

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

KNUST

***IN-VITRO* PERFORMANCE OF THE ADSORPTION OF ACRYLAMIDE USING
ALUMINO-SILICA COMPLEXES**

**A Thesis submitted to the Department of Food Science and Technology, in Partial
fulfillment of the requirements for the award of the degree of**

Master of Science in Food Quality Management

By

ESTHER ADU (BSc. BIOCHEMISTRY)

MAY 2017

CERTIFICATION

I hereby proclaim that this submission is my own work with respect to this MSc. and that, to the best of my knowledge, it has no material published earlier by another person or material which has been accepted for the award of any other degree of the University, except where due credit has been made in the text.

Esther Adu

(PG4367415)

Signature

Date

Isaac W. Ofosu

(Supervisor)

Signature

Date

Dr. F .D. Wireko-Manu (Mrs.)

(Head of Department)

Date

Signature

ABSTRACT

Acrylamide is a contaminant formed in food when preparation of food uses above 120 °C of temperature and low moisture content of food. Several methods have been used in the mitigation of acrylamide in foods due to the health risk it poses to people especially children. In this study, the mitigation method used was a binder (alumino- silica complexes -ASC) which adsorbed acrylamide content based on response surface design for the analysis. The binder which had previously been synthesized, required factors such as heating time of 0 to 4 h of the complex, heating temperature of 0 to 1400 °C and alumina component running from 20% to 60% and that of silica running from 40 % to 80 %. The highest percentage reduction of acrylamide as quantified by HPLC, was 88.75% and the least was 53.33%. According to the predicted optimization conditions, the complex; alumina: silica ratio of 60: 40, obtained at a heating time of 3.6 h, with a heating temperature of 1400 °C and agitation time of 4 h, gave the best adsorbed acrylamide content.

TABLE OF CONTENT

CERTIFICATION
ii ABSTRACT
iii TABLE OF CONTENT
..... iv LIST OF TABLES
..... vi LIST OF FIGURES
..... vii
ACKNOWLEDGEMENT
viii
DEDICATION.....	ix
CHAPTER 1.....	1
INTRODUCTION	1
1.1 Background	1
1.1.1 Acrylamide	2
1.1.2 Binders.....	3
1.1.3 Silica.....	3
1.1.4 Alumina	5
1.2 Problem statement and justification.....	6
1.3 Objectives	8
CHAPTER 2.....	9
LITERATURE REVIEW	9
2.1 Acrylamide.....	9
2.1.1 Sources of human acrylamide exposure.....	10
2.1.2 Mechanism of acrylamide formation	13
2.1.3 Uses of acrylamide	14
2.1.4 Effects of acrylamide on humans and animals.....	14
2.1.5 Metabolism of acrylamide in humans	15
2.1.6 Occurrence of acrylamide in foods	17
2.1.7 Mitigation of acrylamide in foods.....	17
2.2 Factors affecting acrylamide formation	19
2.2.1 Effect of pH during acrylamide formation using Maillard reaction.....	20
2.2.2 Effect of storage temperature and heating temperature during acrylamide formation..	20

2.3 The use of alumina and silica as adsorptive materials	21
2.3.1 Alumina	21
2.3.2 Silica	22
CHAPTER 3.....	24
MATERIALS AND METHODS	24
3.1 Materials	24
3.1.1 Alumino-silica complexes	24
3.1.2 Acrylamide substrate	24
3.2 Method	25
3.2.1 Design of Experiment.....	25
3.1.2 Treatment of ASC with the acrylamide substrate	25
3.1.3 HPLC Determination of residual acrylamide content	26
3.2 Data analysis	26
CHAPTER 4.....	29
RESULTS AND DISCUSSION.....	29
4.1 Time-aluminium-silicate complexes performance	29
4.2 Optimization conditions for adsorption of acrylamide using alumino--silica complex ...	32
4.3 Effect of Temperature on acrylamide Adsorption of ASC compositions	33
4.4 Effect of ASC components on adsorption of acrylamide content	34
CHAPTER 5.....	36
CONCLUSION, LIMITATIONS AND RECOMMENDATION	36
5.1 Conclusion	36
5.3 Recommendation	36
REFERENCES	38
APPENDICES.....	51

LIST OF TABLES

Table 1: Physical and chemical properties of acrylamide	9
Table 2: Indicative values for acrylamide in foodstuffs according to Commission Recommendation 2013/647/EU	11
Table 3: Summary of alumina and silica proportions with the process factors.	26
Table 4: Varying levels of the factors for the synthesis of ASC and their respective residual acrylamide percentage reduction.	28
Table 5: A table of constraint indicating set goals and importance of synthesis factors of ASC and response factor for optimizing acrylamide adsorption process.	29
Table 6: A table of Analysis of variance for acrylamide reduction response using the treatment levels of ASC.	32
Table 7: A table of response surface quadratic model for acrylamide reduction	32
Table 8: A predicted desirable condition for maximum acrylamide adsorption using previously prepared alumino--silica complex.	34

LIST OF FIGURES

Figure.1: Ridge Contour graph showing relationship between alumina: silica ratios and time at a constant temperature of 1397.70 °C	30
Figure.2: Three Dimensional Rotatable Quadratic (Y) graph showing relationship between alumina: silica ratios and time at a constant temperature of 1397.70 °C	31
Figure 3: Response Surface “Saddle” Contour (R) plot indicating interactions between temperature and alumina: silica ratios at fixed time of 3.60 h	35
Figure. 4: Plot (T) graph displaying Quadratic function interactions between fixed time of 3.6 and temperature of 1397.7 °C and alumina: silica proportion	36



ACKNOWLEDGEMENT

I would like to thank the Almighty God for giving me the grace and ability to be able to finish this research. Secondly, I would like to thank my husband, Mr. Enoch Padi Adu for believing in me and encouraging me throughout my studies. A big thank you to my supervisor, Mr. Isaac W. Ofosu of the Department of Food Science and Technology for his support, patience, advice, encouragement throughout my studies and for being a father to me. Special thanks to Miss Michelle Oppong Siaw, Miss Abigail Honey Ntiamoah, Mr. Mighty Akley and Mr. William Appaw, all at the department of Food Science and Technology, for helping me during my research.



DEDICATION

I dedicate this work to the Almighty God, for beginning and ending with me through this research and also to my lovely husband and family for their support during this period.



CHAPTER 1

INTRODUCTION

1.1 Background

Risk, which is the probability, frequency and occurrence of a well-defined hazard, and the degree of the effects of the occurrence (Royal Society, 1992; HMSO, 1995) associated with food, has made consumers lose confidence. In developing nations, there is an influence of diet to cancer risk which is nearly about 20 % (Miller, 2001) and so the discovery of this contribution of diet to cancer risk has been of much essence to public health. Thus, research into food hazards and or contaminants has been on the increase.

Chemical contaminants occur during food industry operations, the use of chemical additives and agricultural processes and controls. Toxicants formed during food preparation or processing and packaging are polycyclic aromatic hydrocarbons (PAH), polycyclic aromatic amines (PAA), Nnitrosamines, acrylamide and chemicals produced during food irradiation (Shibamoto and Bjeldanes, 2009). According to Sorgel *et al.* (2002), the main routes of exposure for these contaminants are through eating of food, dermal contact, and inhalation. According to Brown *et al.* (1980a) and Howard (1989), acrylamide is ingested through food and water (H₂O) – when there is treatment of water using polyacrylamides flocculent, which are very soluble in the water. For dermal route of acrylamide exposure, the utilization of polyacrylamide in cosmetic products, gardening products, paper and pulp products, coatings, and textiles leave small quantities of acrylamide on the skin, which is then absorbed into the body (Manson *et al.*, 2005). Also, the production of polyacrylamide and acrylamide leave traces on the skin. Inhalation of cigarette smoke; dust and vapor during acrylamide and polyacrylamide production also introduce

acrylamide into humans (IARC, 1986). The intake of acrylamide in food is common to everybody and its mean estimate range from 0.8 to 6.0 $\mu\text{g/kg bw/day}$ for near-term exposure and 0.3 to 0.8 $\mu\text{g/kg bw/day}$ for long-term exposure (Tareke *et al.*, 2002). For that of children, the estimated average is about 2 to 3 times that of grown-ups with respect to weight of the person (Tareke *et al.*, 2002).

1.1.1 Acrylamide

The use of fire by early man was a turning point for the preparation of food. The improvement of colour, taste and aroma, all result in the formation of hazards like acrylamide. The discovery of acrylamide concentration in thermally treated foods by Swedish scientists in 2002, occurred when they were originally examining unintentional spillage of grouting agent among tunnel workers, where these scientists observed that workers exposed to acrylamide earlier had their blood containing substantial levels of acrylamide- hemoglobin adducts (Tareke *et al.*, 2002). Thus, foods were investigated to determine the presence of acrylamide in Stockholm where carbohydrate rich-food products had acrylamide, for instance roasted coffee and coffee products. Swedish National Food Administration on the other hand, revealed that protein rich foods had little or no amount of acrylamide; and raw food like raw potatoes have no amount of acrylamide content as indicated by Tareke *et al.* (2002).

Ever since the discovery and formation of acrylamide, a lot of research has been directed into eliminating and reducing acrylamide content in foods. Mottram *et al.* (2002) and Stadler *et al.* (2002) reported that acrylamide could be formed in heated carbohydrate rich-foods where free asparagine and intermediates of Maillard reaction react. The acrylamide content in food differ with the type of food, technique of cooking, increase in temperature i.e. 120 °C (248 °F) or above, how

long the food is cooked, water content of the food and food thickness (Sorgel *et al.*, 2002; WHO, 2003).

1.1.2 Binders

Currently, there are many conventional methods in reducing or eliminating hazards or contaminants such as adsorption on materials, exchange of ions, reverse osmosis, chemical precipitation and electrodialysis. In all these methods, adsorption has been the most feasible, easy and cost effective. Some of the adsorbents are alumina (Ku and Chiou, 2002; Ghorai and Pant, 2005), bone char (Leyva-Ramos *et al.*, 2010) and rare earth oxides (Raichur and Basu, 2001)

1.1.3 Silica

Silicon (Si) is the most copious element found on earth, which is almost around 27.2%. It has essential physical and chemical characteristics; and the complex structure of its compounds makes it of prime interest for scientists from generation to generation. As a result of this, silicon dioxide (SiO_2) has been the most widely studied inorganic compound apart from water (Greenwood and Earnshaw, 1997). Its production begins by alkaline extraction, then by acid precipitation, where the precipitate is washed, followed by dehydration to form silica gel. Treguer *et al.* (1995) reported that silica is found in most water bodies or systems in the orthosilicic acid (H_4SiO_4) form and this compound is important in ecology since it is responsible for rendering silicon to cells of living beings. Hence, it is an indication of its presence in humans and animals.

Another indication of the presence of silicon in humans is in hair (Smith, 1993) and nails (Austin, 1997). It is also a trace element in large quantities in humans since it is abundant in foods in the form of silicate, in free ortho-silicic acid, silicic acids bounded to certain nutrients and silicon

dioxide (Reffitt *et al.*, 1999). Thus, in plant based foods – barley, oats, cereals, polished rice and white wheat flour; and in animal foods – dairy products and meat. In addition, drinking water and mineral water have silicon in them (Bowen and Peggs, 1984.). In a study conducted by Institute of Medicine (2000), the upper intake level (UIL) for amorphous silicon dioxide during a risk assessment was 700 mg/day for adults which corresponds to 12 mg silicon/kg bw/day.

Silica exists in amorphous form – natural and synthetic; and crystalline forms – quartz and porosil. Silica is normally found as quartz and also in different living organisms. Quartz is an abundant and recognized material which is converted to β -quartz, tridymite and cristobalite when there is application of heat. Also, the use of man-made amorphous silica particularly silica nanoparticles (SNPs) in cosmetic, pharmaceutical and food industries as additives on a large scale has received large attention. To add to it, nanoparticles of silica are used in biotechnology and biomedical science in drug delivery, enzyme immobilization, DNA transfection and cancer therapy (Hirsch *et al.*, 2003; Moghimi *et al.*, 2005; Ravi Kumar *et al.*, 2004; Slowing *et al.*, 2008; Vijayanathan *et al.*, 2002). In the food industry of United States of America, nanosilica is used as a food additive for almost half a decade and the maximum limit of its use is 2 % by weight in dried powdered foodstuffs and on the other hand, European Union's maximum level for silica is 1 % (US FDA, 2015).

The use of silica gel includes selective adsorbent in column chromatography, catalyst, desiccant, flattening agent in thin film or coatings for electronic and optical materials, in glass, ceramics and cement. It can also be an adhesive agent or bonding agent during drug, cosmetics and detergents manufacture, using its soluble silicates (Anon., 1997; Laxamana, 1982; Lender and Ruiter, 1990; Brinker and Scherer, 1990).

1.1.4 Alumina

Alumina was first discovered by a man called Marggraf by removing it from natural clay with the utilization of sulphuric acid in 1754 and later discovered in the eastern and south eastern of France where the soil was rich in alumina - bauxite (Davis, 2010). Bauxite which is readily available in Ghana is mainly made up of alumina and in small changing amounts of iron oxides, silica, titanium oxide and alumina silicate. This pure compound is being thermally activated to eliminate chromium (Erden *et al.*, 2004), arsenic (Altundogan *et al.*, 2002) and fluoride (Sujana and Anand, 2010; Lavecchia *et al.*, 2012) from water as an effective adsorbent with a low price. Alumina has chemical, mechanical and electrical properties with purity of 99.7 % which is being used in ceramic production; its hardness makes it useful as an abrasive material and its melting point of 2000 °C as a refractory and as a lining of special furnaces (Davis, 2010). Also, the surface of alumina has terminal hydroxyl groups (Al-OH) which helps in its adsorptive nature. According to Jones-Hughes *et al.* (2013), there was a great effective reduction of arsenic when activated alumina was used as an adsorptive material ($\geq 95\%$) as a form of intervention to remove arsenic contamination of groundwater in developing countries.

