PAH for GHN, NGR and UK were 1.3, 2.9 and 1.7% respectively

Only 0.4% of the 1103 respondents (Table 3.2) were informed on the deleterious effect of acrylamide and the association of the process contaminant with heat-treated foods.

Table 3.2. *Awareness of the formation and health implications of AA in heat-processed foods*

Awareness of the formation and health implications of AA in heat-processed foods	Response Percent	Response Count
Yes	0.4%	4
No	99.6%	1099
Sample size (N)		1103

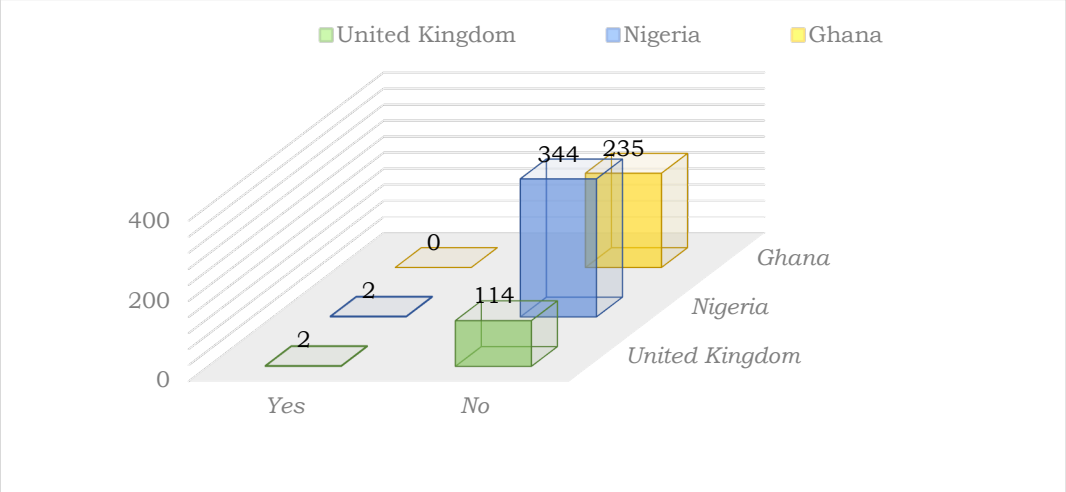

Figure 3.6. *Comparison of the knowledge of GHN, NGR, UK respondents on the presence and health implications of food-borne acrylamide*

For GHN, NGR and UK the percentages of participants informed on the presence and ill effects of acrylamide were 0.0, 0.6 and 1.7% respectively. Statistical analysis of all responses using Chi square test showed no significant difference in the proportion of the WA groups that are aware of the existence and effect of the process contaminant, with p value (0.108) >0.05.

3.4.3 Consumption pattern of important heat-processed WA foods

3.4.3.1 *Consumption of baked WA Foods*

The highest number of responses for frequently consumed baked WA foods was obtained for bread, indicating that it may be the most frequently consumed WA baked food. Approximately forty eight percent (48.2%) of the total responses suggested that WA breads are the most frequently consumed baked WA foods (Figure 3.7).

Other frequently consumed WA baked foods shown by the survey include meat-pie, doughnuts and chin-chin with 17.4, 13.6, and 9.8% responses respectively (Figure 3.7)

The responses from the participants indicated that baked Kokoro and pancake are the least frequently consumed WA baked food. The responses received from GHN, NGR and UK residents are compared in Figure 3.8.

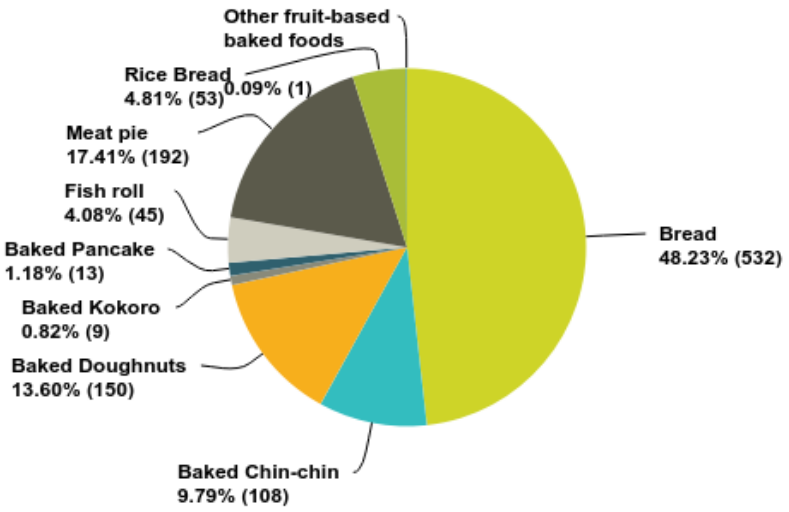

Figure 3.7. *Frequently consumed WA baked foods*

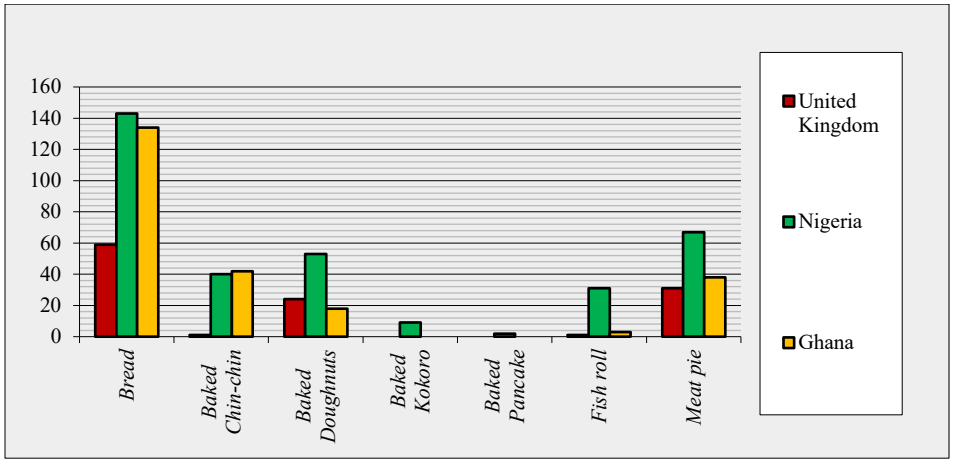

Figure 3.8. *Comparison of frequently consumed WA baked foods for GHN, NGR and UK residents.*

The study indicated that 369 participants representing 33.5% of all the respondents consume WA baked foods as often as once a week. The survey also indicated that the percentages of participants that consume these baked foods twice and thrice a week are 30.9 and 16.6 % respectively (Figure 3.9).

In addition, respondents that consume this category of foods more than thrice a week and occasionally (monthly) are 7.2 and 11.9% respectively. The responses received from GHN, NGR and UK residents are compared in Figure 3.10.

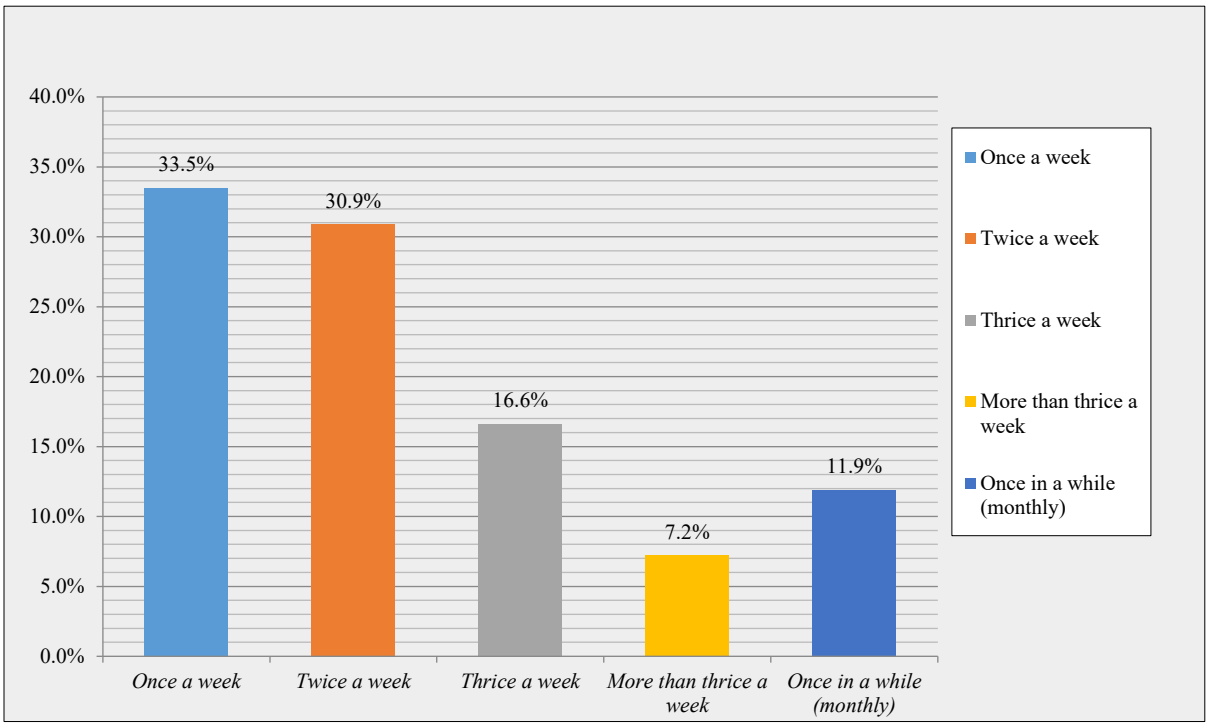

Figure 3.9. *Consumption frequency of WA baked foods*

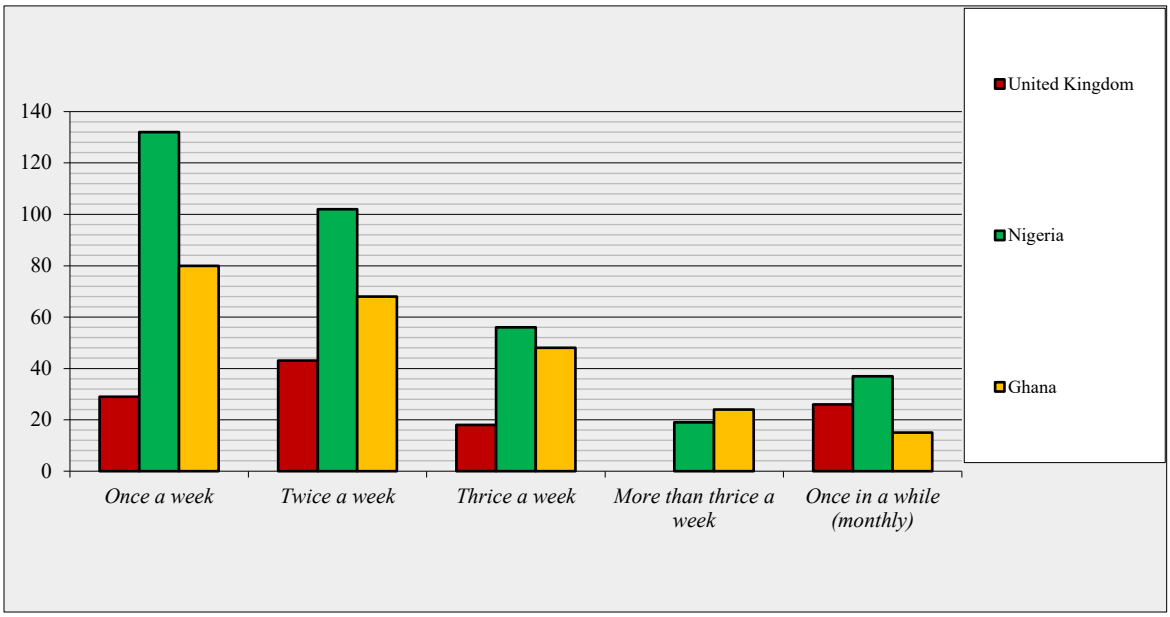

Fig.3.10. *Comparison of the consumption pattern of WA baked foods for GHN, NGR and UK*

3.4.3.2 *Consumption of fried WA foods*

The highest number of responses for frequently consumed fried WA foods was obtained for *akara,* indicating that it may be the most frequently consumed WA fried food (Figure 3.11). Other highly consumed WA fried foods include *chin-chin, plantain* and *yam fries.* The collected

responses indicated that foods such as *kulikuli* and *ojojo* are WA fried foods, which are not consumed so frequently.
The responses received from Ghanaian (GHN), Nigerian (NGR) and UK residents are compared in Figure 3.12.

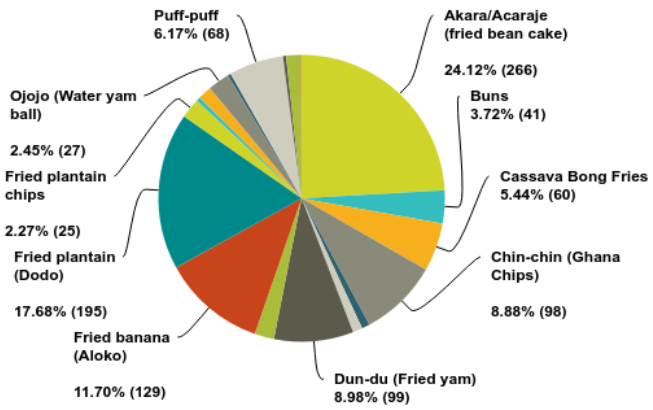

Figure 3.11. *Frequently consumed WA fried foods*

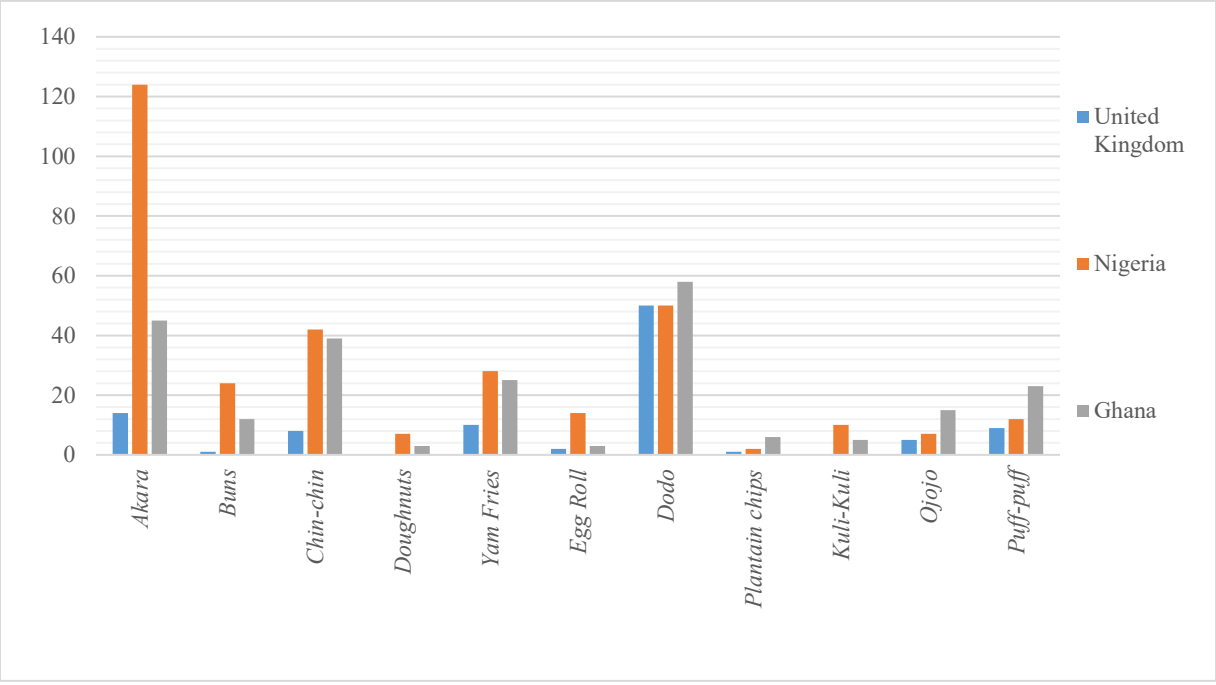

Figure 3.12. *Comparison of frequently consumed WA fried foods for GHN, NGR and UK residents*

According to the results from the survey exercise, 531 respondents representing 48.1% of all the participants consume WA fried foods as often as once a week (Figure 3.13). The collected responses also indicated that the percentages of participants that consume these fried foods weekly and once in a while (monthly) are 21.8 and 28.6 % respectively.

The responses received from Ghanaian, Nigerian and UK residents are compared in Figure 3.14.

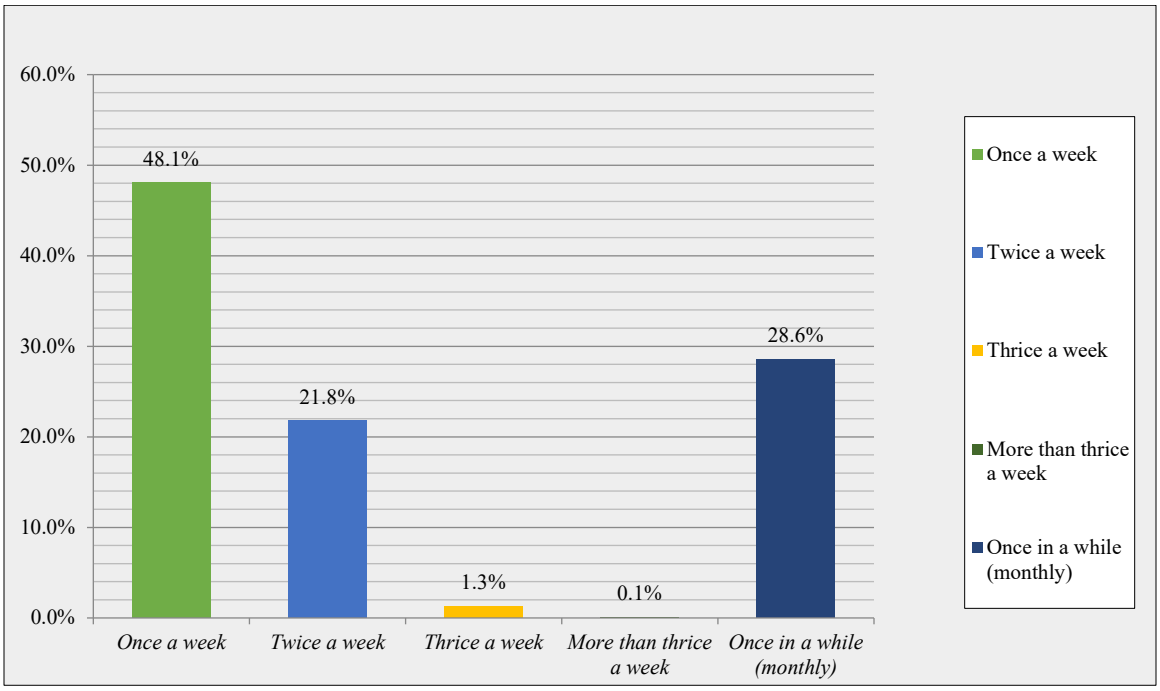

Figure 3.13. *Consumption frequency of WA fried foods*

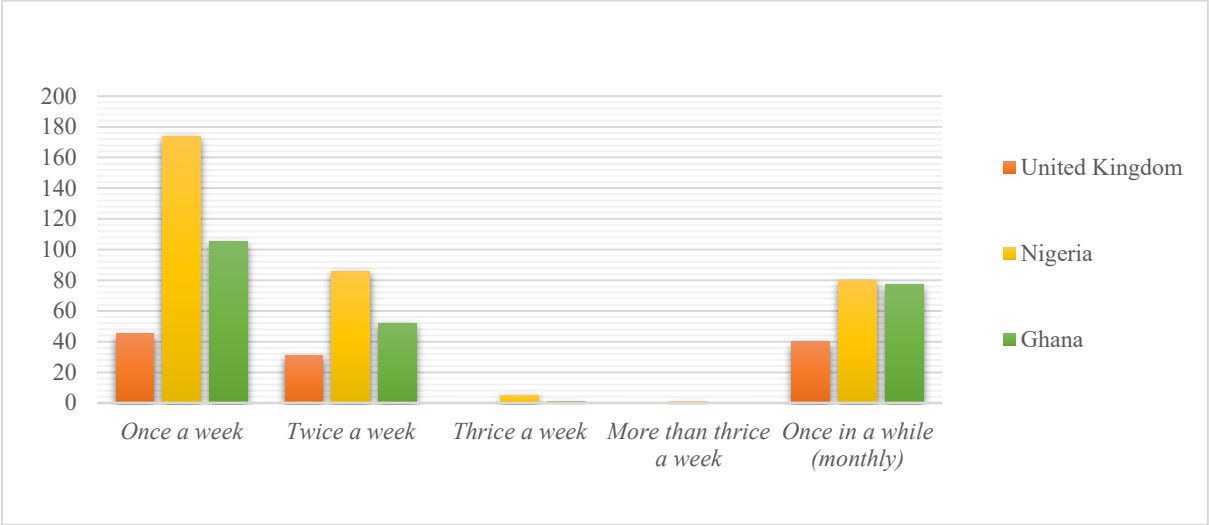

Figure 3.14. *Comparison of the consumption pattern of WA fried foods for GHN, NGR and UK residents*

3.5 Discussion

Various explanations can be given for the unequal proportions of the responses obtained from the WA groups. The results from the survey could be partly attributed to demographical factors, which affect changes in immigration and emigration for these groups of individuals, irregular electric power supply and internet unavailability. According to the report by the Office for National Statistics on the population, Ghana and Nigeria has the highest proportion of African-born UK residents, both representing 89% of the total (O 'brien & Potter-Collins, 2015). In addition, there are reports that Nigeria and Ghana contribute majorly to the population of foreign-born UK residents (UNDESA, 2015).

The feedback received from the participants indicated that majority of the WA participants were not cognisant of the link between heat-treatment of foods and production of hazardous chemicals. This is seen in Figure 3.3 with only 5.1% of the 1103 participants cognisant of the association between heat-processing of foods and release of toxic chemicals. For the evaluation of the knowledge on the link between chemical hazards and heat processing of foods, the percentage of informed participants for GHN, NGR and UK were 2.1, 7.8, and 5.2 respectively.

According to the awareness survey, most West Africans have not heard of the existence and possible adverse health consequences of process contaminants including furans, N-nitrosamines and PAHs. Only 2% of the participants were informed on the presence and ill effects of these process contaminants (Table 3.1). The percentage of participants informed on the presence and deleterious effects of N-nitrosamines, furans and PAH for GHN, NGR and UK were 1.3, 2.9 and 1.7% respectively.

Furthermore, very few percentage of the WA respondents were informed on the deleterious effect of acrylamide and the association of the toxic compound with heat-treated foods. Only 0.4% of the 1103 respondents had knowledge of the occurrence and ill effects of the process contaminant (Table 3.2). Only 0.6 and 1.7% of participants from NGR and UK affirmatively responded to having knowledge of the existence and ill effects of acrylamide. This findings may be an indication that most WA are only concerned about the sensory and nutritional aspects of food consumption and not conscious of the risk of frequent consumption of certain foods.

The low level of awareness of the WA respondents with respect to dietary acrylamide and other process contaminants may be due to the lack of an effective public health education,

communication and campaign on process contaminants by food control agencies. It could also be a reflection of the insignificant value placed on matters related to food safety and consumption.Although the general level of awareness on the existence and adverse health effects of dietary acrylamide is low, the study indicated that WA residing in UK were slightly more informed**.**

The assessment of the knowledge of the WA participants on the existence and toxic effects of dietary acrylamide suggests that WA sources of information dissemination such as television stations and newspapers pay little attention to matters of food safety and control. This is unlike UK, where awareness on the occurrence and effects of dietary acrylamide was created by sources such as BBC and the Telegraph newspaper (The Telegraph, 2017). In addition, Food Drink Europe (FDE) created toolboxes for food businesses to be able to mitigate the amount of acrylamide in their food products.
The feedback (Figure 3.7) from the consumption studies, indicated that the WA breads are the most consumed, thus in agreement with other similar studies, bread being the main stable food. WA breads are quintessential ready-to-eat baked foods, which are often eaten with sauce containing vegetables, meat or fish. They are also combined with foods such as roasted groundnuts, Akara (fried bean cake), and fried eggs. Doughnuts came a close second. Kokoro was only popular in WA, and the consumption rate in the diaspora was low.

According to the survey, the highest responses for frequently consumed baked WA foods including *bread*, *doughnuts* and *meat-pie* came from Nigerian residents (Figure 3.10) and then followed by the Ghanaians. These observation could be explained partly by the relatively higher number of WA participants from these two national groups. The result may also suggest the higher preference and affordability of the food items in these parts of the world. Figure 3.9 shows that most of the participants consumed baked WA at least once a week. The total percentage of responses for weekly consumption of baked WA food (i.e. 81.0%) is an indication that baked WA foods might be the most important dietary source of acrylamide for WA. Comparatively, the participants in the UK, consumed baked WA foods ‘twice in a week’ than ‘once in a week’ (Figure 3.10). This may be partly attributed to the busy lifestyle of people in this part of the world, which requires frequent consumption of ready- to-eat baked snacks.

3.6 Conclusions

Statistical analysis of all responses using Chi square test showed no significant difference in the proportion of the WA groups that are aware of the existence and effect of the process contaminant, with p value (0.108) >0.05. Generally, the level of awareness of the WA participants regarding the presence and health implications of process contaminants such as furans, n-nitrosamines, PAHs and acrylamide is very low.

Based on the results, there is a need for the creation of awareness for the presence and possible ill effects of dietary acrylamide and other process contaminants. It can also be concluded that the there is a steady consumption of heat-treated carbohydrate-rich WA foods. Hence, baked WA foods such as breads, doughnuts and meat pies may be important dietary source of acrylamide for WA consumers.

Finally, the results justify the necessity for investigations on the determination of acrylamide, the propensity of acrylamide development and the factors that influence the synthesis of acrylamide in relevant WA food and food products.

3.7 Summary

The study aimed to evaluate the level of awareness of dietary acrylamide, its toxicity and consumption of heat-treated WA foods; bread, chin-chin, kokoro, doughnut, pancake, fish roll and meat pie, through an online survey questionnaire.

Responses were received from 1103 individuals from 7 WA countries and UK, with 89.5% and 10.5% responses, respectively. Generally, the survey indicated that 98.0% of the respondents were not aware of the occurrence of food processing contaminants and 99.6% were unaware of the presence and possible adverse health impact of acrylamide. The percentage of participants cognisant of the link between chemical hazards and heat-treatment of foods for GHN, NGR and UK were 2.1, 7.8, and 5.2% respectively. The percentage of respondents informed on the presence and deleterious effects of nitrosamines, furans and PAH for GHN, NGR and UK were 1.3, 2.9 and 1.7% respectively. Only 0.6 and 1.7% of participants from NGR and UK affirmatively responded to having knowledge of the existence and ill effects of acrylamide. The survey also showed that 81.0% of the participants consume baked WA foods on weekly bases and that the consumption trend is similar for consumers in Nigeria, Ghana and amongst the WA population in the UK. Chi square test showed no significant difference in the proportion of the WA groups that are aware of the existence and effect of the process contaminant, with p value (0.108) >0.05.

Chapter 4

Spectrophotometric determination of glucose levels in West African foods

CHAPTER 4. SPECTROPHOTOMETRIC DETERMINATION OF GLUCOSE LEVELS IN WEST AFRICAN FOODS

4.1.Overview

This chapter discusses the spectrophotometric quantitation of glucose in popular WA foods. The study also compares the glucose concentrations and the acrylamide forming potentials of various WA foods.

4.2. Introduction

Estimating and monitoring the reducing sugar concentration of foods is very important as this group of sugars are involved in the formation of acrylamide via non- enzymic browning. Therefore, it is expected that products with lesser reducing sugars will contain lower concentrations of acrylamide compared to those with higher levels of the precursor.
For this reason, food business operators using raw materials such as potatoes for their production are advised to select and use varieties with low glucose and fructose levels (FDE, 2016).

