KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

ACRYLAMIDE RESULTING FROM HEAT-TIME TREATMENT IN PIGEON PEA, A NEGLECTED AND UNDERUTILIZED LEGUME

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

MASTER OF SCIENCE IN FOOD SCIENCE AND TECHNOLOGY

BY

RITA DANKWAH (BSc. BIOCHEMISTRY)

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CERTIFICATION PAGE

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

RITA DANKWAH	……	……
(PG 6118011)	Signature	Date

Certified by:

MR. ISAAC W. OFOSU	…….	……

Certified by:

PROF. (MRS) I. ODURO	……	……
(Head of Department)	Signature	Date

ABSTRACT

The influence that heat-time treatment has on the concentration of acrylamide in roasted pigeon pea was analysed. The study focussed on optimising the roasting conditions using Response Surface Methodology (RMS) to minimise concentration of acrylamide in roasted Pigeon pea. The treatment conditions used were temperatures in the range of 80 – 120 ^{o}C and time of 10 - 60 min, additive of 0.1-1.0 g and soaking solvent prepared with citric acid or phosphoric acid. Analysis of the data showed that the concentration of acrylamide content significantly increased as the temperature and time of processing increased ($p<0.005$). Citric acid and phosphoric acid used as additives in soaking the Pigeon pea had no significant effect on acrylamide formation. The optimization of process parameters to give low level of acrylamide resulted in roasting temperature of 80ºC for 10 min and 0.1g mass of additive with citric acid as the soaking solvent having a desirability of 0.972. The roasted Pigeon pea produced using the optimized conditions resulted in a concentration of acrylamide (1.76g/kg).

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

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DEDICATION

This work is dedicated to my mother, Madam Agartha Baidoo, for her support.

CHAPTER 1

1.0 INTRODUCTION

1.1 BACKGROUND

Acrylamide is a water-soluble low-molecular weight compound built up of a reactive ethylenic double bond that is linked with a carboxamide group (Eriksson and Karlsson, 2005). Its presence in food was highlighted in April, 2002 when Swedish scientists reported high levels of the compound in carbohydrate-rich foods that were heated to very high temperatures (Graf *et al*., 2006).

The procedures with which food is prepared for consumption has been part of us and one of the reasons is to produce safe food with best possible sensory properties and the minimum content of possibly harmful substances but processing of food at high temperatures has been shown to generate various kinds of cooking toxicants (Skog *et al*., 1998).

International Agency on Research Cancer (IARC) has classified acrylamide as a probable human carcinogen and exposure to high levels has been found to cause damage to the nervous system (IARC, 1994). Acrylamide as an antinutrient and a food toxicant has been considered as a potential cause of cancer with recent epidemiological studies highlighting its association between dietary acrylamide and an increased risk of some types of cancer (Tornqvist, 2005).

It is formed during heating of foods at high temperatures and low moisture conditions that are associated with frying, baking, and roasting although these cooking practices have been used for so many years. Since the discovery of acrylamide, researchers are concerned about making the effort to find diverse ways to help reduce its levels to a minimum.

The formation of acrylamide in food products has been related to the interaction of reducing sugars with asparagine under high heat and low moisture conditions (Mehrajfatema *et al*., 2011) although recently some breakdown fat products mainly acrolein and acrylic acid have also been found to be precursors for acrylamide formation (Weibhaar,2004). Among the different food products analyzed, high levels of acrylamide have been found in potato chips, deep-fat fried foods, crisp bread, biscuits, crackers, and some breakfast cereals (Tareke *et al.*, 2002). However, none of the works published so far has indicated a direct effect of heat-time treatment on Pigeon pea by roasting.

Pigeon pea is an underutilized legume and serves as inexpensive and valuable source of food protein which could offer a partial solution to increasing protein supplies. Apart from being rich in protein, it contains some amount of carbohydrate, high fibre, and low fats. The utilization of pigeon pea need to be increase since it contains an appreciable amount of protein of about 21% (Abdel- Rahman *et al.*, 2010) and can serve as a supplement to animal proteins.

Proteins are made up of amino acids that provide nutrition to both humans and animals and it is feared that certain amino acids found in proteins could be neurotoxin when they undergo certain reactions under intense heat application during processing. Apart from making these amino acids unavailable, toxic compounds can also be formed. Coultate (2009), has reported that prolong heating of legumes such as soya bean have shown significant loss of amino acids due to Millard reactions. Acrylamide, a food toxicant has also been identified to be formed as a result of Millard reaction (Pedreschi *et al.*, 2004). Therefore, the need for further study into cooking conditions

in terms of temperature-time combinations and their impact on acrylamide formation cannot be over emphasized.

Thus, it is very important to properly process these neglected and underutilized legumes to reduce the antinutritional components to levels that will pose no health threat to consumers as well as ensuring minimal loss of nutrients or desirable quality factors. It is believed that, antinutrients could successfully be removed or reduced to an appreciable limit by employing certain processing methods. It has been reported by researchers such as Chi-Fai *et al.* (1997) that different processing methods and traditional treatments such as fermentation, dehulling, cooking, soaking, and germination have been used to improve the nutritional quality of food legumes to various degrees. Therefore finding ways to prevent the formation of acrylamide or lower its levels of in foods has not been straight forward since different food models might need different approaches due to its composition and method of processing.

Numerous studies and research activities have been developed by various researchers to help understand the reduction of acrylamide levels (Salazar *et al*., 2012; Graf *et al*., 2006, Pedreschi *et al*., 2004 and Cummins *et al*., 2008). This research therefore concentrates on the use of a model to control conditions in order to minimize the amounts of acrylamide that would be formed at the end of roasting treatment of a neglected and underutilized legume in Ghana. The importance of modelling this antinutrient is to prepare safe food from a neglected and underutilized legume (NUL) with low acrylamide content.

1.2 Statement of the problem

There have been concerns arising from heating of food due to the formation of compounds which are dangerous to human health (Capuano and Fogliano, 2011).

Food products left under uncontrolled conditions during processing might form antinutritive substances such as acrylamide. There have been reports of the presence of acrylamide in a range of baked and fried foods and these have caused worldwide concern because it has been classified as a carcinogenic compound in humans (Rosen and Hellenas, 2002).

Pigeon pea is mostly grown by subsistence small scale female farmers (Omoikhoje, 2008). It is one of the underutilized legumes in Ghana due to the presence of antinutrients in it, although it could help solve protein energy problems in Ghana. For effective utilization of this legume in various food products there is the need to minimise the presence of these antinutrients particularly the toxic ones in it.

1.3 Justification

Acrylamide formation is related to factors such as processing conditions which includes pre-treatments, time and temperature. Modifying the time and temperature of cooking method appears to be a practicable approach to control acrylamide levels in the final product that would be produced (Cummins *et al*., 2008). To lower the formation of acrylamide levels in foods, there is a need to better understand the effects of different temperature–time combinations for processing foods. Investigating the effect of time and temperature in roasting pigeon pea would help know the specific time and temperature that would produce minimum acrylamide in it.

Several organic acids have been reported to lower acrylamide formation in different food systems (De Vleeschouwer *et al*., 2006; Low *et al*., 2006; Jung *et al*., 2003). Since most of these additives are already being used in the food industry, they can be easily applied to mitigate the formation of acrylamide in Pigeon pea as well. It is

therefore important to determine the effect of citric acid and phosphoric acid in the treatment of Pigeon pea.

This study therefore seeks to monitor the acrylamide formation during roasting of Pigeon pea at temperatures ranging from 80 – 120 ^{o}C and time from 10 – 60 min and relate the time temperature histories to the resultant acrylamide levels by using scientific models.

1.4 Goal

The goal is to make the thermal processing of neglected and underutilized legumes (NULs) such as Pigeon pea, acrylamide-free.

1.5 Objective

To determine the optimum temperature and time for reducing acrylamide formation in Pigeon peas.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Acrylamide in Foods

Acrylamide is formed in foods due to excessive dry heating during processing of foods. Different levels have been detected due to differences in formulation, processing conditions and preparations. It has been found in cocoa powder, prunes, potato chips, bread, chocolate, baby foods, home cooked foods, roasted soybeans, roasted almonds, fries etc.

Erickson (2005) discovered that acrylamide was formed in different food types, independent of heating or frying done with a frying pan, in an oven, or by microwave heating. He reported levels in Laboratory-fried protein-rich food namely minced beef, Chicken, Cod, as 15 – 22µg/kg, 16 – 41µ/kg and 5 – 11 µg/kg respectively and Laboratory-fried carbohydrate-rich food which were grated potato and grated beetroot as 310 – 780µg/kg and 810 – 890µg/kg accordingly.

Al-Damor (2005) reported that fermented whole flour bread and boiled products were below detectable limit level of 180 µg/ kg of acrylamide. He also reported 2300 µg/kg of acrylamide for both fried bread leavened by sodium bicarbonate and rapidly baked whole flour bread. The level he detected in Arabic coffee ranges from 1200- 1600 µg/kg. He found out that for the traditional foods that he studied, the foods with pH ranging between 6.5 and 8.0 showed high concentrations of acrylamide ranging from 500- 4200 µg/ kg.

Levels of acrylamide in French fries have been reported to be in the range 600 µg/kg and 900 – 1000 µg/kg in potato crisps (Yasuhara *et al.*, 2003). Erickson (2005) found that the highest acrylamide formation in potato was at about pH 8 when heated for 15

minutes in a GC oven at temperatures of 160 and 180 ºC. The mean acrylamide concentration for dry beans is in the range 1.7-14ug/kg with its dietary exposure assessments based on mean body weight of 60 kg reported as 6.1.-15.8g/day (FAO, 2006).

2.2 Effects of processing in foods

Most foods are subjected to a wide range of processes in order to prepare them for consumption. Food processing has been part of us and one of the reasons is to produce good sensory properties and minimum content of possibly harmful substances. According to Sathe *et al.* (2005), the methods used to process foods can have effect on the food depending on the severity of the treatment, time of exposure and the type of food. For example, heat processing can reduce the toxicity of some antinutrients such as lectins as it becomes denatured by heat however low temperature or slow cooking may not be enough to completely eliminate its toxicity (Nelson *et al.*, 1991).

Processing methods can affect the nutritional value of some foods particularly, those that expose foods to high levels of heat, light and oxygen. They either affect them positively or negatively. Vitamin C for example, is destroyed by heat and comparably, canned fruits usually would have lower content of vitamin C than fresh ones (Farhat and Fossian, 2010). According to Thane and Reddy (1997), some foods such as fruits and vegetables which may require processing such as peeling may remove some of the nutrients with the portions that are separated from the peeled products. It is therefore essential to determine proper methods for processing foods as well as evaluating the right cooking points for a specific food product.

With respect to food toxicants, food processing methods can be categorized into two main groups. They are food processing method that accumulates toxicants and food

processing method that reduces or eliminates toxicants. Preparation methods such as thawing, chopping and shredding can affect the composition of certain foods like vegetables by making certain enzymes available to act on their substrates. It has been revealed that glucosinolate content in vegetables analysed can drop by 75 % when the vegetables are shredded (Song and Thornalley, 2006).

Cooking by steaming, microwaving and stir-frying also produced non-significant losses in glucosinolate content whilst boiling produced a significant loss of up to 100% due to leaching into the cooking water. Glucosinolate are metabolized to isothiocyanate which acts as potential agent in reducing the risk of developing lung, bladder and colon cancers and so in order to have dietary isothiocyanates in the vegetables, then excessive boiling of vegetables must be avoided to prevent its loss.

2.2.1 Wet processing of foods

Wet processing methods include soaking, boiling, germination and fermentation. Soaking which is a continuous immersion of a product in liquid has been commonly used domestically and industrially before cooking to reduce the cooking time and many reports have shown that antinutrients such as phytic acid leach out from certain legumes during soaking into the soaking medium.

Soaking in water can result in passive diffusion of water-soluble sodium, potassium, or magnesium phytate, which can then be removed by decanting the water and the extent of reduction depends on the species, pH, and time and conditions of soaking. According to Vijayakumari *et al*. (2007), raw seeds of *Bauhinia purpurea* contained 692 mg/100 g phytic acid but when seeds were soaked in distilled water, a reduction in the phytic acid content by 37% was observed. Igbedioh *et al*. (1993) also,

conducted a research to determine the effect of soaking and boiling and other processing methods on Bambara groundnut and Pigeon pea locally grown in Nigeria.

They observed a decrease in phytic acid content in the boiling method though the reduction observed was not as high as in the soaking treatment. The phytic acid loss was greater for 24 h in soaking period followed by 12 h and subsequently the 6 h period. The greater loss in phytic acid content in soaked seeds was attributed to the leaching of phytic acid in the water. They also reported of a high reducing effect of phytate in Pigeon pea than Bambara groundnut thus revealing that the type of food could also affect its composition during processing.

Effect of germination on phytic acid content was studied by Duhan *et al*. (2002). Soaked seeds were washed with distilled water and kept in an incubator at 30 ºC for 24, 36 and 48 h. As the period of germination was prolonged, a significant and successive reduction in this antinutrient was witnessed after 48 h germination and a loss of up to 45 % was reported. Acrylamide has been determined in different food groups but no acrylamide has been reported in unheated or boiled foods (Al-Damor, 2005).