Currently, nano powders of alumina are used in a lot of technologies like catalysts, adsorbents and for nanoceramics (Khrustaleva and Voronova 2016). The utilization of aluminosilicates as solid-phase catalyst for instance smectites, zeolites and imogolite and sorbents have aided in the use of these aluminosilicates in physiological systems usually through the gut and lungs (Brown, 2010; Godoi *et al.*, 2008; Lomer *et al.*, 2002). Furthermore, White *et al.* (2008) and Desouky *et al.* (2002) found out that there was uptake of aluminum in cells which results in the quick mobilization and buildup of dissolved silica, which forms non-toxic aluminosilicates in the intracellular

compartments of multicellular organisms. Hence, the major purpose of this research was to determine the reduction capacity of alumino- silica complexes on acrylamide and set optimization processing conditions using response surface methodology.

1.2 Problem statement and justification

Hogervorst *et al.* (2009) reported that an increase in the consumption of acrylamide was not associated with lung cancer risk in women and low intake of acrylamide lead to lung cancer risk in women; however in men, the ingestion of dietary acrylamide was not linked with lung cancer risk. Thus, there could be other ways of acrylamide carcinogenesis apart from genotoxicity, which could be hormonal when acrylamide was administered in animal studies (Johnson *et al.*, 1986; Friedman *et al.*, 1995). In relation to the hormonal precursors during acrylamide carcinogenesis, there were endometrial and ovarian cancer risks in association with acrylamide intake (Hogervorst, 2007). In addition, there is an endometrial cancer risk and probably ovarian cancer risk in people who frequently ingest dietary acrylamide products (Wilson *et al.*, 2010). According to Schouten *et al.* (2009) there was a heightened risk of oral cavity cancer during ingestion of dietary acrylamide in women non-smokers but not in men.

Investigations from Sorgel *et al.* (2002) revealed that women who took acrylamide food products had acrylamide in their breast milk and that minute amounts of acrylamide in their breast milk were transferred to their babies. This is as a result of the hydrophilic nature and small size of acrylamide structure which enables it to move to almost all tissues in the body (Friedman, 2003). This is an indication that every tissue and organ could be a target of carcinogenesis which could be a danger to unborn babies since acrylamide could similarly cross the placenta (WHO, 2003).

From all this research, it seems women and children are being affected most, nevertheless, Bongers *et al.* (2012) conducted an epidemiological study which indicated that the intake of acrylamide could heighten the risk of multiple myeloma and follicular lymphoma in men.

Due to the earlier effects and cancer risks of acrylamide in foods stated, there is the need for reducing or eliminating acrylamide in processed food in the food industries and research institutions which will not increase or introduce other process contaminants for public health. Some mitigation methods are the control of time and temperature throughout baking, where applicable, prolong fermentation time, where feasible replace ammonium bicarbonate with other substitutes, reduction or elimination of reducing sugars such as glucose, the use of asparaginase and the last being the improvement of agronomy and plant breeding (controlling the levels of asparagine and sugars) which is a long - term mitigation of acrylamide in food (Konings *et al.*, 2007; Foot *et al.*, 2007). The near -term use of asparaginase is an encouraging means to minimize acrylamide content in food, however the high price of asparaginase will not enable its use in foods for local producers (Ciesarová *et al.*, 2010).

Also, there are disparities of acrylamide content between different production runs of food products, between similar batches, between different manufacturers but the same products, between the same product having varying ingredients from varying crop years. In addition, a food product could have its acrylamide content low but this could increase the dietary acrylamide exposure, if it is eaten regularly or in big quantities, for instance potato chips and coffee as indicated by Lineback *et al.* (2011). Thus, the need for a cheap, an available and an effective method for reducing acrylamide in foods, irrespective of the acrylamide content in the foodstuffs. Binders like silica and alumina are efficient adsorbents used to reduce hazards using different

temperatures since they are known to be good adsorbents of hazards. The present study was aimed at assessing the potential of alumino- silica complexes to reduce acrylamide using different temperatures at a pH of 7.

1.3 Objectives

The main objective of this project was to use previously synthesized alumino-silica complexes (ASC) to remove acrylamide from acrylamide substrate and to predict a model for the optimum adsorption conditions for the acrylamide.



CHAPTER 2

LITERATURE REVIEW

2.1 Acrylamide

The awareness of acrylamide as a contaminant in food was created during the discovery of acrylamide in certain foods that are prepared above temperatures of 120 °C with low moisture in 2012 in Sweden (Tareke *et al.*, 2002). It is also identified as 2-propenamide and has the formula $\text{H}_2\text{CHCCNH}_2\text{O}$. It is referred to as a probable carcinogen to humans (Class 2A), according to International Agency for Research on Cancer (IARC, 1994).

The hydration of acrylonitrile leads to the formation of acrylamide where it exists as odorless, white crystalline solid at room temperature. According to European Food Safety Authority (2010), acrylamide is highly soluble in water, dissolves in methanol, ethanol, acetone, ethyl acetate, and chloroform but insoluble in benzene and heptane.

Table 1: Physical and chemical properties of acrylamide

Property	Information
Molecular weight	71.1
Specific gravity	1.122 at 30 °C/4 °C
Melting point	84.5 °C
Boiling point	192.6 °C
Log K_{ow}	-0.67
Water solubility	371 g/L at 20 °C
Vapor pressure	7×10^{-3} mm Hg at 25 °C
Vapor density relative to air	2.5

Source: Hazardous Substances Data Bank (2009).

In addition, it has a lesser molecular weight as an organic compound. It is biodegradable and can readily move in soil and groundwater (Smith *et al.*, 1996, 1997). According to Smith *et al.* (1996),

acrylamide is stable during normal conditions, on the other hand, when heat is applied or there is exposure of ultraviolet light or outdoor environmental conditions, acrylamide may decompose or polymerize. These physical characteristics can be seen in Table 1.

2.1.1 Sources of human acrylamide exposure

Exposure occurs mainly via ingestion, dermal contact and inhalation routes (Sorgel *et al.*, 2002). Human acrylamide exposure derived from industrial sources has primarily occurred due to workplace exposure especially during the use of acrylamide in the production of polyacrylamide and grouting agents. Major routes of exposure at the workplaces appear to be dermal absorption of acrylamide monomer from solution and inhalation of dry monomer or aerosols of acrylamide solution. There is always an exposure of acrylamide during acrylamide and polyacrylamide manufacture, acrylamide grouting and laboratory preparation of polyacrylamide gels (IARC, 1994). Also, the combustion or burning of tobacco releases acrylamide and hence a major source of human acrylamide exposure among smokers (Urban *et al.*, 2006).

It has also been revealed that during high-temperature processing of carbohydrate-rich foods, acrylamide is formed (Tareke *et al.*, 2002). Ingestion of foodstuffs containing acrylamide appears to be one of the most common methods of exposure for the entire populace. In Ghana, carbohydrate-rich food that contain acrylamide is bread which is mostly consumed by children as reported by Arthur (2014). Approximately, average intake of acrylamide from food sources ranged from 0.8 to 6.0 $\mu\text{g/kg bw/day}$ for short-term exposure and 0.3 to 0.8 $\mu\text{g/kg bw/day}$ for long-term exposure (WHO 2002, 2003). Children may be susceptible to food-borne exposure 2–3 times that of adults

on the basis of body weight (WHO 2002, 2003). Once acrylamide is in the body, acrylamide is widely dispersed by body fluids, and can also cross the placental barrier

(WHO 2003), resulting in exposure to unborn children. Unborn babies' blood brain barrier is partially developed, hence, pregnant women who consume acrylamide containing foods in large amounts could transfer these cancer risks to the fetus.

Table 2: Indicative values for acrylamide in foodstuffs according to Commission

Recommendation 2013/647/EU

Foodstuff	Indicative value (µg/kg)
French fries ready-to-eat	600
Potato crisps from fresh potatoes and from potato dough, Potato based crackers	1000
Soft bread	
-wheat based bread)	80
-soft bread other than wheat based bread	150
Breakfast cereals (excl. porridge)	
- bran products and whole grain cereals, gun puffed grain (gun puffed only relevant if labelled)	400
- wheat and rye based products	300
- maize, oat, spelt, barley and rice based products	200
Biscuits and wafers	500
Crackers with the exception of potato based crackers	500
Crispbread	450
Gingerbread	1 000
Products similar to the other products in this category	500
Roast coffee	450
Instant (soluble coffee)	900
Coffee substitutes	
(a) coffee substitutes mainly based on cereals	2000
(b) other coffee substitutes	4000

Baby food, other than processed cereal based foods(2)	
(a) not containing prunes	50
(b) containing prunes	80
Biscuits and rusks for infants and young children	200
Processed cereal based foods for infants and young children(3), excl. biscuits and rusks	50

Based on experimental humans and animals, administration of acrylamide in food orally lead to the absorption of acrylamide from the gastrointestinal tract (GIT) and then circulated immediately to the tissues. Due to this rapid distribution, it has the potency to cross the placenta and also be taken up by the breast tissues – thus, the presence of acrylamide in human milk to a minimized extent. Breast milk of mothers with diets high in acrylamide-containing foods can have high amounts of acrylamide as revealed by Sorgel *et al.* (2002). With regards to exposure of acrylamide, infants, toddlers and other children are the most exposed groups due to „baby foods“ which are mostly processed cereal-based foods, some potato fried products, „soft bread“ and biscuits.

At an initial concentration of 10 ppm, carbohydrate-rich foods, such as potato, beetroot, potato products, and crispbread, contained much higher concentrations, ranging from 150 to 4,000 µg/kg. The median acrylamide concentration in fried foods, including beef, chicken, soymeal, grated potatoes, boiled mashed potatoes, and grated beetroot were 17, 28, 16, 447, 172, and 850 µg/kg, respectively. In microwave-heated grated potatoes, the median acrylamide concentration was found to be 551 µg/kg, while microwave-heated cod was less than the detection limit (<5 µg/kg). In restaurant-prepared or purchased foods, the median acrylamide concentrations for hamburger, French fries, potato crisps, and three types of crispbread were 18, 424, 1,739, and 208 respectively. The different food stuffs with their acrylamide concentrations can be observed in Table 2.

2.1.2 Mechanism of acrylamide formation

In the quest to improve taste and appearance of foods, Maillard reaction has been used for this effect. This has led to the initiation of acrylamide formation by the use of free aldehyde or free ketone groups of sugars (predominantly fructose and glucose) with proteins or amino acids especially asparagine in Maillard reaction of foods (Stadler *et al.*, 2002; Mottram *et al.*, 2002) and usually using high temperature cooking which contributes mainly to the induction of acrylamide formation (Yaylayan *et al.*, 2003). There are two pathways for the formation of acrylamide, the major one involves glucose and amino acids whilst the minor pathway involves acrolein (Gertz and Klostermann, 2002).

With respect to the major pathway, the amide moiety of asparagine from the Maillard reaction products combines with the carbonyl group of reducing sugars (especially glucose) which produces the Schiff's base which is then decarboxylated to azomethine ylide through Schiff betaine or intermediary oxazoline-5-one (Yaylayan *et al.*, 2003). Later on, the azomethine ylide gives rise to acrylamide (Zyzak *et al.*, 2003).

The minor pathways for acrylamide formation are the use of acrolein (a lipid degradation product). Lipid undergoes hydration to produce glycerol under high temperatures and glycerol releases water when heat is applied to produce acrolein. Acrolein then undergoes oxidation at high temperatures to produce acrylic acid. Acrylic acid reacts with ammonia group from an amino acid to produce acrylamide (Yaylayan and Stadler, 2005). Acrolein can also form a radical at high temperatures and this acrolein radical form acrylamide with amino radical, which is also formed from an amino acid under high temperature (Shibamoto and Bjeldanes, 2009).

2.1.3 Uses of acrylamide

The major utilization of acrylamide is to make water-soluble polyacrylamides which serve as flocculent during waste water treatment, clarifying drinking water and in oil-well processes, it serves as flow control agents – industrial purposes. Also, acrylamide is used in chemical grouting agents for sealing holes in sewers and manholes. According to Lineback *et al.* (2011), polyacrylamide utilization in soil increases porosity, aeration and decreases compaction and water run-off and these enhance soil stability. In addition, there is high demand for gel electrophoresis during scientific research especially in biotechnology laboratories (IARC, 1994).

2.1.4 Effects of acrylamide on humans and animals

According to JECFA (2011) on acrylamide, acrylamide main health issue in humans and animals is cancer due to its presence in most processed foods. The small and hydrophilic nature of acrylamide makes it easy to diffuse to all other tissues in the body thereby all tissues serves as targets for carcinogenesis. It is mostly regarded as a probable human carcinogen. As a result of this, studies have been conducted on animals to prove its carcinogenicity. Hence, in rodents, it caused benign thyroid – gland tumors, in male rats – it caused tumors of the lining of the testes and benign adrenal – gland tumors. Also, it caused benign tumors of the pituitary gland, oral cavity and clitoral gland; cancer of the uterus and the malignant and benign tumors of the mammary gland (IARC, 1994). The genotoxicity in humans is as a result of extrapolation from the increased chronic doses in rats to the decreased chronic doses in humans during acrylamide risk assessment where an acrylamide intake of 1 μ g/kg body weight per day in humans corresponds to a relative risk of cancer in humans of between 1.006 and 1.05 (Mucci and Adami, 2005)

Also, there have been studies that indicated the genotoxic and neurotoxic effects which may pose health effects for humans (Mojska *et al.*, 2010). The ingestion of foods containing acrylamide give

rise to high risk of cancer of the breast, endometrial, ovaries and kidney in women as stated by Olesen *et al.*, 2008 and Hogervorst *et al.*, 2008. Studies on acrylamide in humans when exposed to high levels of acrylamide mainly caused damage to the nervous system (LoPachin, 2004) and presented carcinogenic and mutagenic characteristics in mammals during in vitro and in vivo research (Dearfield *et al.*, 1995). According to a cohort study, workers who were mainly men had a heightened risk of pancreatic cancers when compared with the period of exposure and also the time of first exposure (Marsh *et al.* 1999, Schulz *et al.* 2001). The intake of acrylamide foods correlates with an increased risk of oral cavity cancer during cohort studies in female nonsmokers only. In addition, an inverse association between dietary acrylamide ingestion and lung cancer in females during the cohort studies conducted (Hogervorst *et al.*, 2009).