Severe thermal treatments of food materials including potato and wheat, which contain high amounts of glucose and asparagine, have been reported to yield huge levels of acrylamide (Amrein *et al.*, 2003; Becalski *et al.*, 2004; Elmore *et al.*, 2007, 2010, Kumar et al., 2014).
The most acceptable explanation for the formation of AA is the Maillard reaction (MR) involving free asparagine (asn) and carbonyl group of reducing sugars such as glucose (glu) and fructose (Coultate, T. P. 2016, Mottram et al., 2002; Stadler et al., 2002), Figure 4.1

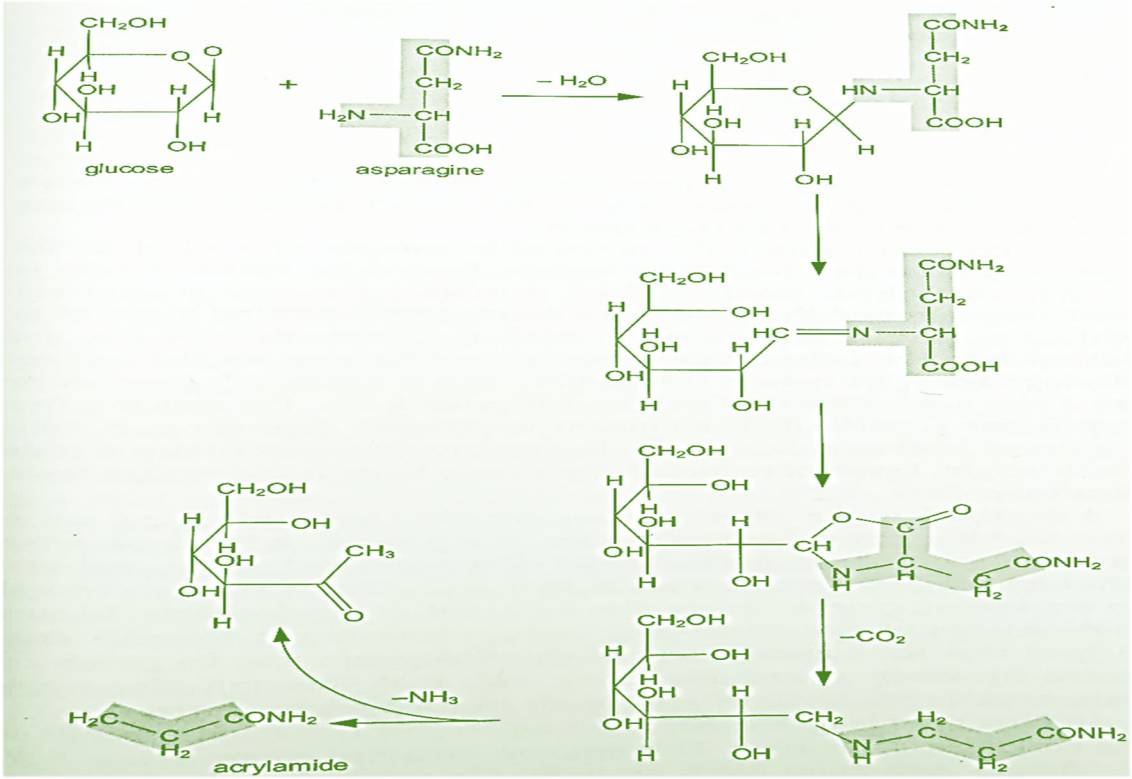

Figure 4.1. *The most acceptable pathway for the formation of acrylamide- "Food-The chemistry of its component"-Coultate, T.P., 2016)*

The association of high levels of acrylamide in potato fries with enormous glucose and asparagine levels in raw potatoes initiated several studies centred on assessing the level of the essential precursors in various foods (European Commission, 2013; EFSA, 2012; Wang et al., 2008).

4.2.1. Problem Statement

In spite of previous studies, which have determined the effect of the amount of glucose on the acrylamide levels in various foods (Grob et al., 2003, Haase et al., 2003 and 2004,Wicklund et al., 2005), there are still no available data on the association between the concentrations of glucose and the level of the contaminant in WA foods. Hence, the potential risk of consuming WA foods such as *akara* and *chin-chin* based on their glucose levels is unknown.

Moreover, even though the amounts of glucose and acrylamide in popular WA foods are unknown, this group of foods are also produced, sold and consumed in countries such as United Kingdom (U.K) and United State of America (U.S.A).

4.2.2. Research Rationale

The study is justified based on the following reasons:

(a) *The toxicity of acrylamide*: The neurotoxic, genotoxic and potential carcinogenic effects of acrylamide have been reported (IARC, 1994; IFST 2017; JECFA 2010).

(b) *The establishment of the strong connection between the heat processing of food materials rich in reducing sugars such as glucose and the formation of AA*

The formation of high amounts of acrylamide in food products made from reducing sugar rich food materials. (Amrein et al, 2005; Croft et al., 2004; European Commission, 2013; EFSA, 2012; IFST, 2017; Leung et al., 2003; Lineback et al., 2005; Murkovic, 2004; Tareke et al., 2002; Roach et al., 2003; Rosén and Hellenäs, 2002).

(c) *The Recommendations by Food Drink Europe*

The Food Drink Europe (FDE), which is a representative of the European food and drink industry, advised food business operators (FBO) to use raw materials with low levels of reducing sugars such as glucose. The recommendations in the FDE "toolbox" tagged "Code of Practice for Managing Acrylamide Formation" also include use of methods such as blanching to reduce the amount of glucose and fructose in food materials (FDE 2016).

4.2.3. Research Questions

Based on the problems stated for the study, the following questions arise:

(i) What is the mean concentration of glucose in each WA food?

(ii) Which WA food material has the highest and lowest glucose concentrations?

(iii) Based on the amount of glucose, which WA food is more likely to contain higher AA?

4.2.4. Research Hypotheses:

The hypotheses for the study are listed below.

(a) Null hypothesis (H_o; [*glu*] = μ) No difference in the concentration of *glu* for the WA foods

(b) Research hypothesis (H_a; [*glu*] ≠ μ) Different WA foods have different amount of *glu*.

(c) Null hypothesis (H_o; [AA] = μ) Based on *glu*, no difference in the AA forming potential for the WA foods

(d) Research hypothesis (H_a; [AA] ≠ μ) Based on *glu*, the WA food have different AA forming potentials.

4.2.5. Research Objectives:

Glucose is one of the precursor compounds required for the development of acrylamide in dry heat processed foods. Therefore a positive correlation between the glucose and acrylamide content of the WA foods is possible and expected.

Based on the questions and hypotheses of the study, the research objectives were to:

(1) Determine the glucose levels in the selected WA food products.
(2) Compare the glucose levels for akara, bread, buns, chin-chin, doughnut, fish-roll, meat-pie, plantain-chips, puff-puff, and yam-chips.
(3) Evaluate the likely levels of acrylamide present in the foods based on their glucose concentrations.

4.2.6 Principle of oxidase method for glucose estimation

The common methods for the determination of reducing sugar include the phenol-sulphuric acid method, Somogyi Nelson method and dinitrosalicalic acid method. Although, these methods are useful, they are not specific enough for particular reducing sugar such as glucose. For this reason, other procedures requiring enzymes such as glucose oxidase are sometimes employed. Enzymatic determination of analytes are specific, reproducible, sensitive, and rapid methods of testing which are widely used by biochemists, microbiologists, pharmacists and food scientists. The high particularity and responsivity of enzymatic tests allows the development of less rigorous quantitative assays, which are rapid and inexpensive.

Glucose oxidase (G-Ox) tests are groups of highly specific and sensitive enzymatic methods used for measuring glucose. The G-Ox tests require the use of instruments such as biosensing electrodes or spectrophotometers. The principle behind spectrophotometric method required for quantifying the amount of glucose in the selected WA food materials is described below. Glucose oxidase (G-Ox) catalyzes the oxidation of the glucose present in the food sample to gluconic acid and hydrogen peroxide. Consequently, an enzyme called peroxidase (P-Ox) in the presence of an electron donor converts the released hydrogen peroxide to water and oxygen.

Several dyes including O-dianisidine, 2-amino-4-hydroxybenzenesulfonic acid (AHBS), and Phenolic solution of 4-aminoophenazone are commonly used as electron donor and indicator substance for the G-Ox test. For the procedure discussed here, the oxygen acceptor and chromogenic substance required is the water soluble dye called 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS. Following the oxidation of ABTS is the appearance of a brilliant blue-green colour, which can be measured at 415nm by a spectrophotometer.

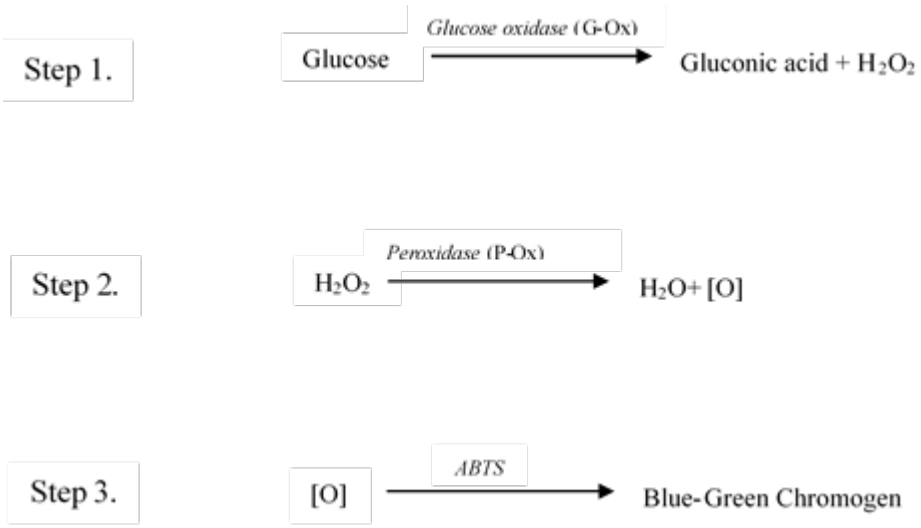

Figure 4.2. *Redox reactions for the conversion of glucose and hydrogen peroxide to a measurable chromogen.* **Step 1** shows the release of hydrogen peroxide from glucose through the action of G-Ox. **Step 2** shows the release of oxygen from H_2O_2 through the action of P-Ox. **Step 3** shows the oxidation of ABTS to a measurable Blue-Green Chromogen

The aim of the study was to determine and compare the amount of glucose present in the selected WA food products using the G-Ox spectrophotometric method.

4.3 Materials and Methods

4.3.1 Food Samples: Popularly consumed WA foods which readily undergo Maillard reaction were selected for the study. Some of these foods have been identified by Apekey et al., 2019, Quayson and Ayernor, 2007 and in Chapter 3. The food samples which include *akara*, *bread*,

buns, *chin-chin*, *doughnuts*, *fish-roll*, *meat-pie*, *plantain-chips*, *puff-puff* and *yam-chips* were collected from different outlets in London markets such as Peckham, Brixton and East Street. For the three locations mentioned, a total of 150 samples of each WA food were obtained.

4.3.2 Reagents: 0.5M Tris buffer of pH=7.0, glucose standard solution (Product No. G 3285) D-glucose**,** Sigma A1888 ABTS; [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt], NATE-0311- glucose oxidase (GOx)- EC 1.1.3.4, 100KU/g from *Aspergillus niger*, peroxidase from horseradish, 450KU/g, and sodium azide [NaN3].

4.3.3 Equipment and Apparatus: Jenway™ model 7315 UV/visible single beam spectrophotometer, cuvettes, 100ml beakers, 500-1000ml volumetric flasks, test tubes (18 mm X 150 mm 4). Pipettes capable of accurately dispensing volumes from 200 µl to 2.0 ml, 10ml syringe and 0.45-µm Fisher brand PTFE filter. Water bath capable of maintaining temperature at 37 ± 1 °C, air operated ultra-turrax T-25 high-speed homogenizer and digital weighing balance.

4.3.4 Preparation of Reagents and Glucose Standards

(a) 0.5M Tris Buffer of pH=7.0:

About 400 ml of deionized water and 30.3g of tris (hydroxymethyl) aminomethane were transferred into a 500ml volumetric flask. The final pH of the solution was checked to be 7.0 $\pm$ 0.05 with a pH meter. For solutions with pH greater than 7, adjustments were made by adding drops of aqueous hydrochloric acid. About 0.5g Sodium azide [NaN_3] was used to preserve the buffer solution. After the stirring of the solution with a glass rod, enough distilled water was added to make a final volume of 500ml. This is stable for 3-4 months, at 2-8°C.

(b) Enzyme-Dye Mix:

To the 500ml of the tris buffer, 0.5g ABTS, 2KU GO_X, and 5KU PO_X [Sigma P 8250] were added.

(c) Stock glucose:

Approximately 900ml distilled water and 0.5g benzoic acid were transferred into a 1L volumetric flask. About 10-30mg of glucose was weighed and dissolved in the benzoic acid solution. To obtain a final volume of 1 litre, more distilled water was carefully added. This solution is stable for six months at room temperature (25-35°C).

4.3.5 Food Sample Treatment and Analysis

A digital weighing balance was used to accurately weigh 0.1-1g food sample, which was consequently transferred into a 100ml beaker.

For the extraction of the analyte, about 10ml de-ionized water was added to the weighed food sample. Solid food samples with very low moisture content such as *chin-chin* were allowed to swell in the de-ionized water at 37°C for 10-20 minutes.

An air operated ultra-turrax T-25 high-speed homogenizer and analytical grade water were thereafter used for the blending and homogenization of the food samples.

Figure 4.3. *Ultra-turrax T-25 high-speed homogenizer used for sample preparation.*

Sufficient DI water was then added to the homogenized sample to make it up to the 100ml graduated mark.

Each homogenized food sample was subsequently deproteinized through the addition of 200µl Carrez reagents (I /II) and gentle stirring of the mix for 1 minute. Alternatively, perchloric acid can be used for the sample deproteination.

The fatty and oily portion of the homogenized food samples were separated through the addition of 3ml hexane or by refrigeration method.

Afterwards, 5ml of each treated food sample was transferred to a 15ml centrifuge tube and centrifuged at 4000 rpm for 10 minutes.

The resulting supernatant was further clarified by using a 10ml syringe and 0.45 μm Fisher brand PTFE filter.

Standard glucose solutions (shown in Table 4.1) were prepared by diluting the stock solution. Accumex pipettes with capacity of 200-1000µl were used to transfer 0-1.0ml of the 3 mg/dl glucose solution to appropriately labelled 13 x 100 mm tubes. Appropriate volumes of distilled water were added to the content of each tube to realize a final volume of 1.0ml.

Table 4.1. *Preparation of standard and test solutions for glucose determination*

Tube	DI Water (ml)	3 mg/dl Glucose Standard (ml)	Colour reagent (ml)	Test Sample (ml)
Reagent Blank	1.0	-	4.0	-
1st Standard (S_1)	0.9	0.1	4.0	-
2nd Standard (S_2)	0.8	0.2	4.0	-
3rd Standard (S_3)	0.7	0.3	4.0	-
4th Standard (S_4)	0.6	0.4	4.0	-
5th Standard (S_5)	0.5	0.5	4.0	-
6th Standard (S_6)	0.4	0.6	4.0	-
7th Standard (S_7)	0.3	0.7	4.0	-
8th Standard (S_8)	-	1.0	4.0	-
Test	0.9	-	4.0	0.1

Approximately 4ml of the enzyme-dye reagent was added to each tube. The resulting solution in each tube was mixed by manually shaking for 20 secs.

The labelled tubes were carefully and orderly arranged in a metallic test tube rack. The tubes were thereafter incubated at 37°C in a water bath for 10 minutes.

The incubated test tubes were removed from the water bath and allowed to cool to room temperature. The blank (B) was used to adjust the absorbance of the spectrophotometer to zero at 415 nm.

A calibration curve for the G-Ox assay was produced after the determination of the absorbance values of the standard solutions (S) with known concentrations.

Consequently, the spectrophotometer was used to measure the absorbance of the test solutions (T) and the concentrations of glucose present in the samples were determined from the values on the calibration curve.

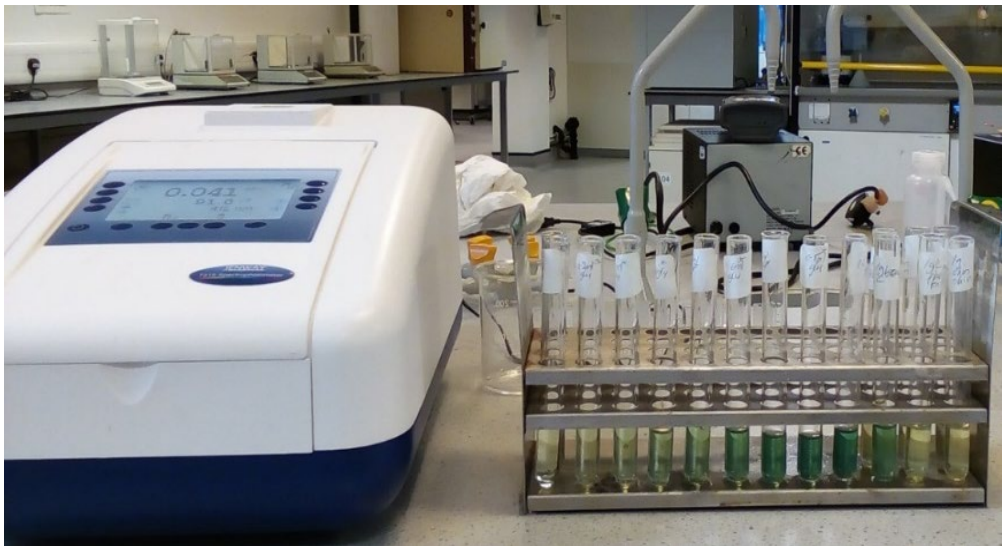

Figure 4.4. *Jenway Spectrophotometer and glucose standard tubes used for the determination of glucose at λ=415nm*

4.3.6 Calibration Curve and Calculation of Glucose Concentrations

EXCEL (2016) software was used to plot the absorbance values of the standard solutions against their respective concentrations. Subsequently, the amount of glucose in each test sample was determined by using the corresponding absorbance value(s) available on the calibration curve. Since the steps for sample tube T and standard tube S_1 are essentially same, the glucose concentration in the test sample was calculated using the formula:

$$\frac{\textit{Test Absorbance}}{\textit{Standard Absorbance}} \times \textit{Concentration of Standard}$$

For diluted food samples, the glucose concentrations determined were multiplied by the dilution factor (s) used during sample preparation.

4.3.7 Statistical Analysis

IBM SPSS Statistics 26 software was used for the data analysis. One-way analysis of variance (ANOVA) method was employed to gain knowledge of the difference in glucose concentrations for the WA foods. The least significance difference (LSD) method was used as the post hoc test for group difference at α=0.05. The data from the test are compared in Table 4.3.

4.4 Results

A typical calibration curve obtained for the glucose determination in shown below. The curve shows a strong determination coefficient and the possible range of linearity. The curve did not originate from zero as deionised water was used as blank.

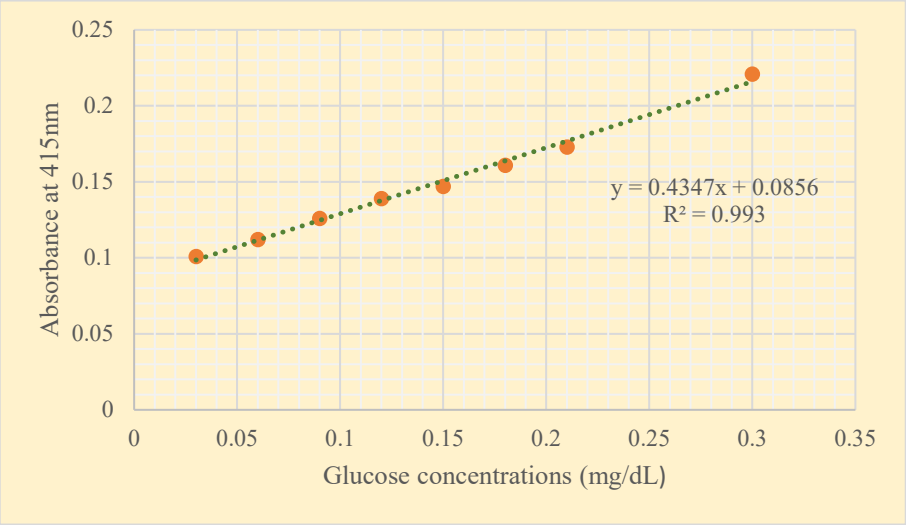

Figure 4.5. *Representative Glucose Calibration Curve*

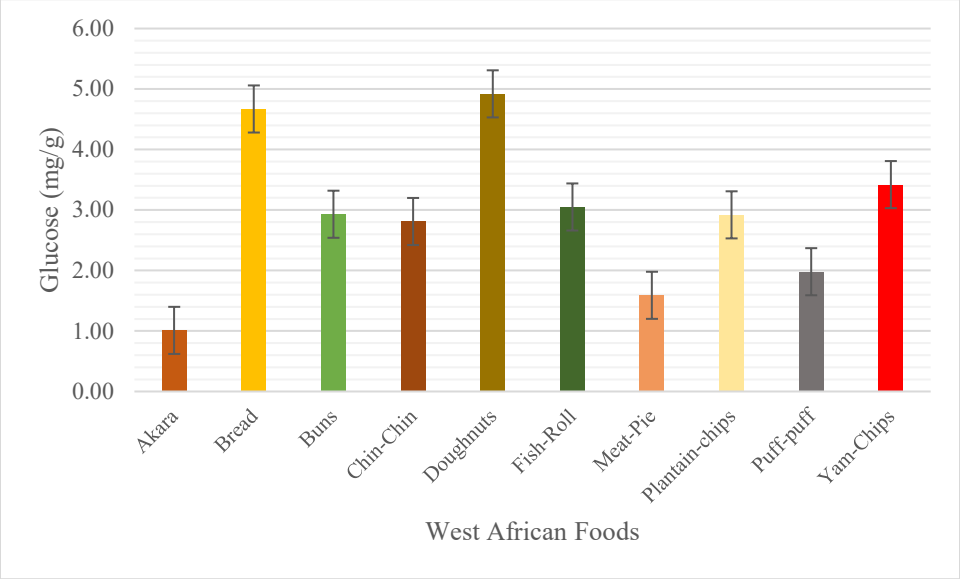

Figure 4.6. *Comparison of the glucose concentrations amongst popular WA foods*

4.4.1. Glucose concentrations for various WA foods

Overall, there was a statistically significant difference in the glucose concentrations for the WA foods at $\alpha=0.05$ i.e. $p< 0.05$. This was particularly the case when the glucose concentrations in Akara was compared to the glucose levels in other WA foods.

The average glucose concentration measured for *akara*, *bread*, *buns, chin-chin*, *doughnuts*, *fish-roll*, *meat-pie*, *plantain-chips*, *puff-puff* and *yam-chips* were 1.01, 4.67, 2.93, 2.81, 4.92, 3.05, 1.59, 2.92, 1.98 and 3.42 mg/g food sample respectively.

The mean, minimum and maximum concentrations of glucose recorded for the WA foods are shown in Table 4.2.

Table 4.2. Glucose values recorded for the selected WA foods

WA food samples	**Mean glucose (mg/g) concentration in food samples**	**Minimum glucose concentration in (mg/g) food sample**	**Maximum glucose concentration in (mg/g) food sample**
Akara	1.01	0.06	2.45
Bread	4.67	2.60	7.80
Buns	2.93	0.08	6.17
Chin-Chin	2.81	0.69	6.32
Doughnut	4.92	2.97	6.94
Fish-Roll	3.05	1.56	4.46
Meat-Pie	1.59	1.02	2.13
Plantain-Chips	2.92	1.28	4.44
Puff-Puff	1.98	1.21	3.15
Yam-Chips	3.42	1.15	6.10

Table 4.3. Significance of difference in glucose concentrations for WA foods

Comparison of Glucose for the WA Foods	**LSD Test of Significant Diff at α=0.05**
Akara Vs Other WA foods	Significant i.e. < 0.001
Bread Vs *Doughnut*	Not Significant (=0.058) i.e. > 0.05
Buns Vs *Chin-Chin*	Not Significant (=0.354) i.e. > 0.05
Buns Vs *Fish-Roll*	Not Significant (=0.407) i.e. > 0.05
Buns Vs *Plantain-Chips*	Not Significant (=0.947) i.e. > 0.05
Chin-Chin Vs *Fish-Roll*	Not Significant (=0.079) i.e. > 0.05
Chin-Chin Vs *Plantain-Chips*	Not Significant (=0.390) i.e. > 0.05
Fish-Roll Vs *Plantain-Chips*	Not Significant (=0.370) i.e. > 0.05
Meat-Pie Vs Other WA foods	Significant i.e. < 0.05
Puff-Puff Vs Other WA foods	Significant i.e. < 0.05
Yam-chips Vs Other WA foods	Significant i.e. < 0.05

4.5 Discussion

The grand mean glucose concentrations recorded for the WA foods showed that *akara* has the least amount of glucose (1.01mg/g), while doughnut has the highest amount of glucose (4.92mg/g). This observation might be a reflection of the ingredients used in the preparation of these foods i.e. *akara* is made mainly from beans and spices, while jam and sugar are additional components of WA *doughnuts*. The high glucose recorded for bread can be explained by the formulations used for its preparation i.e. use of fruit, wheat based recipe which includes high calorie of sugar. This is also an indication that *akara* has less potential for acrylamide formation based on its glucose concentration.

The insignificant difference in the glucose concentration for WA pair of foods such as *bread-doughnuts*, *buns-chin-chin, and chin-chin-fish-roll* is a pointer to the fact that they are prepared from similar ingredients and formulations. Based solely on glucose concentrations, these WA foods are also expected to have similar levels of acrylamide.

To reduce the acrylamide forming potential of foods, Food Drink Europe advised food business operators to use food materials with low reducing sugar (FDE, 2016). Hence, for foods such as doughnut a reduction in jam and sugar content will lead to the formation of less acrylamide.

For foods such as plantain and yam chips, which are similar to potato chips, blanching of the raw material is necessary for a further reduction in glucose concentration.

4.6 Conclusion

The study demonstrated that several WA foods including *akara*, *meat-pie*, *puff-puff* and *yam-chips* are different in their glucose concentrations i.e. $p < 0.05$. This is also an indication that they have varying acrylamide forming potentials.

4.7 Summary

Several studies have linked the existence of high glucose and fructose to elevated levels of dietary acrylamide. The amount of glucose in 10 popular WA foods was determined by spectrophotometric analysis based on the application of oxidase from *Aspergillus niger*.

The analyses of 150 samples of WA foods including *akara*, *bread*, *buns*, *chin-chin*, *doughnuts*, *fish-roll*, *meat-pie*, *plantain-chips*, *puff-puff* and *yam-chips* were conducted at a wavelength of 415nm. The highest mean glucose concentration (4.92mg/g) was found in WA doughnuts, while the lowest mean glucose level (1.01mg/g), was measured in WA Akara.

According to the findings from the study, the selected WA foods have varying acrylamide forming potentials based on their different glucose concentrations i.e. $p < 0.05$.

Chapter 5

Potentiometric determination of asparagine levels in popular West African foods

CHAPTER 5. POTENTIOMETRIC DETERMINATION OF ASPARAGINE LEVELS IN POPULAR WEST AFRICAN FOODS

5.1. Overview

This chapter discusses the potentiometric quantitation of asparagine in popular WA foods. The study also compares the asparagine concentrations and the acrylamide forming potentials of various WA foods.

5.2. Introduction

Asparagine also called 2- amino-3-carbamoylpropanoic acid is a chemical compound with chemical formula $H_2N\text{-}CO\text{-}CH_2\text{-}CH(NH_2)\text{-}COOH$, which is abundant in foods such as egg, milk, meat and potatoes (Science Daily, 2018).Figure 5.1 represents the structural formula of asparagine.

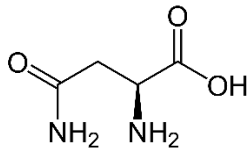

Figure 5.1 *Structure of Asparagine*

Clinical research have demonstrated the anti-tumour potentials of asparaginase in the treatment acute lymphoblastic leukemia (ALL) and lymph sarcoma (Pieters et al., 2011). Varying amounts of the enzyme are found in animals, plants and microorganisms.

Although the growth of normal body cells does not depend on L-asparagine, tumour cells require high levels of the amino acid for their proliferation. Hence, the hydrolysis of L-asparagine into aspartic acid and ammonia by L-asparaginase (EC3.5.1.1) leads to the decline in the formation of tumour cells in rats (Ali et al., 1994, Berenbaum et al., 1970).

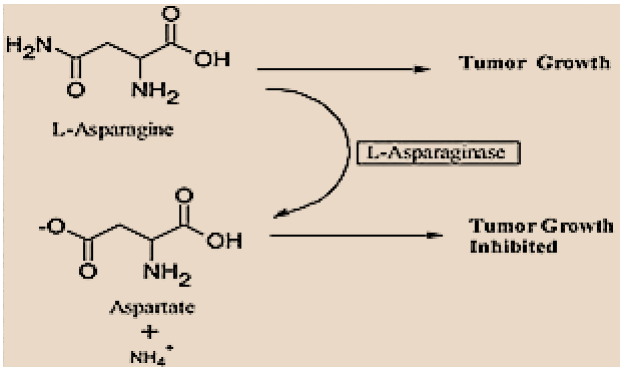

Figure 5.2. *Mitigation of tumour growth by L-asparaginase (Arastoo Badoei-Dalfard, 2016).*

Although L-asparaginase is beneficial to the pharmaceutical industries, it is also an important enzyme to the food industries.

A reaction between asparagine and carbonyl groups of reducing sugars compounds produces acrylamide in several heat-processed foods including French fries, potato chips, and toasted bread. Hence, food grade L-asparaginase is used by food manufacturers to reduce the formation of acrylamide in heat-processed foods such as potato chips and fries (Amrein et al., 2004, Anese et al., 2013, El-Bessoumy et al., 2004, Medeiros Vinci et al., 2012, Pedreschi et al., 2008, 2011)

Methods that are available for the detection of amino acids include spectrophotometric techniques, biosensor based electrochemical procedures (Shvedene et al., 2016, Zahra, et al., 2017), and chromatographic methods.