2.2.2 Dry heat processing of foods

Thermal processes are frequently used in food manufacturing for several reasons which include obtaining products with prolonged shelf-life and quality foods. Baking, toasting, frying, roasting, extrusion result in both desirable and undesirable effects due to various chemical reactions such as the Maillard reaction, caramelisation and lipid oxidation. The major concern arising from detrimental consequences of heating processes comes from the formation of compounds which have harmful effects such

as mutagenic and carcinogenic effects. Such compounds are heterocyclic amines, nitrosamines and polycyclic aromatic hydrocarbons.

Four classes of heterocyclic amines which are pyrido-imidazoles or indoles, quinolines, quinoxalines and pyridines formed in Maillard reaction has been reported to occur in heated meat (Skog *et al*., 2000). They are formed through pyrolyzed amino acids such as tryptophan, glutamic acid, lysine, phenylalanine, creatinine and ornithine and according to Persson *et al*. (2004) addition of small amounts of certain carbohydrates may be a simple and effective way of reducing the amount of heterocyclic amines (HCA) in preparations of beef burgers.

Recently, two neo-formed contaminants have gained much interest because of their high toxicological potential and their wide occurrence in foods. These are acrylamide and 5-hydroxymethylfurfural (HMF) (Capuano and Fogliano, 2011). Hydroxymethylfurfural is formed during the advanced step in Maillard reactions, and can be used as a useful indicator for control of cooking processes in cereal products (Ait Ameur, *et al*., 2004). Hydroxymethylfurfural is reported to be slightly mutagenic, but its toxicological relevance has still not been clarified (Janzowski *et al*., 2000).

Acrylamide is one of the latest discovered neurotoxic and carcinogenic substances in food. The polymers of acrylamide have had a range of applications in water and waste-water treatment, cosmetic additives, textile processing and other areas. A limit of acrylamide not more than 0.05µg per 100g in treated drinking water has been set by Environmental Protection Agency. Risk assessment studies on acrylamide intakes have been conducted in a number of countries and µg/kg body weight daily intakes have been estimated to be between 0.3-0.8 (Petersen, 2002).

2.3 Chemistry of Acrylamide Formation in Foods

Acrylamide is a water-soluble low-molecular compound (79.01 g/mol) built up of a reactive ethylenic double bond linked with a carboxamide group. It is volatile and reactive that can be partially lost after formation. Many research works have shown that acrylamide is formed mainly through the Maillard reaction from free asparagine and a carbonyl source. Mottram *et al*. (2002) illustrated that significant quantities of acrylamide were formed when the amino acid asparagine and the reducing sugar glucose were reacted at 185°C in phosphate buffer. Biedermann *et al*. (2003) also reported that acrylamide formation resulted from the degradation of asparagine by reaction with a carbonyl source most likely from glucose and fructose.

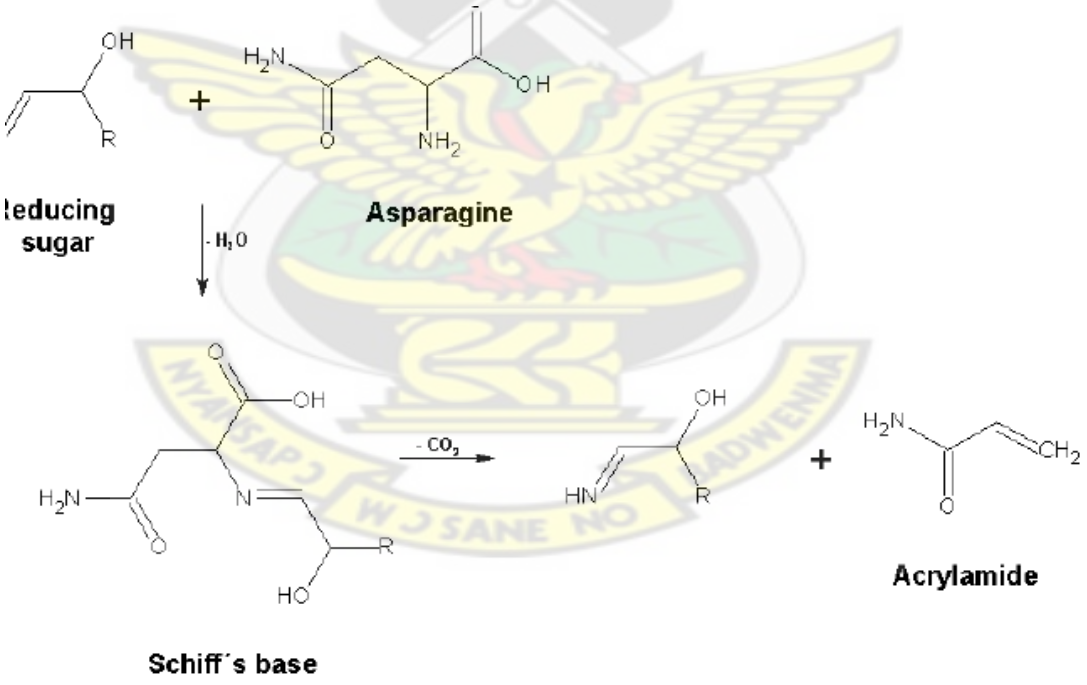


Figure 2.1: Route of Acrylamide formation from Asparagine and reducing sugar

In food model systems depending on its pH, temperature and moisture level, the shift base being the first interaction products can undergo isomerization reactions which increases the Maillard reaction products.

Acrylamide has also been reported to be formed through acrolein or acrylic acid which may be derived from the degradation of lipid as well as through the dehydration or decarboxylation of certain common organic acids such as malic acid, lactic acid and citric acid (Weibhaar, 2004). During frying, lipids heated at high temperature can lead to the formation of acrolein (Umano and Shimoto, 1987) which can further react through oxidation to generate acrylic acid or intermediate acrylic radical (Becalski *et al.*, 2003). Both intermediates would then induce acrylamide formation in the presence of a nitrogen source under favourable reaction conditions (Yasuhara *et al.*, 2003).

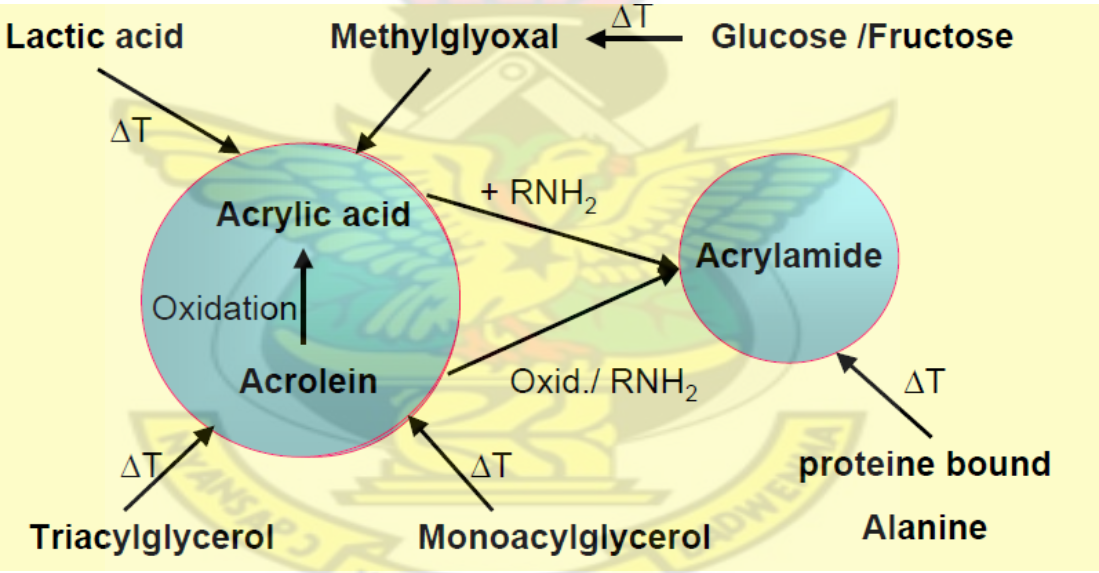


Figure 2.2: Acrylamide formation through Acrylic acid or Acrolein (Weibhaar, 2004)

Mestdagh *et al.*(2005a) reported a significant increase in acrylamide formation in a heated model system containing acrolein and asparagine and reported that the contribution of acrolein to the overall formation of acrylamide appeared negligible in the presence of a reducing sugar.

Zyzak *et al.* (2004) had also demonstrated that 3-aminopropionamide was an intermediate precursor for acrylamide formation between asparagine and reducing sugar. This intermediate has been detected by Granvogi *et al*. (2006) in popcorn, roasted coffee and cocoa. However the 3-aminopropionamide can be formed biochemically in non- heated raw potato through enzymatic decarboxylation of asparagine (Granvogi *et al*., 2004) and according to Granvogi and Schleleber (2006) formation of acrylamide from this route is 12-fold higher than from precursor asparagine.

2.4 Toxicity and Carcinogenicity of Acrylamide

Acrylamide has been added to list of substances of very high concern by European Chemical Agency. Acrylamide has toxic effects on nervous system and fertility. An intake level of 0.5mg/kg body weight/day is required to observed neuropathy. Acrylamide is a carcinogen in laboratory rats. It can cause cancer in the oral cavity, peritoneum, thyroid gland, mammary gland, uterus, clitoris and increases tumour in the nervous system. This had raised concern over human carcinogenicity. A study has found 2.7-fold increase in risk for oestrogen receptor-positive breast cancer for every 10-fold increase of acrylamide in women who adjusted to smoking (Olesen *et al*., 2008). A another study had found a link of acrylamide intake and increase risk of postmenopausal endometrial and ovarian cancer in women aged between 55-69 years

who had adjusted to smoking (Hogervost *et al*., 2009). For carcinogens, risk increases with increasing exposure which implies that acrylamide intake should be low.

2.5 Trends in Acrylamide Formation in Foods

Rydberg *et al*. (2003) found that acrylamide levels in French fries increased as the oven temperature increased from 100-220 °C producing high content of 5000 µg acrylamide per kilogramme of potato fries. However, with prolonged heating at the maximum temperature, acrylamide concentrations decreased due to thermal degradation. The acrylamide formation during food processing as reported by many investigators depends on cooking time and the temperature and to be able to control the reaction process, there is a need to regulate these factors. Karasek *et al*. (2009) investigated whether prolonging the roasting time would further increase the acrylamide content in chestnut. He confirmed that an increase in the roasting time up to 60 min resulted in increased amounts of acrylamide and a decline of acrylamide content after 40 min.

Chestnut which is a seasonal food in Europe around September through to January was eaten in the roasted form or incorporated in other foods. According to Karesek *et al.* (2009) depending upon the temperature and time at which the food was subjected to, there could be high amount of acrylamide produced and therefore they investigated the relationship the roasting time and temperature had on the acrylamide content of roasted chestnut from different sources in Europe. They reported that cooked chestnuts and puree from France contained 36-38 µg/kg and 9 µg/kg respectively whilst chestnut flour and puree from Italy had 159 µg/kg and 4-6 µg/kg accordingly. They also reported of an extreme value of 1278 µg/kg in one of the roasted chestnut from Spain.

Many other researchers have also reported findings of acrylamide in some baked and fried foods. It was reported that a temperature of 120 °C is required for the formation of acrylamide (Becalski *et al*., 2003) and Singh *et al.* (2010) has also confirmed that acrylamide was formed by heating above 120 °C of certain starch-based foods and amino acids. The temperature for acrylamide formation has been investigated by Biedermann *et al*. (2002) that acrylamide can be formed at temperatures below 100 ºC and Gokmen *et al*. (2005), had also suggested that the temperature for the formation of acrylamide need not be higher than 120 °C for acrylamide to form after he investigated on the effect of frying time and temperature on acrylamide formation in the surface and core regions of French fries with frying temperatures being monitored at 150 °C, 170 °C and 190 °C. Prolonged heating time has been shown to decrease acrylamide content (Biedermann *et al*., 2002).

2.6 Reduction of Acrylamide levels in foods

The World Health Organization (WHO) has estimated an intake of acrylamide to be in the range of 0.3–0.8 µg/kg bodyweight per day for an adult which corresponds to 21–56 g/ day for a person weighing 70 kg which serves as the limit for acrylamide intake per day (Petersen, 2002). In order to reduce acrylamide intake, contents in foods must also be reduced to a minimum and many research works have been carried out to determine acrylamide concentrations in food products with different analytical methods. These methods has shown time-temperature to be the most important parameters in its formation (Brathen *et al*., 2005) and therefore, researchers focus on identifying cooking processes that reduces the level of acrylamide to improve food product safety.

Changes in cooking protocols have been in partly successful, particularly lowering of frying and baking temperatures. Pre-treatments have also been used to reduce levels

of acrylamide by prevention of formation or acceleration of destruction. In a study by Erickson (2005), a range of pre-treatment of grilled potato were investigated and compared with surface washing to remove asparagine and reducing sugars.