When considering cancers of the endocrine system due to acrylamide, it is believed that they are not caused by genotoxicity, hence their mode of action could be hormonal or nongenotoxic (Bosetti *et al.*, 2000). an in- vitro study of glycidamide induced gene expression in colon cancer cells and human breast cancer cells indicated a heightened expression of genes which activate androgen and estrogen precursors to testosterone and 17(β)estradiol (Clement *et al.*, 2007).respectively. On the contrary, there was an increased risk in endometrial and ovarian cancers when dietary acrylamide was taken in. As a result of these serious effects, EPA established an oral reference dose (RfD) of 0.002 mg/kg/day for acrylamide (EPA, 2010) and an

concentration (RfC) of 0.006 mg/m³ for acrylamide (EPA, 2010)

2.1.5 Metabolism of acrylamide in humans

By means of acrylamide being a water-soluble molecule, it is distributed immediately to all body tissues without accumulation in tissues. Presence of acrylamide in the body is quickly acted upon

by cytochrome P450 2E1 through a process called epoxidation to produce the epoxide glycidamide, which serves as the more mutagenic metabolite. Acrylamide having active double bond could react with food ingredients like proteins, DNA and RNA. On the contrary, the increased reactivity of acrylamide to food components like amino, hydroxyl and sulfhydryl groups of peptide and proteins of food containing acrylamide might be the cause of reduced bioavailability of acrylamide in the body (Hoenicke and Gatermann, 2005).

The rest of the acrylamide and glycidamide are then conjugated with glutathione as a form of detoxification reaction using glutathione-S-transferase and excreted as mercapturic acid derivatives in urine which is the main excretion route of acrylamide metabolites in humans. These derivatives are detected as cysteine metabolites in urine (Doerge *et al.*, 2007; Doroshyenko *et al.*, 2009; Kopp and Dekant, 2009). Another means where glycidamide can be metabolized is through hydrolysis which forms 2,3-dihydroxypropanamide (glyceramide) and consequently form 2,3-dihydroxypropionic acid (Sumner *et al.*, 2003)

The concentration of acrylamide bound to the N-terminal amino acid in hemoglobin is strongly correlated to the exposure of acrylamide, 3,35–37 while its glycidamide analog correlates to glycidamide DNA adducts, 37 and is considered a bio-marker for the genotoxic dose reflecting the individual ability to activate acrylamide to glycidamide (WHO, 2002). Acrylamide is not expected to significantly bioconcentrate due to its high water solubility and its ability to be degraded by microorganisms (Haberman, 2002; WHO, 2003). Therefore, bioaccumulation through the food chain is expected to be low. No data needs are identified.

2.1.6 Occurrence of acrylamide in foods

There is formation of acrylamide in heated processed foods above 120 °C with low moisture, on the contrary is absent in raw ingredients like raw potatoes (Tareke *et al.*, 2002). Acrylamide content in food product is low but it could also add up to the food acrylamide content exposure when taken often or in big quantities e.g. coffee and potato crisps.

Carbohydrate-rich foods typically contain the highest levels of acrylamide (Muttucumaru *et al.*, 2008; Tareke *et al.*, 2002; WHO 2003), whereas protein-based foods contain smaller amounts (Tareke *et al.*, 2002). Concentrations of acrylamide in food vary with the type of food and method of cooking, and typically increase with temperature and length of heating (Sorgel *et al.*, 2002; WHO, 2002, 2003). As acrylamide content in food appears to be affected by temperature, water content, food thickness, and length of heating, various steps can be taken to minimize the exposure of acrylamide from food sources (Sorgel *et al.*, 2002; WHO, 2002, 2003).

There is great concern in the determination of acrylamide content in food product due to variations between production runs from the same manufacturer, with the same batches, between different manufacturers, between different varieties or cultivars and or producers and between the same products containing different ingredients from different crop years. Also, storage temperatures can affect acrylamide concentration (Biedermann-Brem *et al.*, 2003; Grob *et al.*, 2003).

2.1.7 Mitigation of acrylamide in foods

Essential efforts by scientists and the food industry on the reduction of acrylamide in major foods with high acrylamide content have been carried out. For instance the use of Food Drink Europe (FDE) toolbox in CODEX and other tools by other researchers. The need to control sugar content

of potato-based products by farmers and producers to monitor the best agronomy practices, sorting of variety of potato, harvesting of matured tubers, choosing of tubers due to colour and sugar analysis, storage temperature conditions from farm to fork (Haase, 2006).

To mitigate acrylamide, the prohibition of acrylamide formation when decarboxylation of asparagine is not attained through intramolecular cyclization to form an imide that is maleimide which will not be change to acrylamide (Yaylayan *et al.*, 2003). In avoiding the formation of acrylamide, the Schiff base of *N*-glycosylasparagine – Amadori rearrangement and intramolecular cyclization. At high temperatures, the Amadori rearrangement forms Nsubstituted succinimide. This pathway prevents acrylamide formation using competitive inhibition (Manini *et al.*, 2001).

There is high concentration of asparagine in tubers especially when the soil has high nitrogen and this is leads to an increase in acrylamide content during acrylamide formation (Lea *et al.* 2007). Thus, a reduction of soil nitrogen and potassium will reduce the acrylamide storage, leading to low asparagine for acrylamide formation. There is also the removal of surface reactants like sugars during blanching in the process of French fries industry reduce acrylamide in the final product (Hendriksen *et al.*, 2009). Addition of pyrophosphate to blanching water to avoid discolouration and lowering the pH as a secondary effect to prevent reactants that will lead to Maillard reactions. Also, according to Mestdagh *et al.* (2008), the application of citric acid salts, ascorbic acid, antioxidants, calcium salt and lactic acids to potato matrix in several ways to reduce acrylamide formation during processing. Some of these ingredients can negatively affect the organoleptic characteristics of the final food or product e.g. citric acid.

Furthermore, the use of asparaginase (an enzyme to break down asparagine to aspartic acid and ammonia, although has some reactivity towards glutamine (Pedreschi *et al.*, 2008, 2011). Further laboratory research from Pedreschi *et al.* (2011), revealed that there was 90% reduction of acrylamide when there was combined treatment of blanching and asparaginase on potato slice. On the contrary, Foot *et al.* (2007), observed that, blanching had a negative effect on the texture, flavor, nutritional properties of the fried product (oil content and vitamin C) which is not applied commercially.

With reference to cereals, the use of asparaginase also reduces acrylamide but it can only be very effective in some cereal foods like cereal based dough snacks and semisweet biscuits. Where as in gingerbread, it will aid in the re-introduction of ammonium salts – leading to less or no effect on quality or organoleptic properties of the food products. Thus the efficacy of the use of asparaginase depends on the concentration of the enzyme and contact time. Fermentation of reducing sugars using lactic acid bacteria to produce lactic acid reduces the pH and thus lowers the Maillard reaction which reduces acrylamide formation. Reduction of 90% possible quality/ sensorial properties of final products (Blom *et al.*, 2009). Lastly, the introduction of extra amino acids which will competitively inhibit asparagine during key reaction in the Maillard reaction when plant based protein hydrolysate are used which will mitigate acrylamide (Cook and Taylor, 2005).

2.2 Factors affecting acrylamide formation

During the formation of acrylamide, a lot of factors come into play to enhance its formation.

Factors like the food systems such as carbohydrate-rich or protein-rich matrix (Claeys *et al.*, 2005a). Other factors include concentrations of precursors, heating time, heating temperature and

pH. The concentration of precursors which cause acrylamide formation are affected by farming systems, time of harvest of crops, different cultivars, storage time and pesticide application. To add to it, the effect on food matrices is also essential in the formation of acrylamide (Stadler *et al.*, 2004).

2.2.1 Effect of pH during acrylamide formation using Maillard reaction

pH has been one of the contributing factors which aids in the formation of acrylamide. When Amadori rearrangement is formed during Maillard reaction as stated earlier, pH changes in the system especially pH of 7 and below enables the formation of furfural or hydroxymethylfurfural (HMF). This product undergoes 2,3 –enolization to produce pyruvaldehyde, diacetyl and acetol; and reductones when pH is below 7. All these products undergo several pathways to produce melanoidins which is the last step in Maillard reaction (Martins *et al.*, 2001). Also the decreasing of pH of food matrices reduce acrylamide formation by enabling the α -amino group of asparagine to be protonated hence preventing carbonyl sources from attaching to it (Jung *et al.*, 2003). In addition, Jung *et al.* (2003) revealed that the relationship between acrylamide mitigation and lowering pH may depend on the type of product as a result of different factors or different starting pH values of the products.

2.2.2 Effect of storage temperature and heating temperature during acrylamide formation

During studies conducted by Biedermann-Brem *et al.* (2003) and Grob *et al.* (2003), acrylamide levels in potato cultivar had its concentration increased when the storage of temperatures were below 10 °C as compared to storage temperatures above 10 °C. Also, Mottram *et al.* (2002), indicated the reliance of acrylamide formation on temperatures above 100 °C where a further

increase of temperature from 100 °C did not increase the acrylamide concentration but the acrylamide concentration increased with heating time.

In addition, the process of frying at high temperatures increase acrylamide formation especially when the moisture content of the product is low (Fiselier *et al.*, 2005). Lower moisture content enhances the activation energy of acrylamide formation according to Amrein *et al.* (2006b). An increase in temperature and time of heating increases acrylamide content in French fries (Matthäus, 2002).

2.3 The use of alumina and silica as adsorptive materials

In recent times, the use of conventional methods in reducing or eliminating hazards or contaminants using adsorption on materials, exchange of ions, reverse osmosis, chemical precipitation and electrodialysis. Among all these methods, the easiest and cost effective one is adsorption and in this study alumina and silica are considered.

2.3.1 Alumina

The discovery of alumina was by a man called Marggraf in 1754 where it is obtained from clay when sulphuric acid is added (Davis, 2010). Alumina has chemical, mechanical and electrical properties with purity of 99.7 % which is being used in ceramic production; its hardness makes it useful as an abrasive material and its melting point of 2000 °C as a refractory and as a lining of special furnaces (Davis, 2010). Also, the surface of alumina has terminal hydroxyl groups (AlOH) which helps in its adsorptive nature. According to Jones-Hughes *et al.* (2013), there was a great effective reduction of arsenic when activated alumina was used as an adsorptive material (≥ 95 %) as a form of intervention to remove arsenic contamination of groundwater in developing countries.

Adsorption studies of HF using alumina by Haverkamp *et al.* (1992) where there was no evidence of AlF formation at low temperatures (20 °C – 120 °C) but there was evidence of AlF formation leading to total removal of fluoride when the temperatures were elevated (700 °C – 1000 °C) using alumina as the binder. The use of nanopowders of alumina which has large capacity for the adsorption of eosin as indicated by Khrustaleva and Voronova (2016) also gave a clear indication of the efficient adsorption of alumina when used as a binding material.

2.3.2 Silica

Silicon (Si) is the most copious element found on earth, which is almost around 27.2 %. It has essential physical and chemical characteristics; and the complex structure of its compounds makes it of prime interest for scientists from generation to generation. As a result of this, silicon dioxide (SiO₂) has been the widely studied inorganic compound apart from water (Greenwood and Earnshaw, 1997).

Silica exists in amorphous form – natural and synthetic; and crystalline forms – quartz and porosil. Silica is normally found as quartz and also in different living organisms. Quartz is an abundant and recognized material which is converted to b-quartz, trydimite and cristobalite when there is application of heat. Also, the use of man-made amorphous silica particularly silica nanoparticles (SNPs) in cosmetic, pharmaceutical and food industries as additives on a large scale has received large attention. To add to it, nanoparticles of silica are used in biotechnology and biomedical science in drug delivery, enzyme immobilization, DNA transfection and cancer therapy (Hirsch *et al.*, 2003; Moghimi *et al.*, 2005; Ravi-Kumar *et al.*, 2004; Slowing *et al.*, 2008; Vijayanathan *et al.*, 2002). In the food industry of United States of America, nano silica has been used as a food additive for almost half a decade and the maximum limit of its use is 2 % by weight in dried

powdered foodstuffs and on the other hand, European Union's maximum level for silica is 1 % (US FDA, 2015).

The use of silica gel includes selective adsorbent in column chromatography, catalyst, desiccant, flattening agent in thin film or coatings for electronic and optical materials, in glass, ceramics and cement. It can also be an adhesive agent or bonding agent during drug, cosmetics and detergents manufacture, using its soluble silicates (Anon, 1997; Laxamana, 1982; Lender and Ruiter, 1990; Brinker and Scherer, 1990). The use of kaolinite (aluminosilicate) which has silica and alumina oxides alternating its sheets, in the adsorption of polyacrylamide where there is exchange of cations. Adsorption was very quick, within 15 min of contact time as described by Hollander *et al.* (1981).