The development and application of different potentiometric and biosensor based electrochemical methods of quantification for asparagine has been reported. These include the L-asparaginase-based biosensor with polyimide membrane electrode (Ali et al., 2014, Erdogan et al., 2014, Fatibello-lho et al., 2006), and biosensor constructed from thermostable recombinant asparaginase produced by *Archaeoglobus fulgidus* (Li et al., 2002). Neelem et al., (2012 and 2013) also reported the use of miniaturized fibre optic biosensor constructed from whole cells of *Staphylococcus sp.* containing asparaginase activity and immobilized asparaginase. Kamble et al., 2012 also reported the existence of several microorganisms with asparaginase activity. Important biological sources of L-asparaginase include *Erwinia caratovira*, *Corynebacterium glutamicum*, *Bacillus* sp, *Rhodococcus* sp, *Pseudomonas stutzeri,* and *E. coli* (Davidson et al.,

1977, Dhevendaram and Annie., 1999, Howard and Schwartz., 1968, Mohamed et al., 1994, Peterson and Cieglar., 1996, Shwu, et al., 1998, Soni et al., 2014, Tosa et al., 1971).
The use of asparaginase derived from plants for the construction of biosensors and the determination of asparagine has also been described. Mandeep et al., (2015) utilized the asparaginase from *Solanum nigrum* for the development of a spectrophotometric biosensor which was used to quantify the asparagine in fruit juices. The use of the asparaginase from *Withania somnifera* has also been used for the fabrication of asparagine biosensor (Kuldeep et al., 2013)

Kim et al. 1995 also reported the use of asparaginase from garlic tissue and ammonium gas electrode for the construction of a biosensor for asparagine determination. Asparaginase derived from plants including *Capsicum annum*, *Cannabis sativa* and *Citrus limon* have also been used for the fabrication of asparagine biosensors (Kuldeep et al., 2012, 2013, Pathak et al 2014, 2017)
The aim of this study was to compare the asparagine levels in different WA foods by using simple potentiometric analytical method.
The procedure used was similar to those described by Ali et al., 2014; Erdogan et al., 2014; Fatibello-lho et al., 2006; Li and Bachas, 2002; Neelam et al., 2012, Pathak et al., 2017; and Punia and Kumar 2015.

5.2.1. Problem Statement

No previous study has attempted to evaluate the risk of consuming WA foods such as *chin-chin* and *meat pie* based on the impact of their asparagine level and the associated acrylamide concentrations.

Furthermore, though the amounts of asparagine and acrylamide in popular WA foods are unknown, this group of foods are also produced, sold and consumed in countries including United Kingdom (U.K) and United State of America (U.S.A).

5.2.2 Research Rationale

The study is justified based on the following:

(a) *Acrylamide toxicity*: several studies based on animal models such as rats have established the toxicological effects of AA on genes and the neurons (IARC, 1994; IFST 2017; JECFA

2010).

(b) *The strong connection between the heat processing of food materials rich in amino acids such as asparagine and the formation of AA*

The generation of huge amounts of acrylamide in food products made from asparagine rich food materials. (Amrein et al, 2005; Croft et al., 2004; European Commission, 2013; EFSA, 2012; IFST, 2017; Leung et al., 2003; Lineback et al., 2005; Murkovic, 2004; Tareke et al., 2002; Roach et al., 2003; Rosén and Hellenäs, 2002).

(c) *The guidance given by the FDE in conjunction with the EC*:

The EC regulation on dietary acrylamide, which was updated in 2017 together with the recommendations made by the Food Drink Europe (FDE) are important documents which highlight essential procedures required to reduce acrylamide in food products. The toolbox created for food business operators (FBO) by the FDE recommends the use raw materials with low levels of asparagine The recommendations in the FDE manual tagged "Code of Practice for Managing Acrylamide Formation" also include the use of methods such as blanching and enzymatic hydrolysis to reduce the amount of asparagine in food materials (FDE 2016).

5.2.3 Research Questions

The questions for the study are listed below.

(i) What is the mean concentration of asparagine (asn) in each WA food?
(ii) Which WA food material has the highest and lowest asn concentrations?
(iii)Based on the amount of asparagine, which WA food is more likely to contain higher AA?

5.2.4 Research Hypotheses:

The null and alternative hypotheses for the study are listed below.

(a) Null hypothesis (H_o; [*asn*] = μ) No difference in the concentration of *asn* for the WA foods
(b) Research hypothesis (H_a; [*asn*] ≠ μ) Different WA foods have different amount of *asn*.
(c) Null hypothesis (H_o; [AA] = μ) Based on *asn* no difference in the AA forming potential for the WA foods
(d) Research hypothesis (H_a; [AA] ≠ μ) Based on *asn*, the WA food have different AA forming potentials.

5.2.5 Research Objectives:

Asparagine is a precursor compound in the development of acrylamide in dry heat processed foods. So a positive correlation between the asparagine content and acrylamide content is possible and expected.

Hence, the objectives of the study based on the research questions and hypotheses were to:

(1) Determine the asparagine levels in the WA food samples.

(2) Compare the asparagine levels for *akara, bread, buns, chin-chin, doughnut, fish-roll, meat-pie, plantain-chips, puff-puff*, and *yam-chips.*

(3) Assess the possibility of having high levels of acrylamide in the foods based on their asparagine concentrations.

The common analytical methods for the determination of asparagine in food materials include the HPLC and spectrophotometric techniques. Although, these methods are useful, some of them are expensive and require advance laboratory skills. The sample preparation and treatment for some of these methods also take time. For this reason, an alternative method requiring asparaginase and ion selective electrode was employed. This method is less expensive, more rapid and easier to use.

The principle and application of the potentiometric technique for the quantification of the asparagine concentrations in the selected WA food materials are described below.

5.2.6 Principle of asparagine determination by A-ISE

The presence of NH_4^+ in solution can be determined using various techniques including spectrophotometric (Mandeep et al., 2015), or ion-selective electrode (ISE) methods (Kim et al. 1995). The measurement depends on the hydrolysis of asparagine (*asn*) to aspartic acid and ammonium ion by asparaginase produced by organisms such as *Bacillus sp Escherichia coli, Erwinia sp, Pseudomonas spp, Rhodococcus* sp., and Staphylococcus spp. (Davidson et al., 1977, Dhevendaram and Annie., 1999, Howard and Schwartz., 1968, Mohamed et al., 1994, Peterson and Cieglar., 1996, Shwu, et al., 1998, Soni et al., 2014 and Tosa et al., 1971)**.** The NH_4^+ selective electrode measures the concentration (in ppm [mg/L] and mol/L) of the ammonium ions produced from the enzymatic hydrolysis of the asparagine present in the samples.

The level of *asn* present in the sample is then determined from its stoichiometrical relation to ammonium ion shown in the chemical equation below.

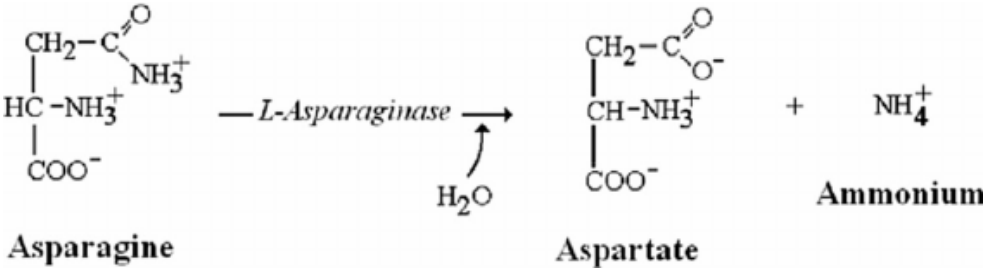

Figure 5.3. *Enzymatic hydrolysis of asparagine to ammonium ions and aspartic acid*

5.2.7 Potentiometric Determination of Asparagine using A-ISE

The molar relationship that exists between the released NH_4^+ and *asn* was used to determine the levels of the contaminant in the samples tested. Ammonium ion-selective electrode (A-ISE) linked to ELIT Ion Analyser (or pH/mV/Ion meter) was used to measure the concentrations of NH_4^+ in mg or mol/L. In addition, since A-ISE is sensitive to the NH_4^+ produced from *asn*, it follows that standard solutions of *asn* that have been treated with asparaginase can also be used to calibrate the ISE system.

The performance of A-ISE is established on the fact that there is a linear relationship between the electrical potential (in mV) developed between an ISE and a reference electrode immersed in the same solution, and the logarithm of the "effective concentration" (or activity**)** of NH_4^+ in the solution. The linear relationship is expressed through the Nernst equation given below:

$$E = E^{o} + (2.303RT/nF) \times Log\ (a)$$

Where E = the total potential (in mV) developed between the reference and sensing electrodes.

E° = is a constant which is characteristic of the particular ISE/reference pair. (It is the sum of all the liquid junction potentials in the electrochemical cell)

2.303 = the conversion factor from natural to base10 logarithm. R = the Gas Constant (8.314 joules/degree/mole).

T = the Absolute Temperature.

n = the charge on the ion (with sign).

F = the Faraday Constant (96,500 coulombs per mole).

Log(a) = the logarithm of the activity of $NH_4^+$from

asparagine

The slope of the equation which is an important diagnostic characteristic of the electrode is decribed by the bracketed contants, 2.303RT/nF.

The tip of A-ISE which comes in contact with the sample to be tested consists of a solid-state PVC polymer matrix membrane. After the placement of the electrode in the sample containing the hydrolysed *asn* and the NH_4^+, an electrical potential develops across the surface of the sensing membrane. The magnitude of the electrical potential is linearly related to the logarithm of the molar concentration of the *asn* and NH_4^+. Hence, higher electronic potentials indicate the existence of higher NH_4^+ and *asn* concentrations in the samples tested.

The membrane potential of the sensing electrode is compared with the univarying potential of another electrode known as the reference electrode. A filling solution present in the reference electrode completes the electrical circuit between the sample and the internal cell of the reference electrode. The 'liquid junction' is the term that describes the point of contact between the sample and the filling solution. The meter serves to display the readout (in millivolts, pH units or NH_4^+ concentration units) representing the difference of the electrical potentials between the reference electrode and the sensingelectrode.

If the background ionic strength is high and constant relative to the NH_4^+ concentration, the activity concentration is constant and activity is directly proportional to concentration. Ionic Strength Adjuster (ISA) was added to all NH_4^+ standards, enzyme treated asparagine standards and samples so that the background ionic strength is high and constant relative to variable concentrations of NH_4^+.The recommended ISA (i.e. 1M aqueous copper sulphate, $CuSO_4$) was used for the measurement of the NH_4^+ released from the hydrolysed *asn*

5.3 Materials and method

5.3.1 Food Samples: WA foods which are commonly consumed and undergoes non-enzymic browning reactions (Apekey et al., 2019; Chapter 3; Quayson and Ayernor, *2007*) including *akara*, *bread*, *buns*, *chin-chin*, *doughnuts*, *fish-roll*, *meat-pie*, *plantain-chips*, *puff-puff* and *yam-chips* were collected from different outlets in London markets such as Peckham, Brixton and East Street. One hundred and fifty samples (150) of each WA food were obtained from the locations mentioned above.

5.3.2. Reagents

Analytical grade reagents and solvents of sufficient purity were used to ensure high accuracy of the determination. These include asparagine (99%), ammonium chloride, de-ionised (DI) water, hexane, Carrez I and II (potassium hexacyanoferrate (II) trihydrate solution, and zinc sulfate heptahydrate solution) or perchloric acid. Calcium chloride, sodium alginate, PHAM 226-asparaginase, 225 IU/mg (EC 3.5.1.1, CAS No. 9015-68-3) from *Escherichia coli.*

5.3.3 Equipment and Apparatus

Weighing balance, volumetric flasks (500-1000ml), 100ml glass beakers, 15 ml centrifuge tubes with caps, air operated homogeniser, 50 ml separating funnel, ELIT 8051 or (Mettler Tolledo™) NH_4^+-ISE, ELIT 003 double junction reference electrode, ELIT dual electrode head (ELIT 201), ELIT ion analyser (or pH/mV/Ion meter) with BNC socket, ammonium ion selective electrode (NH_4^+-ISE) system connected to pH meter or personal computer (PC) interface, standard electrode holder, magnetic stirrer with teflon-coatings set at 100 rpm, 100µl-1000 µl pipettes, 10ml syringes, low-lint tissue, 0.45 μm Fisher brand PTFE filters.

5.3.4 Preparation of Reagents and Standards

Since ammonium ion (NH_4^+) is a monovalent cation with a molar mass of 18.038 grams, and 1000 ppm NH_4^+ is equivalent to 0.055 M NH_4^+, to prepare 1000 ppm NH_4^+, 2.965g anhydrous ammonium chloride (NH_4Cl) was dissolved in 1 litre DI water.

Asparagine standard solution of 0.1M was prepared by dissolving 13.21g asparagine (*asn*) in I litre DI water. Tenfold serial dilution method was then used to prepare 0.01, 0.001, 0.0001 and $1x10^{-5}$M standards.

Carrez I solution was prepared by dissolving 3.6g of potassium hexacyanoferrate (II) trihydrate in 100ml de-ionised water, while Carrez II solution was prepared by dissolving 7.2g of zinc sulfate heptahydrate in 100ml de-ionised water.

Approximately 225 IU/mg of *Creative Enzymes* PHAM 226- asparaginase (EC 3.5.1.1) produced from *Escherichia coli* was added to 1000µl Tris buffer of pH 7.2. The reconstituted enzyme was

mixed with 9ml of 3.0 % sodium alginate solution. For the immobilization of the enzyme, a 10ml syringe was used to transfer drops of the resulting asparaginase-alginate mix into 100ml of 1.5 % CaCl solution.

The resulting asparaginase beads produced were washed with the Tris buffer solution and transferred into a 50ml separating funnel. This served as the reaction vessel for the hydrolysis of any *asn* present in the sample. To remove the leftover analyte after each reaction the *asn-ase* beads were thoroughly washed with the buffer solution.

Figure 5.4. *Asn-ase beads for the degradation of asparagine to ammonium ion.*

5.3.5 Food Sample Treatment and Analysis

Food samples between 1-10g were accurately weighed using a digital weighing balance and transferred into a 100ml beaker.

For the extraction of the analyte, about 10ml de-ionized water was added to the weighed food sample. Solid food samples with very low moisture content such as *Chin-Chin* were allowed to swell in the de-ionized water at 37°C for 2-10 minutes.

An air operated ultra-turrax T-25 high-speed homogenizer and analytical grade water were thereafter used for the blending and homogenization of the food samples.

Sufficient DI water was then added to the homogenized sample to make it up to the 100ml graduated mark.

Each homogenized food sample was subsequently deproteinized through the addition of 200-500µl Carrez reagents (I /II) and gentle stirring of the mix for 1 minute.

The fatty and oily portion of homogenized fat-rich samples such as *meat-pie* were separated through the addition of 3-5ml hexane or by refrigeration. The resulting top oily layer was then discarded.

Approximately 10ml of the treated samples were transferred into15ml centrifuge tubes, which were centrifuged at 4000 rpm for 5-15 minutes.

The resulting supernatant were further cleaned using a 10ml syringe and 0.45 μm Fisher brand PTFE filter.

For the enzymatic release of the analyte, the filtered sample was transferred into the separating funnel with asparaginase beads and left for about 5 min. About 5ml of the resulting sample was collected into a 15ml centrifuge tube or 100ml beaker and immediately treated with a 10% v/v of ionic strength adjustment buffer –ISAB (about 500 μl of 1M $CuSO_4$ for 5ml sample).

The treated sample was further filtered using a 10ml syringe and 0.45 μm Fisher brand PTFE filter.

An ammonium (NH_4^+) ISE which has been preconditioned for 5-10 minutes with 1000ppm ammonium chloride (NH_4Cl) standard solution was used to check the performance of the NH_4^+ ISE-system. The A-ISE system was calibrated using 0.1-1000ppm of NH_4Cl standard solutions to which 10% v/v of ISAB has been added.

Another calibration curve was produced by using $1x10^{-5}$M– 0.1M of enzyme treated asparagine (*asn*) standard solutions. A 10% v/v of the ISAB was also added before recalibrating the NH_4^+ ISE.

The molar concentrations of ammonium ion and *asn* present in the enzyme treated food sample were subsequently measured by using ELIT ion analyser (or pH/mV/Ion meter) connected to a personal computer (PC) data-recording interface.

Figure 5.5. *Reference and NH_4^+ ISE electrodes connected to the ELIT 801 pH/ISE Analyser and a Computer/ Data Recording System.*

The level of *asn* present in the food sample was then determined using the NH_4^+ ISE system and the generated calibration curves.

5.3.6 Expression of results

The concentration of *asn* in food samples is commonly expressed in units such as mg/L or mg/ml. The A-ISE system, which produces output in mg or mol/L was used to determine the NH_4^+ (and *asn*) concentrations per unit volume of solution. Consequently, necessary conversions were made to have final readings in mg/kg.

To express the *asn* concentrations produced by the ELIT ion analyser in mg/kg food sample, the weight of the food sample used in grams was converted to kg after dividing by 1000.

For 5ml feasible sample volume, the mathematical formula given below was applied.

$$\textit{Asn (mg/kg) of food sample} = \frac{\textbf{\textit{ISE Ion Analyser Reading (mg/1000 ml)}}\ \textbf{\textit{x 5ml}}}{\textbf{\textit{Weight of Sample Used}}\ \textbf{(kg)}}$$

5.3.6 Interference:

Apart from NH_4^+, monovalent ions such as potassium (K), sodium (Na), magnesium (Mg), calcium (Ca), lithium (Li) with selectivity coefficients (SC) of 0.1, 0.002, 0.0002, 0.00006 and 0.00003 respectively have been reported to be sensed by A-ISE.

The SC is the approximate apparent increase in the measured concentration caused by 1 unit of the interfering ion. The percentage increase caused by an interfering ion can be calculated using the equation below.

[(*Expected concentration of interfering ion*) x (SC) / (*Expected NH_4^+ concentration*)] x 100.

Considering the SC of the interferents, only K^+ has significant contribution. Hence, the true value of the primary ion was obtained after the *chemtool* menu of the ELIT ISE/pH ion analyser software and ISEs for interfering ions such as K^+ were used to determine the magnitude of interference. The background NH_4^+ not due to the effect of asn-ase was also corrected for in the samples.

Table 5.1. *Ammonium Ion Selective Electrode (A-ISE) Operational Conditions*

Minimum feasible sample volume	5 ml
Preconditioning / Standard solution	1000 ppm NH_4^+ as NH_4Cl
Preconditioning time	At least 5 minutes
Optimal pH range	pH 0 - 8.5
Temperature range	0 to 50° C
Recommended ISAB	1M $CuSO_4$ (Add 10% v/v)
Recommended reference electrode	Double junction (ELIT 003)
Reference electrode outer filling solution	0.1M CH_3COOLi
Electrode slope at 25° C	54 ± 5 mV/ decade
Concentration range	0.03 to 1,800 ppm (2x10-6 to 0.1 Molar)
Response time	< 10 seconds

5.3.7 Statistical Analysis

IBM SPSS Statistics 26 software was used for the data analysis. One-way analysis of variance (ANOVA) method was employed to gain knowledge of the difference in asparagine concentrations for the WA foods. The least significance difference (LSD) method was used as the post hoc test for group difference at $\alpha=0.05$. The result of the comparison is shown in Table 5.3.

5.4 Results

Typical calibration curves obtained for the determination of ammonium ion and asparagine is shown below. The curve shows a strong determination coefficient and the possible range of linearity.

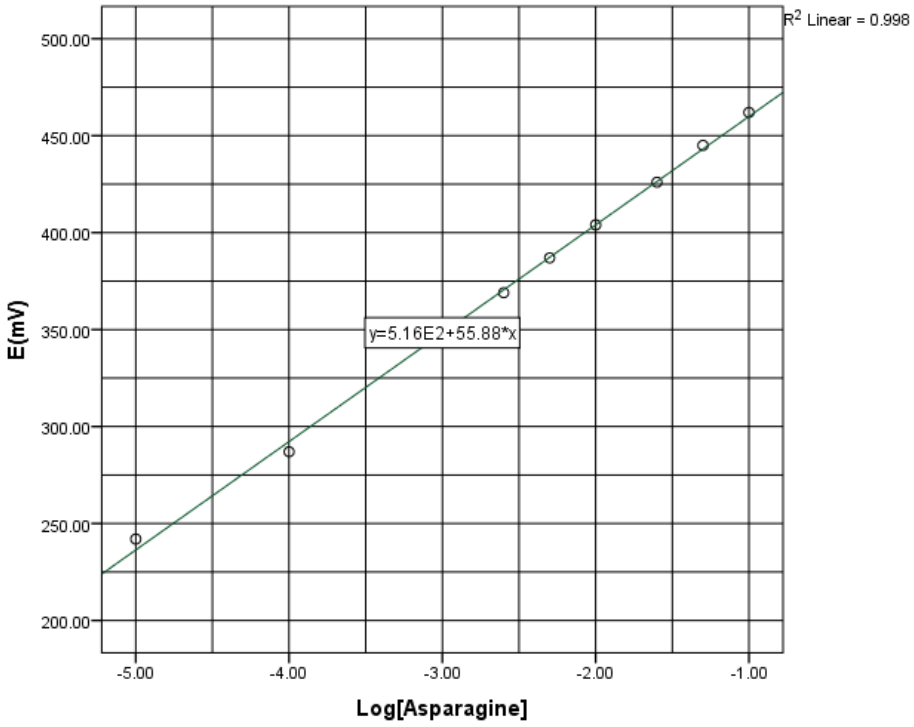

Figure 5.6. *Representative calibration curve for different asparagine standards*

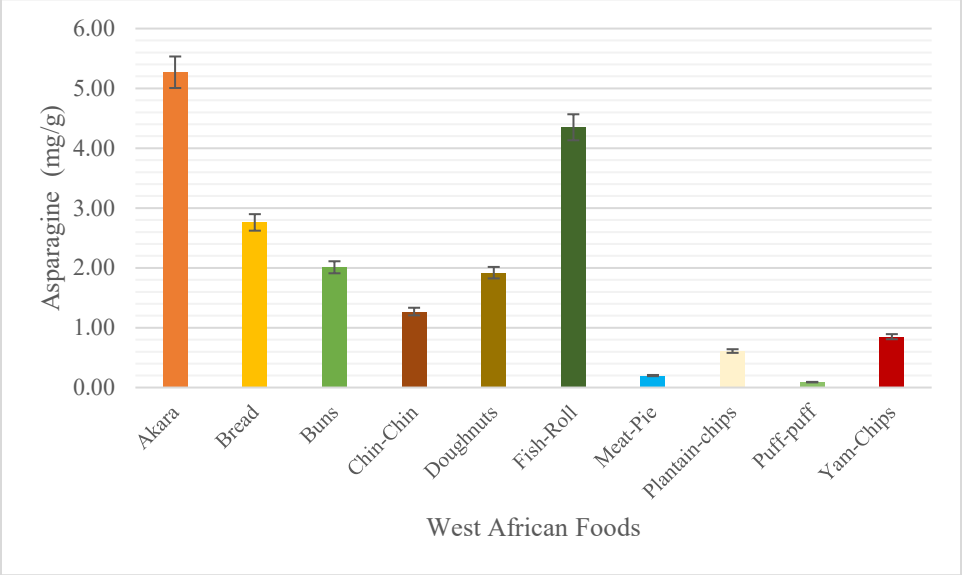

Figure 5.7. *Comparison of the asparagine concentrations amongst popular WA foods*

5.4.1. Asparagine concentrations for various WA foods

The average asparagine concentration measured for *akara*, *bread*, *buns chin-chin*, *doughnuts*, *fish-roll*, *meat-pie*, *plantain-chips*, *puff-puff* and *yam-chips* were 5.27, 2.76, 2.01, 1.27, 1.92, 4.35, 0.20, 0.61, 0.09 and 0.85 mg/g food sample respectively, Table 5.2.

The minimum and maximum concentrations of asparagine recorded for akara, bread, buns chin-chin, doughnuts, fish-roll, meat-pie, plantain-chips, puff-puff and yam-chips are shown in Table 5.2.

Overall, there was a statistically significant difference in asparagine concentrations for the WA foods at $\alpha=0.05$ i.e. $p< 0.05$. This was particularly the case when the asparagine concentrations in akara, bread, chin-chin and fish-roll were compared to the asparagine levels in other WA foods (Table 5.2).

Table 5.2. *Asparagine values recorded for the selected WA foods*

WA food samples	**Average asparagine concentration (mg/g) in food sample**	**Minimum asparagine concentration (mg/g) in food sample**	**Maximum asparagine concentration (mg/g) in food sample**
Akara	5.27	1.95	9.99
Bread	2.76	1.00	4.97
Buns	2.01	0.12	4.65
Chin-Chin	1.27	0.09	4.43
Doughnuts	1.92	0.73	4.07
Fish-Roll	4.35	2.23	7.19
Meat-Pie	0.20	0.04	0.35
Plantain-Chips	0.61	0.23	1.00
Puff-Puff	0.09	0.02	0.19
Yam-Chips	0.85	0.13	2.01

Table 5.3. *Significance of difference in asparagine concentrations for WA foods*

Comparison of Asparagine for WA Foods	**LSD Test of Significant Diff at α=0.05**
Akara vs Other WA foods	Significant i.e. < 0.001
Bread vs Other WA foods	Significant i.e. < 0.001
Buns vs *Doughnut*	Not Significant (=0.462) i.e. > 0.05
Chin-Chin vs Other WA foods	Significant i.e. < 0.001
Fish-Roll vs Other WA foods	Significant i.e. < 0.001
Meat-Pie vs *Puff-Puff*	Not Significant (=0.388) i.e. > 0.05
Plantain-Chips vs *Yam Chips*	Slightly Significant (=0.049) i.e. ≤ 0.05

5.5 Discussion

The overall mean asparagine concentrations recorded for the WA foods showed that *puff-puff* has the least amount of asparagine (0.09mg/g), while *akara* has the highest amount of asparagine (5.27mg/g). This observation might be a reflection of the ingredients used in the preparation of these foods i.e. *akara* is made mainly from beans and spices, while puff-puff is a WA pastry made from all-purpose flour, yeast and sugar. The high asparagine recorded for fish-roll can be explained by the formulations used for its preparation i.e. Wheat and fish based recipe, which is high in protein.

This is also an indication that *akara* and *fish-roll* have high potential for acrylamide formation based on their asparagine concentration, while *puff-puff* has less acrylamide forming potential for the same reason.

The insignificant difference in the asparagine concentration for WA pair of foods such as *buns-doughnuts* and *meat-pie-puff-puff* is a pointer to the fact that they are prepared from similar ingredients and formulations. Based solely on asparagine concentrations, these WA foods are also expected to have similar levels of acrylamide.

To reduce the acrylamide forming potential of foods, Food Drink Europe advised food business operators to treat raw food materials with food grade asparaginase (FDE 2013, 2016). Hence, it is expected that the treatment of the batter of *akara* and *doughnuts* with food grade asparaginase will result in the formation of less acrylamide.

Blanching and soaking of raw potato slices have been reported to reduce the amount of reducing sugars and asparagine which are essential precursors of acrylamide(Grob et al., 2003, Fiselier et al., 2005, Haase et al., 2003, 2004, Pedreschi et al., 2004, 2005, Wicklund et al., 2005). Blanching in warm water was more effective in extracting glucose and asparagine than immersion in cold water (Grob et al., 2003, Kita et al., 2004, Pedreschi et al., 2004).

As an effective acrylamide mitigation strategy, Food Drink Europe (FDE) also recommends the application of blanching and soaking methods for the treatment of food materials. In addition, extraction of the precursors in food grade acids have been shown to be a more effective approach to reducing the acrylamide in food products than water extraction(Kita et al., 2004). There are reports of remarkable reductions in the asparagine and acrylamide levels in foods after the treatment of the raw materials with food grade acids such as acetic and citric acids (Jung et al., 2003). However, the use of citric acid may have an undesirable effect on the taste of the finished product (Gama-Baumgartner et al., 2004). According to

Kita et al., 2004 the use of acetic acid produces more acceptable taste of the final product compared to citric acid.

There are also reports on the effective reduction of the asparagine and acrylamide levels in foods by organisms including yeasts and lactic acid bacteria.

Future studies on the reduction of acrylamide in WA foods such as Akara and Plantain Chips should focus on the effect of the application of methods such as acidification, blanching, fermentation and soaking of food materials.

5.6 Conclusion

The study demonstrated that several WA foods including *akara, bread, chin-chin* and *fish-roll* are different in their asparagine concentrations i.e. $p < 0.05$. This is also an indication that they have varying acrylamide forming potentials.

Future studies on the reduction of asparagine in WA foods such as *akara* and *plantain chips* should focus on the effect of the application of methods such as acidification, blanching, fermentation and soaking of food materials.

5.7 Summary

Various studies have shown the existence of a strong association between the acrylamide and the asparagine in food materials. The amount of asparagine in 10 popular WA foods was determined through potentiometric analytical method based on the application of asparaginase from *Escherichia coli*.