He observed synergies between different treatments, and reductions of up to 40 % were achieved in a non-optimized system. Soaking raw potato slices in water and acidic solution before frying was effective at reducing acrylamide levels in French fries (Pedreschi *et al*., 2004) but there was no significant removal of glucose and asparagine into soaking medium by dipping the slices in 10 and 20 g/l citric acid solutions. Other pre-treatments such as addition of a flavanoid spice mix (Fernandez *et al*., 2003), use of asparginase to breakdown asparagine in the raw product (Zyzak *et al*., 2004) and the use of genetically modified potatoes having a reduced content of soluble sugars have been shown to reduce acrylamide formation(Soyka *et al*., 2004) .

Friedman, (2003) had also suggested several ways to reduce acrylamide content of food by reducing the content of acrylamide precursor asparagine and sugars in food which is carefully selecting appropriate raw materials. For potatoes, it was feasible to select potato variety with low levels of reducing sugars and this variety can be used for cut potato products intended for frying and baking to lower the levels of acrylamide formed. For example, a maximum of 1g/kg reducing sugars in potato has been suggested as a way to significantly diminish the likely formation of acrylamide (European Commission, 2002).

The use of chemical and biochemical tools, example, changes in pH, and the use of hydrolysing enzymes from yeast or lactic acid producing bacteria during bread making, addition of inhibitors and modifiers and changes in recipe of foods has been shown to be other options.

Additionally, factors such as variety, harvest year, growing and storage conditions of food products may also influence the extent of acrylamide formation during processing (AIB International, 2009). Amrien *et al*. (2003) examined the effect of variety and farming systems on glucose, fructose and asparagine content and on subsequent acrylamide levels after frying.

2.7 Production and Processing of Legumes

Inadequate supplies and high shortage of food protein in the world, especially in developing countries has necessitated the search for new sources to supplement or substitute the existing sources of protein (Salma *et al*., 2010). Legumes which are fruits and seeds of pod bearing plants of the family *leguminosae* are widely grown in the world (Maninder *et al*., 2007) and are valuable source of food proteins (Duranti, 2006) which is present from about 20% dry weight in peas, up to 38-40% in soybean and lupin (Gueguen and Cerlertti, 1994). They provide a relative cheaper source of protein as compared to that of animal proteins.

According to Singh and Singh (1992), legumes such as soybean are grown extensively in Northern Europe, USA, Canada, Russia and China whilst legumes like Bambara groundnut (*Vigna subterranean*) and Pigeon pea, African yam bean and Lima bean are of African origin and cultivated throughout Africa, mostly in the semi-arid areas in sub-Saharan Africa (Brough *et al.*, 1993). Interest has shifted in recent years towards the utilization of unconventional legumes in the food industry to improve the quality of diets as well as utilizing the benefits these food products provide (Oboh *et al*., 1998).

Legumes are used in several food preparations. Legumes are used in preparing foods such as infant formulas, bread, doughnuts, spreads etc. This is done mostly by

roasting and milling of legumes. It can either be boiled or crushed and made into balls which are then fried and used in stew preparations.

Legumes are processed by two main traditional processing methods which are the primary processing method called dehulling (Uma, 1994). Dehulling has been shown to cause an appreciable loss in dry matter contents of the legumes but has been shown to have advantage of contributing to pulses being cooked more quickly than undehulled legume (Wang *et al*., 2008) and shows a negligible effect on the total protein content and amino acid composition. Dehulling has also been reported by Tharanathan and Mahadevamma (2003) to remove tannins that lower protein digestibility. Secondary processing generally includes treatments such as soaking, boiling, frying, roasting, puffing, fermentation, germination, extrusion etc., depending on the type of food and the region of consumption (Singh and Singh, 1992).

Soaking of food legumes formed part of bean processing methods which facilitates quicker cooking and precedes methods such as boiling germination and fermentation. Usually, wet processing of foods such as soaking and boiling has been reported by many researchers to reduce antinutrients such as phytic acid, to various degrees in foods (Igbedioh *et al.,* 1993) as well as some undesirable components like flatulence causing oligosaccharides, tannins etc.. Tannins are water soluble and upon investigation conducted by Khattab and Antfield (2009) on the effect of different treatment on antinutritional factors on Kidney bean and Cowpea of Canadian origin. They observed the highest reduction during boiling and long soaking time (16 h) in water resulting in a highly significant reduction of tannins in all legumes.

Heat processing in general, improves the nutritive value of legume proteins, by inactivating trypsin and growth inhibitors and haemagglutinins (Wang *et a*l., 2008).

Roasting, baking and frying are some of the dry heat processing methods that employs high temperature during food preparation and this could have considerable amount of acrylamide produced during the processing of the food.

Lysine is one of the reactive amino acids. Its availability is monitored as an indicator of the severity of processing on the nutritional quality of protein foods. During processing, lysine is involved in Maillard reactions and the formation of lysinoalanine at high pH. Lysinoalanine is formed in a two-step process. Firstly, there is the formation of a dehydroalanine intermediate and secondly the double bond of dehydroalanine reacts with the ε-NH_2 group of lysine to form lysinoalanine. It is reported to induce enlargement of nuclei of rats and mice kidney cells but not in primates (Friedman, 1999).Extensive lysine loss can take place when legume or cereal and legume blends are extruded very high temperature (218 °C) or shear forces of >l00 rpm at low moisture (<15%), and in the presence of reducing sugars such as glucose, fructose, maltose, lactose.

Saalia and Phillips (2011) carried out a research to degrade aflatoxins in peanut meal using extrusion cooking. He recorded a decrease of 0.52 g/kg of lysine in lightly roasted peanut that was extruded compared to the raw defatted peanut meal. He also observed that high moisture conditions provided extrudates with the least in-vitro protein digestibility and lowest available lysine.

Roasting has been reported by Yusuf *et al*. (2008) to increase the water and oil absorption capacities of flours of raw and roasted Nigerian benniseed (*Sesamum indicum*) and Bambara groundnut. Though roasting as a dry heat processing method has its desirable attributes that is impacted to foods, dry heat processing of food at high temperatures has been shown to generate various kinds of cooking toxicants such

as acrylamide (Karesek *et al*., 2009) and lysinoalanine products etc. which when present along a protein chain decreases its digestibility and nutritional quality (Friedman *et al*., 1999).

Among these antinutrients, acrylamide has been a major health concern which has been confirmed by International Organizations such as the Scientific Committee for Food of the European Union and the World Health Organization. Acrylamide has been found to be a carcinogen in laboratory rats and its epoxide functionality containing metabolite, called glycidamide, has also been found to react with DNA (Tornqvist and Landin, 1995). Human exposure to acrylamide has been found to form haemoglobin adducts. Tareke *et al*. (2000) observed a strong increase in the level of haemoglobin adduct in rats fed with animal standard diet fried at high temperature of 180-165 ºC for 1 or 2 months as compared to control rats that were fed with unfried diet.

Since its first findings by the Sweden Scientists, many researchers (Singh *et al.,* 2010; Karasek *et al.,* 2009; Kaplan *et al*., 2009) have also investigated its content in many foods and a way to minimise its amounts in foods. Kaplan *et al.* (2009) investigated the concentrations of acrylamide in grilled foodstuffs of Turkish Kitchen by HPLC-MS.

They observed that addition of citric acid and hydrochloric acid showed a decreased in acrylamide formation and an increase in degradation of formed acrylamide. They also showed that the molecule can be formed at 65-130 ^{o}C whilst prolong heating times decreases acrylamide content. Elevated levels of acrylamide that were identified by Sweden Scientist in April, 2002 in heated potato products and baked foods has

created an awareness and an increased concern of finding its content in especially fried, roasted and baked foods to determine their safety.

2.8 Benefits of Legumes

Legumes are food resources that offer various health benefits. They are sources of complex carbohydrates, proteins, and dietary fiber, as well as significant amounts of vitamins and minerals (Morrow, 1991; Tharanathan and Mahadevamma, 2003). Legumes has been found to play an important role in several favourable physiological responses, such as reducing heart and kidney diseases, lowering the sugar indices of diabetic patients, increasing in satiety, and reducing the occurrence of cancer (Mathres, 2002).

2.9 Pigeon pea

Amongst food legumes is Pigeon pea which is one of the underutilized crops in Ghana and the legume specie whose important contributions to food security have been underestimated. Pigeon pea mainly grown by subsistence small scale female farmers (Omoikhoje, 2008) and are intercropped with many commodities such as maize, millet and sorghum. Adjei-Nsiah (2012), reported that Pigeon pea contains high levels of vitamin A and C and the seed contain 21 % protein and 1.7 % fats (Abdel Rahman *et al.*, 2010). The nutritive value of pigeon pea depends upon the processing methods, presence or absence of anti-nutritional or toxic factors and possible interaction of nutrients with other food components. Pigeon pea contains high levels of protein and the important amino acids are methionine, lysine and tryptophan.

In order to promote the utilization of this legume, it is important to improve its productivity and marketing.

Table 2.1: Nutritional profile of various amino acids within mature seeds of Pigeon pea

Essential amino acid	Available mg/g of protein	Min. required mg/g of protein
Tryptophan	9.76	7
Threonine	32.34	27
Isoleucine	36.17	25
Leucine	71.3	55
Lysine	70.09	51
Methionine+Cystine	22.7	25
Phenylalanine+Tyrosine	110.4	47
Valine	43.1	32
Histidine	35.66	18

Narsimha and Desikachar (1978) observed that chemicals for reducing the cooking time of split pigeon pea were added either to cooking water or soaking water prior to cooking. These chemicals reduced about 50 % cooking time as compared to control (60 min). They observed that pre-soak treatment of different legume seeds at 25 ^{o}C decreased the cooking time substantially. Soaking in solution was found to be more effective method in reducing cooking time than in water alone (Chavan *et al*., 1983).

In the past eleven years, researchers have studied the effect of heat treatment under different conditions such as temperature, time, pH and other factors on acrylamide formation on processed foods. However, most of these investigations were conducted on industrial fried and baked products. However, very little concerns have been addressed and this has led to further investigation into acrylamide in foods (Brunton *et al.*, 2005).

2.10 Research Methodology on Acrylamide Analysis

2.10.1 Response Surface Methodology

It is a statistical tool that uses statistical techniques for modelling and analysing problems where a response of interest is being influenced by many factors to provide an optimized response (Triveni *et al*., 2001). It overcomes the weakness and

limitations of the classical method (Liyana-Pathirana and Shahidi, 2005) by taking into account the possible interrelationship among the test variables while minimizing the number of experiments (Silva *et al.,* 2007).

It has been used by Farah *et al.* (2012) in optimizing cocoa beans roasting process based on concentration of pyrazine and acrylamide with the optimized roasting conditions being able to produce high quality cocoa beans with low concentration of acrylamide. Also, Ku-Madihah *et al.* (2013) used RSM to optimize roasting conditions comprising of temperature and time for Arabica coffee beans in order to study the formation of acrylamide. High quantity of flavour compounds with low level of acrylamide resulted based on optimized roasting temperature and time. It has therefore been an effective method in improving products.

2.10.2 Food Matrices and Acrylamide Analysis

The determination of acrylamide have usually been on few food materials such as cereals (Becalski *et al*., 2003), potato chips, toasted bread (Ahn *et al*., 2002), mushroom (Castle, 1993), tomatoes (Castle *et al.*, 1991), toasted bread (Becalski *et al*., 2003) despite so many food matrices that are available. Rosen and Hellenas (2002) had reported that the results on acrylamide determination would not be constant for all matrices. Therefore, there is the need to determine acrylamide contents of most food products especially those that were subjected to prolong high temperatures.

2.10.3 Extraction of Acrylamide

To determine acrylamide in treated food sample, the whole sample must be homogenized before sampling a portion for analysis. The acrylamide is then extracted from sample with a suitable solvent which could either be cold or hot water

(Pedreschi *et al*., 2005 and Ahn *et al*.,2002), a weak acid or an organic solvent with extraction times varying between 20- 30 min (Wenzi *et al*., 2003). Organic solvents such as formic acid, acetonitrile (Singh *et al*., 2010) has been used in the extraction of acrylamide and has proven to be effective. A combination of water and some organic acids have been also used by other researchers as extractants (Gokmen *et al*., 2005).

During the extraction step, mechanical agitation such as shaking at high temperature on a shaker, swirling or mixing with a blender or on a vortex have been applied to aid the extraction of acrylamide. Depending on the type of food product being analysed, a defatting step is employed either before the extraction or in combination with the extraction step with the use of reagents such as toluene, cyclohexane or hexane. For samples that might contain small amounts of acrylamide, addition of known amount of acrylamide standards has been added prior to extraction to provide complete recovery of acrylamide in the sample (Singh *et al*., 2010).

When enzymes (pepsin, heat stable α-amylase, protease and amyloglucosidase) was used in the extraction procedure the obtained amounts of extractable acrylamide was similar to the yields obtained from extraction without enzymes. Others have confirmed this, that enzymatic treatment with amylase or protease did not show any effects on the results (Erickson, 2005; Biedermann-Brem *et al*., 2003).

Erikson, (2005) has also demonstrated that higher amount of acrylamide was found in food samples by changing pH towards alkaline pH, during the extraction. The method for extraction of food with potato as a food model was studied with regard to yield of acrylamide by Erickson, (2005). He showed that the yield at pH ≥12 increases 3 - 4 times compared to normal water extraction for some food products and extraction at acidic pH or with enzymatic treatment showed no effect on acrylamide yield. In

another study by him, the bioavailability of acrylamide extracted with the normal water extraction and at alkaline pH was compared. He concluded that the extra acrylamide released at alkaline pH gave insignificant contributions to the *in vivo* dose, measured by haemoglobin adducts in mice. At the end of the extraction, centrifugation and filtration have been performed before cleaning-up the extract (Becalski *et al*., 2003).