In conclusion, acrylamide which is a probable carcinogen has led to several research into the mitigation of it where most of the mitigation methods is to prevent the formation of acrylamide. With this study the binder used was alumino-silica binder due to the abrasive nature of alumina and the adsorptive property of silica for column chromatography. The essence of *in – vitro* studies in the reduction of acrylamide levels when alumino--silica complexes are applied is devoid of ethical issues and it is a cheap way to predict the adsorption of acrylamide using alumino – silica complexes *in – vivo*, even though the later studies might not be efficient.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

All reagents used (starch, sodium azide, NaCl, KCl, $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, KH_2PO_4 , acrylamide (99 %) and acetonitrile) in this study were acquired from Sigma Aldrich - USA.

3.1.1 Alumino-silica complexes

The alumino-silica complexes were previously prepared (Iloabuchi, 2016) where silica chippings were obtained from Apromase and alumina from BDH chemicals. The alumino-silica complexes previously prepared at varied proportions of alumina from 20 % to 60 % whilst that of silica was varied from 40 % to 80 % using a temperature range of 0 °C to 1400 °C and time interval of 0 to 4 h.

3.1.2 Acrylamide substrate

Acrylamide substrate was prepared using 0.1 M phosphate-buffered saline (PBS), 560.0 mg of acrylamide, 56.0 g of starch and 1.0 g of sodium azide. In preparing the 0.1 M phosphate-buffered saline (PBS), the Cold Spring Harbor (2014) method was used with slight modifications where 82.0 g NaCl was used instead of 80.0 g, 2.0 g KCl, 21.7 g $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ and 2.59 g KH_2PO_4 were dissolved in 350.0 mL of distilled water. Starch, acrylamide and the sodium azide were added to the buffer solution and topped up to the 1 L mark of the volumetric flask with distilled water at a pH of 7.

3.2 Method

3.2.1 Design of Experiment

The design of experiment used for the synthesis of alumino-silica complexes was based on the method used by Iloabuchi (2016). The design was based on D-optimal quadratic \times quadratic, mixture-process design (Design Expert, 2012), where the variables (Table 3) alumina (A), silica (B), time (C) and temperature (D) were varied according to the limits shown.

Table 3: Summary of alumina and silica proportions with the process factors.

Component	Name	Units	Type	Minimum	Maximum
A	Alumina	%	Mixture	20	60
B	Silica	%	Mixture	40	80
C	Time	h	Numeric	0	4
D	Temperature	°C	Numeric	0	1400

For one particular synthesis, specific weights of alumina and silica were weighed into a crucible and heated in Bionics Muffle furnace (BST/MF/1800, India) according to the required temperature. The synthesized clumped alumino-silica complexes were then ground to powder of size 0.05 mm. In all, a total of 25 runs based on the design was obtained.

3.1.2 Treatment of ASC with the acrylamide substrate

Already prepared alumino-silica complex (ASC) of 30 mg was weighed into a 15 ml tube and topped with the acrylamide stock solution to the 10 ml mark. An orbital shaker was used to agitate the tubes for 4 h and spinning was done at 3000 rpm for 3 min. The supernatant was transferred

into a 1.5 ml Eppendorf tube for HPLC analysis. In preparing the control sample, alumino-silica complex was not added to the acrylamide stock solution/ substrate.

3.1.3 HPLC Determination of residual acrylamide content

The quantitative analysis of acrylamide was obtained from a Cecil-adept binary pump HPLC (CE 4300, UK) with a diode array detector. The quantification was based on what was described by Nagappan *et al.* (2014). An Agilent eclipse plus C18 column (4.6 mm x 150 mm, 3.5 µm) with column oven set at 25 °C and the detector also set at 225 nm. The mobile phase at a flow rate of 1 ml/min, was made up of acetonitrile and water (20:80, v/v) adjusted to pH 3.5 with orthophosphoric acid. A volume of 60 µl was injected into the HPLC for both samples and standards. Calibration curve was constructed using the concentrations 0.001g/L to 0.04g/L of acrylamide standard (Appendix 1). Limit of detection (LOD) and limit of quantification (LOQ) were estimated to be 0.1 µg/kg and 0.3 µg/kg respectively. The concentrations obtained from the HPLC analysis were loaded in the Equation 1 to determine the % reduction of residual acrylamide,

$$\% \text{ Reduction of acrylamide} = \frac{(Q - Y) \times 100}{Q} \dots \dots \text{Equation(1)}$$

where Q = acrylamide stock solution without ASC (control sample) and Y = acrylamide stock solution treated with ASC.

3.2 Data analysis

The input variables for the synthesis of ASC and the residual acrylamide % reduction after treatment with the ASC was presented in Table 4.

Table 4: Varying levels of the factors for the synthesis of ASC and their respective residual acrylamide percentage reduction.

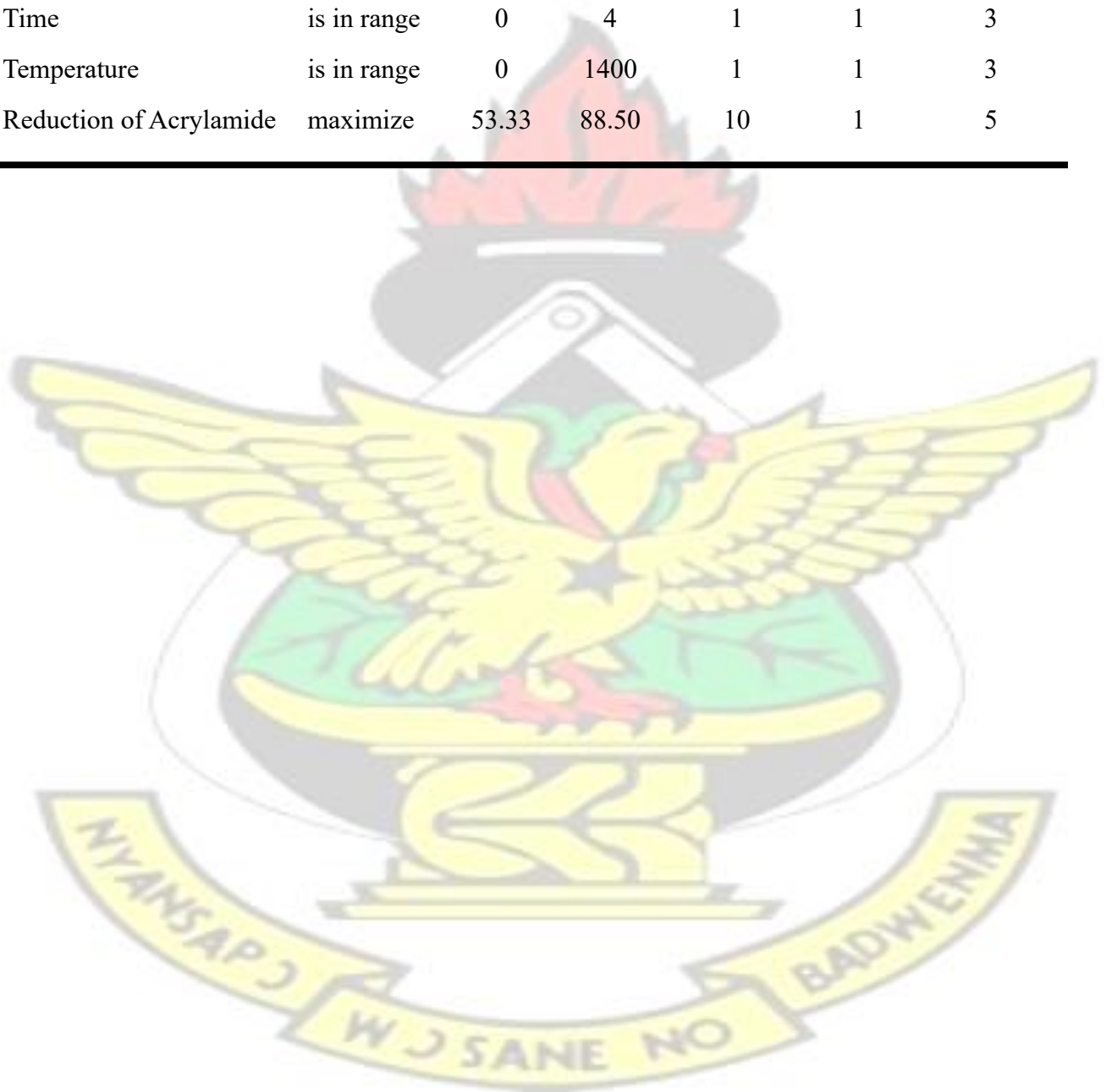
Run	Component A Alumina (%)	Component B Silica (%)	Component C Time (h)	Component D Temperature (°C)	Response Reduction%
1	20	80	4	0	85.46
2	20	80	0	800	70.46
3	60	40	4	800	76.44
4	30	70	4	800	59.92
5	20	80	0	1400	53.33
6	40	60	4	1400	64.52
7	30	70	2	1400	59.65
8	60	40	2	1400	88.49
9	20	80	0	1400	56.78
10	40	60	0	0	72.99
11	40	60	0	800	86.14
12	20	80	4	0	87.45
13	40	60	0	1400	86.95
14	60	40	4	0	59.02
15	60	40	0	1400	87.40
16	40	60	2	800	57.56
17	40	60	2	800	85.24
18	40	60	4	0	61.07
19	20	80	2	0	68.15
20	20	80	0	0	69.21
21	30	70	1	1400	87.70
22	60	40	2	0	86.48
23	60	40	1	800	83.60
24	20	80	0	0	72.14
25	60	40	0	0	58.30

A= Alumina, B= Silica, C= Time, D= Temperature

The data obtained were fitted to models that could explain the behavior of the factors required for the syntheses of alumino-silica complexes within the limits of heating time, heating temperature and proportions of alumino-silica complexes. Optimization was performed to predict the optimum conditions (Table 5) for the synthesis of ASC that would most effectively produce the least residual acrylamide. The analysis of variance (ANOVA) was used to study significant differences between the variables for evidences of interaction and also for the adequacy of the model used in the studies.

Table 5: A table of constraint indicating set goals and importance of synthesis factors of ASC and response factor for optimizing acrylamide adsorption process.

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Alumina	is in range	20	60	1	1	3
Silica	is in range	40	80	1	1	3
Time	is in range	0	4	1	1	3
Temperature	is in range	0	1400	1	1	3
Reduction of Acrylamide	maximize	53.33	88.50	10	1	5



CHAPTER 4

RESULTS AND DISCUSSION

4.1 Time-aluminium-silicate complexes performance

Figure 1, shows the regression contours of the behavior of AS complexes and effectiveness of the complexes ability to bind acrylamide. where the red to yellow colours indicate the most adsorption ability by ASC, the colour green represents the medium adsorption and the blue colour shows the lowest adsorption.

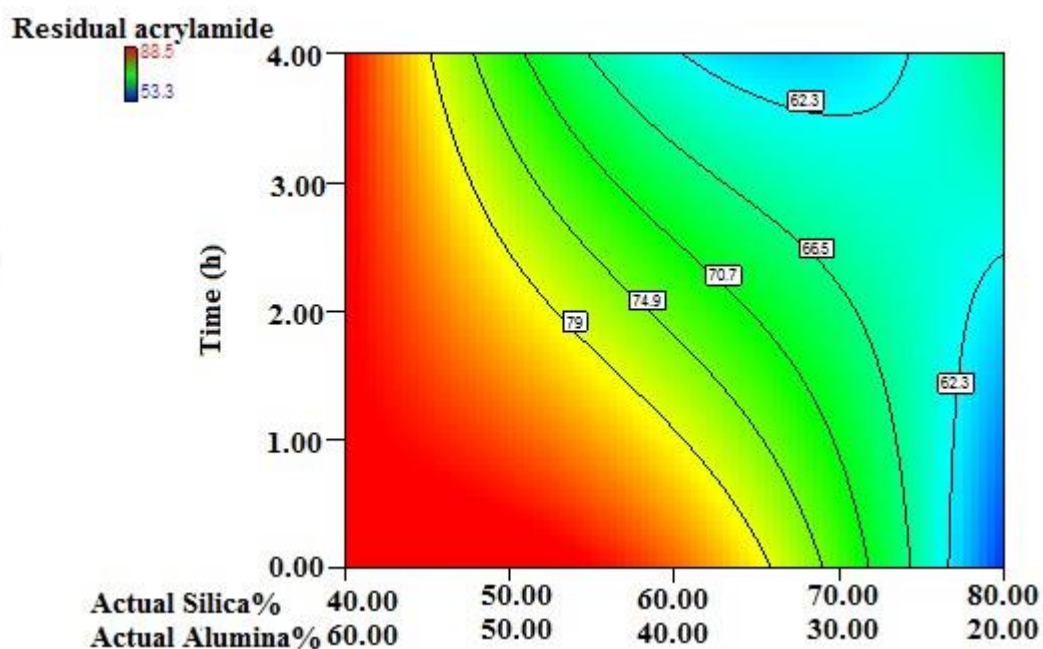


Figure.1: Ridge Contour graph showing relationship between alumina: silica ratios and time at a constant temperature of 1397.70 °C

Hence, from the contour graph (P), the red to yellow colours cover alumina: silica ratio of 60:40, 50:50 and 40: 60 respectively as the highest adsorption capacity of the ASC and curve tapered as the time increased to 4 h. There is also a medium adsorption of acrylamide for ASC of alumina silica ratio of 70:30 which curves extend at the middle (1-3 h) and narrows at 0 – 1 h and 3 – 4 h.

Furthermore, the 3D graph of Figure. 2, indicates the alumina: silica ratio of 20:80 adsorption to be the least and this occurred from 0 to 2 h in addition the alumina: silica ratio of 30:70 was also the lowest adsorption from 3.5 to 4 h.