One hundred and fifty (150) samples of WA foods including *akara*, *bread*, *buns*, *chin-chin*, *doughnuts*, *fish-roll*, *meat-pie*, *plantain-chips*, *puff-puff* and *yam-chips* were tested. The highest mean asparagine concentration (5.27 mg/g) was found in WA *akara*, while the lowest mean asparagine level (0.09 mg/g), was measured in WA *puff-puff*.

According to the study, the selected WA foods have varying acrylamide forming potentials based on their different asparagine concentrations i.e. $p < 0.05$.

Chapter 6

Potentiometric determination of acrylamide levels in popular West African foods

CHAPTER 6. POTENTIOMETRIC DETERMINATION OF ACRYLAMIDE LEVELS IN POPULAR WEST AFRICAN FOODS

6.1. Overview

This chapter discusses the potentiometric quantitation of acrylamide in popular WA foods. The study also compares the acrylamide concentrations amongst popular WA foods.

6.2. Introduction

It is well established that acrylamide can be produced at high levels in high-carbohydrate heat treated foods. In addition, the carcinogenicity, neurotoxicity and genotoxicity of the chemical has been confirmed through in vivo studies of animal models such as rats.

Although, enormous levels of the contaminant has been found in potato chips and French fries, no study has reported the amount of the chemical for WA foods.

Common methods for the quantification of acrylamide are based on liquid and gas chromatography. In addition, electrochemical and potentiometric quantification of acrylamide by different electrodes and biosensors have also been reported. These include the use of ammonium ion selective electrode reconstructed with immobilized *Pseudomonas aeruginosa* containing amidase activity (Silva et al., 2009), the use of platinum electrode (Casella et al., 2006), and the use of carbon-paste electrode modified with haemoglobin-Hb (Krajewska et al., 2008; Stobiecka et al., 2007).

The aim of this study was to measure and compare the acrylamide levels in WA foods by using simple potentiometric analytical method.

6.2.1 Problem Statement

Although the acrylamide levels in several European foods are known, no study has considered the AA concentrations in WA foods. Consequently, no study has compared the acrylamide levels in important WA foods to the regulatory limits for similar EU foods.

6.2.2 Research Rationale

The study is justified based on the following:

(a) *Deleterious effect of acrylamide*: various invivo studies based on models such as rats have established the genotoxic, neurotoxic and potential carcinogenic effects of acrylamide (IARC, 1994; IFST 2017; JECFA 2010).

(b) *The need to know if the AA levels in WA food exceed benchmark levels for similar EU foods*
Following the surveillance of several EU foods, several benchmark levels called indicative values were published (EC, 2013; AFTP, 2019). Since there are no data on acrylamide concentrations for WA foods, there is no basis for statistical comparison to know if WA foods exceed the target levels.

Table 6.1. *The former and most recent AA benchmark levels for some European foods*

EU Foods	AA Bench Mark Levels 2013(µg/kg)	AA Bench Mark Levels 2017(µg/kg)	Similar WA Foods	Benchmark levels(µg/kg)
Potato Crisps	1000	750	*Yam Chips*	-
Soft Breads	150	100	*WA Breads*	-
Crackers	500	400	*Chin-chin*	-

6.2.3 Research Questions

The list of questions for the study are shown below.

(i) What is the average level of AA for each WA food?

(ii) Which WA food products have the highest and lowest AA concentrations?

(iii) Based on the benchmark levels for EU foods, which WA food exceeds the recommended limits for AA?

6.2.4 Research Hypotheses:

The null and alternative hypotheses for the research are shown below.

(a) Null hypothesis (H_o; $[\mu] = [B_L]$ or H_o; $[\mu] \leq [B_L]$) No difference between the benchmark AA level for EU foods and the AA level in similar WA foods

(b) Research hypothesis (H_a; $[\mu] \neq [B_L]$ or H_a; $[\mu] > [B_L]$) There is a difference between the benchmark AA level for EU foods and the AA level in similar WA foods

6.2.5 Research Objectives:

Based on the research questions and hypotheses, the objectives of the study were to:

(1) Determine the acrylamide levels in the selected WA foods.

(2) Compare the acrylamide levels for *akara, bread, buns, chin-chin, doughnut, fish-roll, meat-pie, plantain-chips, puff-puff,* and *yam-chips.*

(3) Compare the acrylamide levels in the WA foods to the benchmark levels for similar EU foods.

6.2.6 Principle of Acrylamide Determination by Ammonium-ISE

Several methods including colorimetry and potentiometry have been used for the detection and quantification of NH_4^+ in aqueous solutions. In the case of the A-ISE technique, the quantitation depends on the hydrolysis of acrylamide to acrylic acid and ammonium ion by acrylamide amidohydrolase produced by organisms such as *Aspergillus nidulans*, *Bacillus clausii*, *Helicobacter pylori*, *Moraxella osloensis or Pseudomonas putida* (Settanni et al., 2016). The ammonium ions produced from the enzymatic hydrolysis of the acrylamide present in the samples are detected and measured in ppm (mg/L) and mol/L by the NH_4^+ ISE system.

The level of acrylamide present in the sample is then determined from its stoichiometrical relation to ammonium ion shown in the chemical equation below.

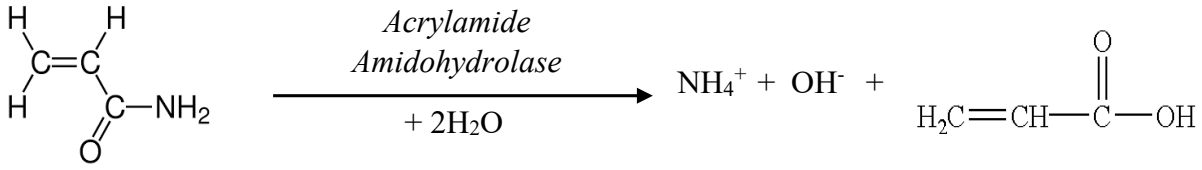

Figure 6.1. *Enzymatic hydrolysis of acrylamide to ammonium ions and acrylic acid*

6.2.7 Potentiometric Determination of Acrylamide using A-ISE

Ammonium ion-selective electrode (A-ISE) linked to ELIT Ion Analyser (or pH/mV/Ion meter) was used to measure the concentrations of NH_4^+ in mg and mol/L. Consequently, the molar relationship that exists between the released NH_4^+ and acrylamide was employed to determine the

levels of the contaminant in the samples tested. Consequently, the standard solutions of acrylamide that have been treated with amidohydrolase can also be used to calibrate the ISE system.
The direct association between the electrical potential (in mV) formed between an ISE and the Logarithm of the "effective concentration" (or activity) of the ions in the solution is used to validate the performance of the ISE.

An electrical potential develops across the surface of the sensing solid-state PVC polymer matrix membrane after the placement of the A-ISE tip in the sample containing the hydrolyzed acrylamide and the NH_4^+.

The logarithm of the molar concentration of the hydrolyzed acrylamide is directly related to the magnitude of the electrical potential produced. Hence, the presence of higher NH_4^+ and AA concentrations in the samples tested causes the development of higher electronic potentials across the sensing membrane.
The relationship is expressed through the Nernst equation described in chapter 5.

6.3 Materials and method

6.3.1 Food Samples: commonly consumed WA foods which undergo non-enzymic browning (Apekey et al., 2019; Chapter 3; Quayson and Ayernor, 2007) including *akara*, *bread*, *buns*, *chin-chin*, *doughnuts*, *fish-roll*, *meat-pie*, *plantain-chips*, *puff-puff* and *yam-chips* were obtained from different outlets in London markets such as Peckham, Brixton and East Street. A total of 150 samples of each WA food were obtained from the three locations mentioned.

6.3.2 Reagents

Analytical grade reagents and solvents of sufficient purity were used to ensure high accuracy of the determination. These include Acrylamide (99%), ammonium chloride, de-ionised (DI) water, hexane, Carrez I and II (potassium hexacyanoferrate (II) trihydrate solution, and zinc sulfate heptahydrate solution) or perchloric acid. Calcium chloride, sodium alginate, NATE-0809-acylamide amidohydrolase, 200 units/mg (EC 3.5.1.4, CAS No. 9012-56-0) from *Pseudomonas aeruginosa.*

6.3.3 Equipment and Apparatus

Weighing balance, volumetric flasks (500-1000ml), 100ml glass beakers, 15 ml centrifuge tubes with caps, air operated homogeniser, 50 ml separating funnel, ELIT 8051 or (Mettler Tolledo™) NH_4^+-ISE, ELIT 003 double junction reference electrode, ELIT dual electrode head (ELIT 201), ELIT ion analyser (or pH/mV/Ion meter) with BNC socket, ammonium ion selective electrode (NH_4^+-ISE) system connected to pH meter or personal computer (PC) interface, standard electrode holder, magnetic stirrer with teflon-coatings set at 100 rpm, 100µl-1000 µl pipettes, 10ml syringes, low-lint tissue, 0.45 μm Fisher brand PTFE filters.

6.3.4 Preparation of reagents and standards

To obtain 1000 ppm NH_4^+, 2.965g anhydrous ammonium chloride (NH_4Cl) was dissolved in 1 litre DI water, since ammonium ion (NH_4^+) is a monovalent cation with a molar mass of 18.038 grams and 1000 ppm NH_4^+ is equivalent to 0.055 Molar.

Acrylamide standard solution of 0.1M was prepared by dissolving 7.11g acrylamide (AA) in 1 litre DI water. Tenfold serial dilution method was then used to prepare 0.01, 0.001, 0.0001 and $1x10^{-5}$M standards.

Carrez I solution was prepared by dissolving 3.6g of potassium hexacyanoferrate (II) trihydrate in 100ml de-ionised water, while Carrez II solution was prepared by dissolving 7.2g of zinc sulfate heptahydrate in 100ml de-ionised water.

Approximately 200 IU/mg of *Creative Enzymes* NATE-0809 acylamide amidohydrolase (AA-ase, EC 3.5.1.4) produced from *Pseudomonas aeruginosa* was added to 1000µl Tris buffer of pH 7.2. The reconstituted enzyme was immobilized by methods similarly described by Sathesh and Thatheyus in 2007. The immobilization was done by adding 1ml of the enzyme-buffer mix to 9ml of 2.5% sodium alginate solution, and transferring drops of the AA-*ase* -alginate mix into 100ml of 2.0% CaCl solution, using a 10ml syringe.

The resulting AA-*ase* beads produced were washed with the Tris buffer solution and transferred into a 50ml separating funnel. This served as the reaction vessel for the hydrolysis of any AA present in the sample. To remove the leftover analyte after each reaction the AA-ase beads were thoroughly washed with the buffer solution.

6.3.5 Food Sample Treatment and Analysis

A digital weighing balance was used to accurately weigh 1-10g food sample, which was consequently transferred into a 100ml beaker.

For the extraction of the analyte, about 10ml de-ionized water was added to the weighed food sample. Solid food samples with very low moisture content such as *chin-chin* were allowed to swell in the de-ionized water at 37°C for 2-10 minutes.

An air operated ultra-turrax T-25 high-speed homogenizer and analytical grade water were thereafter used for the blending and homogenization of the food samples.

Sufficient DI water was then added to the homogenized sample to make it up to the 100ml graduated mark.

Each homogenized food sample was subsequently deproteinized through the addition of 200-500µl Carrez reagents (I /II) and gentle stirring of the mix for 1 minute.

The fatty and oily portion of homogenized fat-rich samples such as *meat-pie* were separated through the addition of 3-5ml hexane or by refrigeration. The resulting top oily layer was then discarded. N.B It is advisable to separate the fat content of the food samples using cold temperature method as the PVC membrane of the ISE may be damaged by organic solvents such as hexane.

Approximately 10ml of the treated samples were transferred into15ml centrifuge tubes, which were centrifuged at 4000 rpm for 5-15 minutes.

The resulting supernatant were further cleaned using a 10ml syringe and 0.45 μm Fisher brand PTFE filter.

For the enzymatic release of the analyte, the filtered sample was transferred into the separating funnel with AA-ase beads and left for 5-10 min.

About 5ml of the resulting sample was collected into a 15ml centrifuge tube or 100ml beaker and immediately treated with a 10% v/v of ionic strength adjustment buffer –ISAB (about 500 μl of 1M $CuSO_4$ for 5ml sample).

The treated sample was further filtered using a 10ml syringe and 0.45 μm Fisher brand PTFE filter.

An ammonium (NH_4^+) ISE which has been preconditioned for 5-10 minutes with 1000ppm ammonium chloride (NH_4Cl) standard solution was used to check the performance of the NH_4^+ ISE-system. The A-ISE system was calibrated using 0.1-1000ppm of NH_4Cl standard solutions to which 10% v/v of ISAB has been added.

Another calibration curve was produced by using $1x10^{-5}$M– 0.1M of enzyme treated AA standard solutions. A 10% v/v of the ISAB was also added before recalibrating the NH_4^+ ISE. The molar concentrations of ammonium ion and AA present in the enzyme treated food sample were subsequently measured by using ELIT ion analyser (or pH/mV/Ion meter) connected to a personal computer (PC) data-recording interface.
The level of AA present in the food sample was then determined using the NH_4^+ ISE system and the generated calibration curves.

6.3.6 Expression of results

The concentration of acrylamide in food samples is commonly expressed in units such as mg/kg (ppm) or µg/kg (ppb). The A-ISE system, which generates results in mg or mol/L was used to determine the NH_4^+ (and AA) concentrations per unit volume of solution. Consequently, necessary calculations were done to have final readings in *mg/kg* or *µg/kg*.

To convert the AA concentrations produced by the ELIT Ion analyzer to mg/kg food sample, the weight of food sample used in *g* was divided by 1000.

For a minimum feasible sample volume of 5ml, the mathematical equation given below was applied.

$$\textit{AA (mg/kg) of food sample} = \frac{\textit{ISE Ion Analyser Reading (mg/1000 ml)} \times \textit{5ml}}{\textit{Weight of Sample Used}\ \textbf{(kg)}}$$

AA (µg/kg) of food sample= AA (mg/kg) of food sample X 1000

6.3.7 Interference:

The ammonium ion selective electrode biosensors based on immobilized *Pseudomonas aeruginosa* containing amidase activity may also have selectivity for amides such as acetamide and formamide (Silva et al., 2009). Hence, these cross reactions may limit the use of A-ISE for the quantification of acrylamide.

In addition, potassium ions with selectivity coefficients (SC) of 0.1 have been reported to interfere with ammonium ion measurements. The use of the *chemtool* section of the ELIT ISE/pH ion analyser software and K^+ ISE were used to determine the contributions of the

interfering ion to the readings obtained for the primary ion. Measured NH_4^+ not due to the effect of AA-ase was also corrected for in the samples.

6.3.8 Statistical Methods

IBM SPSS Statistics 26 software was used for the data analysis. One-way analysis of variance (ANOVA) method was employed to gain knowledge of the difference in acrylamide concentrations for the WA foods. The least significance difference (LSD) method was used as the post hoc test for group difference at α=0.05 (Table 6.2).

One-sample t-test (at α=0.05) was used to compare the benchmark levels of acrylamide for potato crisps, soft bread and cracker to similar WA foods including yam-chips, WA bread and *chin-chin* respectively (Table 6.1).

6.4. Results

Typical calibration curves obtained for the determination of ammonium ion and acrylamide is shown below. The curve shown in Figure 6.2 depicts the possible range of linearity for the measured ion.

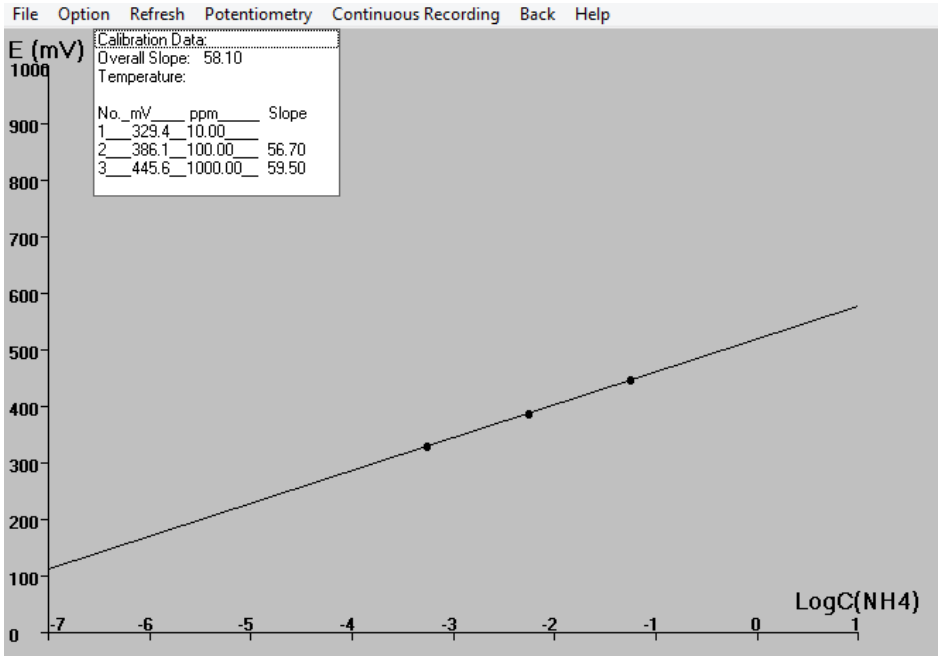

Figure 6.2. *A typical calibration curve for ammonium ion* (NH_4^+)

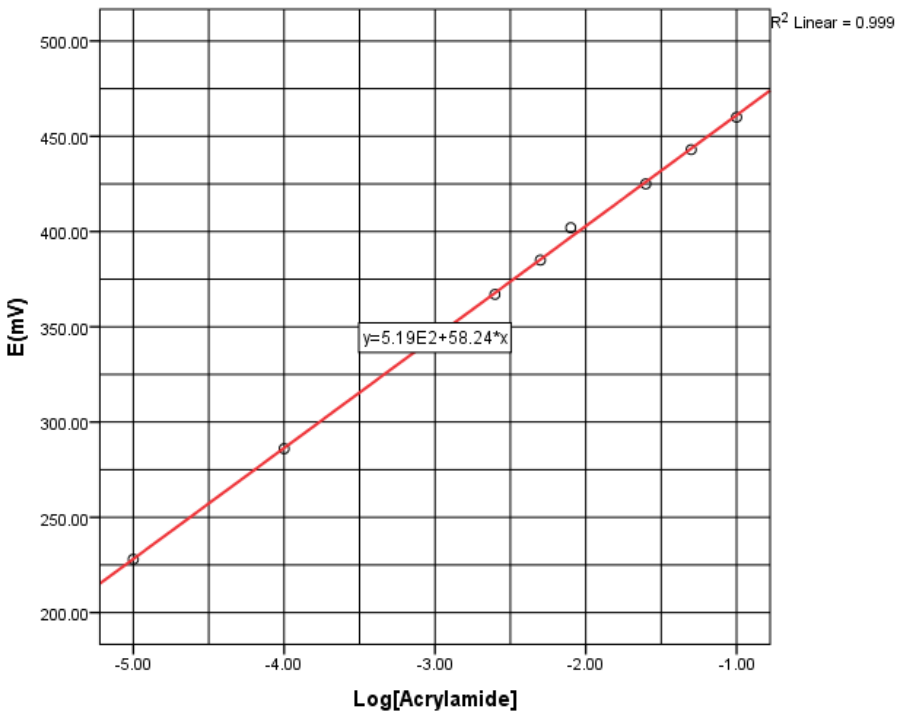

Figure 6.3. *Representative calibration curve for different acrylamide standards*

Overall, the calibration curves obtained showed a strong correlation coefficient and a good linear relationship for the logarithm of acrylamide concentration and the potentiometric values from the NH_4^+ released, Figure 6.2. According to Nernst equation, the sensitivity of the instrument is represented by the slope of the curve, which is 58.24 mV/log [AA].In addition, the instrument response time was determined to be about 15s.

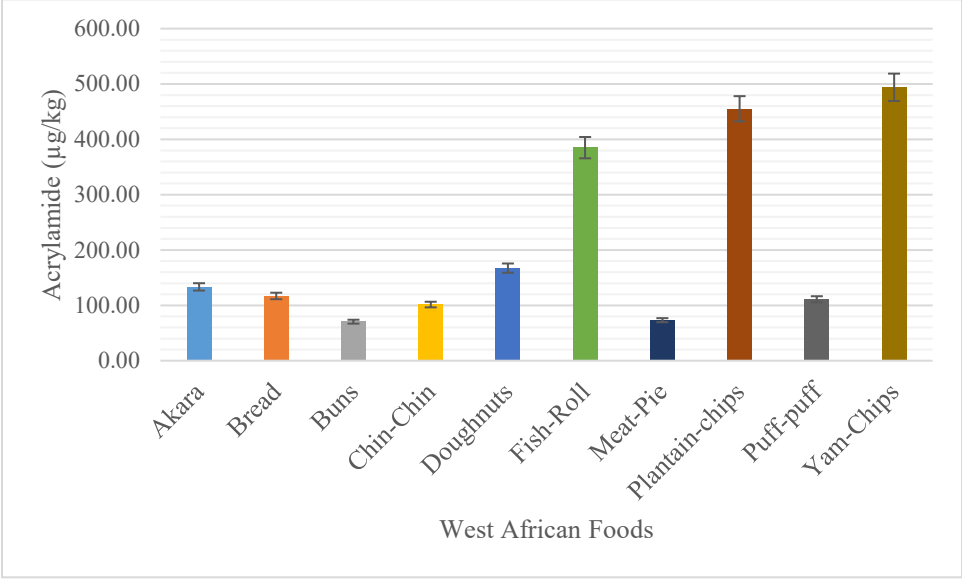

Figure 6.4. *Comparison of the acrylamide concentrations amongst popular WA foods*

6.4.1 Acrylamide concentrations for various WA foods

The mean acrylamide concentration measured for *akara*, *bread*, *buns, chin-chin*, *doughnuts*, *fish-roll*, *meat-pie*, *plantain-chips*, *puff-puff* and *yam-chips* were 133, 117, 71, 101, 167, 385, 73, 455, 111 and 494 µg/kg food sample respectively.

The minimum and maximum concentrations of acrylamide recorded for *akara*, *bread*, *buns chin-chin*, *doughnuts*, *fish-roll*, *meat-pie*, *plantain-chips*, *puff-puff* and *yam-chips* are shown in Table 6.2.

6.4.2 Comparison of acrylamide concentrations amongst various WA foods

Generally, there was a statistically significant difference in acrylamide concentrations for the WA foods at $\alpha=0.05$ i.e. $p< 0.05$. This was particularly the case when the acrylamide concentrations in *doughnut*, *fish-roll*, *plantain* and *yam-chips* were compared to the acrylamide levels in other WA foods (Table 6.3)

6.4.3 Comparison of the indicative values for soft breads, crackers, and potato crisps to the acrylamide levels in WA bread, chin-chin and yam-chips respectively

Two sets of indicative acrylamide values issued by the EC in 2013 and 2017 for EU foods including bread, crackers, and potato crisps were compared against similar WA foods used in this study. These values are not safety thresholds, but are to be used to investigate, identify and control steps in food processing which may lead to enormous formation of the contaminant (EC, 2013).

The descriptive statistics for the acrylamide concentrations obtained from the analyses of 120 samples of WA *bread* (BR), *chin-chin* (CN) and *yam-chips* (YC) are shown in Appendix C.

Overall, the use of t- test gave results which suggested that WA *bread, chin-chin* and *yam-chips* have acrylamide levels which are all different from the benchmark levels set by the European commission for similar foods.

For the former benchmark level set for soft breads in 2013, analysis of 120 samples showed that WA bread has acrylamide levels lower than the benchmark target set i.e. 150 µg/kg However, WA bread exceeded the recent indicative value set in 2017 for soft breads i.e. 100 µg/kg. Appendix D and Table 6.1.

In the case of WA *chin-chin*, the acrylamide levels measured were below both indicative values set for crackers in 2013 and 2017. Appendix E and Table 6.1.

Also, for WA yam-chips, the acrylamide levels measured were below both indicative values set for potato crisps in 2013 and 2017. Appendix F and Table 6.1.

Since the EC list of indicative acrylamide values for foods do not cover home-made foods (EC, 2011) such as buns, meat-pies and puff-puff, there was no benchmark level to indicate if their acrylamide concentrations were high or low.

Although there are no indicative values for some EU foods similar to certain WA foods considered in this study, the acrylamide levels in closely related Caribbean foods have been studied (Bent et al., 2012). **Table 6.2** compares the AA levels in WA foods including akara, bread, buns, meat-pie, puff-puff and plantain-chips to similar Caribbean foods.

Table 6.2 *Comparison of the acrylamide levels in some WA foods to similar Caribbean foods (Bent et al., 2012)*

WA Foods	**AA (µg/kg)**	**Similar Caribbean Foods**	**AA (µg/kg)**
Akara	133	*Peanut cake*	500
Bread	117	*Bread*	70-150
Buns	71	*Rock Buns*	300
Meat-pie	73	*Beef-patty*	2270
Puff-puff	111	*Dumplings*	2440-3360
Plantain-chips	455	*Plantain-chips*	120-140

Table 6.3. *Acrylamide values recorded for the selected WA foods*

WA Food Samples	**Minimum Acrylamide Concentration in (µg/kg) food sample**	**Maximum Acrylamide Concentration in (µg/kg) food sample**
Akara	32	243
Bread	53	200
Buns	20	127
Chin-Chin	31	151
Doughnuts	31	387
Fish-Roll	76	630
Meat-Pie	23	160
Plantain-Chips	94	727
Puff-Puff	23	416
Yam-Chips	77	729

Table 6.4. *Significance of difference in acrylamide concentrations for West African foods*

Comparison of Acrylamide for WA Foods	**LSD Test of Significant Diff at α=0.05**
Akara Vs *Bread*	Not Significant (=0.243) i.e. > 0.05
Akara Vs *Puff-Puff*	Not Significant (=0.110) i.e. > 0.05
Bread Vs *Chin-chin*	Not Significant (=0.269) i.e. > 0.05
Bread Vs *Puff-Puff*	Not Significant (=0.667) i.e. > 0.05
Buns Vs *Meat-Pie*	Not Significant (=0.846) i.e. > 0.05
Chin-Chin Vs *Puff-Puff*	Not Significant (=0.499) i.e. > 0.05
Doughnut Vs Other WA foods	Significant i.e. < 0.05
Fish-Roll Vs Other WA foods	Significant i.e. < 0.05
Plantain-Chips Vs Other WA foods	Significant i.e. < 0.05
Yam Chips Vs Other WA foods	Significant i.e. < 0.05

6.5 Discussion

Following the analysis of the WA foods, buns was found to be the food with the least amount of acrylamide (70 μg/kg), while *yam-chips* was noted to be the food with the highest amount of acrylamide (494 μg/kg). Several studies on the acrylamide levels in potato chips , fries and many other carbohydrate rich foods supports this observation (Amrein et al., 2003, 2004, Halford et al., 2012a, Muttucumaru et al., 2008).

The high acrylamide recorded for *fish-roll* can be explained by the main ingredients used for its preparation i.e. Wheat and fish which are known to be high in asparagine.

The high acrylamide recorded for *plantain* and *yam chips* can be explained by the presence of high carbohydrate and glucose in the materials required for their preparation.

The insignificant difference in the acrylamide concentration for WA pair of foods such as *bread-puff-puff* and *buns-meat-pie* is a pointer to the fact that they are prepared from similar ingredients and formulations.

Most of the WA foods had acrylamide levels, which are below the benchmark levels set by the European Commission in 2013 and 2017 for similar foods. For example, yam chips was found to contain acrylamide levels lower than both benchmark levels set for potato crisps in 2013 and 2017. This observation suggests that certain processes and materials used for the production of the WA foods do not support enormous release of the contaminant. Previous studies on the acrylamide levels in closely related Caribbean foods (Bent et al., 2012) also support these findings.

However, the relatively higher levels of AA in WA bread compared to the indicative value for soft bread (European Commission, 2017) and the concentrations of the contaminant in Caribbean breads (Bent et al., 2012), suggests that the process for production of the WA food needs to be investigated to identify and control any step which may increase the production of the contaminant.

For the mitigation of acrylamide in heat-processed foods, Food Drink Europe recommends that food business operators should treat raw food materials with food grade asparaginase, and use starting materials, which have been blanched and soaked in solvents such as warm water (FDE 2013, 2016). Although the mitigation strategies have not been considered for WA foods such as *fish-roll* and *plantain chips*, this is an important area of study to explore.

Previous studies also supports the use of methods including blanching and soaking of raw potato slices for the reduction of the levels of reducing sugars and asparagine which are essential precursors of acrylamide (Grob et al., 2003; Fiselier et al., 2005; Haase et al., 2003, 2004; Pedreschi et al., 2004, 2005; Wicklund et al 2005). Compared to warm water, blanching in cold water was shown to be less effective in extracting glucose and asparagine (Grob et al., 2003; Kita et al., 2004; Pedreschi et al., 2004). Also, compared to water extraction, the use of food grade acids for the extraction of asparagine and glucose have been shown to be a more effective strategy for the reduction of acrylamide levels in food products Kita et al., 2004). The treatment of food materials with food grade acids such as acetic and citric acids have been reported to cause remarkable reductions in the asparagine and acrylamide levels of the final food products (Jung et al., 2003). However, it must be noted that the use of citric acid may have an undesirable effect on the taste of the food products (Gama-Baumgartner et al., 2004). Compared citric acid, the treatment of food materials with acetic acid have been reported to produces more acceptable taste of the food product (Kita et al., 2004). The treatment of food materials with fermentative organisms including yeasts and lactic acid bacteria have also been reported to effectively reduce levels of asparagine and acrylamide levels in food products.