2.10.4 Purification of acrylamide extract

Researchers have employed several procedures in order to clean up the acrylamide extract so as to minimise interferences during acrylamide analysis. Defatting of sample has been employed either prior to the extraction or during the extraction to remove fat from samples with chemicals such as hexane (Jezussek and Schieberle, 2004), 1-propanol (Biedermann *et al*. 2002) and toluene by Singh *et al*. (2010). Purification of acrylamide extracts before analysis had been performed in three different ways either by purification with SPE columns, chemical purification, such as deproteination and defatting or with no purification before derivatization (Erickson, 2005).

In order to remove proteins from samples, chemical deproteination has been performed by the use of Carrez solutions I and II ($K_4[Fe(CN)_6]$ and $ZnSO_4$ salt solutions respectively) prior to Gas chromatography (GC) and Liquid chromatography (LC) analysis (Gökmen *et al*., 2005).

2.10.5 Analytical methods for analyzing acrylamide in foods

Acrylamide can be measured by using colorimetry, gas chromatographic (GC) methods or Liquid Chromatographic methods. The measurement of acrylamide with

colorimetric method is not specific for only acrylamide since there can be interference by other organic compounds.

Measurement of acrylamide with GC-MS requires derivatization of the molecule with either bromine, hydrogen bromide or potassium bromide, to form Dibromopropionamide with improved GC-properties. According to Wenzi *et al.* (2003), the bromination is usually performed at or slightly above the freezing point of water. The brominated derivative was extracted into ethyl acetate and analysed by GC with an electron capture detector which was then identified by its retention time and by the ratio of characteristic MS ions. GC-MS method with bromination has been published by Castle *et a*l. (1991) for the determination of acrylamide in tomatoes that was grown on polyacrylamide gel.

The lowest level that could be measured when using GC-MS is in the range of 5 to 10 µg/kg (Kaplan *et al*., 2009). Possible artefact formation during the bromination when using GC-MS raised concerns for direct analysis of the molecule without derivatization. Besides Liquid and gas chromatography, a thin layer chromatography method with fluorescence detection after derivatization with dansulfinic acid was reported (Alpmann and Morlock, 2008).

Without derivatization, acrylamide analysis by GC-MS has been performed by researchers such as Hamlet *et al.* (2004) and Hoenicke *et al*. (2004b) and the major drawback of GC-MS analysis without derivatization was the lack of selective fragmentation patterns. Co-extracted substances such as maltol or heptanoic acid showed nearly the same fragmentation pattern and may therefore interfere (Biedermann *et al*., 2003).

The LC-MS/MS procedures were developed for analysis of acrylamide without derivatization of the acrylamide molecule. The limit of measurement using LC-MS/MS was about 20 to 50 μg/k. but the identification of acrylamide in foodstuffs using the two methods is said to be highly reliable (FAO/WHO, 2002). Gokmen *et al.* (2005) used the LC-MS procedure to determine relation between the acrylamide formation and time–temperature history of surface and core regions of French fries prepared from potato that was obtained from Turkey whilst Paleologos and Kontominas, 2005 used LC-UV to determine acrylamide concentration. Singh *et al.* (2010) determine the concentration of acrylamide in processed food products available in open market at Chennai using high performance liquid chromatography.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Sources of Materials

Pigeon pea was obtained from an outgrower in the Sunyani Metropolis and all chemicals used in this research were procured from Sigma Aldrich Company Limited in the United States of America.

3.1.2. Preparation of Materials

Samples were sorted and cleaned to remove dust and foreign materials. The samples were weighed to 50 g and kept in containers prior to treatment.

3.2. Methods

3.2.1 Determination of Treatment Factors and Response

The effect of four factors: soaking solvent, mass of additive, temperature and time on response was studied. Design Expert (2009) was used to randomize the factors. Table 3.1 indicates the specified levels at which each factor was varied during the experiment. A total of thirty four runs were generated to study the acrylamide concentration.

3.2.2. Soaking Treatment

Each of the experimental runs was soaked overnight with tap water in containers at room temperature. An additive of either phosphoric acid or citric acid of concentration 0.1-1g was added with respect to the experimental runs generated. The soaked legumes were then drained, dried in a solar dryer for 3 days and stored at room temperature for roasting.

Table 3.1: Constraint for Treatment Factors

Factors	Level of variation
Soaking	H_3PO_4 /Citric acid
Mass of additive	0.1 – 1.0 g
Roasting temperature	80 – 120 ^{O}C
Roasting time	10 – 60 min

3.2.3. Roasting Treatment

For studies of roasting, the oven was preheated to the desired temperatures and maintained for roasting. The dried samples were roasted in batches in a Melano-60-oven (Model: DCGML 6/P) at a temperature of 80-120 ^{o}C and time interval of 10-60 min as represented in Table 4.1. After roasting, the legumes were taken out of the oven and cooled to room temperature. All treated samples were then milled with Panasonic blender (Model No MX-1515P1) to a particle size of 600 µm.

3.2.4 Preparation of Defatted Pigeon pea Flour

The flour of treated Pigeon pea was defatted using the cold extraction method by soaking the flour (tied in a cheese cloth) in hexane using a ratio of 1:10 w/v with respect to flour and solvent. The set up was then sealed and left for 3 days at room temperature after which it was then solar dried for 72 h (3days) to expel residual solvent and packed in polyethylene bags for further analysis .

Table 3.2: Summary of RSM Design for roasting of Pigeon pea

	Factor 1	Factor 2	Factor 3	Factor 4
Run	A:Mass of add g	B:Roasting time min	C:Roasting temp oC	D:soaking solv
1	0.10	60.00	100.00	H_3PO_4
2	0.55	35.00	100.00	H_3PO_4
3	0.10	10.00	100.00	H_3PO_4
4	0.55	35.00	100.00	Citrate
5	1.00	35.00	80.00	H_3PO_4
6	1.00	60.00	100.00	Citrate
7	0.55	35.00	100.00	Citrate
8	0.10	10.00	100.00	Citrate
9	0.55	60.00	80.00	Citrate
10	0.55	35.00	100.00	H_3PO_4
11	0.55	35.00	100.00	Citrate
12	1.00	35.00	80.00	Citrate
13	0.55	10.00	120.00	Citrate
14	0.55	35.00	100.00	H_3PO_4
15	0.55	60.00	120.00	Citrate
16	0.10	35.00	120.00	Citrate
17	0.55	35.00	100.00	H_3PO_4
18	1.00	35.00	120.00	Citrate
19	0.55	10.00	80.00	Citrate
20	0.55	10.00	80.00	H_3PO_4
21	0.55	60.00	120.00	H_3PO_4
22	1.00	10.00	100.00	H_3PO_4
23	0.55	35.00	100.00	H_3PO_4
24	0.55	10.00	120.00	H_3PO_4
25	0.55	35.00	100.00	Citrate
26	1.00	10.00	100.00	Citrate
27	0.10	60.00	100.00	Citrate
28	1.00	60.00	100.00	H_3PO_4
29	1.00	35.00	120.00	H_3PO_4
30	0.10	35.00	120.00	H_3PO_4
31	0.55	35.00	100.00	H_3PO_4
32	0.10	35.00	80.00	Citrate
33	0.55	60.00	80.00	H_3PO_4
34	0.10	35.00	80.00	H_3PO_4

3.2.5 Determination of Acrylamide

3.2.5.1 Extraction of acrylamide

Extraction of acrylamide was adopted from previous published methods by Zhang and Zhang (2007) and Gokmen *et al.* (2005). A mass of 1 g portion of defatted treated Pigeon pea sample was measured into a 50 mL polypropylene tube with cap. A volume of 500 µl Carrez I and 500 µl Carrez II solution were added to precipitate proteins and carbohydrates in order to minimize interferences. The volume was adjusted to 10 ml with 0.2 mM acetic acid (Gokmen *et al.*, 2005).

A volume of 30 ml of 70 % of acetonitrile was added and vortex briefly (Zhang and Zhang, 2007). Afterwards the mixture was then shaken for 60 min on a horizontal shaker without heat to maximum sample extractant agitation speed of 100 rpm as described by Karasek *et al*. (2009) and centrifuge for 3000 rpm for 30 min.

3.2.5.2HPLC analysis

The supernatant was then decanted with a microfiber filter paper and a volume of 2 µl transfer with auto-sampler vial for HPLC analysis. The analysis was performed on HPLC-UV detector mode using an Atlantis dC18 column (150 ×2.1 mm, 5 mm, USA). Six standard acrylamide solutions were prepared (Appendix A2) and measured according to the HPLC-UV conditions.

3.2.5.3 Identification of acrylamide

The determination of acrylamide in samples was based on the peak area and retention time of acrylamide in a chromatographic run. The calibration curve was obtained by plotting the peak area against concentration of acrylamide.

Once the acrylamide in the sample was identified, the quantification of the acrylamide was made based on its calibration curve (appendix A1) y = bx + a, where a = -0.28848, b = 0.02179 with a correlation coefficient of 0.9951. The peak areas for the experimental runs that were used in calculating the corresponding acrylamide concentrations are shown in appendix A4.

3.2.6 Statistical Analysis

3.2.6.1 Fitting the data collected

The response data for acrylamide obtained from the analysis was loaded and fitted to models using Design Expert (2009). The model that best fit the data was identified by determining the adequacy of the model by evaluating regression parameters such as regression (R^2), adjusted regression (adj. R^2), predicted regression (pred. R^2), and adequate precision (adeq. precision).

When a model had been selected, analysis of variance was calculated to find out how well the model represented the data. P and F – values were also determined to identify the variations between the factors and data obtained. The P-values and the interactions among factors of treatment were tested against $P< 0.05$. When all the model statistics and diagnostic plots were evaluated to be good, the model graphs were plotted and performance of the factors and response were made. The optimum condition generated was then used to treat the pigeon pea and acrylamide concentration was then determined.

3.2.6.2 Optimization of process

The research requires minimizing the acrylamide content of the treated sample and therefore goals were set by imposing constraints on processing and response factors to obtain optimum conditions desired to process the NUL. This is critical for the

statistical tool to select the optima according to the constraints imposed. Therefore the response factor was set to minimum whilst the process factors; mass of additive, roasting time, roasting temperature were set in ranges.

Table 3.3: A constraint table showing the importance of factors and response goals set for optimization

	Goal	**Lower limit**	**Upper limit**	**Importance**
Process factors				
Mass of additive				
Roasting temperature	is in range	10 min	60 min	3
Roasting time	is in range	80 ºC	120 ºC	3
Soaking solvent	is equal to	H_3PO_4	Citrate	3
Response factor				
Acrylamide	minimize	X_1	X_2	5

Where X_1 and X_2 are unknown acrylamide concentrations from low to high.

CHAPTER 4

4.0 RESULTS AND DISCUSSION

4.1 Data Analysis

The dependent variable, which is acrylamide concentration and the independent variables; mass of additive, soaking solvent, roasting temperature and roasting time were run in the Design Expert(2009) package to obtain a regression model that predicted the response within the given data set. The regression equation for the treatment is as follows;

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots\ldots + \beta_q X_q, \qquad i = 1, 2, 3, \ldots, q$$

where:

Y= Dependent variable (acrylamide concentration)
βi =Coefficient of independent variables
Xi= Independent variables (processing factors)

4.1.1 ANOVA for Acrylamide Formation

Table 4.1: ANOVA for response surface linear model for produced acrylamide

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	35.43	4	8.86	2.89	0.0418	significant
A-Mass of add	1.67	1	1.67	0.54	0.4672	
B-Roasting time	13.29	1	13.29	4.34	0.0472	
C-Roasting temp	16.38	1	16.38	5.35	0.0289	
D-soaking solv	5.13	1	5.13	1.67	0.2070	
Residual	79.62	26	3.06			
Lack of Fit	48.73	18	2.71	0.70	0.7483	not significant
Pure Error	30.90	8	3.86			

The p-value was used as a tool to check the significance of each of the coefficients. The smaller the p- value the more significant the regression. ANOVA showed that

there was a non-significant ($p > 0.05$) lack of fit which validates the model (Ku-Madihah, 2013).

The actual equations for both soaking solvents are given as:

Acrylamide formation using $Citric\ acid = -2.069 + 0.745A + 0.041B + 0.054C$

Acrylamide formation using $H_3PO_4 = -2.069 + 0.745A + 0.041B + 0.054C$

The adequacy of the model was determined by using model analysis, lack of fit test and coefficient of determination (R^2). The significance of the equation parameter was assessed by F value at probability ($p > F$) less than 0.05 (Zaibunnisa *et al*., 2009). The regression model is significant at the considered confidence level (95%) since the regression has p-value of 0.0418 and F value of 2.89. Lack of fit test has p-value of 0.74 83 and an F-value of 0.70 (Table 4.1).