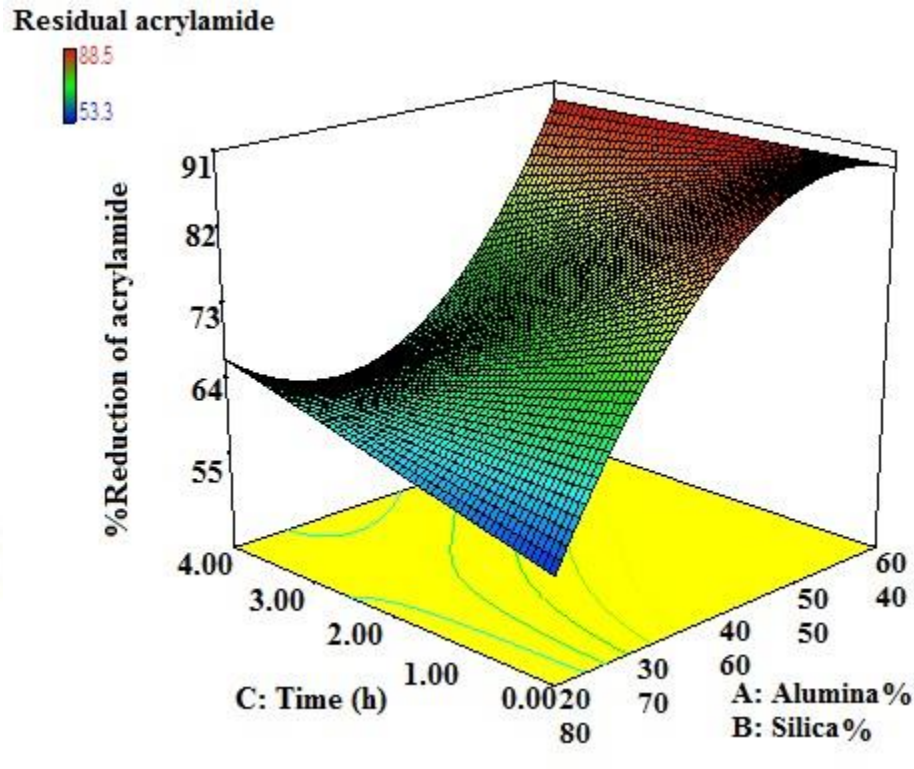


Figure.2: *Three Dimensional Rotatable Quadratic (Y) graph showing relationship between alumina: silica ratios and time at a constant temperature of 1397.70 °C*

Table 6: *A table of Analysis of variance for acrylamide reduction response using the treatment levels of ASC.*

Source	Sum of Squares	df	Mean Square	F Value	p-value
Model	2327.50	8	290.94	3.25	0.02*
Linear Mixture	199.16	1	199.16	2.23	0.16
AB	16.30	1	16.30	0.18	0.68
AC	0.23	1	0.23	0.00	0.96
AD	500.60	1	500.60	5.60	0.03*
BC	139.63	1	139.63	1.56	0.23
BD	319.15	1	319.15	3.57	0.08
ABC	616.41	1	616.41	6.89	0.02*
ABD	1.50	1	1.49	0.02	0.90
Lack of Fit	1036.21	12	86.35	0.87	0.62

Zaibunnisa *et al.* (2009) suggested that R^2 should be at least 0.80 to have good fit of the model.

The closer the value of R^2 is to unity, the better the empirical model fits the actual data. According to Table 7, the "Pred R-Squared" of 0.15 is not as close to the "Adj R-Squared" of 0.43 as expected.

Table 7: A table of response surface quadratic model for acrylamide reduction

Measure	Value
Std. Dev.	9.46
Mean	72.98
R-Squared	0.62
Adj R-Squared	0.43
C.V. %	12.96
Pred R-Squared	0.15
PRESS	3186.30
Adeq Precision	5.94

This may represent a large block effect or a possible problem with the model and/or data. Thus, model reduction, transformation of response, outliers, etc. could be considered. "Adeq Precision"

measures the signal to noise ratio which is greater than 4 is desirable. The ratio of 5.94 indicates an adequate signal and hence, this model can be used to navigate the design space. The R squared was 0.62 as indicated in Table 7, was not close to one (1) but still represents a good empirical model that fits the actual data. Even though, interactions among some independent variables were not significant the model was significant and hence from this statistical analysis % reduction of residual acrylamide was adsorbed by previously prepared ASC from the substrate.

4.2 Optimization conditions for adsorption of acrylamide using alumino--silica complex

As a result of the quadratic model that was used for the response surface, it was important to carry out optimization and this aided the location of the optimum response. Optimizing the adsorption of ASC for acrylamide from the acrylamide substrate as in Table 8 provides a higher temperature of 1397.67 °C with heating time of 3.6 h which is a little expensive, on the other hand, the abundance, inexpensive and availability of silica and alumina makes it economical and easy to use. This optimizations process indicates that for an effective adsorption the percentage of alumina proportion should be higher than that of silica during its synthesis for the best removal of acrylamide.

Table 8: A predicted desirable condition for maximum acrylamide adsorption using previously prepared alumino--silica complex.

Alumina(%)	Silica(%)	Heating Time(h)	Temperature(°C)	% Reduction of Acrylamide
60	40	3.64	1397.67	88.75

4.3 Effect of Temperature on acrylamide Adsorption of ASC compositions

With respect to Figure. 3, the blue colour had the lowest adsorption ability for acrylamide content using alumina: silica ratio of 30:70 and 40:60 respectively when the time was fixed at 3.6 h with the synthesizing temperatures of 0 to 1400 °C. The red colour also signifies the highest adsorption at temperature of 1050 °C to 1400 °C indicating that temperature has an effect on the adsorptability of ASC during formation of ASC. Thus, high temperatures of formation of ASC with a heating time of 3.6 h would give the highest adsorption of acrylamide level when alumina were 50 % and 60 % and silica were 50 % and 40 %. These changes of adsorption may be due to physical changes as reported by Iloabuchi (2016), when high temperatures of 1200 °C and 1400 °C during the formation of ASC made the complexes stick together which made it uneasy to remove them from the crucibles. Also, the colour of silica was transformed from brown to peach. This behavior of the complexes could also lead to the high adsorption property at high temperatures of 1050 °C to 1400 °C.

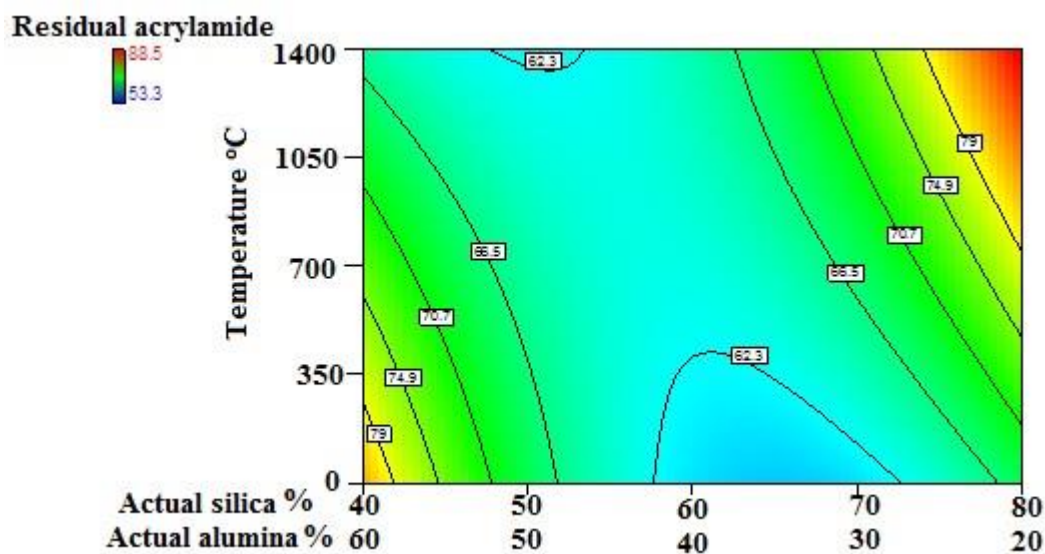


Figure 3: Response Surface “Saddle” Contour (R) plot indicating interactions between temperature and alumina: silica ratios at fixed time of 3.60 h

4.4 Effect of ASC components on adsorption of acrylamide content

Relationship between ASC and adsorption of acrylamide content is very essential in this model, thus, the graph below, Figure. 4, indicates this interaction. Alumina: silica ratio of 50:50 and 60:40 respectively during synthesis of alumino-silica complexes shows best adsorption of acrylamide content (from 75.5 % to 89 %). In general, the adsorption of acrylamide content decreased with decreased alumina content from 40 % to 20 % and inversely with the content of silica from 60 % to 80 %. Hence, alumina was preferred over silica in terms of % reduction of acrylamide. With these differences, it is a clear indication that alumina has an adsorptive rate during acrylamide adsorption which is higher than that of silica as in relation to alumina and activated carbon during dye adsorption. Adsorption rate of alumina and activated carbon were $6.42 \cdot 10^{-5} \text{ cm}^2/\text{s}$ and $2.37 \cdot 10^{-6} \text{ cm}^2/\text{s}$ respectively during dye adsorption but achieved approximately the similar maximum dye removal (Moreira *et al.*, 1997).

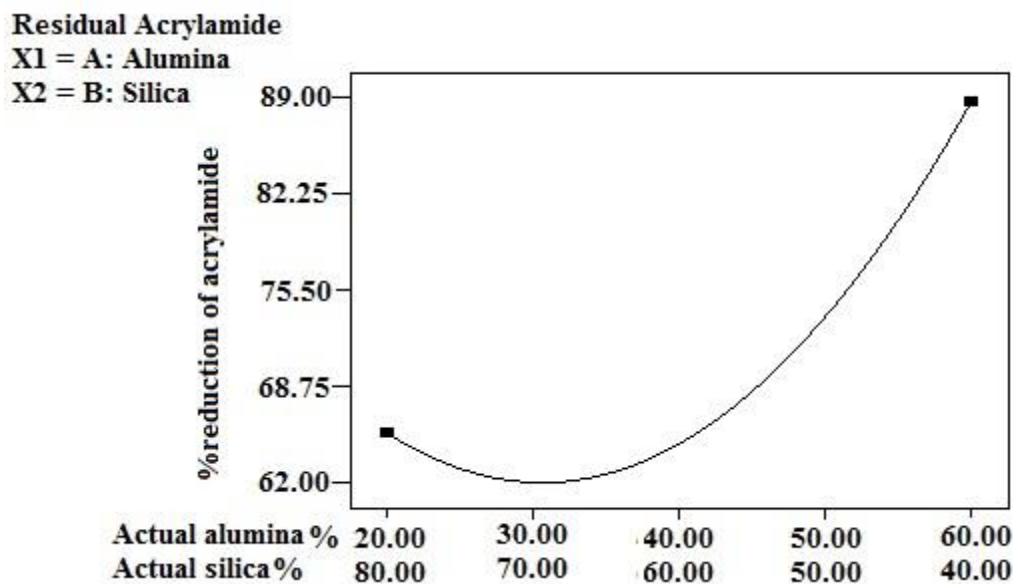


Figure. 4: Plot (T) graph displaying Quadratic function interactions between fixed time of 3.6 and temperature of 1397.7 °C and alumina: silica proportion



CHAPTER 5

CONCLUSION, LIMITATIONS AND RECOMMENDATION

5.1 Conclusion

The predicted optimum conditions for maximum adsorption of acrylamide in the substrate under study was 88.75%, at alumina: silica ratio of 60: 40 with heating time of 3.64 h, and a temperature of 1397.7 °C.

5.3 Recommendation

Alumina and silica has been used as separate binding agents in ground water for hydrogen fluoride and desalination of water respectively. In using both as a binding agent, it is an innovative method to bind other hazards in foods and feed in –vitro and in-vivo. Also, studies on how ASC can affect the human system and essential nutrients as a binding agent could be studied. Organoleptic properties of food could also be studied when ASC is applied to food in order to mitigate acrylamide. To add to it, the period (shaking time – 4 h) between the ASC and the test samples could have been varied to obtain the effect of the shaking time on the adsorptive capacity.

This research provided a significant indication of the efficient adsorptive property of aluminosilica complexes on acrylamide. Thus, this adsorptive property can be taken advantage of by using it in the reduction of dietary acrylamide *in – vivo* by putting them in food during food preparation to prevent the risk of obtaining cancers.

The optimum conditions can be applied in the food industries during preparation of foods by using alumino-silica complexes as binding materials to reduce acrylamide during the preparation of the

foods. Findings from this study will enable further work to be carried out on the reduction of acrylamide in – vivo using alumino-silica complexes.