Consequent studies will explore the role of the application of mitigation methods such as enzymatic hydrolysis, acidification, blanching, fermentation and soaking of food materials on the reduction of acrylamide in WA foods such as *akara* and *plantain chips.*

6.6 Conclusion

The study demonstrated that several WA foods including *chin-chin, doughnuts, fish-roll, plantain* and *yam-chips* are significantly different in their acrylamide concentrations when compared to other WA foods i.e. $p < 0.05$. This is also a reflection of the varying concentrations of glucose and asparagine present in these WA foods.

Future studies on the reduction of acrylamide in the WA foods discussed should focus on the effect of the application of methods such as acidification, blanching, fermentation and soaking of food materials.

6.7 Summary

The level of acrylamide in 10 popular WA foods was determined through potentiometric analytical method based on the application of acylamide amidohydrolase- EC 3.5.1.4 from *Pseudomonas aeruginosa*. One hundred and fifty (150) samples of *akara*, *bread*, *buns*, chin-chin, *doughnuts*, *fish-roll*, *meat-pie*, *plantain-chips*, *puff-puff* and *yam-chips* were tested. Generally, the mean acrylamide (70 μg/kg) for *buns* was the lowest while the average acrylamide (494 μg/kg) for *yam-chips* was the highest. Significant differences in AA levels (i.e. $p < 0.05$) were obtained after comparison of the WA foods. Also, the descriptive statistics and t-tests for 120 samples of WA foods including bread, chin-chin and yam chips showed that they contain acrylamide levels lower than the indicative values set for similar EU foods.

Chapter 7

Effects of different cooking methods, times and temperatures on the level of acrylamide in common heat-processed West African foods

CHAPTER 7. EFFECTS OF DIFFERENT COOKING METHODS, TIMES AND TEMPERATURES ON THE LEVEL OF ACRYLAMIDE IN COMMON HEAT-PROCESSED WEST AFRICAN FOODS

7.1 Overview

This chapter discusses the impact of different cooking methods, times and temperatures on the formation of acrylamide in popular WA foods.

7.2. Introduction

Different cooking conditions have been reported to increase the levels of process contaminant such as furans, n-nitrosamines and polycyclic aromatic hydrocarbons (PAHs).
An important furanic compound called 5-hydroxymethylfurfural (HMF) is also formed through Maillard Reaction (MR) when foods rich in carbohydrate are subjected to high thermal treatments and dehydration (Ames, 1992;Kroh, 1994).

Although the level of HMF drastically increases as the cooking or storage temperatures increase, its formation has also been reported at lower temperatures under acidic conditions (Lee and Nagy, 1990)

HMF is also used for monitoring the thermal conditions applicable to cereal products such as pasta drying (Resmini, *et al.,* 1993), bread baking (Ramírez-Jiménez, *et al.*, 2000a; Ramírez-Jiménez, *et al.,* 2000b), bread slices toasting (Ramírez-Jiménez, 1998) as well as extrusion of baby cereals (Fernández–Artigas, *et al.*, 1999; Ramírez-Jiménez, *et al.,* 2003) and breakfast cereals (García-Villanova, *et al.*, 1993).
In addition, potentially hazardous compounds called melanoidins, which are also derivative of MR are widely distributed in thermally processed food (Morales, 2002; Bekedam *et al.,* 2006)
The most recently identified MR derived process contaminant called acrylamide (AA) is also formed during frying, roasting, and baking of foods (Rosen and Hellenas, 2002).
Acrylamide is well known to be produced by the reaction of asparagine with carbonyl groups of reducing sugars and bioactive compounds.
It was first associated with municipal water in 1997, before it was detected in heat-processed foods in 2002. Its presence in the water body was as a result of a large water leakage into the environment from a tunnel under construction in Sweden. This led to the death of large

number of fish and paralysis of cattles rared close to the building site.

Acrylamide has been observed to elicit neurotoxic, genotoxic and carcinogenic effect in rat models. It is not usually found in non-thermally processed foods and highest levels have been found in fried potato products, bread and coffee. The presence of AA was also reported in foods such as hazelnuts, almonds, olives (Amrein, *et al.*, 2005) and foods not subjected to severe thermal treatments, such as dried plums, pears, and apricots(Amrein, et al., 2007). Heat-treated foods derived from animal tissues such as meat and fish, generally exhibit low or inconsiderable levels of acrylamide (Swedish National Food Administration, 2002; EFSA, 2009).

The hypothesis that heating of foods would contribute to the acrylamide levels in foods originated from observed increments in the contaminant after certain foods were heated in ovens, frying pans and microwaves at temperatures above 160°C. (Castle and Eriksson 2005; Tareke et al., 2000, 2002).

According to Burch (2007), acrylamide content of foods is determined by the time and temperature of food processing, and not the food processing method itself. For examples, different processing methods such as, frying, roasting, baking and blanching does not necessarily result in different levels of acrylamide production. This 'cooking time effect' is thought to be a result of a combination of decreasing moisture levels towards the latter stages of cooking time (Palazoğlu et al., 2010), and increased surface temperature of the food product after a longer cooking time. Romani et al., (2008) also associated an exponential increase in acrylamide concentrations to longer period of thermal treatments of carbohydrate-rich foods. For WA countries, the processing and preservation of foods depends largely on thermal methods including baking, frying, roasting and smoking of foods.

In addition, most of these foods are rich in carbohydrates and protein precursors necessary for acrylamide formation (Chapter 4 and 5). These heat-dependent methods of preservation are important since electric power supply is unreliable in this part of the world.

Many studies conducted after the discovery of AA in potato fries and chips have focused on the influence of thermal methods and conditions on the presence, levels and mitigation of the toxicant in several foods excluding popular WA foods (European Commission, 2013; EFSA, 2012; Wang et al., 2008).

Consequently, this study considers the impact of different thermal methods and treatments on the AA in common WA foods, such as Chin-chin.

7.2.1. Problem Statement:

Although several WA heat-processed foods are produced in several regions of the world, no study has considered the impact of commonly used cooking methods, times and temperatures on the formation of the process contaminant in these WA foods. In addition, in spite of the unknown concentrations of the contaminant in these WA foods they are been sold and consumed in and out of WA countries.

7.2.2 Research Rationale

The justifications for the study are listed below.

(a) *The substantiation of the toxic properties of acrylamide*: The categorization of AA as a group 2A carcinogenic substance and the in vivo demonstration of its neurotoxic effects (IARC, 1994; JECFA, 2010) supports preceding studies on the toxic features of the contaminant.

(b) *Progressive studies on the presence of acrylamide in heat-treated foods*: Since 2002 when AA was first discovered in thermally processed foods several studies have considered the impact of different heat treatments on carbohydrate-rich foods(Amrein et al, 2005; Croft et al., 2004; European Commission, 2013; EFSA, 2012; IFST, 2017; Leung et al., 2003; Lineback et al., 2005; Murkovic, 2004; Ono et al., 2003; Tareke et al., 2002; Roach et al., 2003; Rosén and Hellenäs, 2002).

(c) *The public awareness campaign by FSA*: The intense effort of the Food Standard Agency (FSA) in creating public awareness on the formation and health implications of this toxic compound in heat-treated foods. The FSA warnings on the potential cancer risk of consuming browned toast and crispy roast potatoes (The Telegraph, 2017).

(d) *Absence of advices for WA food producers and consumers*: The FDE toolbox tagged "Code of Practice for Managing Acrylamide Formation" contain important information for food business operators on the appropriate use of thermal conditions to reduce acrylamide formation in European foods. For WA countries, there is no manual with information to assist food business operators (FBO) in reducing the acrylamide content of their products.

(e) *The unknown impact of different cooking methods, times and temperatures on the acrylamide levels in WA foods:*

Since the discovery of acrylamide in heat-treated foods, no research has focused on the influence of the use of different cooking methods, times and temperatures on the amount of acrylamide produced in popular WA heat-treated foods such as *Akara* and *Chin-chin* to mention a few. This study aims to provide WA food suppliers important advices concerning the correct use of cooking methods, temperatures and times to reduce the formation of dietary acrylamide.

7.2.3 Research Questions

The questions for the study are listed below.

(i) How does the use of different cooking methods (e.g. baking, frying, roasting) affect the AA content of these WA foods?
(ii) Is a particular cooking method better than others, with respect to the amount of acrylamide released?

7.2.4 Research Hypotheses

The alternative and null hypotheses based on the research questions are listed below.

(a) Null hypothesis (H_o; $[\mu_b] = [\mu_f] = [\mu_r]$) No difference in the concentration of AA produced by baking, frying or roasting;
(b) Research hypothesis (H_a; $[\mu_b] \neq [\mu_f] \neq [_r]$) Different cooking methods produce different amount of AA.

Thus, this study aimed to evaluate the contributions of different cooking methods and temperatures to the formation of the contaminant in WA foods including *akara, bread, buns, chin-chin, doughnut, fish-roll, meat-pie, plantain-chips, puff-puff,* and *yam-chips*.

The study evaluated the effect of traditional cooking methods such as baking, frying and roasting on the acrylamide content of quint-essential WA foods. Samples assessed include *akara (fried bean cake)*, *bread, buns, chin-chin, doughnut, fish roll, meat-pie, plantain chips. puff-puff,* and *yam chips.*

7.2.5 Research Objectives:

Based on the research questions and hypotheses, the objectives of the study were to:

(1) Preparation of the aforementioned WA foods using recognized cooking methods such as baking, frying, and roasting
(2) Estimation of the acrylamide produced as a result of using different cooking methods and temperatures.
(3) Use of statistical analytical methods e.g. ANOVA to compare the concentrations of acrylamide produced using the different cooking methods and temperatures.
(4) Determination of which method is best for each type of food with regards to acrylamide formation.

7.3. Materials and methods

7.3.1 Food sample preparation:

Different bakeries and restaurants producing WA foods in London were consulted to produce the selected foods using the pre-selected temperatures of cooking. The materials listed in Table 1.1 were used for the preparation of the foods. Equal portions of each WA foods were prepared by baking and frying at 150, 180 and 210 °C. The study also evaluated the effect of cooking at each of the selected temperatures for 5, 10 and 15 mins.

A total of 150 samples of each WA food were used for each cooking method studied using the three temperatures and a particular period of time.

7.3.2 Food Sample Analysis:

The level of the contaminant was determined by potentiometric analytical method based on the use of ammonium ion selective electrode and immobilised acrylamide amidohydrolase.

The effect of baking and frying at the different temperatures was studied. For each cooking temperature considered, 50 samples of the selected WA foods were prepared. Same portions and dimensions of food materials were processed using the different cooking methods. Unheated formulations were used as control. For equipment, apparatus, materials and procedures for acrylamide analysis see Chapter 6.

7.3.3. Statistical Analysis:

For the WA food samples obtained through different cooking conditions, the mean acrylamide and the standard deviation were calculated using IBM SPSS statisitics 26 and MS Excel 2016 softwares. The independent T- test for equality of sample means and ANOVA tests for significant differences at $\alpha=0.05$ were calculated for acrylamide concentrations resulting from the use of two main cooking methods and the three temperature respectively. The effect of interaction between cooking methods and temperatures used for each WA food was also determined using 2-way ANOVA at $\alpha=0.05$.

7.4. Results

Generally, independent T-test for equality of sample means at $\alpha=0.05$ showed no statistical significant difference ($p > 0.05$) in the AA produced by using baking and frying at the same temperature. However, ANOVA for the AA concentrations resulting from varying the cooking temperature indicated that increasing baking and frying temperatures effectively raised the amount of the process contaminants i.e. ($p < 0.05$).

7.4.1 Mean acrylamide concentration for WA foods processed by different cooking methods and temperatures.

The determination of acrylamide in the WA foods processed at 150, 180, 210 °C by different cooking methods and the statistical analysis by MS Excel 2016 software gave results shown in Table 7.1. Highest readings for the contaminant was noted for WA foods processed at 210°C, while the lowest measurements were obtained for those processed at 150°C. the lowest amounts of acrylamide (25±8 µg/kg) was detected in *buns* baked at 150°C, while the highest levels of the contaminant equal to 703±27 and 706±13 µg/kg respectively were noticed in yam and plantain chips fried at 210°C.

Table 7.1. *Acrylamide levels in popular heat-processed WA foods*

WA Food Samples	**Mean AA±S.D (µg/kg) at 150 °C**	**Mean AA±S.D (µg/kg) at 180 °C**	**Mean AA±S.D (µg/kg) at 210 °C**	**Mean AA±S.D (µg/kg) for Control**
Baked Akara	36±3	172±17	194±17	7±3
Baked Bread	72±7	184±9	195±3	8±2
Baked Buns	25±8	90±9	97±15	7±2
Baked Chin-Chin	53±18	119±11	134±8	8±3
Baked Doughnut	60±23	117±27	326±26	9±3
Baked Fish-Roll	233±56	404±57	519±42	6±2
Baked Meat-Pie	28±4	81±14	112±18	7±3
Baked Plantain-Chips	140±35	563±86	603±84	7±2
Baked Puff-Puff (Bofrot)	46±23	120±33	168±61	8±3
Baked Yam-Chips	211±56	578±72	693±43	10±2
Fried Akara	69±7	174±16	191±20	5±2
Fried Bread	70±7	185±9	195±3	6±3
Fried Buns	26±10	91±9	94±18	8±2
Fried Chin-Chin	70±7	122±19	138±16	9±2
Fried Doughnut	57±22	109±17	328±29	7±2
Fried Fish-Roll	138±16	396±40	523±39	8±2
Fried Meat-Pie	30±5	85±13	115±19	7±3
Fried Plantain-Chips	189±63	576±70	706±13	12±4
Fried Puff-Puff (Bofrot)	47±24	119±31	183±77	7±2
Fried Yam-Chips	203±50	576±70	703±27	10±4
Roasted Plantain-Chips	222±66	577±71	500±212	12±3
Roasted Yam-Chips	244±43	574±69	705±16	10±3

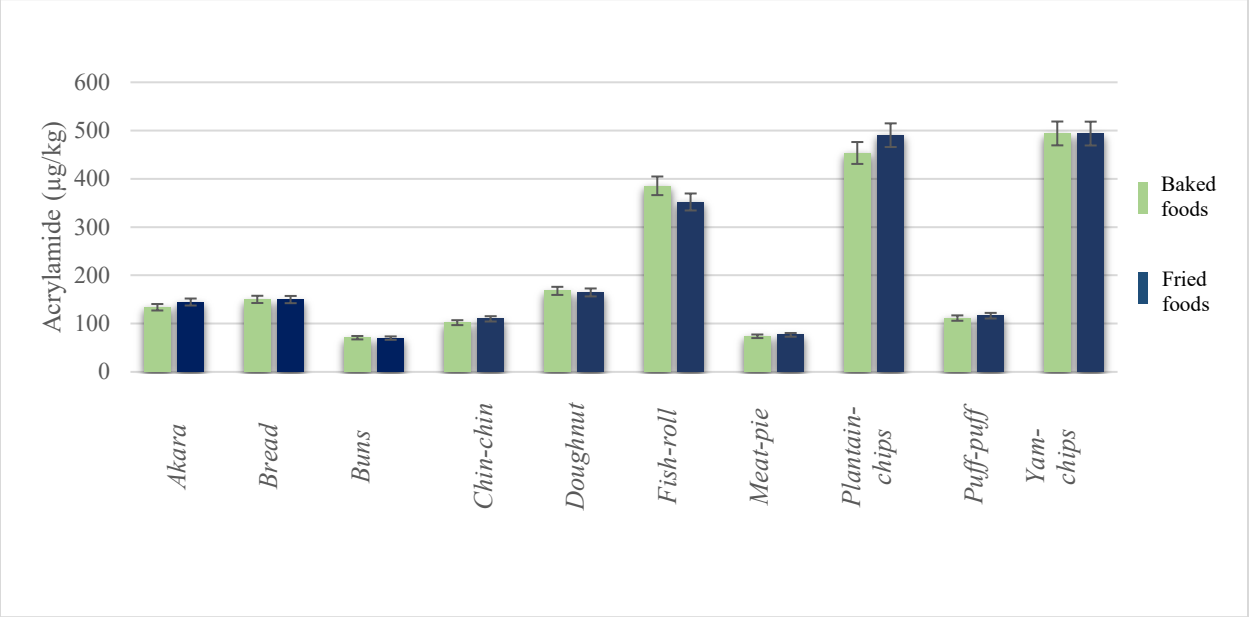

Figure 7.1. *Overall effect of baking and frying on the AA levels in WA foods*

7.4.2 Overall effect of cooking methods on the acrylamide formed in the WA foods

The main cooking methods used for the preparation of the WA foods were baking and frying. The overall effect of the two cooking methods on the production of acrylamide in the WA foods is shown in Figure 7.1. The graph shows slight contribution of cooking methods to the formation of acrylamide in WA foods such as *chin-chin* and *fish roll*. For most of the WA foods the effect of cooking method was insignificant i.e. p> 0.05, Table 7.2.

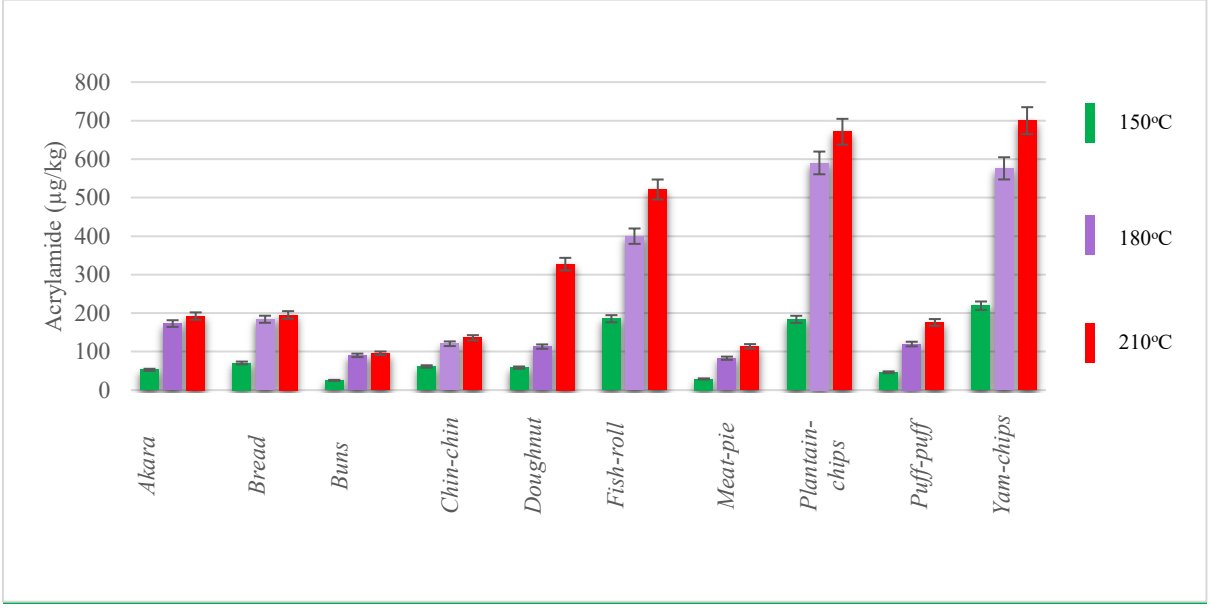

Figure 7.2. *Overall effect of processing temperatures on the AA levels in WA foods*

7.4.3 Overall effect of processing temperatures on the acrylamide formed in the WA foods

The temperatures selected for the preparation of the WA foods were 150, 180, 210 °C. For each cooking method and WA food, the different processing temperatures were applied. The overall effect of the selected temperatures on the production of acrylamide in the WA foods is shown in Figure 7.2. The chart shows the remarkable contribution of higher temperatures to the formation of acrylamide in the WA foods. For all the WA foods studied, the effect of increasing the temperatures was significant i.e. $p < 0.05$, Table 7.2.

Table 7.2. *Significance of the acrylamide contributed to each WA food by the cooking methods and processing temperatures*

WA Foods	**Significance of 2-tailed T-Test for the cooking methods at α=0.05**	**Significance of ANOVA for the processing temperatures at α=0.05**	**Significance of ANOVA for the interaction between cooking methods and processing temperatures at α=0.05**
Akara	0.145	*<0.001*	*<0.001*
Bread	0.956	*<0.001*	*0.275*
Buns	0.831	*<0.001*	*0.386*
Chin-Chin	0.054	*<0.001*	*0.001*
Doughnuts	0.814	*<0.001*	*0.28*
Fish-Roll	0.050	*<0.001*	*<0.001*
Meat-Pie	0.500	*<0.001*	*0.699*
Plantain-Chips	0.157	*<0.001*	*<0.001*
Puff-Puff	0.547	*<0.001*	*0.412*
Yam-Chips	0.815	*<0.001*	*0.017*

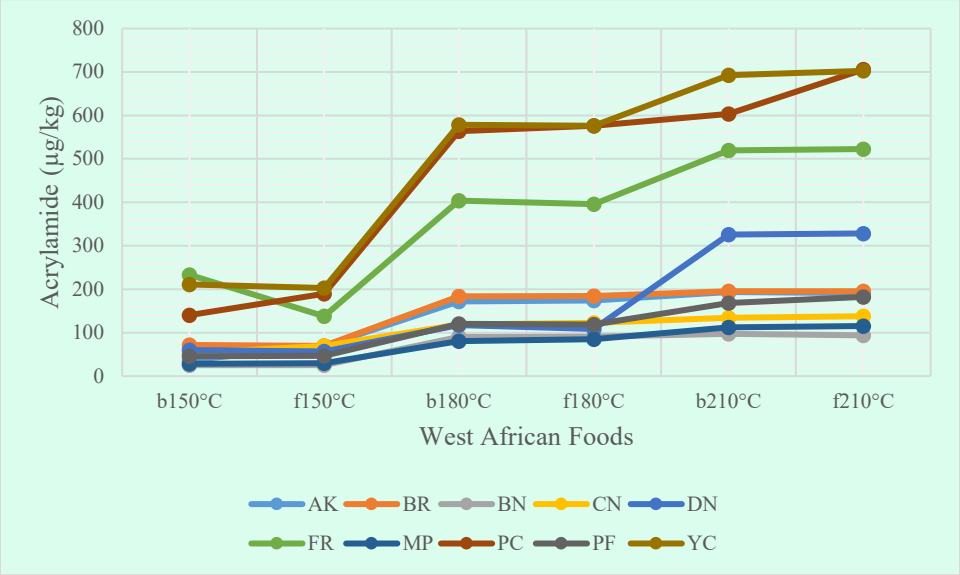

Figure 7.3. *Combined effect of cooking methods and processing temperatures on the acrylamide formed in the WA foods.*

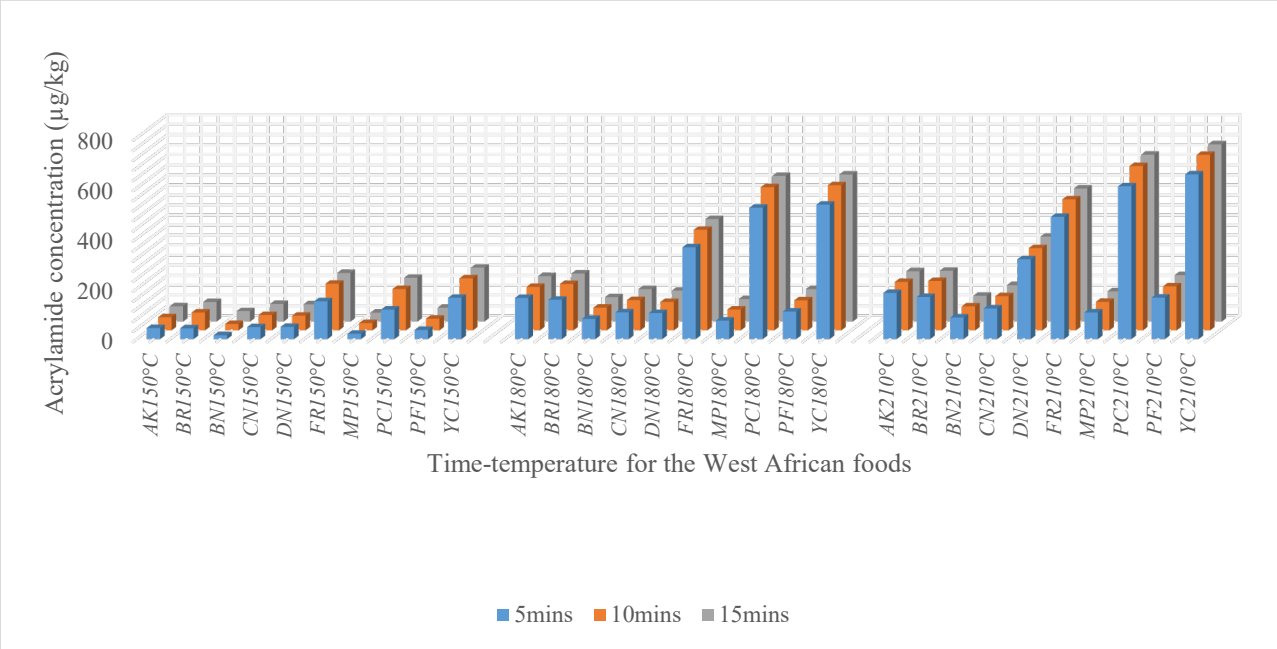

Figure 7.4. *Overall effect of processing times on the AA levels in WA foods*

Table 7.3. Time-temperature effect on the acrylamide produced in the WA foods

WA Food Samples	Time (t) in mins	Mean±SD [AA] from baking at 150°C (µg/kg)	Mean±SD [AA] from frying at 150°C (µg/kg)	Mean±SD [AA] from baking at 180°C (µg/kg)	Mean±SD [AA] from frying at 180°C (µg/kg)	Mean±SD [AA] from baking at 210°C (µg/kg)	Mean±SD [AA] from frying at 210°C (µg/kg)
Akara	5	27±4	61±9	163±17	165±16	185±17	182±21
	10	36±3	69±7	172±16	174±11	194±17	191±20
	15	44±4	78±8	180±17	182±16	202±18	199±19
Bread	5	44±8	42±8	156±9	157±9	167±5	168±5
	10	72±6	70±7	184±9	185±7	195±3	198±6
	15	79±7	77±8	191±8	192±9	202±4	203±4
Buns	5	15±6	16±7	80±9	81±8	88±15	84±18
	10	25±8	26±10	90±8	91±9	97±14	94±16
	15	33±7	51±13	97±9	98±8	105±15	101±19
Chin-chin	5	39±16	56±8	105±12	108±18	120±8	124±17
	10	53±18	69±7	119±11	122±19	134±9	138±16
	15	62±19	79±9	128±12	131±19	143±8	147±18
Doughnut	5	50±24	47±22	108±27	99±17	316±26	319±29
	10	60±23	57±21	117±26	109±18	326±25	328±27
	15	71±23	67±22	128±26	119±17	337±28	339±29
Fish-roll	5	198±57	103±18	369±61	361±43	484±44	488±40
	10	233±56	138±16	404±57	396±40	519±42	523±39
	15	241±55	146±17	412±58	404±40	528±43	531±38
Meat-pie	5	21±5	22±7	73±14	74±13	105±18	107±18
	10	28±4	30±5	81±14	85±13	112±17	115±19
	15	35±5	37±6	88±13	92±14	119±17	122±20
Plantain-chips	5	93±35	142±63	516±84	529±68	556±84	659±17
	10	140±34	189±62	563±86	576±70	603±83	704±13
	15	150±35	199±63	573±87	586±69	613±83	715±12
Puff-puff	5	36±24	37±25	110±34	109±31	158±61	172±77
	10	46±23	47±24	120±33	119±30	168±63	182±78
	15	55±24	56±23	130±32	129±31	178±61	192±76
Yam-chips	5	169±56	160±52	536±74	533±70	650±44	660±27
	10	211±55	203±50	578±72	576±69	693±43	703±26
	15	219±56	211±51	586±73	584±71	700±43	710±27

7.4.4 Synergistic effect of cooking methods and processing temperatures on the acrylamide formed in the WA foods

The combined effect of the cooking methods and temperatures used for the study is shown in Figure 7.3.

The chart shows the effect of the interaction between processing temperatures and cooking methods on formation of acrylamide in the WA foods. For WA foods such as *fish-roll*, and *plantain-chips* a significant interaction effect was observed i.e. $p < 0.05$, Table 7.2.

7.4.4.1 Acrylamide formation in akara prepared using different cooking conditions

Although there was no statistically significant difference in the amount of acrylamide contributed by the cooking methods, the frying of akara at 150°C produced nearly twice the amount contributed by baking. In addition, the use of 210°C contributed more than fivefold increase in the amount of acrylamide compared to the processing temperature of 150°C. In comparison to the use of 180°C, the application of the processing temperature of 210°C for WA akara produced almost 20 µg/kg additional acrylamide.