However, according to Tan *et al*. (2012) when R^2 value in the range of 0–1.0 and nearing 1.0, the more fit the model is deemed to be. Similarly, according to (Zaibunnisa *et al*., 2009), for a good fit of a model, R^2 should be at least 0.80 thus approaching 1. In this study, low R^2 value of 0.31 and adjusted R^2 of 0.20 was obtained. This result indicates that the presence of acrylamide was not influenced by soaking solvent and mass of additive. Roasting time and roasting temperature are significant model terms. The predicted R-squared of 0.04 was in reasonable agreement with adjusted-R-squared 0.20 with the difference being less than 0.2(Appendix B3).

For a model to be used to navigate the design space there should be adequate signal which is measured as adequate precision showing the signal to noise ratio. A ratio

greater than 4 is desired and the adequate precision measured was 6.2 indicating adequate signal (Appendix B3).

4.1.2 Acidulant Treatment of Pigeon pea

Legumes contain nutrients (proteins, oils, vitamins, mineral substances, carbohydrates and dietary fibres) which positively affect nutritional value and antinutrients that negatively affect diets. However, it has been observed that the effects of antinutrients may disappear or decrease when legumes are properly prepared (Onder and Kahraman, 2009). Antinutrients such as acrylamide have been reported to decrease when potato slices were soaked in acidic medium prior to frying (Predreschi *et al*., 2004). The relationship between temperature, time and mass of additive in determining acrylamide level using citric acid and H_3PO_4 is shown in Figure 4.1 and 4.2.

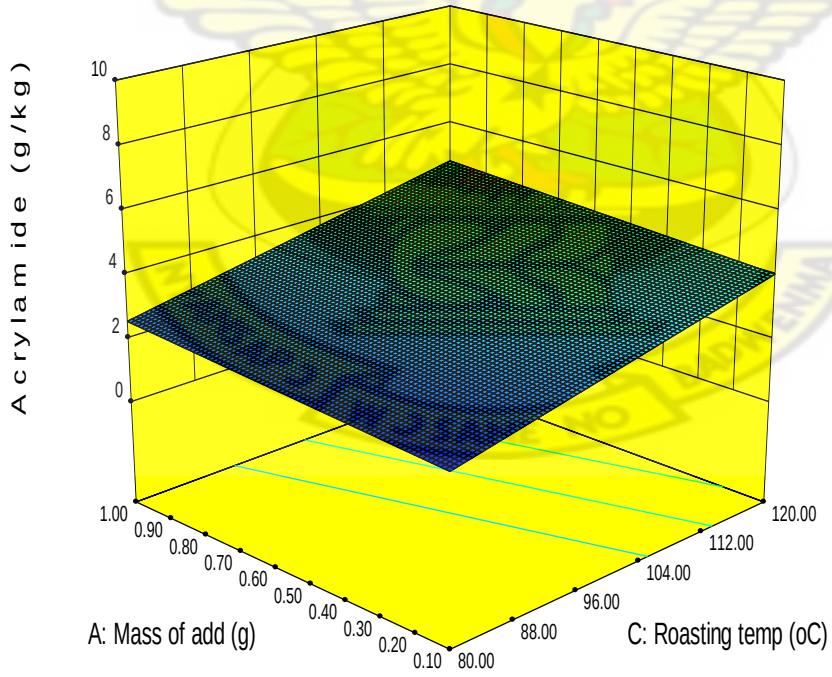


Figure 4.1: *Response surface plot showing the three dimensional (3D) image of acrylamide produced during roasting in relation with temperature and mass of citrate additive.*

The graph indicates the relationship between mass of additive and temperature and their influence on acrylamide level. Increase in acrylamide level was observed as mass of additive and temperature increase from 0.1 to 1.0 g and 80 to 120 ^{O}C respectively. However, increasing the mass of additive did not have a significant increase in the level of acrylamide. At a temperature of 80 ^{o}C and mass of additive of 0.1 g, 2 g of acrylamide was formed in per kilogramme Pigeon pea and increased to 4 g/kg as roasting temperature increases up to 120 ^{O}C.

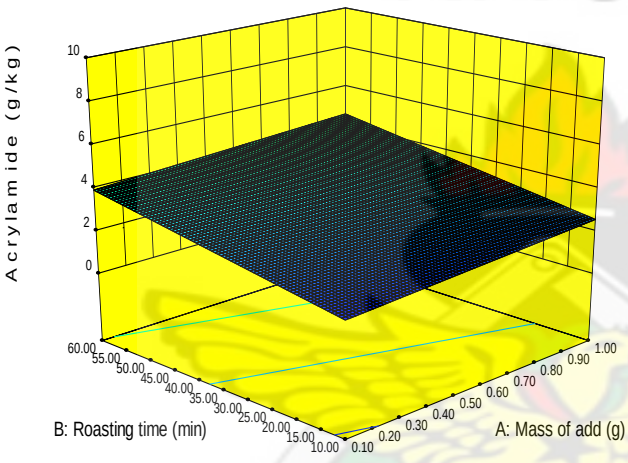


Figure 4.2a: Response surface plot showing the three dimensional (3D) image of acrylamide produced during roasting in relation with roasting time and mass of citrate additive.

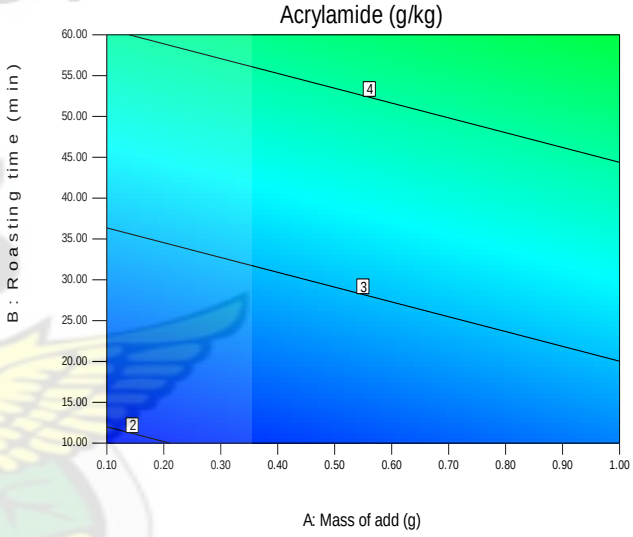


Figure 4.2b: Response surface plot showing the contour plot of acrylamide produced in Figure 4.2a.

The graph indicates the relationship between roasting time and mass of additive and their influence on acrylamide levels (Figure 4.2a and b). Increase in acrylamide formation was observed when roasting time and mass of additive was increased from 10 to 60 min and 0.1 to 1.0 g respectively. Acrylamide concentration of 2 g acrylamide /kg of roasted Pigeon pea was observed when mass of additive was 0.1 g and a roasting time of 10 min and increases close to 4 g/kg when time was increased.

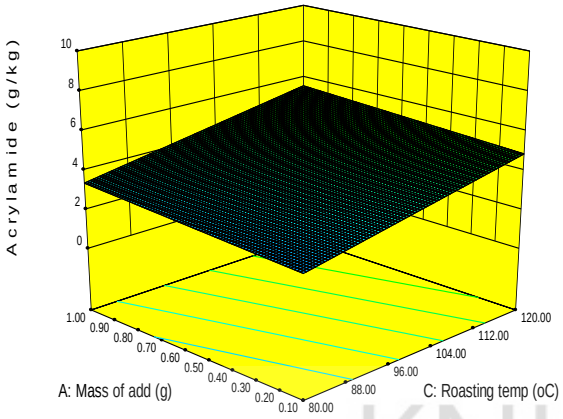


Figure 4.3a: Response surface plot showing the three dimensional (3D) image of acrylamide produced during roasting in relation with roasting temperature and mass of H_3PO_4 additive.

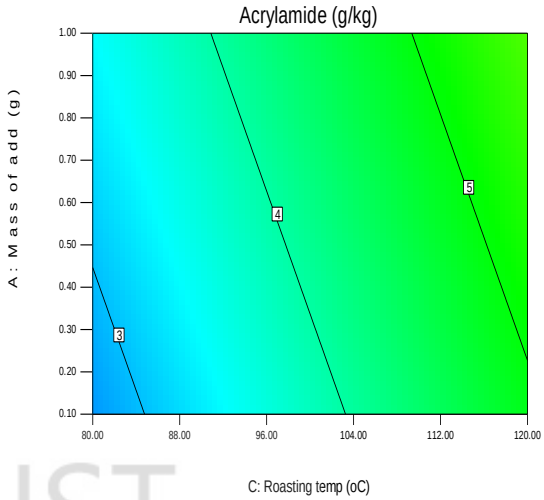


Figure 4.3b: Response surface plot showing the contour plot of acrylamide produced in Figure 4.3a.

The relationship between mass of additive and roasting temperature on the acrylamide level is shown in Figure 4.3a and b. Increase in acrylamide level was observed as mass of additive and roasting temperature increase from 0.1 to 1.0 g and 80 ^{O}C to 120 ^{O}C. Concentration of 3 g acrylamide /kg of pigeon pea was observed at 0.1 g mass of additive and 80 ^{O}C roasting temperature. Influence of mass of additive on acrylamide level was not significant as compared to roasting temperature.

Figure 4.4a and b shows the relationship between roasting time and mass of additive on acrylamide level. The level of acrylamide detected was 3 g acrylamide / kg of roasted pigeon pea at time of roasting of 10 min and mass of additive at 0.1 g. At higher roasting time and mass of additive (60 min and 1.0 g respectively), acrylamide level increased above 4 g/kg. However, mass of additive had no significant difference on the level of acrylamide.

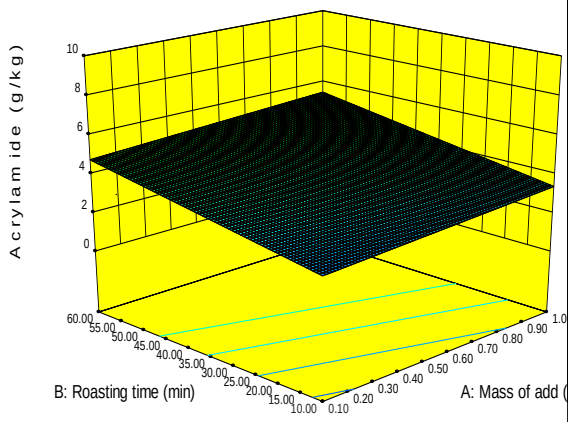


Figure 4.4a: Response surface plot showing the three dimensional (3D) image of acrylamide produced during roasting in relation with roasting time and mass of H_3PO_4 additive.

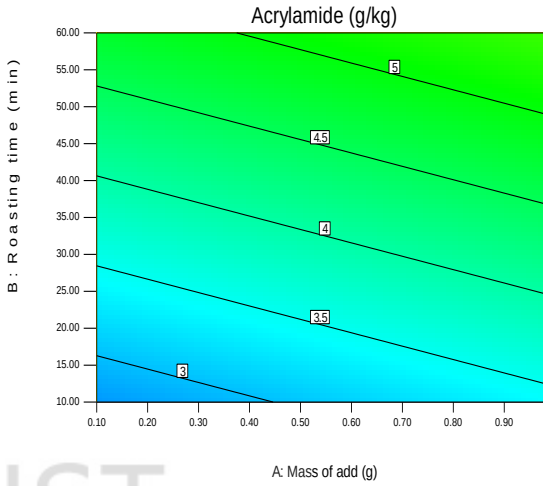


Figure 4.4b: Response surface plot showing the contour plot of acrylamide produced in Figure 4.4a

4.2. Effects of Time-Temperature Relation on Acrylamide Level in Roasted Pigeon Pea

Sanganyodo *et al*. (2011) reported mean concentration of 70 µg acrylamide/kg roasted soybean from Zimbabwe. The mean concentration of acrylamide in this study was 4.7g acrylamide/kg Pigeon pea. Previously, Friedman, (2003) had reported concentration of 25 µg acrylamide /kg roasted Soybeans.

According to Sanganyodo *et al*. (2011), the amounts of acrylamide in the samples he analysed was underestimate due to the fact that samples spent about four weeks in storage during their shipment before analysis whilst studies have shown that acrylamide contents usually decrease with storage time (Hoenicke and Gatermann, 2005) . In this study, lower roasting temperature of 80 ^{o}C was capable of producing lower acrylamide level in Pigeon pea using citric acid and H_3PO_4 as soaking solvents. Under the studied roasting conditions, acrylamide concentration of 1.758 - 8.368g/kg was detected in roasted Pigeon pea (Appendix B1). Higher levels of acrylamide have

been detected in cocoa products (17-23 g/100g) from countries such as Norway, Sweden, United Kingdom and United State of America (FAO/WHO, 2005).

The roasting temperature has a significant impact on the acrylamide content of Pigeon pea (*p-value* 0.0289) as does roasting time (*p value* - 0.04) as shown in Table 4.1 and therefore controlling these two factors would have influence on acrylamide formation. This is evident in a report by Zhang *et al*. (2011) that controlling the roasting temperature resulted in low acrylamide levels at all roasting times that he evaluated.