KNUST



REFERENCES

- Altundogan**, H. S., Altundogan, S., Tümen, F. and Bildik, M. (2002). Arsenic adsorption from aqueous solutions by activated red mud. *Waste Management*, **22**: 357–363.
- Amrein**, T. M., Limacher, A., Conde-Petit, B., Amado, R. and Escher, F. (2006b). Influence of thermal processing conditions on acryl- amide generation and browning in a potato model system. *Journal of Agricultural and Food Chemistry*, **54**:5910–5916.
- Anon**. (1997). Soluble Silicates and their Applications. Crossfield Publication, Crossfield, Warrington, UK. Issue No. 2.
- Arthur, A. (2014). Dietary risk assessment of acrylamide in food commonly consumed among children, pp. 54 - 55.
- Austin**, J. H. (1997). Silicon levels in human tissues. *Nobel Symposium*, 255–268.
- Biedermann-Brem**, S., Noti, A., Grob, K., Imhof, D., Bazzocco, D. and Pfefferle, A. (2003). How much reducing sugar may a potato contain to avoid excessive acrylamide formation during roasting and baking? *European Food Research and Technology*, **217**:369–373.
- Blom**, H., Baardseth, P., Sundt, T.W. and Slinde, E. (2009). Lactic acid fermentation reduces acrylamide formed during production of fried potato products. *Aspects of Applied Biology*, **97**:65–71.
- Bongers**, M.L., Hogervorst, J. G. F., Schouten, L. J., Goldbohm, R. A., Schouten, H. C. and van den Brandt, P. A. (2012). Dietary acrylamide intake and the risk of lymphatic malignancies: The Netherlands cohort study on diet and cancer. *PLoS ONE*, **7**(6).
- Bosetti**, C., Negri, E., Franceschi, S., (2000). Risk factors for oral and pharyngeal cancer in women: a study from Italy and Switzerland. *British Journal of Cancer*, **82**(1):204–207.
- Bowen**, H. J. M. and Peggs, A. (1984) Determination of the silicon content of food. *Journal Science for Food and Agriculture*, **35**:1225–1229.

- Brinker**, C. J., Scherer, G. W., (1990). The Physics and Chemistry of Sol-Gel Processing. Applications In: Sol-Gel Science, Academic Press, San Diego, California, pp. 839-880.
- Brown**, L., Rhead, M. M., Bancroft, K. C. C. and Allen, N. (1980a). Case studies of acrylamide pollution resulting from industrial use of acrylamides. *Water Pollution Control* **79**(4): 507-510.
- Brown**, S. H. (2010). Zeolites in catalysis: handbook of green chemistry. Wiley and Sons, New Jersey.
- Ciesarová**, Z., Kukurová, K. and Benešová, C. (2010). Enzymatic elimination of acrylamide in potato-based thermally treated foods. *Nutrition and Food Science*, **40**(1), 55–63.
- Claeys**, W. L., de Vleeschouwer, K. and Hendrickx, M. E. (2005a). Quantifying the formation of carcinogens during food processing: acrylamide. *Trends in Food Science and Technology*, **16**:181–193.
- Clement**, F. C., Dip, R. and Naegeli, H. (2007) . Expression profile of human cells in culture exposed to glycidamide, a reactive metabolite of the heat-induced food carcinogen acrylamide . *Toxicology*, **240** (1 – 2): 111 – 124
- Cold Spring Harbor** (2014). Cold spring harbor laboratory press. Available at: http://m.cshprotocols.cshlp.org/content/2014/12/pdb.rec085126.full?text_only=true
- Cook**, D. J., and Taylor, A. J. (2005). On-line MS/MS monitoring of acrylamide generation in potato- and cereal-based systems. *Journal of Agricultural and Food Chemistry*, **53**:8926–8933.
- Davis**, K., 2010. Material Review: Alumina (Al_2O_3). School of Doctoral Studies. *European Union Journal*, pp.109–114. Available at: [http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Material+Review+:+Alumina+\(+Al+2+O+3+\)+#0](http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Material+Review+:+Alumina+(+Al+2+O+3+)+#0).

- Dearfield, K. L., Douglas, G. R., Ehling, U. H., Moore, M. M., Sega, G. A. and Brusick, D. J.** (1995). Acrylamide: A review of its genotoxicity and an assessment of heritable genetic risk. *Mutation Research*, **330**: 71–99.
- Design Expert** (2012). Stat Ease Inc., Hennepin Square, Suite 480, 2021E. Hennepin Ave., Minneapolis, MN55413-2726.
- Desouky, M., Jugdaohsingh, R., McCrohan, C. R., White, K. N. and Powell, J. J.** (2002). Aluminum-dependent regulation of intracellular silicon in the aquatic invertebrate *Lymnaea stagnalis*. *Proceedings of the National Academy of Sciences of the United States of America*, **99**: 3394-3399.
- Doerge, D. R., Twaddle, N. C., Boettcher, M. I., McDaniel, L. P. and Angerer, J.** (2007). Urinary excretion of acrylamide and metabolites in Fischer 344 rats and B6C3F(1) mice administered a single dose of acrylamide. *Toxicology Letters*, **169**: 34–42.
- Doroshenko, O., Fuhr, U., Kunz, D., Frank, D., Kinzig, M., Jetter, A., Reith, Y., Lazar, A., Taubert, D., Kirchheiner, J., Baum, M., Eisenbrand, G., Berger, F. I., Bertow, D., Berkessel, A., Sorgel, F., Schomig, E. and Tomalik-Scharte, D.,** (2009). *In-vivo* role of cytochrome P450 2E1 and glutathione-S-transferase activity for acrylamide toxicokinetics in humans. *Cancer Epidemiology, Biomarkers and Prevention*, **18**: 433– 443.
- Environmental Protection Agency,** (2010). USEPA: Integrated Risk Information System Toxicological Review and Summary Documents. Washington. Available at <http://www.epa.gov/iris/>.
- Erden, M., Altundogan, H. S. and Turner, F.** (2004). Removal of hexavalent chromium by using heat-activated bauxite. *Minerals Engineering*, **17**: 1045–1052.
- European Food Safety Authority,** (2010). Scientific Opinion on Arsenic in Food. *European Food Safety Authority Journal*, **7**(10): 1351.

- FAO/WHO** (Joint FAO/WHO Expert Committee on Food Additives), (2011). Evaluation of certain Food Contaminants. Seventy-second report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Reports Series 959.
- Fiselier, K., Bazzocco, D., Gama-Baumgartner, F. and Grob, K.** (2005). Influence of the frying temperature on acrylamide formation in French fries. *European Food Research and Technology* **222**: 414–419.
- Friedman, M. A., Dulak, L. H. and Stedham, M. A.** (1995). A lifetime oncogenicity study in rats with acrylamide. *Fundamental and Applied Toxicology*, **27**: 95–105.
- Friedman, M.** (2003). Chemistry, Biochemistry, and Safety of Acrylamide. A review. *Journal of Agricultural and Food Chemistry*, **51**: 4504–4526.
- Foot, R. J., Haase, N. U., Grob, K. and Gondé, P.** (2007). Acrylamide in fried and roasted potato products: A review on progress in mitigation. *Food additives and contaminants*, **24** (1): 37–46.
- Gertz, C. and Klostermann, S.** (2002). Analysis of acrylamide and mechanisms of its formation in deep-fried foods. *European Journal of Lipid Science and Technology*, **104**: 762–771.
- Ghorai, S. and Pant, K. K.** (2005). Equilibrium, kinetics and breakthrough studies for adsorption of fluoride on activated alumina. *Separation and Purification Technology*, **42**: 265–271.
- Godoi, R. H., Braga, D. M., Markarorska, Y., Alfoldy, B., Carvalho, F. M. A.** (2008) Inhale particulate matter from lime industries: chemical composition and deposition in human respiratory tract. *Atmospheric Environment*, **42**: 7027-7033.
- Greenwood, N. N. and Earnshaw, A.** (1997). Chemistry of the elements. 2nd edition. Oxford: Butterworth-Heinemann.
- Grob, K., Biedermann, M., Biedermann-Brem, S., Noti, A., Imhof, D., Amrein, T., Pfefferle, A.**

- and Bazzocco, D. (2003). French fries with less than 10 mg/kg acrylamide. A collaboration between cooks and analysts. *European Food Research and Technology*, **217**:185–194.
- Haase**, N. U. (2006). The formation of acrylamide in potato products. In: Skog, K. and Alexander, J. editors. *Acrylamide and other hazardous compounds in heat-treated foods*. Cambridge, Woodhead. pp 41–59.
- Habermann**, C. E. (2002). Acrylamide. In *Kirk-Othmer Encyclopedia of Chemical Technology*, vol. 1. John Wiley and Sons, New York. pp. 288-304.
- Haverkamp**, R. G. et al., 1992. Adsorption of hydrogen fluoride on alumina. *Surface and Interface Analysis*, **19** (1–12):139–144.
- HSDB**, 2009. Hazardous Substances Data Bank: Acrylamide. National Library of Medicine. Available at <https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~selNxb:1>
- Hendriksen**, H. V., Kornbrust, B. A., Østergaard, P. R. and Stringer, M. A. (2009). Evaluating the potential for enzymatic acrylamide mitigation in a range of food products using an asparaginase from *Aspergillus oryzae*. *Journal of Agricultural and Food Chemistry*, **57**: 4168–4176.
- Hirsch**, L. R., Stafford, R. J., Bankson, J. A., Sershen, S. R., Rivera, B., Price, R. E., Hazle, J. D., Halas, N. J. and West, J. L. (2003). Nanoshell-mediated near-infrared thermal therapy of tumors under magnetic resonance guidance. *Proceedings of the National Academy of Sciences of the United States of America*, **100**:13549-13554.
- Hoenicke**, K., and Gatermann, R. (2005). Studies on the stability of acrylamide in food during storage. *Journal of AOAC International*, **88**:268–273.
- Hogervorst**, J. G., Schouten, L. J., Konings, E. J., Goldbohm, R. A. and van den Brandt, P. A. (2007) A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiol Biomarkers Prevention* **16**(11): 2304– 2313

- Hogervorst, J. G., Schouten, L. J., Konings, E. J., Goldbohm, R. A. and van den Brandt, P. A.** (2008). Dietary acrylamide intake and the risk of renal cell, bladder, and prostate cancer. *The American Journal of Clinical Nutrition*, **87**(5): 1428–1438.
- Hogervorst, J. G. F., Schouten, L. J., Konings, E. J., Goldbohm, R. A. and van den Brandt, P. A.** (2009). Lung cancer risk in relation to dietary acrylamide intake. *Journal of the National Cancer Institute*, **101**(9), pp.651–662.
- Hollander, A. F., Somasundaran, P. and Gryte, C. C.** (1981). Adsorption characteristics of polyacrylamide and sulfonate-containing polyacrylamide copolymers on sodium kaolinite. *Journal of Applied Polymer Science*, **26**(7), 2123–2138.
- Howard, P. H.** (1989). Acrylamide. In Handbook of Environmental Fate and Exposure Data for Organic Chemicals, vol. 1. Chelsea, MI: Lewis Publishers. pp. 13-19.
- HMSO,** (1995). A Guide to Risk Assessment and Risk Management for Environmental Protection, Department of the Environment, HMSO, pp. 77-78.
- IARC,** (1986). Acrylamide. In Some Chemicals Used in Plastics and Elastomers. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. *Lyon, France: International Agency for Research on Cancer*, **39**: 41-66.
- IARC,** (1994). Acrylamide. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. *Some Industrial Chemicals*, **60**: 389–433.
- Iloabuchi, C. G.** (2016). Synthesis and interventive performances of alumina, silica and their complexes in aflatoxin adsorption *in-vitro*. pp. 25 - 37.
- Institute of Medicine,** (2000). Food and Nutrition Board: Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. National Academy Press, Washington.
- Johnson, K. A., Gorzinski, S. J., Bodner, K. M., Campbell, R. A., Wolf, C. H., Friedman, M. A.**

- and Mast, R. W. (1986). Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking-water of Fischer 344 rats. *Toxicology and Applied Pharmacology*, **85**: 154–168
- Jones-Hughes**, T., Peters, J., Whear, R., Cooper, C., Evans, H., Depledge, M and Pearson, M. (2013). Are interventions to reduce the impact of arsenic contamination of groundwater on human health in developing countries effective? A systematic review. *Environmental Evidence*, **2**(1): 11.
- Jung**, M. Y., Choi, D. S. and Ju, J. W. (2003). A novel technique for limitation of acrylamide formation in fried and baked corn chips and in French fries. *Journal for Food Science*, **68**: 1287–1290.
- Khrustaleva**, K. and Voronova, G. (2016). Investigation of adsorption properties of alumina produced by vacuum spray method. *IOP Conference Series: Materials Science and Engineering*. **116**:12019. Available at:
<http://stacks.iop.org/1757899X/116/i=1/a=012019?key=crossref.3da2fe5cb93a840336a632728d58409d>.
- Konings**, E. J. M., Ashby, P., Hamlet, C. G. and Thompson, G. A. K. (2007). Acrylamide in cereal and cereal products: a review on progress in level reduction. *Food additives and contaminants*, **24**(1): 47–59.
- Kopp**, E. K. and Dekant, W. (2009). Toxicokinetics of acrylamide in rats and humans following single oral administration of low doses. *Toxicology and Applied Pharmacology*, **235**: 135–142.
- Ku**, Y. and Chiou, H. M. (2002). The adsorption of fluoride ion from aqueous solution by activated alumina, *Water Air Soil Pollution*, **133**: 349–361.
- Lavecchia**, R., Medici, F., Piga, L., Rinaldi, G. and Zuurro, A. (2012). Fluoride removal from water by adsorption on a high alumina content bauxite. *Chemical Engineering Transactions*, **26**: 225–230.