Also, nearly 10 µg/kg additional acrylamide was formed by increasing the processing time from 5 to 10mins, and 10 to 15 mins.

7.4.4.2 Acrylamide formation in bread prepared using different cooking conditions

For bread, the use of frying did not produced any remarkable difference in the level of acrylamide compared to baking. However, the baking of bread at 150°C produced 2 µg/kg more acrylamide than frying. The use of 180°C caused the formation of acrylamide which is more than twice the amount produced at 150°C. Further increase of the processing temperature to 210°C generated almost 10 µg/kg additional acrylamide.

In addition, about 30 µg/kg additional acrylamide was formed by increasing the processing time from 5 to 10mins, while the change of processing time from 10 to 15 mins caused an increase in the production of acrylamide by levels greater than 5µg/kg.

7.4.4.3 Acrylamide formation in buns prepared using different cooking conditions

The difference in the amount of acrylamide contributed by the cooking methods alone was not statistically significant. However, the baking of buns at 210°C produced about 3µg/kg more acrylamide than frying. In addition, the use of 210°C contributed more than threefold the amount of acrylamide produced using the processing temperature of 150°C. The change of processing temperature from 180°C to 210°C only resulted in slight increment in acrylamide of about 5 µg/kg.

Approximately 10 µg/kg additional acrylamide was formed by increasing the processing time from 5 to 10mins, while the transition in processing time from 10 to 15 mins produced acrylamide concentrations greater than 5 µg/kg.

7.4.4.4 Acrylamide formation in chin-chin prepared using different cooking conditions

In the case of chin-chin, a slight statistical significant difference in the amount of acrylamide contributed by the cooking methods was obtained. The amount of acrylamide contributed by frying of chin-chin at 150°C was about 17 μg/kg more than that produced by baking at the same temperature. In addition, the acrylamide produced by frying at 180°C and 210°C were about 3 and 4 μg/kg more than those formed through baking at the same temperatures.

The application of the processing temperature of 210°C contributed more than twofold increase in the amount of acrylamide compared to the processing temperature of 150°C.
Acrylamide production in chin-chin was reduced by almost 20 μg/kg with the use of 180°C instead of 210°C.
The amount of acrylamide formed by increasing the processing time from 5 to 15mins was greater than 13 μg/kg. The changing of processing time from 5 to 10 mins created acrylamide levels greater than 4 μg/kg.

7.4.4.5 Acrylamide formation in doughnut prepared using different cooking conditions

For doughnut, the difference in the amount of acrylamide contributed by the cooking methods was statistically insignificant**.** However the amount of acrylamide formed by baking of doughnut at 180°C was about 8 μg/kg more than that produced by frying at the same temperature.

The processing of doughnut at 210°C contributed more than fivefold the amount of acrylamide produced using 150°C. Also, the amount of acrylamide produced at 150°C was about half of that produced at 180°C.
About 10 μg/kg less acrylamide was produced in doughnut by applying processing time of 5 min instead of 10 mins. Similar effect was noted by using processing time of 10 mins instead of 15mins.

7.4.4.6 Acrylamide formation in fish-roll prepared using different cooking conditions

For fish-roll, there was a notable change in the acrylamide concentration by applying same cooking method at different processing temperatures, and a slight statistical significance for the difference in the amount of acrylamide produced by the cooking methods at same temperature. Although the difference in acrylamide concentrations resulting from the use of the cooking methods was statistically insignificant, the baking of fish-roll at 150°C produced about 95 μg/kg

more acrylamide than frying at same temperature. Also, baking fish-roll at 180°C created approximately 8 µg/kg more acrylamide than frying at the same temperature. At higher temperature of 210°C, frying produced about 4 µg/kg more acrylamide in fish-roll than baking.

Overall, the amount of acrylamide formed at 150°C was less than half of the concentrations produced at 210°C. The application of 180°C instead of 150°C for the processing of fish-roll also increased the acrylamide concentration by more than 170 µg/kg.
In addition, about 30 µg/kg additional acrylamide was formed by increasing the processing **time** from 5 to 10mins, while the change of processing time from 10 to 15 mins caused an increase in the production of acrylamide by levels greater than 5µg/kg.

The use of the processing time of 5 mins produced about 35 and 43 µg/kg less acrylamide concentrations compared to the heating period of 10 and 15 mins respectively.

7.4.4.7 Acrylamide formation in meat-pie prepared using different cooking conditions
The effects of cooking methods on the acrylamide formation in meat-pie were noticed, but not as statistically significant as the influence of processing temperatures and times.
For the three temperatures (150°C, 180°C and 210°C) frying contributed about 3 µg/kg more acrylamide than baking at the same temperatures.
The application of the processing temperature of 210°C resulted in the formation of about four times the amount of acrylamide produced at 150°C.
In comparison to the use of 210°C for the processing of meat-pie, acrylamide formation was reduced by approximately 30 µg/kg with the use of 180°C.
The concentrations of acrylamide resulting from increasing the processing **period** from 5 to 15mins was about 14 µg/kg. Additional concentrations of acrylamide close to 8 µg/kg were formed by changing the processing time from 5 to 10 mins.

7.4.4.8 Acrylamide formation in plantain-chips prepared using different cooking conditions
The use of same cooking method at different processing temperatures produced remarkable changes in the acrylamide concentrations for plantain-chips. Also, the application of the different cooking methods at same temperature caused notable, but statistically insignificant differences in levels of acrylamide formed. The amount of acrylamide formed by frying of the plantain-chips at 150°C was about 49 µg/kg more than that produced by baking at the same temperature. The

effect of frying was more pronounced during processing at 210°C and slightly impactful at 180°C. For the processing temperatures 180°C and 210°C, frying produced 13 and 103 μg/kg acrylamide more than baking. Compared to baking and frying, roasting was more effective at 150°C than other processing temperatures. The acrylamide contributed by roasting at 150°C was 33 and 82 μg/kg more than the amount produced at the same temperature through frying and baking respectively.

Overall, the processing of plantain-chips at 210°C contributed about fourfold the amount of acrylamide produced using 150°C. Also, the amount of acrylamide produced at 150°C was less than half of that produced at 180°C.

For the different cooking methods, about 43 μg/kg less acrylamide were produced in plantain-chips by applying processing time of 5 mins instead of 10 mins. In addition, about 10 μg/kg additional acrylamide was formed by increasing the processing time from 10 to 15mins.

7.4.4.9 Acrylamide formation in puff-puff prepared using different cooking conditions

Overall, the effect of the cooking methods on the acrylamide formation in puff-puff were noticed, but not as statistically significant as the influence of processing temperatures and times. However, frying of puff-puff at 210°C produced about 15 μg/kg more acrylamide than baking at the same temperatures.

For the processing temperatures, the use of 210°C resulted in the formation of about four times the amount of acrylamide produced at 150°C.

In comparison to the use of 210°C for the processing of puff-puff, acrylamide formation was reduced by more than 50 μg/kg with the use of 180°C.

In addition, the concentrations of acrylamide resulting from increasing the period of processing from 5 to 15mins was about 20μg/kg. Additional concentrations of acrylamide close to 10 μg/kg were formed by changing the processing time from 5 to 10 mins.

7.4.5.0 Acrylamide formation in yam-chips prepared using different cooking conditions

The application of same cooking method at different processing temperatures produced notable changes in the acrylamide concentrations for yam-chips. In addition, the use of the different cooking methods at same temperature caused remarkable, but statistically insignificant differences in amount of acrylamide formed. The amount of acrylamide formed by frying of the yam-chips at 210°C was about 10μg/kg more than produced by baking at the same temperature. The effect of frying was less pronounced during processing at 150°C and 180°C. At 150°C and

180°C, baking produced about 8 and 2 µg/kg more acrylamide respectively than frying. Compared to baking and frying, roasting was more effective at 150°C than other processing temperatures. The acrylamide contributed by roasting at 150°C was 44 and 33 µg/kg more than the amount produced at the same temperature through frying and baking respectively. Generally, the processing of yam-chips at 210°C contributed about threefold the amount of acrylamide produced using 150°C. Also, the amount of acrylamide produced at 180°C was more than twice of that produced at 150°C.

For the different cooking methods, over 40 µg/kg more acrylamide were produced in yam-chips by applying processing time of 10 mins instead of 5 mins. In addition, about 8 µg/kg less acrylamide was formed by reducing the processing time from 15 to 10mins.

7.5. Discussion

Varying amounts of acrylamide were noticed in all the WA foods prepared by the different cooking methods, temperatures and times.

For some of the WA foods, the contribution of frying compared to baking was slightly higher. The increment in acrylamide due to frying for *akara*, *chin-chin*, *meat-pie*, *plantain-chips*, and *puff-puff* were 8.09, 7.71, 3.97, 8.12, 4.40 % respectively. In comparison to frying, baking contributed 0.24, 1.21, 1.96, and 9.52% additional acrylamide for *bread*, *buns*, *doughnut*, *fish-roll*, and *yam-chips* respectively.

The range of AA concentration resulting from preparing *akara, bread, buns, chin-chin, doughnut, fish-roll, meat-pie, plantain-chips, puff-puff,* and *yam-chips* by different methods and at the selected temperatures were 211, 142, 107, 164, 361, 472, 137, 654, 400, 652 µg/kg respectively. The AA concentration range obtained after baking *akara*, *bread, buns, chin-chin, doughnut, fish-roll, meat-pie, plantain-chips, puff-puff*, and *yam-chips* at the highest temperature (210ºC) were 94, 11, 74, 26, 77, 143, 66, 196, 333, 198 µg/kg respectively. The range of AA concentration observed after frying *akara, bread, buns, chin-chin, doughnut, fish-roll, meat-pie, plantain-chips, puff-puff,* and *yam-chips* at the highest selected temperature were 98, 11,74, 70, 82, 141, 66, 50, 339,189 µg/kg respectively.

Although there was a slight influence of the cooking methods on the acrylamide formed in the WA foods, generally, independent T-test for equality of mean showed no statistically significant effect between the contributions of baking and frying i.e. (p-value > 0.05).

Temperatures higher than 150ºC contributed remarkable amount of acrylamide to all the WA foods studied. The magnitude of thermal effect was particularly obvious has the cooking temperature changed from 150ºC to 180ºC. For *doughnut* (DN), the change in temperature from 180ºC to 210ºC raised the acrylamide level by 189.50%, while the change in temperature from 150ºC to 180ºC resulted in 93.59% increment in acrylamide formed. Apart from DN, the transition from 150ºC to 180ºC was more effective in raising the acrylamide levels in other WA foods studied. The switch in cooking temperature from 180ºC to 210ºC for *akara*, *bread*, *buns*, *chin-chin*, *doughnut*, *fish-roll*, *meat-pie*, *plantain-chips*, *puff-puff* and *yam-chips* resulted in 11.2, 6.09,5.77, 12.82,30.29, 37.33,13.72,46.90, and 21.51% increment in acrylamide respectively. In contrast, the use of 180ºC instead of 150ºC for the preparation of WA *akara*, *bread*, *buns*, *chin-chin*, *fish-roll*, *meat-pie*, *plantain-chips*, *puff-puff* and *yam-chips* resulted in 227.78, 160.80, 256.55, 96.24, 115.21, 185.79, 220.88, 157.63, 162.69 % increment in acrylamide respectively. Overall, the lowest and highest acrylamide concentrations measured in the WA foods resulted from the use of the cooking temperatures of 150ºC for 5mins and 210ºC for 15 mins respectively.

Similar influence of baking and frying temperatures were obtained by Palazoğlu (2010). According to the study, an increase in frying temperature from 170ºC to 190ºC raised the acrylamide levels in potato chips. Another study by Granda, Moreira & Tichy (2004) demonstrated that vacuum frying at a low temperature of 118ºC caused a 94% reduction in acrylamide content compared to traditional frying at 165ºC and normal atmospheric (CIAA, 2014). A drop in acrylamide formation by 68% and 88% was also observed by Palazoğlu & Gökmen (2008b) after the reduction in potato frying temperatures from 190ºC to 170ºC and 150ºC respectively (Pedreschi, Kaack & Granby, 2004).

ANOVA test of significant difference also showed that the three cooking temperatures were remarkably unequal in their contributions to the acrylamide content of the WA foods studied (p-value < 0.05) i.e. increasing baking and frying temperatures effectively raised the amount of the process contaminants. Also, higher readings of AA were observed as the time of thermal processing increased. The lowest amounts of acrylamide (25±8 μg/kg) was detected in *buns* baked at 150ºC, while the highest levels of the contaminant (703±27 and 706±13 μg/kg) were noticed in *yam* and *plantain chips* fried at 210ºC.

7.6. Conclusion

Generally, independent T-test for equality of sample means at α=0.05 showed no statistical significant difference (P > 0.05) in the AA produced by using baking and frying at the same temperature. However, ANOVA for the AA concentrations resulting from varying the cooking temperature indicated that increasing baking and frying temperatures effectively raised the amount of the process contaminants i.e. (P < 0.05).

Although cooking methods have minute effect on the AA produced, the temperature and time of cooking are the major determinant of the level of AA formed in heat-processed WA foods.

7.7. Summary

Acrylamide (AA) is a neurotoxic, genotoxic and potentially carcinogenic compound known to be produced by the reaction of asparagine with carbonyl groups of reducing sugars and bioactive compounds. Many studies have reported its presence in a wide range of foods, particularly heat-treated carbohydrate-rich foods. Although many such foods of WA origin are also produced in Western countries including U.S.A and UK, no study has considered the impact of commonly used cooking methods and temperatures on the formation of the process contaminant in these WA foods.

Thus, this study aimed to evaluate the contributions of different cooking methods, temperatures and times to the formation of the contaminant in WA foods including *akara, bread, buns, chin-chin, doughnut, fish-roll, meat-pie, plantain-chips, puff-puff,* and *yam-chips*.

The level of the contaminant was determined by potentiometric analytical method based on the use of ammonium ion selective electrode and immobilised acrylamide amidohydrolase.

The effects of baking and frying at 150, 180 and 210ºC for 5, 10 and 15mins were studied. For each cooking temperature considered at a particular time, 50 samples of the selected WA foods were prepared. Same portions and dimensions of food materials were processed using the different cooking methods.

Generally, independent T-test for equality of sample means at α=0.05 showed no statistical significant difference (p > 0.05) in the AA produced by using baking and frying at the same temperature for a particular time. However, ANOVA for the AA concentrations resulting from varying the cooking temperature indicated that increasing baking and frying temperatures

effectively raised the amount of the process contaminants i.e. ($p < 0.05$). Highest readings for the contaminant were noted for WA foods processed at 210ºC, while the lowest measurements were obtained for those processed at 150ºC. The lowest amounts of acrylamide (25±8 µg/kg) was detected in *Buns* baked at 150ºC, while the highest levels of the contaminant (703±27 and 706±13 µg/kg) were noticed in *yam* and *plantain chips* fried at 210ºC.

According to the study, though cooking methods have minute effect on the AA produced, the temperature and time of cooking are the major determinants of the level of AA formed in heat-processed WA foods.

Chapter 8

Impact of precursors on the acrylamide levels in important West African foods

CHAPTER 8. IMPACT OF PRECURSORS ON THE ACRYLAMIDE LEVELS IN IMPORTANT WEST AFRICAN FOODS

8.1. Overview

This chapter discusses the influence of precursors including asparagine and glucose on the formation of acrylamide in popular WA foods. The associations between the precursors and the acrylamide produced in the WA foods are also described.

8.2. Introduction

Following the recognition of AA as a carcinogenic, neurotoxic and mutagenic substance (Calleman et al., 1993; Stadler & Scholz, 2004), considerable number of studies focused on understanding its formation and strategies for its mitigation in several heat processed foods (Friedman, 2003; Medeiros et al., 2012).

Yasuhara et al., 2003 demonstrated that dietary AA is partly formed in heat processed, lipid-rich foods by the reaction between ammonia and acrolein. Some findings also suggested that the type of oil used for deep-frying contributes to the formation of AA (Becalski et al., 2003). Another study also claimed that the release of acrylamide depends on the sugar content of food materials processed at high temperatures (Biedermann et al. 2002).

The most prominent explanation for the formation of AA reported after many studies is the Maillard reaction (MR) otherwise called non-enzymatic reaction involving free asparagine (asn) and reducing carbonyl compounds such as glucose (glu) and fructose (Mottram et al., 2002; Stadler et al., 2002). Although MR was originally recognised as a complicated cascade of reactions, which are required for enhancing the aroma, colour, and flavour of foods such as bread dough processed above 160ºC , it is now identified as the major pathway leading to the formation of process contaminants such as hydroxymethylfurfural (HMF) and AA (Lindenmeier and Hofmann 2010).

In addition, the strong and positive correlation between asn and AA for food materials such as wheat and potato led to the identification of asparaginase as a food grade enzyme with the potential to reduce the levels of AA in bread (Kumar et al., 2014).

After the discovery of high levels of asparagine in potatoes and enormous AA levels in potato chips and fries in 2002, many studies have focused on the presence, levels and mitigation of the toxicant in several traditional foods produced in different parts of the world (European Commission, 2013; EFSA, 2012; Wang et al., 2008)

8.2.1 Problem Statement

Although various studies have exhibited the substantial link between AA in food products and the asparagine-glucose levels in intercontinental food materials such as potato, cereals, wheat and rye (Biedermann et al., 2002; Curtis et al., 2010; Elmore et al., 2015; Fei et al., 2016, Halford et al., 2012; Ohara-Takada et al., 2005; Vinci et al., 2012), no study has attempted to determine the level of the process contaminant and its precursors in WA food materials such as those used for *Akara* and *Chin-Chin* production.

Hence, no study has considered the strength and nature of the association between the concentrations of the contaminant in WA foods and the level of its precursors (i.e. asparagine and glucose) in WA food materials.

Furthermore, even though the amounts of AA and its precursors in popular WA foods are unknown, this group of foods are also produced, sold and consumed in countries such as United Kingdom (U.K) and United State of America (U.S.A).

8.2.2 Research Rationale

The study is justified based on the following:

(a) *The confirmation of the toxic effects of acrylamide*: Many reports from animal and human studies have established the neurotoxic, genotoxic and potential carcinogenic effects of acrylamide (IARC, 1994; IFST 2017; JECFA, 2010).

(b) *The establishment of the strong connection between the formation of AA and the heat processing of food materials rich in asparagine and reducing sugars.*

The findings from the testing of different groups of food materials and products by governmental and non-governmental agencies give credence to the acclaimed formation of high amounts of acrylamide in food products made from heat-treated asparagine and reducing sugar rich food materials. (Amrein et al, 2005; Croft et al., 2004; European Commission, 2013; EFSA, 2012; IFST, 2017; Leung et al., 2003; Lineback et al., 2005; Murkovic, 2004; Ono et al., 2003; Tareke et al., 2002; Roach et al., 2003; Rosén and Hellenäs, 2002).

(c) *The efforts of Food Drink Europe and European Commission*: The Food Drink Europe (FDE), which is a representative of the European food and drink industry, sets specific requirements for food business operators (FBO) in a "toolbox" manual designed to help in reducing the acrylamide content of food products. In May 2016, The FDE toolbox tagged "Code of Practice for Managing Acrylamide Formation" was revised (FDE, 2016) and requires FBO using raw potatoes in their products to select varieties that are reasonably low in reducing sugars and asparagine.

(d) Recognition of the relationship between AA levels and its precursors for WA foods may lead to the identification of effective approaches for mitigating the process contaminant.

8.2.3 Research Questions

The list of questions based on the rationale for the study are:

(i) What is the nature and strength of the relationship between the concentration of precursors such as reducing sugars, asparagine and the AA levels in these WA foods?

(ii) Could reducing particular precursors be an important mitigating factor?

8.2.4 Research Hypotheses:

The null and alternative hypotheses for the study are shown below.

(a) Null hypothesis (H_o; $[r_{PA}]= 0$) There is no relationship between the levels of precursors and the AA concentration in the WA foods.

(b) Research hypothesis (H_a; $[r_{PA}] \neq 0$) There is a relationship between the levels of precursors and the AA concentration in the WA foods.

8.2.5 Research Objectives:

The objectives of the study based on the research questions and hypotheses were to:

(1) Determine the asn-glu levels in the food materials required for the preparation of selected WA foods.

(2) Prepare the popular WA foods including *akara, bread, buns, chin-chin, doughnut, fish-roll, meat-pie, plantain-chips, puff-puff,* and *yam-chips* using indigenous methods of preparation.

(3) Establish if there is a correlation between the concentration of precursors such as asparagine, glucose and the concentration of acrylamide produced.

(4) Evaluate the nature and strength of the relationship between the concentration of precursors and the AA levels in the selected WA foods.
(5) Use correlation and regression analysis to explore the different factors, which may be used reduce the production of acrylamide in heat-processed foods.

8.3 Materials and method

8.3.1 Food sample preparation

Since the focus of this chapter is not on the effects of cooking methods or extrinsic cooking conditions such as temperature and time, different African bakeries and restaurants in London markets were consulted to prepare the selected WA foods using the common methods for each WA foods. For example, *akara* which is usually prepared by frying was fried. The WA foods were prepared at 180^{o}C for about 15 minutes. Ingredients similar to those listed in Table 1.1 were used to prepare 150 samples of each WA food.

8.3.2 Food sample analysis

8.3.2.1 Determination of the amount of glucose in the WA food samples

Spectrophotometric analysis based on an enzyme catalysed redox reaction was used to measure the amount of glucose in the unprocessed WA food materials.

NATE-0311- glucose oxidase (GOx)- EC 1.1.3.4, 100KU/g from *Aspergillus niger*, glucose standard (product no. G 3285), ABTS; [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt], Sigma G7141, peroxidase from horse radish, 450KU/g, and 0.5M Tris buffer were used for the determination of the levels of glucose in the WA foods (Barham, D. and Trinder, P., 1972, Rajko et al., 1992,Trinder, P.,1969). The principle behind this method is explained in Chapter 4.

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Reagents and standards were prepared as described in Chapter 4. Four ml of the prepared enzyme dye reagent were added to 1ml of the diluted test samples test tube. The blank, standards and test samples were incubated in water at 37±1°C for 10 min to develop a blue-green colour (Figure 8.1). After the incubation, the absorbance was recorded with a spectrophotometer (Jenway^{TM} model 7315 UV/visible single beam spectrophotometer) at 415 nm.

Figure 8.1. *Test tubes being heated at approximately 37°C to develop the blue-green colour for quantification of glucose.*

A standard curve was prepared using different glucose concentrations (Figure 4.5). EXCEL 2016 software was used to plot the absorbance values of the standard solutions against their respective concentrations. The standard curve was then used for the determination of the amount of glucose in the test sample.

8.3.2.2 Determination of the amount of asparagine in the WA food samples

Several studies have reported the potentiomentric or electrode based determination of amino acids (Shvedene et al., 2016, Zahra et al., 2017. In addition, asparaginase from *Escherichia coli*, Serratia spp., *Pseudomonas aeruginosa*, *Bacillus spp., Aeromonas* species and *Proteus spp*. have been characterized (Kamble et al., 2012).

The asparagine content of the unprocessed WA food materials was determined by potentiometric analytical method based on the use of ammonium ion selective electrode and immobilised asparaginase.

The procedure used was similar to those described by Ali et al., 2014; Erdogan et al., 2014; Fatibello-lho et al., 2006; Li et al., 2002; Neelam et al., 2012, Pathak et al., 2017; Punia and Kumar 2015.

Weighed samples of the selected WA foods were homogenised, cleaned and analysed as described in Chapter 5. Carrez I and II reagents were used for precipitation of interfering proteins. The samples were centrifuged at 4000rpm for 5-15 mins. The samples were further cleaned by 0.45μm nylon filter and consequently treated with asparaginase which was immobilised by beads formed by the reaction of calcium chloride with sodium alginate (Punia and Kumar, 2015). The method for asparagine analysis and the principle behind its application are discussed in Chapter 5.

8.3.2.3 Determination of the amount of acrylamide in the WA food samples

The amount of acrylamide in the different WA food samples was measured by potentiometric analytical method based on the use of ammonium ion selective electrode and immobilised acrylamide amidohydrolase (described in Chapter 6).

8.3.2.4 Determination of the relationship between precursors in raw food materials and the acrylamide in the WA foods

Correlation and regression analyses of 150 samples of each WA food were used to evaluate the nature and strength of the connection between the contaminant and the precursors.

8.3.3 Statistical analysis

IBM SPSS Statistics 26 software was used for the data analysis. Correlation and regression analyses of data from 150 samples of each WA food were conducted to gain knowledge of the strength and nature of the relationship between the precursors and the acrylamide concentrations. An α-level of 0.01 was used to obtain the significance of the observed relationship.

8.4.Results

Generally, a significant positive relationship ($p < 0.01$) was observed between AA present in the food products and the precursors measured in the unprocessed food materials.

The strongest and weakest determination coefficients of 0.878 and 0.295 were observed for WA plantain-chips and bread respectively. The regression equations and coefficients describing the relationship between the precursors and the acrylamide in each WA food are shown in Table 8.1.

Table 8.1. *Regression equations for acrylamide and its predictors- glucose and asparagine*

Heat Processed WA Foods	**Regression Equation (with Unstandardized Coefficients –[$Y_{AA} = a + bglu + casn$]) of Acrylamide (AA) with Glucose (glu) and Asparagine (asn)**	**Regression Equation (with Standardized Coefficients –[$Y_{AA} = bglu \pm casn$]) of Acrylamide (AA) with Glucose (glu) and Asparagine (asn)**	**Coefficient of Determination-r^2 and Significance Level for the Dependent Variable (AA) and the Predictors (glu and asn)**
Akara	$Y_{AA}=7.29 + 7.36glu + 22.35asn$	$Y_{AA}=0.073glu + 0.64asn$	0.604, (P<0.01)
Bread	$Y_{AA}= 31.98 + 5.82glu + 20.65asn$	$Y_{AA}=0.15glu + 0.44asn$	0.295, (P>0.01)
Buns	$Y_{AA}=38.54 + 0.28glu + 15.39asn$	$Y_{AA}=0.016glu + 0.58asn$	0.575, (P<0.01)
Chin-chin	$Y_{AA}=61.38 + 7.84glu + 13.86asn$	$Y_{AA}=0.39glu + 0.43asn$	0.723, (P<0.01)
Doughnuts	$Y_{AA}=16.94 + 11.91glu + 46.51asn$	$Y_{AA}=0.091glu + 0.39asn$	0.693, (P<0.01)
Fish-Roll	$Y_{AA}=59.18 + 40.90glu + 45.40asn$	$Y_{AA}=0.22glu + 0.41asn$	0.738, (P<0.01)
Meat-Pie	$Y_{AA}=47.79 + 7.77glu + 67.38asn$	$Y_{AA}=0.064glu + 0.15asn$	0.813, (P<0.01)
Plantain Chips	$Y_{AA}=66.74 + 62.85glu + 331.13asn$	$Y_{AA}=0.21glu + 0.29asn$	0.878, (P<0.01)
Puff-Puff	$Y_{AA}=44.83 + 28.50glu + 102.45asn$	$Y_{AA}=0.24glu + 0.067asn$	0.785, (P<0.01)
Yam Chips	$Y_{AA}=105.94 + 62.76glu + 203.58asn$	$Y_{AA}=0.37glu + 0.49asn$	0.666, (P<0.01)

The regression equations predict the acrylamide formation in the WA foods as a function of the glucose (glu) and asparagine (asn) content of the WA food material processed.

8.4.1. Impact of precursors on the acrylamide produced in WA *akara*

The knowledge of the strength and nature of the relationship between the precursors and the acrylamide concentrations for WA *akara* was gained through the correlation and regression analysis of 150 samples. An α-level of 0.01 was used to obtain the significance of the observed relationship.

For WA *akara*, a significant and positive relationship ($p < 0.01$) was observed between AA present in the food product and the precursors measured in the unprocessed food materials. Although the acrylamide in WA *akara* was positively linked to both asparagine and glucose levels in the unprocessed batter, a stronger relationship (indicated by the correlation coefficients in Table 8.2 and the 3D surface plot shown in Figure 8.2) was observed between glucose and acrylamide.