The results clearly indicate that substantial amounts of acrylamide was generated at temperatures lower than 120 °C and this was contrary to the report that 120 ^{o}C was required for the formation of acrylamide (Becalski *et al.*, 2003). Also this indicates that the acrylamide level in roasted Pigeon pea could be very high, especially when it remains in the roasting vessel for quite a long time without keeping an exact time or applying too high temperatures (Karesek *et al.*, 2009). The data agrees with those published by several investigators (Gökmen, 2005, Erickson, 2005, Mottram, 2002) regarding the relationship between cooking temperature and acrylamide formation in foods. An increase in roasting time results in increased amounts of acrylamide in roasted Pigeon pea as shown in Figure 4.2b and 4.4b.

Roasting experiments under different process conditions showed that acrylamide increases with time and temperature. Temperature had a much stronger effect on acrylamide formation than time and a similar trend was reported by Amrein *et al*. (2005).

4.3 Optimization of Processing Condition

Numerical optimization was carried out in order to determine the optimum condition for roasting Pigeon pea. The goals were set in range for all process factors and minimum for acrylamide. The optimal condition for roasting which depends on the process factors was obtained using predicted equation determined by using RSM. The optimization solution which was based on minimum level of acrylamide was obtained at a roasting temperature of 80 °C, roasting time of 10 min with 0.1g additive, using citric acid and phosphoric acid as soaking solvent (Table 4.2). Soaking with citric acid and phosphoric acid gave a desirability of 0.97 and 0.84 as shown in the Figure 4.5 and 4.6 respectively.

Table 4.2: Predicted Optimum Acrylamide Concentration for Pigeon pea Treated with Citrate and Phosphoric acid

Acidulant level (g/kg)	Optimum condition			Minimum acrylamide
Treatment	Temperature (^{o}C)	Time (min)	Additive (g)	Pigeon pea
Citrate	80	10	0.1	1.91
Phosphoric acid	80	10	0.1	2.74

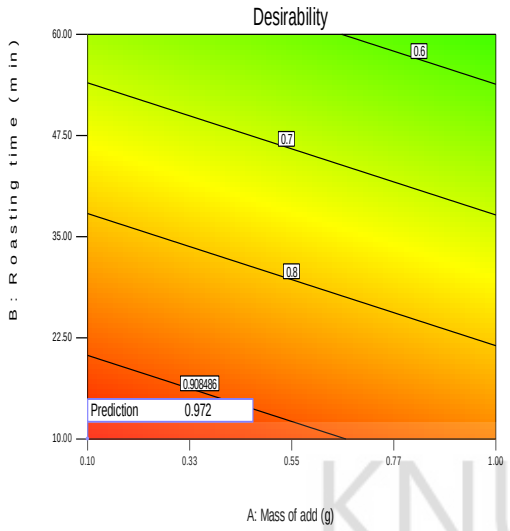


Figure 4.5: Desirability graph showing predicted level of acrylamide using citric acid for soaking

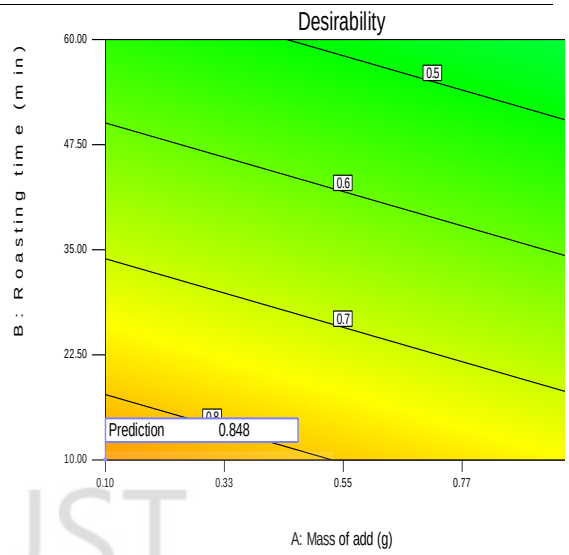


Figure 4.6: Desirability graph showing minimum undesired acrylamide level at optimum condition with phosphoric acid.

Acidulants are reported to reduce acrylamide levels by interfering with Maillard reaction. At optimum conditions (80 ^{o}C, 10 min, mass of additive of 0.1) acrylamide produced was lower with citric acid (Figure 4.5) than phosphoric acid (Figure 4.6) which indicates that citric acid has the ability to lower acrylamide concentration than phosphoric acid but could have had a significant impact at a higher concentration. From desirability graphs, it was evident that the desired soaking solvent for processing Pigeon pea to obtain minimum acrylamide is citric acid. Citric acid has been reported by Mestdagh *et al.* (2005) to reduce acrylamide formation in potato. Low *et al.* (2006) reported that when processed food was treated with citric acid and a combination of acetic acid and glycine, the mixtures containing citric acid had a lower acrylamide content compared to other mixtures with acetic acid. This therefore agrees with Low *et al.* (2006) that citric acid has a stronger effect on acrylamide. Under the

study, low amount of acrylamide was detected (1.76 g/kg) using citric acid as the soaking solvent with roasting conditions (80 °C, and 10 min).

However, researchers noted that lower concentrations of citric acid would further reduce the effect on volatile flavour compounds, but still significantly reduce the formation of acrylamide as was evident in a potato model system cooked at 180 ^{o}C for 10-60 min but that affected the volatile profiles particularly the alkylpyrazines in roasted coffee (Mestdagh *et al*., 2005).

CHAPTER 5

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Pigeon pea was processed with varying mass of additive (0.1-1 g), roasting temperature (80- 120^{o}C) and time (10-60 min) using citric acid and phosphoric acid as soaking solvent. Acrylamide occurred in roasted Pigeon pea with unexpectedly high levels at different roasting temperatures, mass of additives, roasting times and soaking solvents. Increasing roasting time and roasting temperature resulted in an increased acrylamide concentration. It was found out that acrylamide was formed during heating of Pigeon pea even below 120 °C.

The roasting temperature and time had significant impact on the formation of acrylamide. Citric acid had a stronger effect of reducing acrylamide formation than phosphoric acid although no significant effects were observed from addition of citric acid or phosphoric acid to the soaking medium. Soaking of raw Pigeon pea in acidic medium was shown to aid in minimising acrylamide formation. From this study, it was observed that acrylamide content can be lowered if concentration of the mass of additives could have been higher.

The model with equation; -2.069 + 0.745A + 0.041B + 0.054C, gave the optimized roasting condition for roasting of Pigeon pea to produce low acrylamide. The optimized condition was found at temperature of 80 °C, roasting time of 10 min and 0.1 g of citric acid with desirability of 0.97. Under this condition, acrylamide concentration 1.76 g/kg was observed. The experimental result was close to the predicted response which validated this model experimentally

5.2 Recommendation

It is recommended that further research into the use of high amounts of citric acid and its effects on both acrylamide and the composition of the roasted legume should be investigated.

REFERENCES

Abd El-Hady, E. A., Habiba R. A. (2003). Effect of soaking and extrusion conditions on antinutrients and protein digestibility of legume seeds. *Lebensm.-Wiss U.-Technology*, 36:285–293.

Abdel-Rahman, S. M. E., Ali, A. O. and Rea, H. (2010).The chemical composition of Pigeon pea seed and functional properties of protein isolate. *Pakistan Journal of Nutrition*, 9 (11): 1069-1073.

Adjei-Nsiah, S. (2012). Evaluating sustainable cropping sequences with cassava and three grain legume crops: Effects on soil fertility and maize yields in the semi-deciduous forest zone of Ghana. *Journal of Soil Science and Environmental Management*, 3(2): 49-55.

Ahn, J. S., Castle, L., Clarke, D. B., Lloyd, A. S., Philo, M. R. and Speck, D. R. (2002). Verification of findings of acrylamide in heated foods. *Food Additives and Contaminants*, 19:1116–1124.

Ait Ameur, L., Zude, M., Trystram, G. and Birlouez-Aragon, I. (2004). Hydroxy-methyl furfural: An indicative parameter of heat damage in cereal products. *Czech Republic Journal of Food Sciences*, 22 : 99-101.

Al-Damor H.M. (2005). Determination of acrylamide levels in selected traditional food stuffs and drinks in Jordan. Journal of Food, *Agricultural and Environment*, 3(2):77-80.

Alpmann, A., and Morlock, G. (2008). Rapid and sensitive determination of acrylamide in drinking water by planar chromatography and fluorescence detector after derivitization with disulfinic acid. *Journal of Separation science*, 31(1): 71-77.

Amrein, T. M., Schönbächler, B., Rohner, F., Lukac, H., Schneider, H., Keiser, A., Escher, F. and Amadò, R. (2004). Potential for acrylamide formation in potatoes: Data from the 2003 harvest. *European Food Research and Technology*, 219: 572-578.

Amrein, T., Bachmann, S., Noti, A., Biedermann, M., Barbosa, M., Biedermann, B., Grob, K., Keiser, A., Realini, P., Escher, F. and Amado, R. (2003). Potential of acrylamide formation, sugars, and free asparagine in potatoes: A comparison of variety and farming systems. *Journal of Agricultural and Food Chemistry* 51: 5556-5560.

.

Apata, D. F. and Ologhobo, A. D. (1994). Biochemical evaluation of some Nigerian legume seeds. *Food Chemistry*, 49:333-338.

Arusha (2003). FAO/WHO Seminar on Acrylamide in Food, EU Commission perspective on acrylamide in food. www.fao.org/ag/agn/jecfa/acrylamide/slayne/ts1d001.htm.(accessed 2 April, 2011) 2010)

Becalski, A., Lau, B. P-Y., Lewis, D. and Seaman, S. W. (2003). Acrylamide in foods: Occurrence, sources, and modeling. *Journal of Agricultural and Food Chemistry*; 51:802–808.

Biedermann, M., Biedermann-Brem, S., Noti, A. and Grob, K. (2002b). Methods for determining the potential of acrylamide formation and its elimination in raw materials for food preparation, such as potatoes. *Mitteilungen aus Lebensmitteluntersuchung und Hygiene*, **93**, 653-667.

Biedermann-Brem, S., Noti, A., Grob, K., Imhof, D.,Bazzocco, D. and Pfefferle, A. (2003). How much reducing sugar may potatoes contain to avoid excessive acrylamide formation during roasting and baking? *European Food Research and Technology*: 217:369–373.

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

Brough, H. S., Azam-Ali, N. S. and Taylor, J. A. (1993). The potential of bambara groundnut (*Vigna subterranea*) in vegetable milk production and basic protein functionality systems. *Food Chemistry*, 47:277-283.

Brunton, N., Gormley, R. and Murray B. (2005), Status Report on Acrylamide in Potato products. http:/www.teagasc.ie/research/reports/foodprocessing/5265/eor5265.pdf.(Accessed 1 January, 2011).

Capuano, E. and Fogliano, V. (2011). Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism, toxicity, occurrence in food and mitigation strategies. *Food Science and Technology*, 44:793-810.

Castle, L. (1993). Determination of acrylamide monomer in mushrooms grown on polyacrylamide gel. *Journal of Agricultural and Food Chemistry*, 41: 1261-1263.

Castle, L., Campos, M-J. and Gilbert, J. (1991). Determination of acrylamide monomer in hydroponically grown tomato fruit by capillary gas chromatography-mass spectrometry. *Journal of the Science of Food and Agriculture*, 54: 549-555.

Chavan, J. K., Jawale, H. K., Shere, D. M., Jadhav, S. J. and Kadam, S. S. (1983). Effect of presoak treatments on the cooking quality of legume Dhal. *Indian Food Packer*, 37: 78-81.

Chi-Fai, C., Peter, C. K. C. and Yum-Shing, W. (1997). Effect of cooking on content of amino acids and antinutrients in the Chinese indigenous legume seed. *Journal of Food Science and Agriculture*, 75: 447-452.

Coultate, T. P. (2009). Food: The chemistry of its components. Fifth edition, Published by The Royal Society of Chemistry, Thomas Graham House, Science Park, Milton Road, Cambridge, UK, P 128.

Cummins, E., Butlera, F., Gormley, R. and Brunton, N. (2008). A methodology for evaluating the formation and human exposure to acrylamide through fried potato crisps. LWT-*Food Science and Technology*, 41: 854–867.

Dolores del Castillo, M., Corzo, N., Carmen Polo, M., Pueyo, E., and Olano, A. (1998). Changes in the amino acid composition of dehydrated orange juice during accelerated non-enzymatic browning. *Journal of Agricultural and Food Chemistry*, 46, 277–280.

Delgado-Andrade, C., Mesías, M., Morales, F. J., Seiquer , I. and Navarro, M. P. (2012). Assessment of acrylamide intake of Spanish boys aged 11-14 years consuming a traditional and balanced diet. LWT - *Food Science and Technology*, 46:16-22.

Design Expert (2009). Stat- Ease Inc., Hennepin Square, Suite 480, 2021E. Hennepin Ave., Minneapolis, MN55413-2726.

De Vleeschouwer, K., Van der Plancken, I., Van Loey, A. and Hendrickx, M. E. (2006) Impact of pH on the kinetics of acrylamide formation/elimination reactions in model systems. *Journal of Agricultural and Food Chemistry*, 54: 7847–7855.

Dost, K. and Tokul, O**.** (2006). Determination of phytic acid in wheat and wheat products by reverse phase in plants: phytases and their action on phytic acid. *Plant Biology* 9: 77-92.