- Laxamana, N. B.** (1982). Binders from rice hull ash low-cost housing materials. Forbride-Dig. College: Forest Products Research and Industries Development Commission. **11**: 27-30.
- Lea, P. J. and Azevedo, R. A.** (2007). Nitrogen use efficiency. 2. Amino acid metabolism. *Annals of Applied Biology*, **151**: 269–275.
- Lender, P. W. and Ruiter, R.** (1990). Novel inorganic materials and heterogeneous catalysis: Preparation and properties of high surface area silicon carbide and silicon oxynitrides. In: Sheats, J. E., Carraher, C. E., Pittman, C. U., Eldin, M. and Currell, B. (Eds.). *Inorganic and Metal-Containing Polymeric Materials*, Plenum Press, New York. pp. 187195.
- Leyva-Ramos, R., Rivera-Utrilla, J., Medellin-Castillo, N. A., Sanchez-Polo, M.** (2010). Kinetic modeling of fluoride adsorption from aqueous solution onto bone char. *Chemical Engineering Journal*, **158**: 458–467.
- Lineback, D. R., Coughlin, J. R. and Stadler, R. H.** (2011). Acrylamide in Foods: A Review of the Science and Future Considerations. pp. 1-21.
- Lomer, M. C., Thompson, R. P. and Powell, J. J.** (2002). Fine and ultrafine particles of the diet: influence on the mucosal immune response and association with Crohn's disease. *Proceedings of Nutrition Society*, **61**: 123-130.
- LoPachin, R. M.** (2004). The changing view of acrylamide neurotoxicity. *NeuroToxicology*, **24**:617–630.
- Manini, P., d'Ischia, M. and Prota, G.** (2001). An unusual decarboxylative Maillard reaction between L-DOPA and D-glucose under biomimetic conditions: Factors governing competition with Pictet-Spengler condensation. *Journal of Organic Chemistry*, **66**:5048–5053.
- Manson, J., Brabec, M. J., Buelke-Sam, J., Carlson, G. P., Chapin, R. E., Favor, J. B., Fischer, L. J., Hattis, D., Lees, P. S. J., Perreault-Darney, S., Rutledge, J., Smith, T. J., Tice, R. R. and Working, P.** (2005). NTP-CERHR expert panel report on the reproductive and

developmental toxicity of acrylamide. *Birth Defects Research Part B-Developmental and Reproductive Toxicology*, **74**(1): 17-113.

Marsh, G. M., Lucas, L. J., Youk, A. O. and Schall, L. C. (1999). Mortality patterns among workers exposed to acrylamide: 1994 follow up. *Occupational and Environmental Medicine*, **56**: 181–190

Martins, S. I. F. S., Jongen, W. M. F. and van Boekel, M. A. J. S. (2001). A review of Maillard reaction in food and implications to kinetic modeling. *Trends in Food Science and Technology*, **11**: 364–373.

Matthäus, 2002. BAGKF, Bundesanstalt für Getreide- Kartoffel und Fettforschung. Available at:http://www.bfr.bund.de/cm/343/acrylamidgehalte_von_im_backofen_zubereiteten_pommes_frites_und_von_reibekuchen.pdf

Mestdag, F., De Wilde, T., Delporte, K., Van Peteghem, C. and De Meulenaer, B. (2008). Impact of chemical pre-treatments on the acrylamide formation and sensorial quality of potato crisps. *Food Chemistry*, **106**: 914–922.

Miller, A. B. Diet in cancer prevention. http://www.who.int/ncd/cancer/publications/abstracts/abs9810_05 (accessed 2001)

Moghimi, S. M., Hunter, A. C., Murray, J. C. (2005) Nanomedicine: current status and future prospects. *Federation of American Societies for Experimental Biology Journal*, **19**:311330.

Mojska, H., Gielecińska, I., Szponar, L. and Ołtarzewski, M. (2010). Estimation of the dietary acrylamide exposure of the Polish population. *Food and Chemical Toxicology*, **48**: 2090–2096.

Moreira, R. F. P. M., Peruch, M. G. and Kuhnen, N. C. (1997). Adsorption of textile dyes on alumina. Equilibrium studies and contact time effects. *Brazilian Journal of Chemical Engineering*, **15**(1)

- Mottram, D. S., Wedzicha, B. L. and Dodson, A. T. (2002).** Food chemistry: acrylamide is formed in the Maillard reaction. *Nature* **419**:448–449.
- Mucci, L. A. and Adami, H. O. (2005)** The role of epidemiology in understanding the relationship between dietary acrylamide and cancer risk in humans. *Advances in Experimental Medicine and Biology*, **561**: 39 – 47.
- Muttucumaru, N., Elmore, J. S., Curtis, T., Mottram, D. S., Parry, M. A. J. and Halford, N. G. (2008).** Reducing acrylamide precursors in raw materials derived from wheat and potato. *Journal of Agricultural and Food Chemistry*, **56**(15): 6167–6172.
- Myers, R. H. and Montgomery, D.C. (1995).** Response Surface Methodology. New York: John Wiley and Sons.
- Nagappan, K.V., Meyyanathan, S. N., Karthik, Y., Hemnath, E., Satiesh, K. R and Usha, K (2014).** A simple and validated RP HPLC Method for the estimation of acrylamide in potato chips. *World Journal of Pharmacy and Pharmaceutical Sciences*, **3**(5): 1468 - 1478.
- Olesen, P. T., Olsen, A., Frandsen, H., Frederiksen, K., Overvad, K. and Tjønneland, A. (2008).** Acrylamide exposure and incidence of breast cancer among postmenopausal women in the Danish Diet, Cancer and Health Study. *International Journal of Cancer*, **122**(9): 2094–2100.
- Pedreschi, F., Kaack, K. and Granby, K. (2008),** „The effect of asparaginase on acrylamide formation in French fries“, *Food Chemistry*, **109**: 386-392.
- Pedreschi, F., Mariotti, S., Granby, K. and Risum, J. (2011).** Acrylamide reduction in potato chips by using commercial asparaginase in combination with conventional blanching. *LWT-Food Science and Technology*, **44**(6): 1473-1476.
- Ravi-Kumar, M. N. V., Sameti, M., Mohapatra, S. S., Kong, X., Lockey, R. F., Bakowsky, U., Lindenblatt, G., Schmidt, C. H. and Lehr, M. (2004).** Cationic Silica Nanoparticles as Gene

Carriers: Synthesis, Characterization and Transfection Efficiency *In vitro* and *In vivo*.
Journal of Nanoscience and Nanotechnology, **4**:876-881.

Raichur, A. M. and Basu, M. J. (2001). Adsorption of fluoride onto mixed rare earth oxides,
Separation and Purification Technology, **24**: 121–127.

Reffitt, D. M., Jugdaohsingh, N. R., Thompson, R. P. H., Powel, J. J (1999) Silicic acid: its
gastrointestinal uptake and urinary excretion in man and effects on aluminium excretion.
J Inorg Biochem, **76**(2):141–147.

Robert, F., Vuataz, G., Pollien, P., Saucy, F., Alonso, M. I., Bauwens, I. and Blank I. (2004).
Acrylamide formation from asparagine under low-moisture Maillard reaction conditions.
1. Physical and chemical aspects in crystalline model systems. *Journal of Agricultural and
Food Chemistry*, **52**:6837–6842.

Royal Society, (1992). Risk: Analysis, Perception and Management. The Royal Society, London.

Sanganyado, E., Parekh, C. T. and Eriksson S. (2007). Analysis of acrylamide in traditional
foodstuffs in Zimbabwe. *African Journal of Food Science*, **5**(17):.910–913. Available at:
[http://www.academicjournals.org/AJFS/abstracts/abstracts/abstract2011/30
Dec/Sanganyado et al.htm](http://www.academicjournals.org/AJFS/abstracts/abstracts/abstract2011/30Dec/Sanganyado%20et%20al.htm).

Schouten, L. J., Hogervorst, J. G. F., Konings, E. J. M. R., Goldbohm, A. and van den Brandt P.
A. (2009). Dietary acrylamide intake and the risk of head-neck and thyroid cancers: results
from the Netherlands cohort study. *American Journal of Epidemiology*, **170**(7):
873–884.

Schulz, M. R, Hertz-Picciotto, I., Van Wijngaarden, E., Hernandez, J. C., Ball, L. M. (2001).
Correspondence: Dose- response relation between acrylamide and pancreatic cancer.
Occupational and Environmental Medicine, **58**(9): 609.

Shibamoto, T. and Bjeldanes, L. (2009). Introduction to Food Toxicology. Food Science and
Technology, International Series, (2nd Edition) Academic Press. pp. 269 – 273.

- Slowing**, I. I., Vivero-Escoto, J. L., Wu, C. W., Lin, V. S. (2008). Mesoporous silica nanoparticles as controlled release drug delivery and gene transfection carriers. *Advance Drug Delivery Reviews*, **60**:1278-1288.
- Smith**, R. (1993) Environmental economics and the new paradigm. *Chemistry and Industry Newsletter*, November.-December, 8-9
- Smith**, E. A., Prues, S. L., and Oehme, F. W. (1996). Environmental degradation of polyacrylamides. 1. Effects of artificial environmental conditions: temperature, light, and pH. *Ecotoxicology and Environmental Safety*, **35**:121–135.
- Smith**, E. A., Prues, S. L., and Oehme, F. W. (1997). Environmental degradation of polyacrylamides. *Ecotoxicology and Environmental Safety*, **37**:76–91. Springer.
- Stadler**, R. H., Blank, I., Varga, N., Robert, F., Hau, J. and Guy, P. A. (2002). Food Chemistry: Acrylamide from Maillard reaction products. *Nature*, **419**:449–450.
- Stadler**, R. H., Robert, F., Riediker, S., Varga, N., Davidek, T., Devaud, S., Goldmann, T., Hau, J. and Blank, I. (2004). In-depth mechanistic study on the formation of acrylamide and other vinyllogous compounds by the Maillard reaction. *Journal of Agricultural and Food Chemistry*, **52**:5550–5558.
- Stadler**, R. and Scholz, G. (2004). Acrylamide: An update on current knowledge in analysis, levels in food, mechanisms of formation, and potential strategies of control. *Nutrition Reviews*, **62**: 449– 467.
- Sorgel**, F., Weissenbacher, R., Kinzig-Schippers, M., Hofmann, A., Illauera, M., Skott, A. and Landersdorfer, C. (2002) Acrylamide: increased concentrations in homemade food and first evidence of its variable absorption from food, variable metabolism and placental and breast milk transfer in humans. *Chemotherapy*, **48**: 267–274
- Sumner**, S. C., Williams, C. C., Snyder, R. W., Krol, W. L., Asgharian, B. and Fennell, T. R. (2003). Acrylamide: a comparison of metabolism and hemoglobin adducts in rodents following dermal, intraperitoneal, oral, or inhalation exposure. *Toxicological Sciences*, **75**, 260–270.

- Sujana, M. G. and Anand, S.,** (2010). Fluoride removal studies from contaminated ground water by using bauxite. *Desalination*, **267**, 222–227.
- Tareke, E., Rydberg, P., Karlsson, P., Erickson, S. and Törnqvist, M.** (2002). Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *Journal of Agricultural and Food Chemistry*, **50**:4998–5006
- Treguer, P., Nelson, D. M., Van Bennekom, A. J., DeMaster, D. J., Leynaert, A. and Queguiner, B.** (1995). The silica balance in the world ocean: A reestimate. *Science*, **268**(5209):375–379.
- Urban, M., Kavvadias, D.; Riedel, K.; Scherer, G. and Tricker, A. R.** (2006). Urinary mercapturic acids and a hemoglobin adduct for the dosimetry of acrylamide exposure in smokers and nonsmokers. *Inhalation Toxicology*, **18**(10): 831–839.
- US FDA** (2015). United States Food and Drug Administration. Code of Federal Regulations Title 21, 21CFR172.480. 2015. Available at: <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=172.480>.
- Vijayanathan, V., Thomas, T., Thomas, T. J.** (2002). DNA nanoparticles and development of DNA delivery vehicles for gene therapy. *Biochemistry*, **41**:14085-14094.
- White, K. N., Ejim, A. I., Walton, R. C., Brown, A. P., Jugdaohsingh, R. et al.** (2008). Avoidance of aluminum toxicity in freshwater snails involves intracellular silicon–aluminum biointeraction. *Environmental Science and Technology*, **42**: 189-194.
- Wilson, K. M., Mucci, L. A., Rosner, B. A. and Willet, W. C.** (2010). A prospective study on dietary acrylamide intake and the risk for breast, endometrial, and ovarian cancers. *Cancer epidemiology, biomarkers and prevention*, **19**(10): 2503–15. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2952046&tool=pmcentrez&ndren>

World Health Organization Meeting, (2002). Food Safety Consultations. Report of a Joint FAO/WHO Consultation WHO Headquarters, Geneva, Switzerland. Available from: http://www.who.int/foodsafety/publications/chem/en/acrylamide_full.pdf

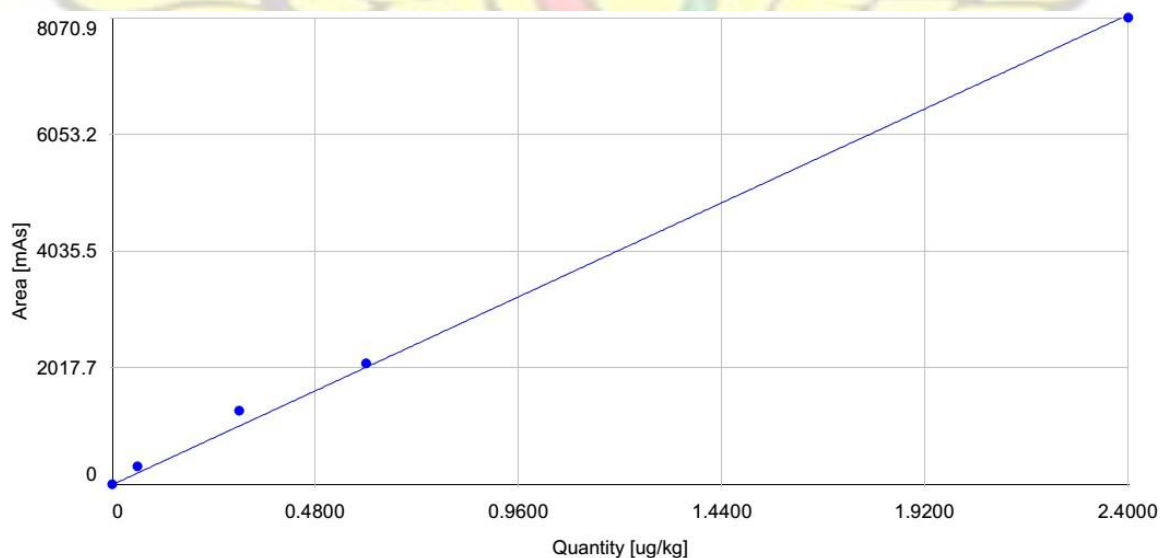
Yaylayan, V.A., Wnorowski, A. and Locas, C.P. (2003). Why asparagine needs carbohydrates to generate acrylamide. *Journal of Agricultural and Food Chemistry*, **51**: 1753–1757.