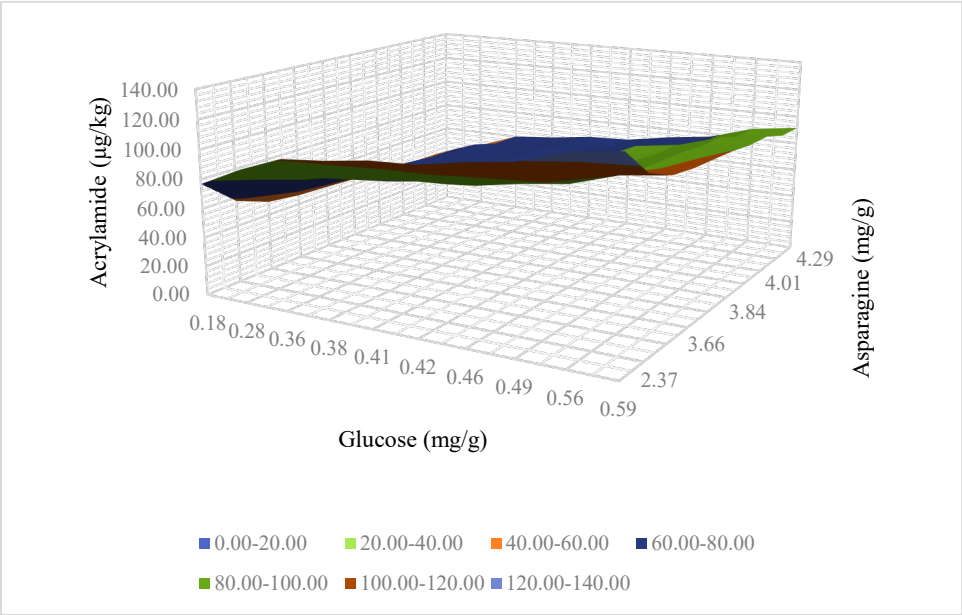

Figure 8.2. *3D surface plot depicting the relationship between the precursors and the acrylamide in WA Akara*

8.4.1.1 Concentration of glucose, asparagine and acrylamide for WA *akara*

The analysis of the precursors in the unprocessed materials and the acrylamide in the final products gave results shown in Table 8.2. The mean glucose, asparagine and acrylamide concentrations measured for WA *akara* were 1.06, 5.32 mg/g and 134 µg/kg food sample respectively.

The correlation coefficients, minimum and maximum values of the precursors are shown in Table 8.2.

Table 8.2. *Correlation coefficients and descriptive statistics for WA Akara*

WA AK	*Glu (mg)*	*Asn (mg)*	*Acrylamide (µg/kg)*
Corr. r with AA	0.76	0.69	1
Mean	1.06	5.32	134
S.E	0.06	0.17	6
Med.	0.85	4.97	176
Mode	0.91	3.30	179
S.D	0.70	2.05	71
Range	2.39	8.04	211
Min.	0.11	2.00	32
Max.	2.50	10.04	243

8.4.2. Impact of precursors on the acrylamide produced in WA bread

Correlation and regression analysis of 150 samples was conducted to gain knowledge of the strength and characteristic of the association between the precursors and the acrylamide concentrations for WA bread. An α-level of 0.01 was used to obtain the significance of the observed relationship.

For WA bread, a positive and direct relationship was observed between AA present in the food product and the precursors measured in the unprocessed food materials (Figure 8.3). Although the acrylamide in WA bread was positively linked to both asparagine and glucose levels in the unprocessed dough, the coefficient of determination (r^2) and P-value for the regression equation (Shown in Table 8.1) depicts a weak and statistically insignificant relationship P>0.01. In addition, though the relationship between acrylamide and the precursors for WA bread was weak, a slightly stronger relationship (indicated by the correlation coefficients in Table 8.3) was observed between asparagine and acrylamide.

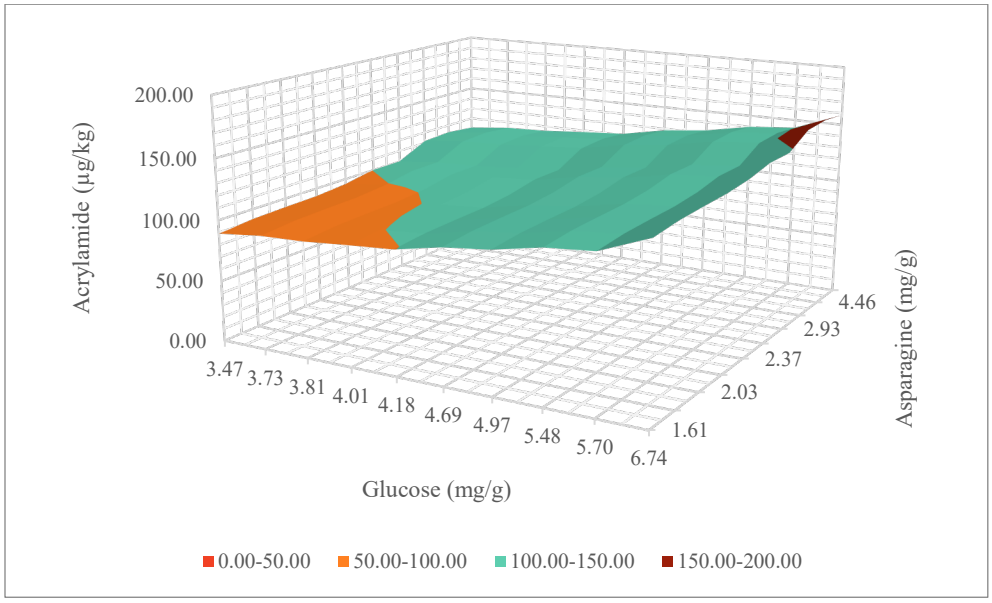

Figure 8.3. *3D surface plot depicting the relationship between the precursors and the acrylamide in WA bread*

8.4.2.1 Concentration of glucose, asparagine and acrylamide for WA bread

The analysis of the precursors in the unprocessed materials and the acrylamide in the final products gave results shown in Table 8.3. The mean glucose, asparagine and acrylamide concentrations measured for bread were 4.72, 2.81 mg/g and 118 µg/kg food sample respectively.

The correlation coefficients, minimum and maximum values of the precursors are presented below.

Table 8.3. *Correlation coefficients and descriptive statistics for WA bread*

WA BR Statistics	*Glu (mg)*	*Asn (mg)*	*Acrylamide (µg/kg)*
Corr. r with AA	0.53	0.54	1
Mean	4.72	2.81	118
S.E	0.10	0.09	4
Median	4.64	2.56	94
Mode	5.48	1.61	176
S.D	1.26	1.04	49
Range	5.20	3.97	147
Min.	2.65	1.05	54
Max.	7.85	5.02	201

8.4.3. Impact of precursors on the acrylamide produced in WA *buns*

The knowledge of the strength and direction of the relationship between the precursors and the acrylamide concentrations for WA *buns* was gained through the correlation and regression analysis of 150 samples. An α-level of 0.01 was used to obtain the significance of the observed relationship.

For WA *buns*, a significant and positive relationship ($p < 0.01$) was observed between AA present in the food product and the precursors measured in the unprocessed food materials. Although the acrylamide in WA *buns* was positively associated with both asparagine and glucose levels in the unprocessed batter, a stronger relationship (indicated by the correlation coefficients in Table 8.4 and the 3D surface plot shown in Figure 8.4) was observed between asparagine and acrylamide.

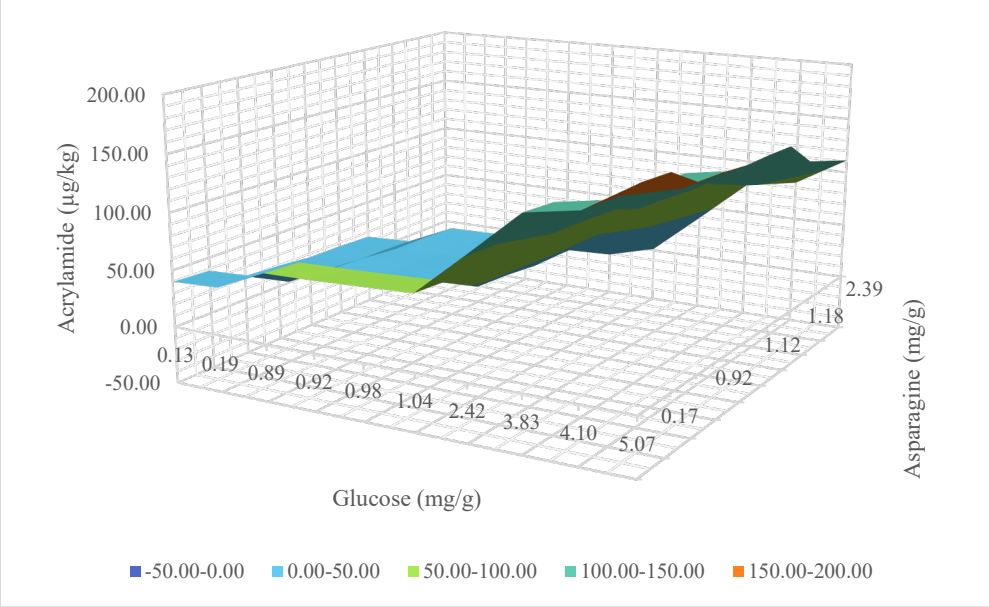

Figure 8.4. *3D surface plot depicting the relationship between the precursors and the acrylamide in WA Buns*

8.4.3.1 Concentration of glucose, asparagine and acrylamide for WA *buns*

The analysis of the precursors in the unprocessed materials and the acrylamide in the final products gave results shown in Table 8.4. The mean glucose, asparagine and acrylamide

concentrations measured for WA *buns* were 2.98, 2.06 mg/g and 71 µg/kg food sample respectively.

The correlation coefficients, minimum and maximum values of the precursors are shown below.

Table 8.4. *Correlation coefficients and descriptive statistics for WA buns*

WA BN Statistics	*Glu (mg)*	*Asn (mg)*	*Acrylamide (µg/kg)*
Corr. ***r*** with AA	0.58	0.70	1
Mean	2.98	2.06	71
S.E	0.16	0.11	3
Med.	3.35	1.96	88
Mode	0.94	1.13	21
S.D.	2.01	1.30	34
Range	6.09	4.53	107
Min.	0.13	0.17	20
Max.	6.22	4.70	128

8.4.4. Impact of precursors on the acrylamide produced in WA *chin-chin*

The state of the strength and direction of the relationship between the precursors and the acrylamide concentrations for WA *chin-chin* was identified by the correlation and regression analysis of 150 samples. An α-level of 0.01 was used to obtain the significance of the observed relationship.

For WA *chin-chin*, a significant and positive relationship ($p < 0.01$) was observed between AA present in the food product and the precursors measured in the unprocessed food materials. Although the acrylamide in WA *chin-chin* was positively linked to both asparagine and glucose levels in the unprocessed batter, a stronger relationship (indicated by the 3D surface plot shown in Figure 8.5 and the correlation coefficients in Table 8.5) was observed between asparagine and acrylamide.

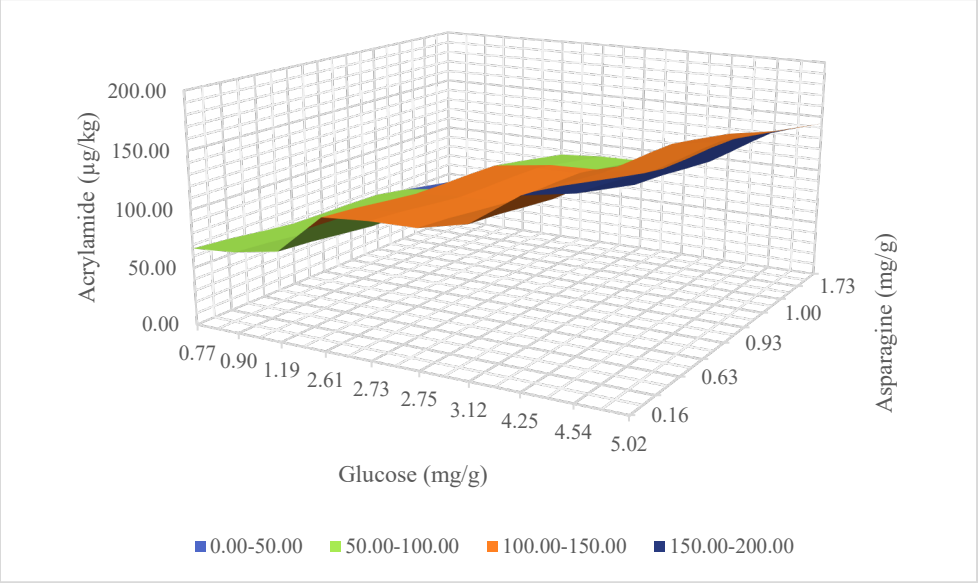

Figure 8.5. *3D surface plot depicting the relationship between the precursors and the acrylamide in WA Chin-chin*

8.4.4.1 Concentration of glucose, asparagine and acrylamide for WA *chin-chin*

The analysis of the precursors in the unprocessed materials and the acrylamide in the final products gave results shown in Table 8.5. The mean glucose, asparagine and acrylamide concentrations measured for WA *chin-chin* were 2.86, 1.32 mg/g and 102 µg/kg food sample respectively.

The correlation coefficients, minimum and maximum values of the precursors are shown below.

Table 8.5. *Correlation coefficients and descriptive statistics for WA Chin-Chin*

WA CN Statistics	*Glu (mg)*	*Asn (mg)*	*Acrylamide (µg/kg)*
Corr. ***r*** with AA	0.71	0.84	1
Mean	2.86	1.32	102
S.E	0.15	0.09	3
Med.	2.39	0.86	121
Mode	1.13	0.63	130
S.D	1.88	1.16	38
Range	5.63	4.34	120
Min.	0.74	0.14	31
Max.	6.37	4.48	151

8.4.5. Impact of precursors on the acrylamide produced in WA doughnuts

The knowledge of the strength and nature of the relationship between the precursors and the acrylamide concentrations for WA doughnuts was obtained through the correlation and regression analysis of 150 samples. An α-level of 0.01 was used to obtain the significance of the observed relationship.

For WA doughnuts, a significant and positive relationship ($p < 0.01$) was observed between AA present in the food product and the precursors measured in the unprocessed food materials. The strength of the relationship observed between glucose and acrylamide was approximately the same as the one noted for asparagine and acrylamide. The regression equations, coefficient of determination in Table 8.1 and the 3 D surface plot shown in Figure 8.6 represent the association between the precursors and the contaminant.

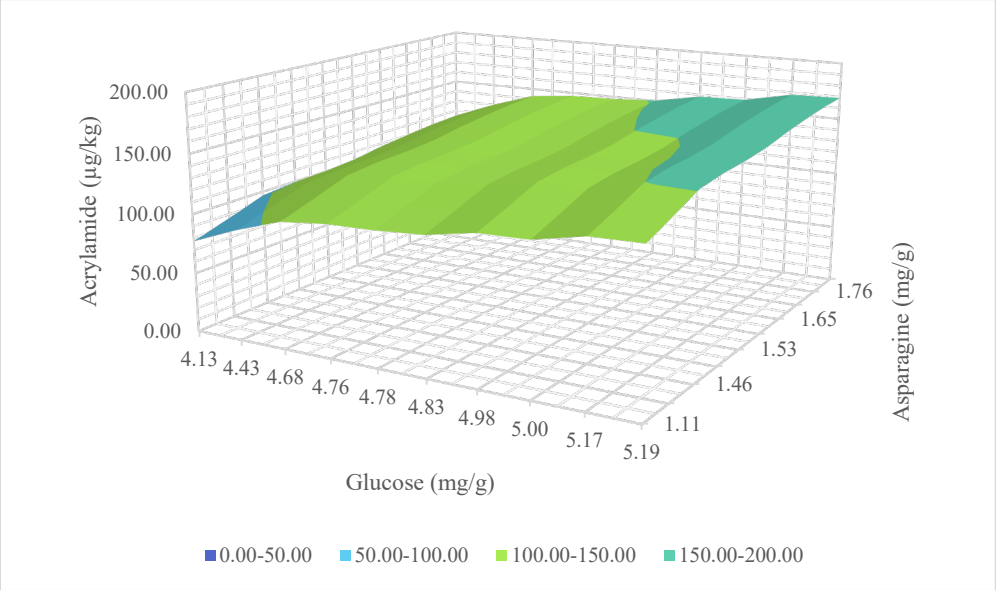

Figure 8.6. *3D surface plot depicting the relationship between the precursors and the acrylamide in WA doughnut*

8.4.5.1 Concentration of glucose, asparagine and acrylamide for WA doughnut

The analysis of the precursors in the unprocessed materials and the acrylamide in the final products gave results shown in Table 8.6. The mean glucose, asparagine and acrylamide concentrations measured for WA doughnuts were 4.97, 1.97 mg/g and 168 µg/kg food sample respectively.

The correlation coefficients, minimum and maximum values of the precursors are presented below.

Table 8.6. *Correlation coefficients and descriptive statistics for WA Doughnut*

WA DN Statistics	*Glu (mg)*	*Asn (mg)*	*Acrylamide (µg/kg)*
Corr. ***r*** with AA	0.82	0.82	1
Mean	4.97	1.97	168
S.E	0.07	0.08	10
Med.	5.00	1.68	118
Mode	4.68	1.57	312
S.D	0.90	0.97	117
Range	3.97	3.34	356
Min.	3.02	0.78	31
Max.	6.99	4.12	387

8.4.6. Impact of precursors on the acrylamide produced in WA *fish-roll*

Correlation and regression analysis of 150 samples was conducted to evaluate the strength and direction of the relationship between the precursors and the acrylamide concentrations for WA *fish-roll*. An α-level of 0.01 was used to obtain the significance of the observed relationship.

For WA *fish-roll*, a significant and positive relationship ($p < 0.01$) was observed between AA present in the food product and the precursors measured in the unprocessed food materials.

The acrylamide in WA fish-roll was positively associated with both asparagine and glucose levels in the unprocessed batter. The 3D surface plot shown in Figure 8.7 and the correlation coefficients in Table 8.7 depicts the relationship between the precursors and the contaminant.

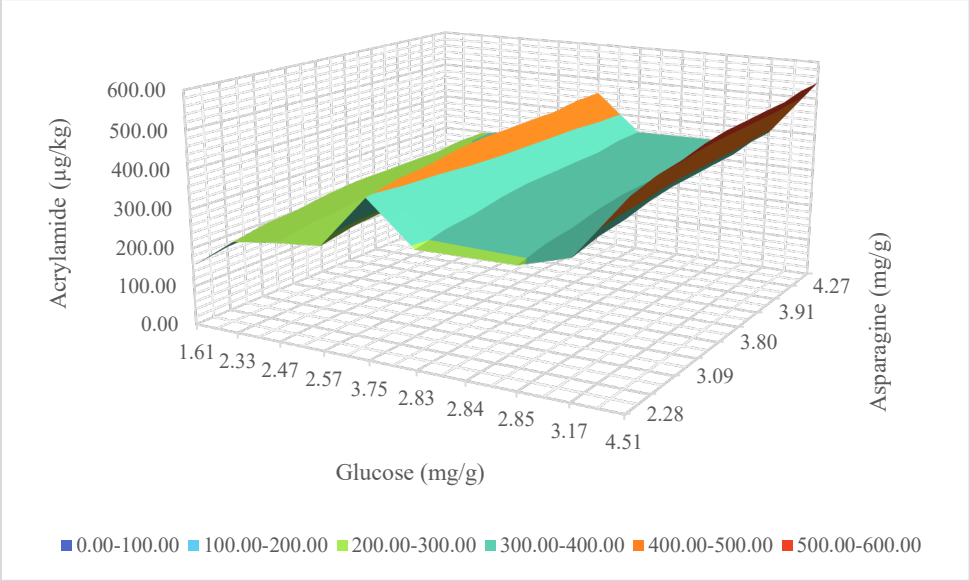

Figure 8.7. *3D surface plot depicting the relationship between the precursors and the acrylamide in WA fish-roll*

8.4.6.1 Concentration of glucose, asparagine and acrylamide for WA *fish-roll*

The analysis of the precursors in the unprocessed materials and the acrylamide in the final products gave results shown in Table 8.7. The mean glucose, asparagine and acrylamide concentrations measured for WA fish-roll were 3.10, 4.40 mg/g and 386 µg/kg food sample respectively.

The correlation coefficients, minimum and maximum values of the precursors are shown below.

Table 8.7. *Correlation coefficients and descriptive statistics for WA Fish-Roll*

WA FR Statistics	*Glu (mg)*	*Asn (mg)*	*Acrylamide (µg/kg)*
Corr. ***r*** with AA	0.85	0.79	1
Mean	3.10	4.40	386
S.E	0.06	0.10	11
Med.	3.10	4.24	386
Mode	2.33	4.31	532
S.D	0.71	1.17	129
Range	2.90	4.96	554
Min.	1.61	2.28	77
Max.	4.51	7.24	631

8.4.7. Impact of precursors on the acrylamide produced in WA *meat-pie*

The knowledge of the strength and direction of the association between the precursors and the acrylamide concentrations for WA *meat-pie* was obtained through the correlation and regression analysis of 150 samples. An α-level of 0.01 was used to obtain the significance of the observed relationship.

For WA *meat-pie*, a significant and positive relationship ($p < 0.01$) was observed between AA present in the food product and the precursors measured in the unprocessed food materials. Although the acrylamide in WA meat-pie was positively and linearly connected to both asparagine and glucose levels in the unprocessed batter, a stronger association (indicated by the 3 D surface plot shown in Figure 8.8 and the correlation coefficients in Table 8.8) was observed between glucose and acrylamide.

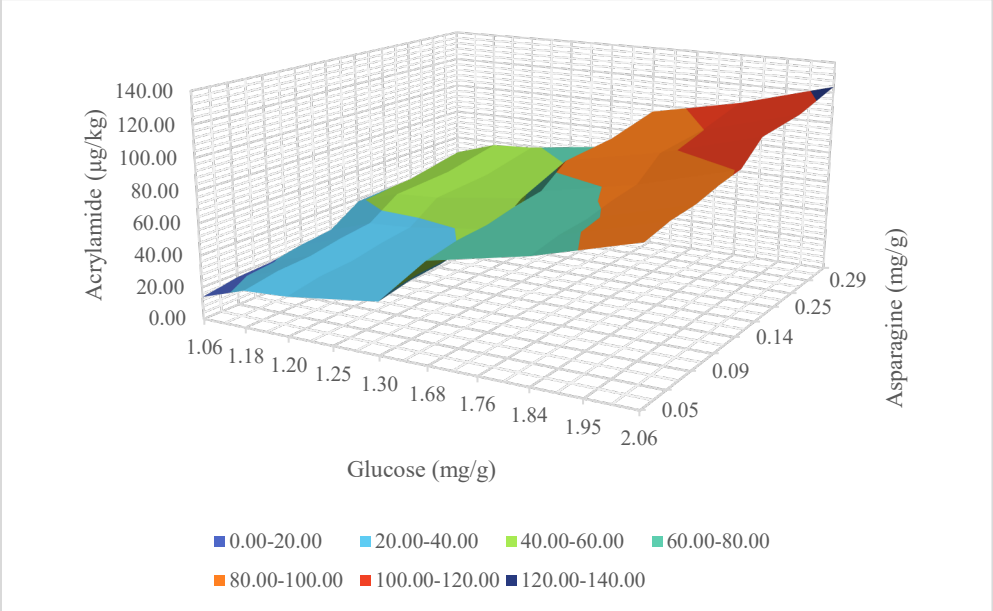

Figure 8.8. *3D surface plot depicting the relationship between the precursors and the acrylamide in WA meat-pie*

8.4.7.1 Concentration of glucose, asparagine and acrylamide for WA *meat-pie*

The analysis of the precursors in the unprocessed materials and the acrylamide in the final products gave results shown in Table 8.8. The mean glucose, asparagine and acrylamide concentrations measured for WA *meat-pie* were 1.60, 0.20 mg/g and 74 µg/kg food sample respectively.

The correlation coefficients, minimum and maximum values of the precursors are shown below.

Table 8.8. *Correlation coefficients and descriptive statistics for WA Meat-Pie*

WA MP Statistics	*Glu (mg)*	*Asn (mg)*	*Acrylamide (µg/kg)*
Corr. ***r*** with AA	0.90	0.88	1
Mean	1.60	0.20	74
S.E	0.02	0.01	3
Med.	1.61	0.21	83
Mode	1.24	0.26	95
S.D	0.31	0.08	37
Range	1.11	0.31	137
Min.	1.02	0.04	24
Max.	2.13	0.35	161

8.4.8. Impact of precursors on the acrylamide produced in WA *plantain-chips*

Correlation and regression analysis of 150 samples was conducted to recognize the direction and strength of the relationship between the precursors and the acrylamide concentrations for WA *plantain-chips*. An α-level of 0.01 was used to obtain the significance of the observed relationship.

For WA *plantain-chips*, a significant and positive relationship ($p < 0.01$) was observed between AA present in the food product and the precursors measured in the unprocessed food materials. The strength of the relationship observed between glucose and acrylamide was approximately the same as the one noted for asparagine and acrylamide. The regression equation, coefficient of determination in Table 8.1 and the 3 D surface plot Figure 8.9 represent the association between the precursors and the contaminant.

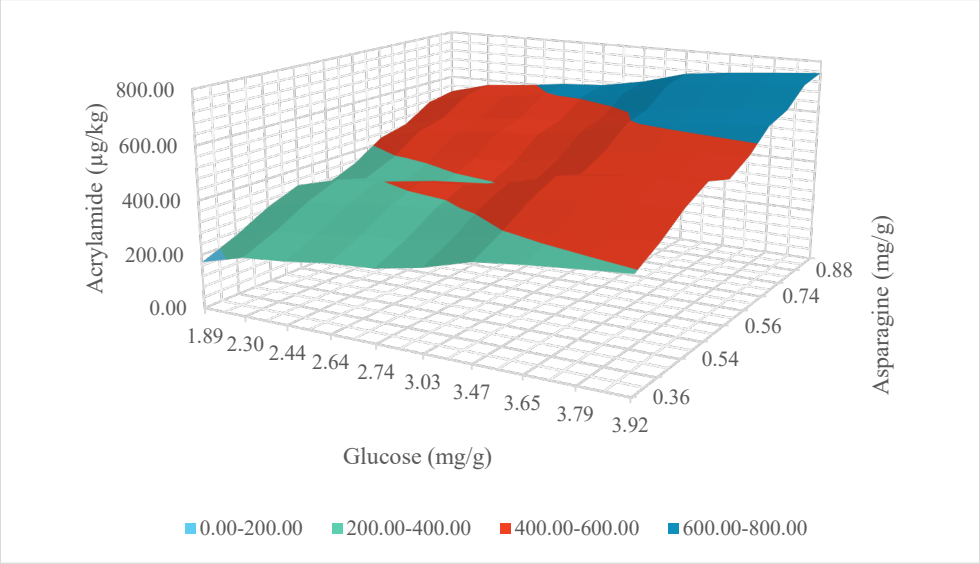

Figure 8.9. *3D surface plot depicting the relationship between the precursors and the acrylamide in WA plantain-chips*

8.4.8.1 Concentration of glucose, asparagine and acrylamide for WA *plantain-chips*

The analysis of the precursors in the unprocessed materials and the acrylamide in the final products gave results shown in Table 8.9. The mean glucose, asparagine and acrylamide concentrations measured for WA *plantain-chips* were 2.93, 0.62 mg/g and 456 µg/kg food sample respectively.

The correlation coefficients, minimum and maximum values of the precursors are presented below.

Table 8.9. *Correlation coefficients and descriptive statistics for WA plantain-chips*

WA PC Statistics	*Glu (mg)*	*Asn (mg)*	*Acrylamide (µg/kg)*
Corr. ***r*** with AA	0.93	0.93	1
Mean	2.93	0.62	456
S.E	0.06	0.02	19
Med.	2.99	0.63	536
Mode	3.65	0.61	723
S.D	0.78	0.21	236
Range	3.16	0.77	633
Min.	1.28	0.23	95
Max.	4.44	1.00	728

8.4.9. Impact of precursors on the acrylamide produced in WA *puff-puff*

The knowledge of the strength and nature of the relationship between the precursors and the acrylamide concentrations for WA *puff-puff* was gained through the correlation and regression analysis of 150 samples. An α-level of 0.01 was used to obtain the significance of the observed relationship.

A significant linear and positive relationship ($p < 0.01$) was observed between the AA present in WA *puff-puff* and the precursors measured in the unprocessed dough.

The strength of the relationship observed between glucose and acrylamide was lesser than the one noted for asparagine and acrylamide. The regression equation, coefficient of determination shown in Table 8.1 and the 3 D surface plot depicted by Figure 8.10 represent the relationship between the precursors and the contaminant.

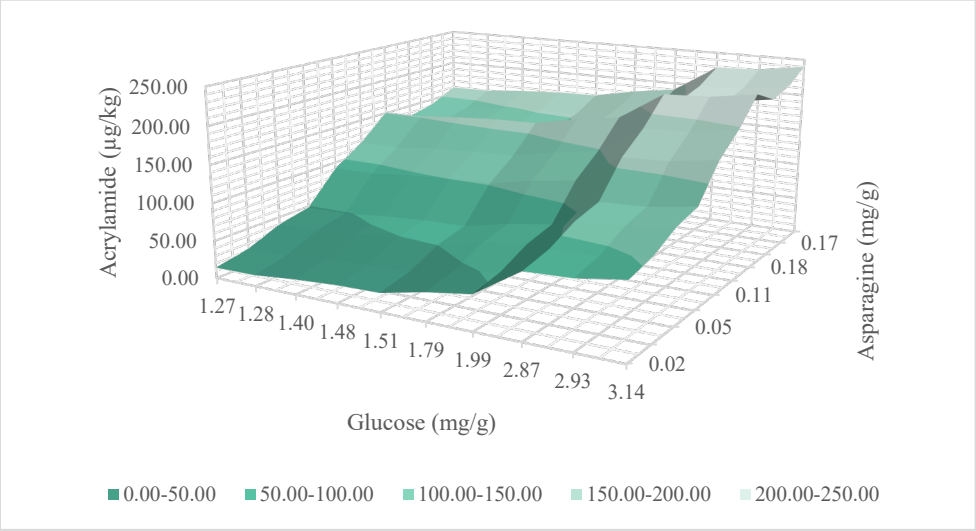

Figure 8.10 *3D surface plot depicting the relationship between the precursors and the acrylamide in WA Puff-puff*

8.4.9.1 Concentration of glucose, asparagine and acrylamide for WA *puff-puff*

The analysis of the precursors in the unprocessed materials and the acrylamide in the final products gave results shown in Table 8.10. The mean glucose, asparagine and acrylamide concentrations measured for WA *puff-puff* were 1.99, 0.10 mg/g and 111 µg/kg food sample respectively. The correlation coefficients, minimum and maximum values of the precursors are shown below.