Duhan, A., Khetarpaul, N. and Bishnoi, S. (2002). Content of phytic acid and HCl-extractability of calcium, phosphorus and iron as affected by various domestic processing and cooking methods. *Food Chemistry* 78:9–14.

Duranti, M. (2006). Grain legume proteins and nutraceutical properties. *Fitoterapia*, 77:67-82

Eriksson, S. (2005). Acrylamide in food products: Identification, formation and analytical methodology [PhD thesis]. Department of Environmental Chemistry, Stockholm University, Stockholm, Sweden.

Eriksson, S. and Karlsson, P. (2005). Some analytical factors affecting measured levels of acrylamide in food products. In: Friedman M. and D. Mottram, Chemistry and safety of acrylamide in food, Springer Science and Business Media, New York, pp 285-291.

European Commission (2002). European Union risk assessment report on Acrylamide, In:

Erickson S., Karlson P. and Tornqvist M. (2007). Measurement of evaporated acrylamide during heat treatment of food and other biological materials. *LWT- Food Science and Technology*, 40(4): 706-712.

Faist, V., Drusch S., Kiesner, C., Elmadfa, I. and Erbersdobler, H. F. (2000). Determination of lysinoalanine in foods containing milk protein by high-performance chromatography after derivatisation with dansyl chloride. *International Diary Journal*, 10(5-6):339-346.

Farah, D.M.H., Zaibunnisa, A. H. and Misnawi, J. (2012). Optimization of cocoa beans roasting process using Response Surface Methodology based on concentration of pyrazine and acrylamide. *International Food Research Journal* 19(4):1355-1359

Farhat G. A. and Fossian T. M. (2010). Effects of cooking methods and processing stages on vitamin C in traditional Lebanese meals". *Nutrition and Food Science*, 40(5): 504 – 514.

FAO/WHO (2006). Food standards programme with CODEX committee on food additives and contaminants on 24 April 2006. www.codexalimentarius.org/input/download/report 657/fa38_ 01e.pdf.(Accessed 15/12/2012)

FAO/ WHO (2005). Summary and conclusions of evaluation performed by the joint FOA/WHO Expert Committee on Food Additives (JECFA).Sixty-fourth meeting, 8-17 February (Rome).http://.who.int/icps/food/jecfa/en/. (Accessed 1/1/2012)

FAO/WHO(2002).Consultation on the Health Implications of Acrylamide in Food Geneva, 25-27 June 2002. http://www.ntp.niehs.gov/ntp/htdocs/Chem_Background/ExSumPdf/Acrylamide_508.pdf (Accessed 5/15/2013)

Fernandez, S., Kurppa, L. and Hyvonen, L. (2003). Content of acrylamide decreased in potato chips with addition of a proprietary flavonoid spice mix (Flavomare) in frying. *Innovations in Food Technology*, 18: 24-26.

Friedman, M. A., Tyl, R. W., Marr, M. C., Myers, C. B., Gerling, F. S. and Ross, W. P. (1999). Effects of lactational administration of acrylamide on rat dams and offspring. *Reproductive Toxicology*, 13: 511-520.

Friedman, M. (2003). Chemistry, biochemistry, and safety of acrylamide. A review. *Journal of Agricultural and Food Chemistry*, 51:4504–4526.

Garcia, M. C., Marina, M. L., Laborda, F. and Torre, M. (1998). Chemical characterization of commercial soybean products. *Food Chemistry*, 62: 325-331.

Giovanna, B. and Anna A. (2011). Legumes as valuable sources of tocopherols. *Food chemistry*, 127:1199- 1203.

Gökmen, V., Şenyuva, H. Z., Acar, J. and Sarıoğlu, K. (2005). Determination of acrylamide in potato chips and crisps by high-performance liquid chromatography. *Journal of Chromatography A*, 108:193-199.

Gormley, T. R. and Mee, P. M. (2003). Acrylamide in food: an emerging public health concern? *Farm and Food*, 13: 22-27.

Graf, M., Amreina, T. M., Graf, S., Szalay, R-K., Eschera, F. and Amado, R. (2006). Reducing the acrylamide content of a semi-finished biscuit on industrial scale. LWT – *Food Science and Technology*, 39:724–72.

Grob, K., Biedermann, M., Biedermann-Brem, S., Noti, A., Imhof, D., Amrein, T., Pfefferle, A. and Bazzocco, D. (2003). French fries less than 100 µg/kg acrylamide. A collaboration between cooks and analysts. *European Food Research and Technology*, 217: 185-194.

Granvogl, M. and Schieberle, P. (2007). Quantification of 3-aminopropionamide in cocoa, coffee and cereal products. *European Food Research and Technology*, 225:857–863.

Granvogl, M. and Schieberle, P. (2006).Thermally generated 3-aminopropionamide as a transient intermediate in the formation of acrylamide. *Journal of Agriculture and Food Chemistry*, 54(16):5933–5938.

Granvogl, M., Jezussek, M., Köhler, P., Schieberle, P. (2004) Quantitation of 3-aminopropionamide in potatoes—a minor but potent precursor in acrylamide formation. *Journal of Agriculture Food Chemistry*, 52:4751–4757.

Gueguen, J. and Cerletti, P. (1994). New and Developing Sources of Food Proteins of some legume seeds: soybean bean, pea, fababean and lupin. Chapman and Hall London, UK. 145-193.

Habiba, R. A. (2002). Changes in anti-nutrients, protein solubility, digestibility, and HCl-extractability of ash and phosphorus in vegetable peas as affected by cooking methods. *Food Chemistry*, 77:187-192.

Hamlet, C. G., Jayratne, S. M. and Sadd, P. A. (2004). Rapid, sensitive and selective analysis of acrylamide in cereal products using bromination and GC/MS/MS. *Czech Journal of Food Science*, 22: 290-293.

Hoenicke, K. and Gatermann, R. (2005). Studies on the stability of acrylamide in food during storage. *Journal of Association of Official Agricultural Chemists International*, 88(1): 268-273.

Hoenicke, K., Gatermann, R., Harder, W. and Hartig, L. (2004b). Analysis of acrylamide in different foodstuffs using liquid chromatography – tandem mass spectrometry and gas chromatography – tandem mass spectrometry. *Analytica Chimica Acta*, 520: 207-215.

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 Epidemiology Biomarkers and Prevention,* 16 (11): 2304–2313.

IARC. (1994). Monographs on the evaluation of carcinogenic risks to humans, some industrial chemicals. *International Agency for Research on Cancer*, 60: 389–433.

Igbedioh, S. O., Olugbemi, K.T. and Akpapunam, M. A.(1993). Effects of processing methods on phytic acid level and some constituents in Bambara groundnut (*Vigna subterranea*) and pigeon pea (*Pigeon Pea*), *Food Chemistry,* 50:147-151.

Ijarotimi, O. S., Oyewo, M. T. and Oladeji, B. S. (2009). Chemical, functional and sensory properties of roasted Bambara groundnut (*Vigna subterranean L. Verdc)* and cooking banana (Musa spp., ABB genome) weaning diet. *African Journal of Food Science,* 3(5): 139-146.

Janzowski, C., Glaab, V., Samimi, E., Schlatter, J. and Eisenbrand, G. (2000). 5-Hydroxymethylfurfural: assessment of mutagenicity, DNA-damaging potential and reactivity towards cellular glutathione. *Food and Chemical Toxicology*, 38: 801-809.

Jägerstad, M. and Skog, K. (2005). Genotoxicity of heat-processed foods. *Mutation Research*, 574: 156-172.

Jezussek, M. and Schieberle, P. (2004). Derivatization with 2-Mercaptobenzoic acid - a new method for LC-MS determination of acrylamide in foods. *Lebensmittelchemie,* 58(1): 5-6.

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 of Food Science*, 68: 1287-1290.

Kaplan, O., Kaya, G., Ozcan, C., Ince, M. And Yaman, M. (2009). Acrylamide concentrations in grilled foodstuffs of Turkish kitchen by high performance liquid chromatography-mass spectrometry. *Microchemical Journal*, 93: 173–179.

Karasek, L., Wenzi, T. and Anklam, E. (2009). Determination of acrylamide in roasted chestnuts and chestnut-based foods by isotope dilution HPLC-MS/MS. *Food Chemistry,*114:1555-1558.

Khattab, R. Y and Arntfield, S. D. (2009). Nutritional quality of legumes seeds as affected by some physical treatments. Part 1: Protein quality evaluation. *LWT- Food Science and Technology*, 42: 1107-1112.

Ku Madihah, K. Y., Zaibunnisa, A. H., Norashikin, S., Rozita, O. and Misnawi, J. (2013). Optimization of roasting conditions for high-quality Arabica coffee. *International Food Research Journal,* 20(4):1623-1627.

Liyana-Pathirana, C. and Shahidi, F. (2005).Optimization of extraction of phenolic compounds from wheat using response surface methodology. *Food Chemistry,* 93: 47-56.

Low, M. Y., Koutsidis, G., Parker, J. K., Elmore, J. S., Dodson, A. T. and Mottram, D. S. (2006). Effect of citric acid and glycine addition on acrylamide and flavour in a potato model system. *Journal of Agriculture and Food Chemistry*, 54: 5976-5983.

Maninder K., Sandhu K.S. and Singh, N. (2007), Comparative study of the functional, thermal and pasting properties of flours from different field (*Pisum sativum* L.) and pigeon pea (Pigeon Pea L.) cultivars. *Food Chemistry*, 104(1): 259-263.

Mathres, J. C. (2002). Pulses and carcinogenesis: potential for the prevention of colon, breast and other cancers. *British Journal of Nutrition*, 88: 273-279.

Mehrajfatema Z. Mulla, Vikas R. Bharadwaj, Uday S. Annapure, Rekha S. Singhal (2011). Effect of formulation and processing parameters on acrylamide formation on extrusion of blends of potato flour and semolina. *Journal of Food Science and Technology, 44: 1643-1648.*

Mestdagh, F. J., De Wilde, T., Casrelein, P., Nemeth, O. and Van Peteghem, C.(2008). Impact of the reducing sugars on the relationship between acrylamide and Maillard browning in French fries. *European Food Research and Technology*, 227(1): 69-76.

Mestdagh F., Maertens, J. , Cucu, T., Karel Delporte, K., Van Peteghem C. and De Meulenaer, B.(2005). Impact of additives to lower the formation of acrylamide in a potato model system through pH reduction and other mechanisms. *Food Chemistry,* 107: 26–31.

Mestdagh, F. J., De Meulenaer, B., Van Poucke, C., Detavernier, C., Cromphout, C. and Van Peteghem, C. (2005a). Influence of oil type on the amounts of acrylamide generated in a model system and in French fries. *Journal of Agricultural and Food Chemistry*, 53: 6170–6174.

Mestdagh, F., De Meulenaer, B., Van Petegham, C., Cromphout, C. and Thas, O. (2004). Towards a better understanding in acrylamide formation, degradation and reduction in model systems (and foodstuffs). *Czech Journal of Food Science*, 22:11-14.

Morrow, B. (1991). The rebirth of legumes: legume production, consumption and export are increasing as more people become aware of legumes nutritional benefits. *Food Technology*, 9: 96-121.

Mottram, D. S., Wedzicha, B. L. and Dodson, A. T. (2002). Acrylamide is formed in the Maillard reaction. *Nature*, 419: 448–449.

Mulla, M. Z., Bharadwaj, V. R., Annapure, U. S. and Singhal, R. S. (2011). Effect of formulation and processing parameters on acrylamide formation on extrusion of blends of potato flour and semolina. *Journal of Food Science and Technology, 44: 1643-1648.*

Narsimha, H. V. and Desikachar, H. S. R. (1978). Simple procedures for reducing the cooking time of split red gram (*Cajanus cajan*). *Journal of Food Science and Technology*, 15(4): 149.

Nelson, G. A., Calderon de la Barca, A. M., and Mauro, E. V. (1991). Effect of different heat treatments on the antinutritional activity of Phaseolus vulgaris (variety Ojo de Cabra) lectin. *Journal of Agricultural and Food Chemistry*, 39(9): 1627-1630.

Oboh, A. H., Muzquiz, M., Burbano, C., Cuadrado, C., Pedrosa, M. M., Ayet, G. and Osagie, U. A. (1998). Anti-nutritional constituents of six underutilized legumes grown in Nigeria. *Journal of Chromatography A*, 823:307–312.

Olesen, P.T., Olsen, A., Frandsen, H., 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.

Omoikhoje, S. O. (2008). Assessment of the nutritive value of Bambara groundnut as influenced by cooking time, *Livestock Research for Rural* Development,20(4).

Onder, M. and Kahraman, A. 2009. Antinutritional factors in Food Grain Legumes. 1st International symposium on sustainable Development, Sarajevo.

Paleologos, E. K. and Kontominas, M. G. (2005). Determination of acrylamide and methacrylamide by normal phase high performance liquid chromatography and UV detection. *Journal of Chromatography A*, 1077: 128-135.

Pedreschi, F., Mariotti, M. S. and Granby, K. (2014), Current issues in dietary acrylamide: formation, mitigation and risk assessment. *Journal of Food Science and Agriculture*, 94: 9–20.