Yaylayan, V.A. and Stadler, R.H. (2005). Acrylamide formation in food: a mechanistic perspective. *Journal of AOAC International*, **88**: 262–267.

Zyzak, D. V., Sanders, R. A., Stojanovic, M., Tallmadge, D. H., Eberhart, B. L., Ewald, D. K., Gruber, D. C., Morsch, T. R., Strothers, M. A., Rizzi, G. P. and Villagran, M. D. (2003). Acrylamide formation mechanism in heated foods. *Journal of Agricultural and Food Chemistry*, **51**:4782–4787.

APPENDICES

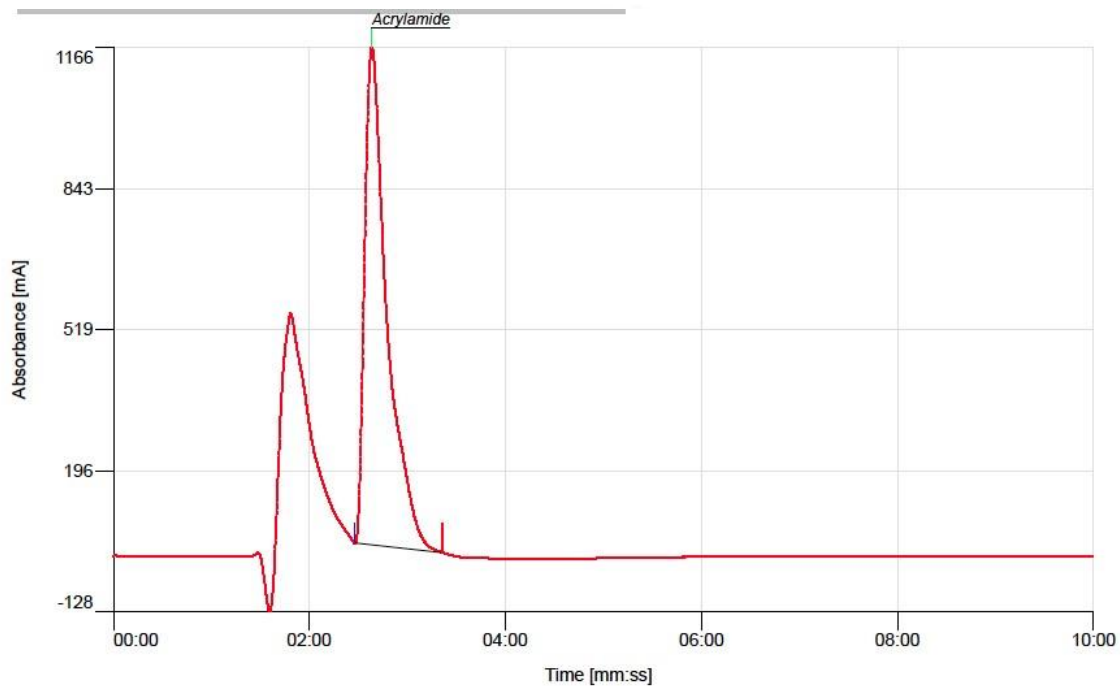
A1: Acrylamide standard calibration curve.



Equation of Curve:

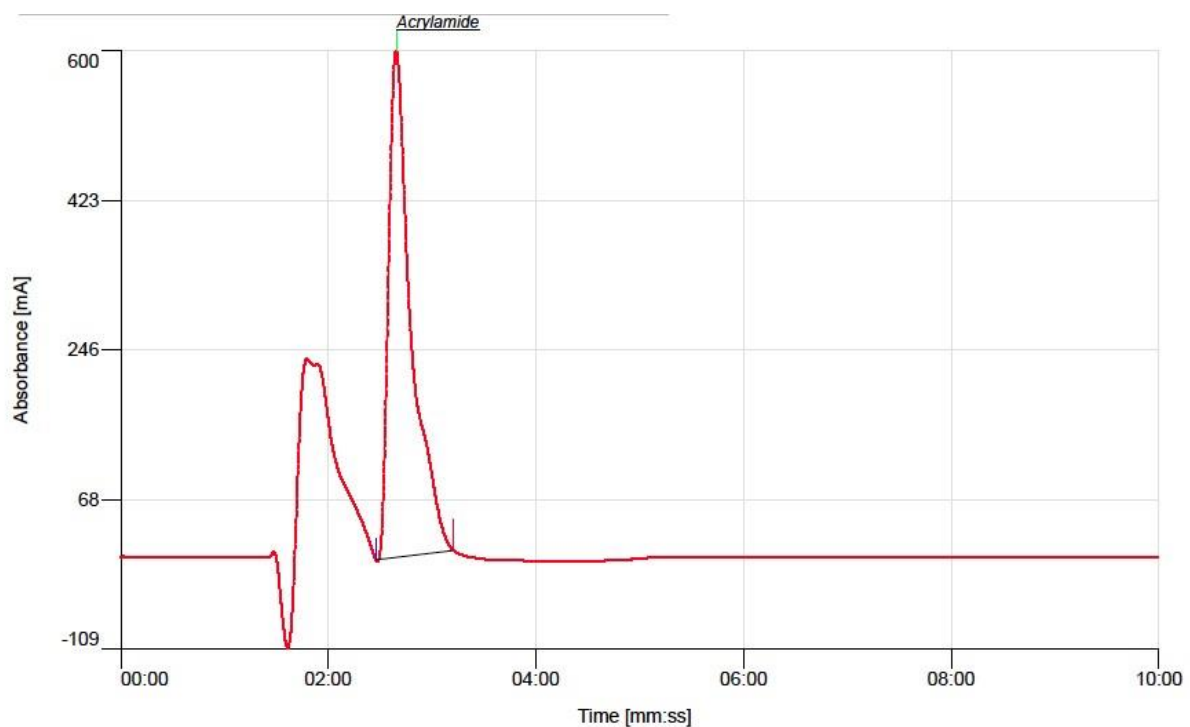
Quantity (ug/kg) = 0 + 0.295239 x Area (mAs), where correlation coefficient (r^2) = 0.998205

A2: Chromatogram report of alumina silica ratio of 40:60 for a heating time and heating temperature of the ASC of 0 h and 0 °C respectively



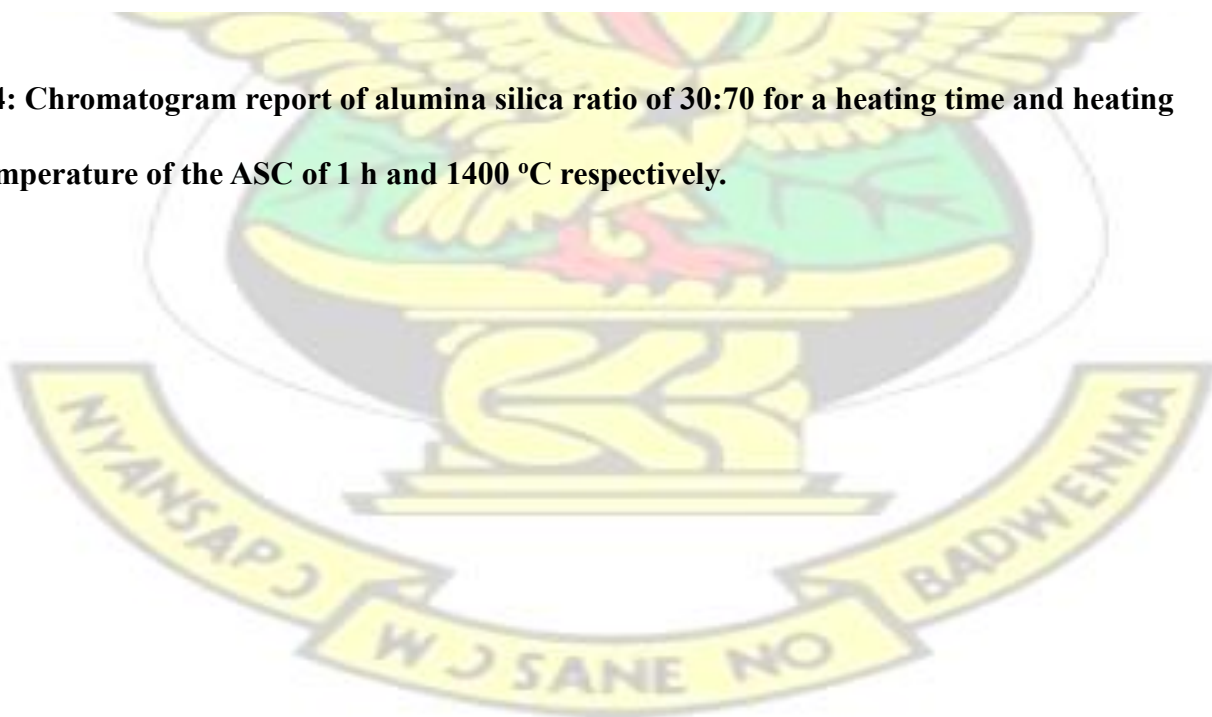
No.	Peak Name	Ret. Time [mm:ss]	Start Time [mm:ss]	End Time [mm:ss]	Area [mAs]	Height [mA]	Qty [ng/g]
001	Acrylamide	02:38.3	02:27.4	03:21.4	18131.4	1140.9	5.3531

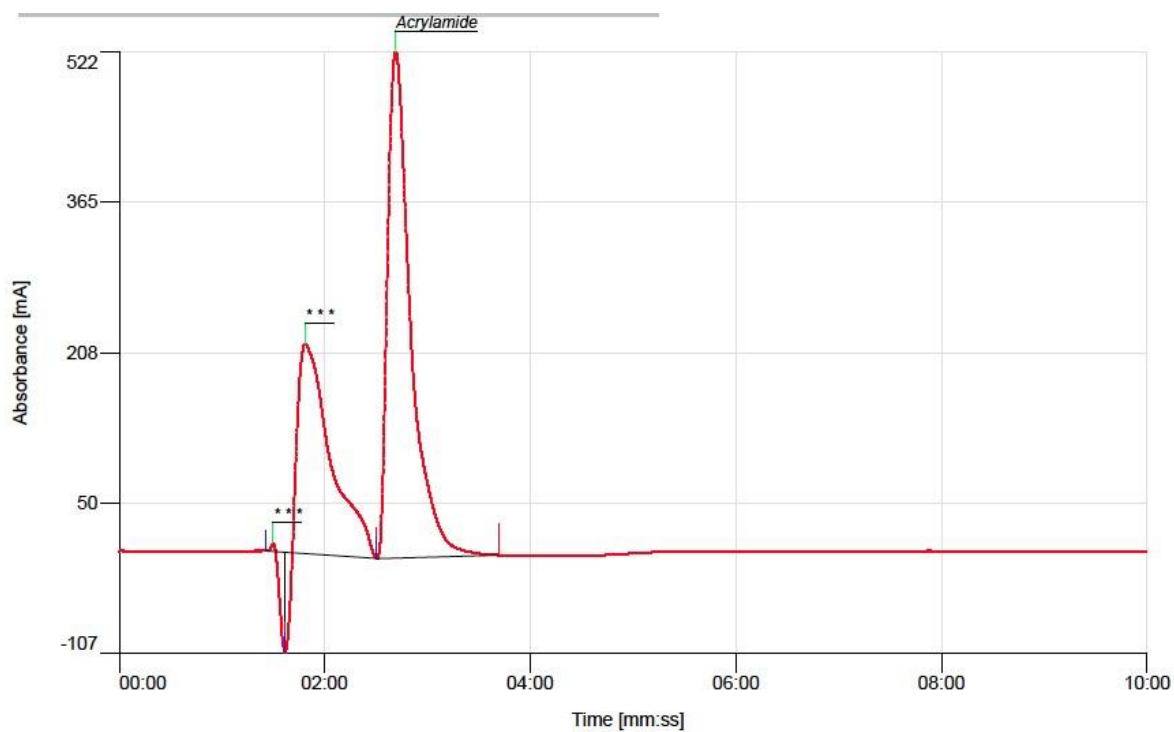
A3: Chromatogram report of alumina silica ratio of 60:40 for a heating time and heating temperature of the ASC of 2 h and 0 °C respectively.



No.	Peak Name	Ret. Time [mm:ss]	Start Time [mm:ss]	End Time [mm:ss]	Area [mAs]	Height [mA]	Qty [ng/g]
001	Acrylamide	02:39.3	02:27.4	03:12.5	9078.1	600.4	2.6802

A4: Chromatogram report of alumina silica ratio of 30:70 for a heating time and heating temperature of the ASC of 1 h and 1400 °C respectively.





No.	Peak Name	Ret. Time [mm:ss]	Start Time [mm:ss]	End Time [mm:ss]	Area [mAs]	Height [mA]	Qty [ng/g]
001	***	01:30.0	01:25.3	01:36.8	-290.1	9.0	N/A
002	***	01:48.6	01:36.8	02:30.4	4593.5	219.1	N/A
003	Acrylamide	02:41.4	02:30.5	03:41.4	8258.5	529.3	2.4382