Table 8.10 *Correlation coefficients and descriptive statistics for WA puff-puff*

WA PF Statistics	*Glu (mg)*	*Asn (mg)*	*Acrylamide (µg/kg)*
Corr. ***r*** with AA	0.84	0.87	1
Mean	1.99	0.10	111
S.E	0.04	0.00	5
Med.	1.88	0.09	99
Mode	1.51	0.09	154
S.D	0.54	0.04	66
Range	1.94	0.17	393
Min.	1.21	0.02	24
Max.	3.15	0.19	417

8.4.10. Impact of precursors on the acrylamide produced in WA *yam-chips*

Correlation and regression analysis of 150 samples was conducted to understand the nature and strength of the association between the precursors and the acrylamide concentrations for WA *yam-chips*. An α-level of 0.01 was used to obtain the significance of the observed relationship. A significant positive relationship ($p < 0.01$) was observed between the AA present in WA *yam-chips* and the precursors measured in the unprocessed dough.

The strength of the relationship observed between glucose and acrylamide was slightly less than the one noted for asparagine and acrylamide. The 3 D surface plot shown in Figure 8.11, the regression equation and correlation of determination in Table 8.1 represent the association between the precursors and the contaminant.

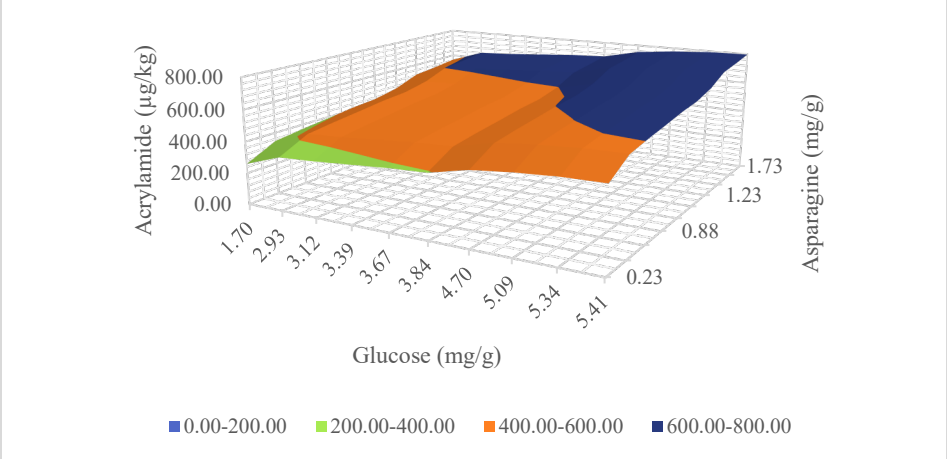

Figure 8.11 *3D surface plot depicting the relationship between the precursors and the acrylamide in WA yam chips*

8.4.10.1 Concentration of glucose, asparagine and acrylamide for WA *yam-chips*

The analysis of the precursors in the unprocessed materials and the acrylamide in the final products gave results shown in Table 8.11. The mean glucose, asparagine and acrylamide concentrations measured for WA yam-chips were 3.42, 0.85 mg/g and 494 µg/kg food sample respectively.

The correlation coefficients, minimum and maximum values of the precursors are shown below

Table 8.11 *Correlation coefficients and descriptive statistics for WA yam-chips*

WA YC Statistics	*Glu (mg)*	*Asn (mg)*	*Acrylamide (µg/kg)*
Corr. ***r*** with AA	0.75	0.78	1
Mean	3.42	0.85	494
S.E	0.10	0.04	17
Med.	3.40	0.89	538
Mode	2.55	0.35	533
S.D	1.27	0.52	214
Range	4.95	1.88	652
Min.	1.15	0.13	77
Max.	6.10	2.01	729

8.5.Discussion

The establishment of the relationship between acrylamide in WA foods and the precursors present in the WA food materials is critical for the effective control of the process contaminant by food producers.

Varying strength of relationships were noted between the acrylamide in the WA foods and the two precursors considered. Generally, a significant linear and positive relationship ($P < 0.01$) was noted for the AA present in the food products and the precursors measured in the food materials. The strongest and weakest determination coefficients (r^2) of 0.878 and 0.295 were observed for WA *plantain-chips* and *bread* respectively.

These findings further confirms the reports from previous studies on the relationship between acrylamide and its precursors. According to Becalski et al., 2003 and Matsuura-Endo et al., 2006, a direct correlation exists between the amount of acrylamide formed in heated food products and

the level of asparagine in the food material. Other studies on the precursors of acrylamide also demonstrated that glucose and asparagine are essential determining factors for the formation of acrylamide (Amrein *et al.*, 2003; Becalski *et al.*, 2004; Elmore *et al.*, 2007, 2010).
Among the WA foods studied, *Akara* had the least average glucose concentration (1.06 mg/g), while doughnut had the highest mean glucose level (4.97mg/g). This observation might be a reflection of the ingredients used in the preparation of these foods i.e. *akara* is made mainly from beans and spices, while jam and sugar are additional components of WA doughnuts. Hence, glucose is a limiting precursor required for acrylamide formation in *Akara*. Previous studies also indicated that the level of reducing sugars may directly limit the concentration of acrylamide formed in foods (Amrein et al., 2003; Oruna-Concha et al., 2001).

This may also explain the low concentration of acrylamide formed in *akara* (134 μg/kg) compared to the relatively high amount of acrylamide produced in *doughnut* (168 μg/kg).

The closeness in the levels of acrylamide produced in *bread* and *puff-puff* can be explained by considering the similar ingredients and formulation required for the preparation of both WA foods. Previous studies also indicated that raw materials used in the preparation of foods could affect the total acrylamide formed in the final food product (Amrein et al., 2003, 2004, Halford et al., 2012a, Muttucumaru et al., 2008).

A comparison of the mean asparagine concentrations for the WA foods showed that *puff-puff* had the least amount of asparagine (0.10 mg/g), while *akara* and *fish-roll* had the highest amount of asparagine (5.32 and 4.40 mg/g respectively). This observation is a reflection of the ingredients used in the preparation of these foods i.e. *puff-puff* also called *bofrot* is a WA pastry made from all-purpose flour, yeast and sugar, while *akara* and *fish-roll* are made from protein-rich materials such as beans, pepper, wheat and fishes. Hence, asparagine is a limiting precursor required for acrylamide formation in *WA puff-puff*. This may also explain the low mean amount of acrylamide formed in WA *puff-puff* (111 μg/kg) compared to the relatively high amount of acrylamide produced in WA *akara* and *fish-roll* (134 and 386 μg/kg respectively). Previous studies also corroborate the significance of asparagine during the formation of acrylamide (Curtis *et al.*, 2009, 2010; Granvogl *et al.*, 2007;
Muttucumaru *et al.*, 2006; Postles *et al.*, 2013).

The high acrylamide recorded for *plantain* and *yam chips* can be explained by the presence of high carbohydrate and glucose in the materials required for their preparation. Several studies on

the acrylamide levels in potato chips , fries and many other carbohydrate rich foods supports this observation**.**

To reduce the acrylamide forming potential of foods, Food Drink Europe advised food producers to use food materials and varieties with low level of precursors including glucose, fructose and asparagine (FDE 2013, 2016). Other strategies that have been reported to successfully reduce the amount of acrylamide in foods include blanching, soaking, enzymatic hydrolysis of asparagine, acidification with citric and acetic acid, fermentation of food materials by lactic acid bacteria and yeasts.

Blanching and soaking of raw potato slices have been reported to reduce the amount of reducing sugars and asparagine which are essential precursors of acrylamide (Grob et al., 2003, Fiselier et al., 2005, Haase et al., 2003, 2004, Pedreschi et al., 2004, 2005, Wicklund et al 2005). Blanching in warm water was more effective in extracting glucose and asparagine than immersion in cold water (Grob et al., 2003, Kita et al., 2004, Pedreschi et al., 2004).

In addition, extraction of the precursors in food grade acids have been shown to be a more effective approach to reducing the acrylamide in food products than water extraction (Kita et al., 2004). There are reports of remarkable reductions in the asparagine and acrylamide levels in foods after the treatment of the raw materials with food grade acids such as acetic and citric acids (Jung et al., 2003). However, the use of citric acid may have an undesirable effect on the taste of the finished product (Gama-Baumgartner et al., 2004). According to Kita et al. 2004, when compared to citric acid, the use of acetic acid produces final products with more acceptable taste.

Alternatively, enzymatic hydrolysis of asparagine may help in reducing the acrylamide levels in heated foods.

Asparaginase derived from *Aspergillus oryzae* has been reported to hydrolyse asparagine into aspartic acid and ammonia (Amrein et al., 2004). For this reason, the enzyme is used to reduce the level of acrylamide in foods without altering the taste and appearance of the final product (Medeiros Vinci et al., 2012). The enzyme has been successfully used for potato products such as French fries and potato chips (Pedreschi et al., 2011).

Acrylamide reduction by asparaginase was confirmed both in model systems and real food matrixes (Anese et al., 2013). The dipping of potato strips and slices in asparaginase containing

solutions was reported to result in 30% and 15% acrylamide reductions in French fries (Pedreschi et al., 2008) and potato chips (Pedreschi et al., 2011) respectively.

For foods such as plantain and yam chips, which are similar to potato chips, blanching of the raw material may be necessary for a further reduction in glucose and asparagine concentrations (FDE, 2016). Modification of varieties of WA food materials such as tubers of yam by genetic alterations may also help to reduce the acrylamide in WA foods (Rommens *et al.*, 2008; Chawla *et al.*, 2012).

8.6.Conclusion

The precursor compounds for the development of acrylamide in foods are asparagine and the reducing sugars; glucose and fructose. Formation of acrylamide is not possible in the absence of the precursors. Furthermore, there a positive correlation between the content of these precursors and the content of acrylamide. So the focus was on the analytical determination of these precursors in the ingredients used in the preparation of the WA foods

The findings supported the research hypothesis that claims the existence of a relationship between the contaminant and its precursors. In general, a strong and significant relationship ($P< 0.01$) with a positive direction was obtained between the precursors and acrylamide in the WA food materials. Thus, future studies on the reduction of acrylamide in WA foods such as *akara* and *plantain chips* should focus on decreasing the precursor compounds in the ingredients or raw materials by enzyme treatment, acidification, blanching and fermentation.

8.7.Summary

Acrylamide (AA) is a group 2A carcinogenic toxicant known to be produced by the reaction of some free amino acids with carbonyl groups of bioactive compounds and reducing sugars.

Many studies have reported a strong connection between AA and heat processing of plant materials with high levels of precursors including asparagine, fructose and glucose.

Although the relationship between AA and the precursors in several intercontinental food materials have been studied, no study has considered the association between the formation of the process contaminant and the level of its precursors in popular WA food materials.

In this study, popular WA foods including *akara*, *bread*, *buns*, *chin-chin*, *doughnut, fish-roll*, *meat-pie*, *plantain-chips*, *puff-puff*, and *yam-chips* were prepared using indigenous methods of preparation.

The level of the contaminant in these foods was determined by potentiometric analytical method based on the use of ammonium ion selective electrode and immobilised acrylamide amidohydrolase. Recognised methods requiring glucose oxidase and asparaginase were used for measuring the amount of glucose and asparagine in the unprocessed food materials.

Consequently, the level of dependence of the contaminant on the essential precursors was evaluated by correlation and regression analysis of 150 samples of each food.

Generally, a significant positive relationship ($P < 0.01$) was observed between AA present in the food products and the precursors measured in the food materials.

The strongest and weakest determination coefficients (r^2) of 0.878 and 0.295 were observed for WA *plantain-chips* and *bread* respectively.

According to the study, the asparagine and glucose levels in popular WA food materials have a strong and positive link to the amount of AA in the heat-processed WA food products.

Chapter 9

Conclusions and Recommendations

CHAPTER 9. CONCLUSIONS AND RECOMMENDATIONS

9.1 Overview

This research considered common WA foods and their potential contributions to dietary acrylamide intake. Essential factors affecting the level of the contaminant in the selected WA foods were also examined. In this chapter, the findings from the study are reviewed and recommendations are provided for future research in this area.

9.2 Conclusions

For the first time, an extensive study on the presence and levels of acrylamide in popular WA foods was conducted. The effects of parameters such as processing methods, temperatures and times, amount of asparagine and glucose on the formation of the contaminant in the WA foods were considered.

The initial phase of the study, which was an online survey evaluating the knowledge of 1103 different WA participants on the presence of well-known process contaminants in heat-processed foods, indicated that very few (5.1%) recognize the association between heat-treatment of foods and production of hazardous chemicals.

Furthermore, majority of the WA respondents (99.6%) were not informed on the presence of dietary acrylamide in thermally processed foods and its potential toxic effects.

Although Chi square test of the data obtained from the survey showed an insignificant difference in the proportion of the WA groups that are aware of the existence and toxicity of the process contaminant (p value =0.108) >0.05), the study indicated that WA residing in UK (1.7%) were slightly more informed than other WA groups**.**

In addition, the survey demonstrated the importance of baked WA foods as dietary source of acrylamide with 81.0% of the participants consuming them on weekly bases.

Another part of the study considered the role of different cooking methods such as baking and frying on the formation of acrylamide in WA foods including *akara, bread, buns, chin-chin, doughnut, fish-roll, meat-pie, plantain-chips, puff-puff,* and *yam-chips*. The effects of using different cooking temperatures of 150, 180 and 210°C for 5, 10 and 15 mins were also investigated.

The application of 180 and 210°C for the processing of the foods were shown to have high impact on the formation of acrylamide. Also, the cooking of the foods at these high temperatures for 10 and 15 mins significantly increased the levels of dietary acrylamide.

Overall, the use of the different cooking methods under the same cooking conditions resulted in insignificant difference ($P > 0.05$) in the formation of acrylamide. However, the use of frying compared to baking for WA foods including *akara*, *chin-chin*, *meat-pie*, *plantain-chips*, and *puff-puff* increased the level of the contaminant by 8.09, 7.71, 3.97, 8.12, 4.40 % respectively. For *bread*, *buns*, *doughnut*, *fish-roll*, and *yam-chips* the use of baking in comparison to frying created 0.24, 1.21, 1.96, and 9.52% additional acrylamide respectively.

Similar to the findings of previous studies, the amount of asparagine and glucose in popular WA food materials were shown to be positively related to the amount of AA in heat-processed WA food products. The positive association existing between the AA present in the food products and the precursors measured in the food materials was also shown to be significant ($p < 0.01$). Amongst all the WA foods studied, *plantain-chips* had the highest coefficient of determination (r^2) of 0.878.

In order to establish the importance of the amount of acrylamide measured in the WA foods, the indicative values set for similar foods consumed in European countries were used as target marks for comparison.

One-sample t-test of the indicative values for soft breads, crackers, and potato crisps compared to the mean concentration of acrylamide in *bread*, *chin-chin* and *yam-chips* respectively showed that the WA foods had acrylamide levels, which are significantly different from the benchmark levels set by the European commission.

Although most of the WA foods had acrylamide concentrations lower than the target levels set by the European Commission, WA bread exceeded 100 μg/kg, which is the recent indicative value set in 2017 for soft breads, Appendix D.

A comparison of the WA foods to similar Caribbean foods which have been previously studied (Bent et al., 2012), also agrees with the level of acrylamide detected in the WA foods.

9.3 Recommendations

Based on the findings from the various aspects of the study which have been discussed, the following are recommended

- The application of temperatures and times less than 150°C and 5mins respectively should be used by WA food businesses producing heat processed foods such as akara and puff-puff.
- The government of each WA country should establish and fund food and drink organizations to facilitate effective dissemination of information on the formation of acrylamide in heat-processed WA foods, and the risk of consuming such foods
- The members of the African Union should organize a team of food science experts to advise the public and to monitor the level of hazardous chemicals such as acrylamide in WA foods.
- The government and research agencies in each WA country should work together to provide training manuals for small, medium and large scale food businesses on the appropriate method of food production to mitigate the amount of the contaminant in WA foods
- Media including WA television stations and newspapers should promote dietary acrylamide awareness programs and campaigns.
- The African Union should work in conjunction with a committee of food science experts to create a database for the acrylamide content of popular WA foods.
- When it is impossible to use temperatures lower than 150°C for domestic or industrial preparation of WA foods, cooking times less than 15 mins should be used.
- More studies based on the use of a larger number of food samples and other analytical methods should be conducted to evaluate the effect of the cooking methods, temperatures and precursors on the acrylamide levels in WA foods.
- Educational programs and trainings on the mitigation strategies for acrylamide should be organized at both rural and urban levels in WA countries.
- Future studies should focus on identifying susceptible age groups through risk assessment.
- Consequent studies should explore the effect of raw materials on the acrylamide levels in WA foods. For example, the implications of using varieties of plant materials, different flour and oil for the preparation of WA foods.
- In addition, future work should include the evaluation of the risks and benefits of using mitigation strategies such as acidification, blanching, enzymatic treatment, fermentation and soaking for WA foods.

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APPENDIX A- ETHICS APPROVAL LETTER

London South Bank University

School of Applied Sciences

Direct line: 0207 815 5422
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Ref: SAS1730

Monday 21st May 2018

Dear Timothy,

RE: Determination of the acrylamide content in some West African foods

Thank you for submitting your application.

I am pleased to inform you that full Chair's Approval has been given by Dr. Lynne Dawkins, on behalf of the School of Applied Sciences.

I wish you every success with your research.

Yours sincerely,

L. Dawkins

Dr. Lynne Dawkins
Chair, Research Ethics Coordinator
School of Applied Sciences

APPENDIX B- QUESTIONNAIRE

AN AWARENESS SURVEY ON THE PRESENCE AND IMPLICATIONS OF ACRYLAMIDE IN WEST AFRICAN FOODS

1. What is your country of residence?

- ○ Benin
- ○ Burkina Faso
- ○ Gambia
- ○ Ghana
- ○ Guinea
- ○ Guinea-Bissau
- ○ Island of Saint Helena
- ○ Ivory Coast
- ○ Liberia
- ○ Mali
- ○ Mauritania
- ○ Niger
- ◉ Nigeria
- ○ Other African Countries
- ○ São Tomé and Príncipe
- ○ Senegal
- ○ Sierra Leone

- Togo
- United Kingdom
- United States
- Other (Please specify)

2. Which of the listed WA fried food do you eat most often?

- Akara/Acaraje/ Koose (fried bean cake)
- Buns
- Cassava Bong Fries
- Chin-chin (Ghana Chips)
- Dodo-Ikire/Kelewele (spicy fried plantain)
- Doughnuts
- Dun-du (yam fries)
- Egg roll (African Scotch)
- Fried banana (Aloko)
- Fried plantain (Dodo)
- Fried plantain chips
- Kokoro (Corn-meal snack)
- Kuli-Kuli (Deep-fried groundnut cake)
- Ojojo (Water yam ball)

- Pancake (African Style)
- Puff-puff
- Robo (Deep-fried melon seed snack)
- Sinasir/Masa (Rice Pancake)
- Other (please specify)

3. How often do you eat the WA fried food?

- Once a week
- Twice a week
- Thrice a week
- More than thrice a week
- Once in a while (monthly)
- Once in a while (yearly)

4. Which of the listed WA roasted food do you eat most often?

- Boli (Roasted Plantain)
- Garri
- Roasted Maize
- Roasted Yam/Cocoyam
- Suya/Kebab (Roasted Skewered Meat)
- Roasted fish

- Other cereal-based roasted foods
- Other fruit-based roasted foods
- Other tuber or root-based baked foods
- Other (please specify)

5. How often do you eat the WA roasted food?

- Once a week
- Twice a week
- Thrice a week
- More than thrice a week
- Once in a while (monthly)
- Once in a while (yearly)

6. Which of the listed WA baked food do you eat most often?

- Bread
- Baked Chin-chin
- Baked Doughnuts
- Baked Kokoro
- Baked Pancake
- Fish roll
- Meat pie
- Rice Bread

- Other cereal-based baked foods
- Other fruit-based baked foods
- Other tuber or root-based baked foods
- Other (please specify)

7. How often do you eat the WA baked food?

- Once a week
- Twice a week
- Thrice a week
- More than thrice a week
- Once in a while (monthly)
- Once in a while (yearly)

8. Do you know any hazard associated with foods processed by baking, frying or roasting?

- Yes
- No

9. Have you heard about contaminants such as furans, nitrosamines and polycyclic aromatic hydrocarbons (PAHs) in heat-processed foods?

- Yes
- No

10. Are you aware of the formation and health consequences of "Acrylamide" in heat-processed foods?

- Yes
- No

APPENDIX C

Descriptive statistics for the acrylamide levels in WA Bread, Chin-chin and Yam-chips

AA in Bread (µg/kg)		**AA in Chin-chin (µg/kg)**		**AA in Yam-chips (µg/kg)**	
Mean	*117.01*	*Mean*	*100.84*	*Mean*	*483.80*
Standard Error	*4.54*	*Standard Error*	*3.57*	*Standard Error*	*20.26*
Median	*93.35*	*Median*	*121.05*	*Median*	*535.00*
Mode	*175.86*	*Mode*	*151.37*	*Mode*	*533.19*
Standard Deviation	*49.76*	*Standard Deviation*	*39.07*	*Standard Deviation*	*221.91*
Sample Variance	*2476.52*	*Sample Variance*	*1526.63*	*Sample Variance*	*49243.43*
Kurtosis	*-1.46*	*Kurtosis*	*-1.07*	*Kurtosis*	*-1.50*
Skewness	*0.49*	*Skewness*	*-0.65*	*Skewness*	*-0.39*
Range	*146.95*	*Range*	*119.97*	*Range*	*652.04*
Minimum	*53.80*	*Minimum*	*31.40*	*Minimum*	*76.69*
Maximum	*200.75*	*Maximum*	*151.37*	*Maximum*	*728.72*
Sum	*14041.30*	*Sum*	*12100.47*	*Sum*	*58055.91*
Count	*120.00*	*Count*	*120.00*	*Count*	*120.00*
Largest(1)	*200.75*	*Largest(1)*	*151.37*	*Largest(1)*	*728.72*
Smallest(1)	*53.80*	*Smallest(1)*	*31.40*	*Smallest(1)*	*76.69*

APPENDIX D

Comparison of the 2013/17 EC indicative values for soft bread to the AA levels in WA bread.

One-Sample t-Test	
Acrylamide in WA Bread Vs Benchmark Level=150 (µg/kg)	
Mean	117.01
Variance	2476.52
Observations	120
Hypothesized Mean	150
df	119
t Stat	-7.262
P(T<=t) one-tail	< 0.001
t Critical one-tail	1.662
P(T<=t) two-tail	< 0.001
t Critical two-tail	1.978

One-Sample t-Test	
Acrylamide in WA Bread Vs Benchmark Level=100 (µg/kg)	
Mean	117.01
Variance	2476.52
Observations	120
Hypothesized Mean	100
df	119
t Stat	3.744
P(T<=t) one-tail	0.000140
t Critical one-tail	1.662
P(T<=t) two-tail	0.000280
t Critical two-tail	1.978

APPENDIX E

Comparison of the 2013/17 EC indicative values for crackers to the acrylamide levels in WA Chin-chin.

One-Sample t-Test	
Acrylamide in WA Chin-chin Vs Benchmark Level=500 (µg/kg)	
Mean	100.84
Variance	1526.63
Observations	120
Hypothesized Mean	500
df	119
t Stat	-111.911
P(T<=t) one-tail	< 0.001
t Critical one-tail	1.660
P(T<=t) two-tail	< 0.001
t Critical two-tail	1.984

One-Sample t-Test	
Acrylamide in WA Chin-chin Vs Benchmark Level=400 (µg/kg)	
Mean	100.84
Variance	1526.63
Observations	120
Hypothesized Mean	400
df	119
t Stat	-83.875
P(T<=t) one-tail	< 0.001
t Critical one-tail	1.660
P(T<=t) two-tail	< 0.001
t Critical two-tail	1.984

APPENDIX F

Comparison of the 2013/17 EC indicative values for potato crisps to the acrylamide levels in WA yam-chips.

One-Sample t-Test	
Acrylamide in WA Yam-chips Vs Benchmark Level=1000 (µg/kg)	
Mean	483.80
Variance	49243.43
Observations	120
Hypothesized Mean	1000
df	119
t Stat	-25.482
P(T<=t) one-tail	< 0.001
t Critical one-tail	1.658
P(T<=t) two-tail	< 0.001
t Critical two-tail	1.980

One-Sample t-Test	
Acrylamide in WA Yam-chips Vs Benchmark Level=750 (µg/kg)	
Mean	483.80
Variance	49243.43
Observations	120
Hypothesized Mean	750
df	119
t Stat	-13.141
P(T<=t) one-tail	< 0.001
t Critical one-tail	1.658
P(T<=t) two-tail	< 0.001
t Critical two-tail	1.980

APPENDIX G

The nutritional information and the acrylamide levels for various West African food products

	Carbohydrate (g/100g)	Glucose (g/100g)	Protein (g/100g)	Asparagine (g/100g)	Acrylamide (µg/kg)
West African Food Products					
Akara-Brixton	60.00	0.25	22.00	0.99	193.12
Akara-Peckham	44.00	0.19	16.00	0.68	191.52
Akara- East Street	43.00	0.16	14.00	0.62	179.47
Abuja Bread	45.30	0.48	7.99	0.33	92.75
Agege Bread	45.00	0.45	8.00	0.26	75.00
Sabo Bread	47.90	0.55	9.40	0.32	179.50
Uncle Johns Bread	69.00	0.70	8.50	0.43	198.34
Kings Soft Bread-Afro Tasty Bakery	51.70	0.61	8.90	0.39	192.32
Buns-Brixton Bakery	58.30	0.59	7.30	0.44	93.95
Buns-Peckham Bakery	49.70	0.12	9.00	0.12	20.30
Buns-East Street Bakery	52.60	0.45	8.00	0.25	85.52
Jafro Foods Chin-chin	56.80	0.50	6.90	0.21	130.09
Oyebola Chin-chin	60.40	0.64	6.93	0.45	151.37
Original Tropic-Way Chin-chin	49.20	0.26	5.10	0.09	121.25
Tabitha's Original Chin-chin	56.00	0.45	6.00	0.17	129.68
Doughnut- Oluyole Bakery, Camberwell	33.00	0.41	4.80	0.11	77.60
Doughnut-Ades Food, Charlton	44.50	0.54	6.10	0.20	154.58
Doughnut-Agege Bakery, East Street	35.60	0.51	4.40	0.18	122.06
Fish-roll, Brixton	15.00	2.57	6.00	0.36	264.90
Fish-roll, Peckham	12.50	0.23	11.40	0.35	177.60
Fish-roll -East Street	21.30	0.28	7.10	0.38	353.20
Meat-pie, Brixton	24.00	0.18	5.50	0.03	94.25
Meat-pie, Peckham	19.00	0.17	8.10	0.02	83.51
Meat-pie, East Street	25.80	0.19	6.30	0.03	102.38
Meat-Pie, Agege Bakery	36.28	0.21	19.47	0.03	129.68
Ades Plantain Chips	23.00	0.15	1.00	0.03	96.76
Village Pride Salted Plantain-chips	71.00	0.39	4.00	0.09	722.70
Village Pride Sweet Plantain-chips	64.50	0.30	2.40	0.06	538.01
Olu-olu Green Gourmet Plantain Chips	62.00	0.19	3.00	0.04	120.45
Olu-olu Sweet Yellow Plantain Chips	62.00	0.19	4.00	0.04	124.47
Grace Paprika Plantain Chips	64.00	0.27	4.00	0.06	533.19
Grace Chili Plantain Chips	65.00	0.37	4.10	0.08	693.39
Purely plantain Chips	63.00	0.24	1.00	0.05	177.00
Puff-puff- Ades Bakery, Charlton	64.60	0.21	6.50	0.02	124.06
Puff-puff- Agege Bakery, East Street	73.10	0.30	7.20	0.02	216.41
Puff-puff-Mix Bimbolas Bakery	68.68	0.25	9.54	0.01	154.58
Puff-puff- Peckham Bakery	60.20	0.18	6.70	0.01	93.15
Ade's Foods Yam-chips	35.50	0.53	2.10	0.16	716.68
Yam chips, Brixton Market	75.00	0.57	7.40	0.19	721.09
Yam chips, Peckham Market	42.00	0.37	4.00	0.04	676.93
Yam-chips, East Street	33.00	0.30	1.70	0.08	533.19