Pedreschi, F., Granby, K., and Risum, J. (2010). Acrylamide mitigation in potato chips by using NaCl. *Food Bioproces Technology*, 3: 917-921.

Pedreschi, F., Moyano, P., Kaack, K. and Granby, K. (2005). Colour changes and acrylamide formation in fried potato slices. *Food Research International*, 38:1–9.

Pedreschi, F., Kaack, K. and Granby, K. (2004). Reduction of acrylamide formation in potato slices during frying. *LWT – Food Science and Technology*, 37(6): 679–685.

Persson, E., Sjöholm, I., Nyman, M. and Skog, K. (2004). Addition of various carbohydrates to beef burgers affects the formation of heterocyclic amines during frying. *Journal of Agricultural and Food Chemistry*, 52: 7561-7566.

Petersen, B. (2002). Exposure and Biomarkers. JIFSAN/NCFST Acrylamide in food Workshop.http://www.jifsan.umd.edu/Acrylamide/arylamideworkshop.html. (Accessed 30/06/2013)

Rehman, Z., Shah, H. W. (2005). Thermal heat processing effects on antinutritional proteins and starch digestibility of food legumes. *Food Chemistry,* 91: 327-331

Rosen, J. and Hellenas, K. E. (2002). Analysis of acrylamide in cooked foods by liquid chromatography tandem mass spectrometry. *Analyst*, 12: 880–882.

Rydberg, P., Eriksson, S., Tareke, E., Karlsson, P., Ehrenberg, L. and Tornquist, M. (2003). Investigations of factors that influence the acrylamide content of heated foodstuffs. *Journal of Agricultural and Food Chemistry*, 51: 7012-7018

Saalia, F. K. and Phillips R.D. (2010). Reduction of aflatoxins in peanut meal by extrusion cooking in the presence of nucleophiles. *LWT-Food Science and Technology,* 44(6): 1511-1516.

Salazar, R., Arámbula-Villa, G., Hidalgo, F. J. and Zamora, R. (2012). Mitigating effect of piquin pepper (*Capsicum annuum* L. var. Aviculare) oleoresin on acrylamide formation in potato and tortilla chips. *LWT - Food Science and Technology,* 48:261-267.

Salma, H. A., Nahid, A. A., ElShazali, A. M., Isam, A. M. A. and Elfadil, E. B. (2010). Changes in the functional properties as a function of NaCl concentration of legumes protein isolate by transglutaminase cross linking. *International Food Research Journal, 17: 817-824.*

Salmon, P. C., Knize, G. M. and Felton, S. J. (1997). Effects of marinating on heterocyclic amine carcinogen formation in grilled chicken. *Food and Chemical Toxicology,* 35:433-441.

Sanganyado, E., , Parekh, C. T. and Eriksson,S.(2011) Analysis of acrylamide in traditional foodstuffs in Zimbabwe. *African Journal of Food Science*, 5(17): 910-913.

Sathe S. K.,Teuberb S. S. and Roux K. H. (2005). Effects of food processing on the stability of food allergens. *Biotechnology Advances,* 23: 423–429.

Scientific Committee on Food (SCF) (2002). Opinion of the Scientific Committee on Food on new findings regarding the presence of acrylamide in food. SCF/CS/CNTM/CONT/4 Final, 3 July 2002.

Şenyuva, H .Z. and Gökmen, V. (2005a). Survey of acrylamide in Turkish foods by an in-house validated LC-MS method. *Food Additives and Contaminants*, 22: 204-209.

Silva, E. M., Rogez, H. and Larondelle, Y. (2007). Optimization of extraction of phenolics from Inga edulis leaves using response surface methodology. *Separation and Purification Technology,* 55: 381-387.

Singh, P., Singh, P. and Raja, R. B. (2010). Determination of acrylamide concentration in processed food products using normal phase high performance liquid chromatography (HPLC). *African Journal of Biotechnology,* 9(47):8085-8091.

Singh, U., and Singh, B. (1992). Tropical grain legumes as important human food, *Economic botany*, 46(3): 310-321.

Skog, K. (1993). Cooking procedures and food mutagens: a literature review. *Food and Chemical Toxiclogy*, 31(9): 655-675.

Skog, K., Johansson, M.A.E. and Jagerstad, M. (1998). Carcinogenic heterocyclic amines in model systems and cooked foods: a review on formation, occurrence and intake. *Food and Chemical Toxicology,* 36:879–896.

Song, L. and Thornalley, P. J. (2007). Effect of storage, processing and cooking onglucosinolate content of Brassica vegetables, *Food chemical toxicology,* 45(2): 216-224.

Soyka, S., Frohberg, C., Quanz, M. and Essigmann, B. (2004). Process for reducing the acrylamide content of heat-treated foods. European PCT Patent Application, no: WO-2004-040999.

Stadler, R. H., Blank, I.,Varga, N., Robert, F., Hau, J. and Guy, P. A. (2002). Acrylamide from Maillard reaction products. *Nature*, 419: 449–450.

Törnqvist, M. (2005). Acrylamide in food: The discovery and its implications. In Friedman, M. and Mottram, D. Chemistry and safety of acrylamide in food. New Springer Science and Business Media Inc., York, US, pp York, US:1-19.

Thompson, U. L. (1993). Potential health benefits and problems associated with antinutrients in foods. *Food Research International*, 26: 131-149

Tan, Q. P., Xinh N. T. K., Nguyet, H. T. K. and Xuyen, N. T. H. (2012). Application of response surface methodology (RSM) in condition optimization for essential oil production from *Citrus latifolia, Journal of Food and Agriculture*, 24 (1): 25-30.

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S. and Tornqvist, M. (2002). Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *Journal of Agricultural and Food Chemistry*, 50: 4998–5006.

Tareke E, Rydberg P, Karlsson ,P. and Törnqvist, M (2000): Acrylamide a cooking carcinogen? *Chemical Research Toxicology,* 13: 517–522.

Thane, C. and Reddy, S. (1997). Processing of fruit and vegetables: effect on Carotenoids. *Nutrition and Food Science*, 97(2): 58 – 65.

Tharanathan, R. N. and Mahadevamma, (2003). Grain legume- a boon to human nutrition. *Trends in food science and Technology,* 14: 507-518.

Törnqvist, M. (2005). Acrylamide in food: The discovery and its implications. In Friedman M. and Mottram D, *Chemistry and safety of acrylamide in food*, Springer Science and Business Media Inc., New York, pp 1-19.

Tornqvist, M. and Landin, H. (1995). Haemoglobin adducts for in vivo dose monitoring and cancer risk estimation. *Journal of Occupational and Environmental Medicine*, 37: 1077–1085.

Triveni, R., Shamala, T. R. and Rastogi, N. K. (2001). Optimized production and utilization of exopolysaccharide from *Agrobacterium radiobacter*. *Process Biochemistry,* 36: 787-795.

Uma, C. (1994), effect of storage and processing on phytic acid levels in legumes and its interference with the utilisation of protein and iron, [PhD thesis]. Andhra Pradesh Agricultural University.

Umano, K. and Shibamoto, T. (1987). Analysis of acrolein from heated cooking oils and beef fat. *Journal of Agricultural and Food Chemistry*, 35: 909-912.

Vijayakumari, K., Pugalenthi, M. and Vadivel, V. (2007). Effect of soaking and hydrothermal processing methods on the levels of antinutrients and *in vitro* protein digestibility of *Bauhinia purpurea* L. seeds. *Food Chemistry,* 103: 968-975.

Wang, H., Lee, A. W. M., Shuang, S. and Choi, M. M. F. (2008). SPE/HPLC/UV studies on acrylamide in deep-fried flour based indigenous Chinese foods. *Microchemical Journal,* 89: 90–97

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

Wenzl, T., De la Calle, M. B. and Anklam, E. (2003). Analytical methods for the determination of acrylamide in food products: A review, *Food Additive and Contaminant*, 20(10): 885- 902.

Yasuhara, A., Tanaka, Y., Hengel, M. and Shibamoto, T. (2003). Gachromatographic investigation of acrylamide formation in browning model systems. *Journal of Agricultural and Food Chemistry* 51:3999–4003.

Yusuf, A. A., Ayedun, H. and Sanni, O. L. (2008). Chemical composition and functional properties of raw and roasted Nigerian benniseed (*Sesamum indica*) and bambara groundnut (*Vigna subterranean*), *Food Chemistry*, 3: 277-282.

Zaibunnisa, A.H., Norashikin, S., Mamot, S. and Osman, H. (2009). An experimental design approach for the extraction of volatile compounds from turmeric leaves (*Curcuma domestica*) using Pressurised Liquid Extraction (PLE). *Food Science and Technology*, 42: 233–238.

Zhang, Y., Ren, Y., Jiao, J., Li, D. and Zhang, Y. (2011). Ultra high-performance liquid chromatography-tandem mass spectrometry for the simultaneous analysis of asparagine, sugars, and acrylamide in Maillard reactions. *Analytical Chemistry*, 83: 3297-3304.

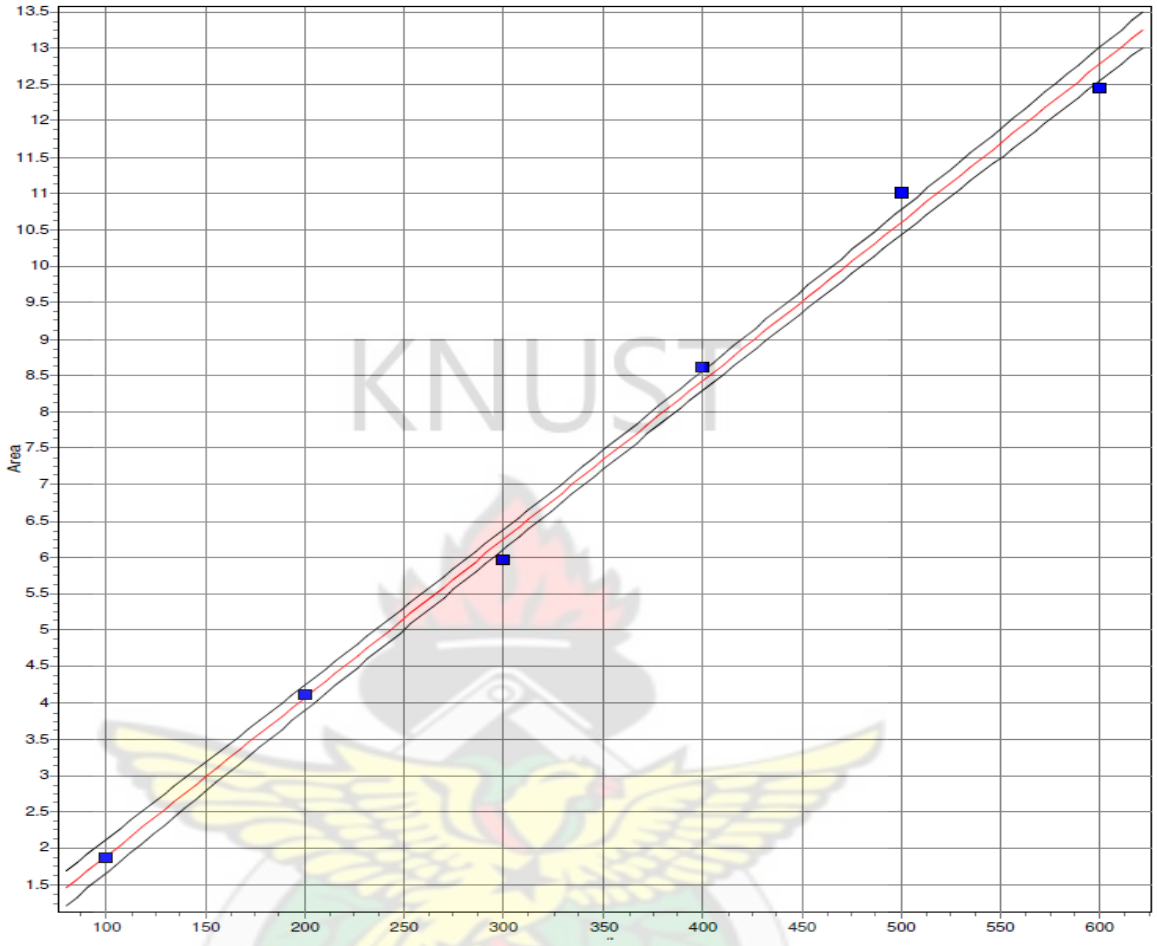
Zhang, Y. and Zhang, Y. (2007). Formation and reduction of acrylamide in Maillard reaction: A review based on the current state of knowledge. Critical Reviews in *Food Science and Nutrition*, 47:521–542.

Zhang, Y., Zhang, G. and Zhang, Y. (2005). Occurrence and analytical methods of acrylamide in heat-treated foods. Review and recent developments. *Journal of Chromatography A*, 1075: 1-21.

Zyzak, D. V., Sanders, R. A., Stojanovic, M., Gruber, D. C., Lin, P. Y. T., Villagran, M. DM., Howie, J. K. and Schafermeyer, R. G. (2004). Method for reducing acrylamide in foods, foods having reduced levels of acrylamide, and article of commerce. United States Patent Application Publication, No. US 2004/0101607.

APPENDICES

Appendix A1: Calibration curve for acrylamide determination



(ppm)

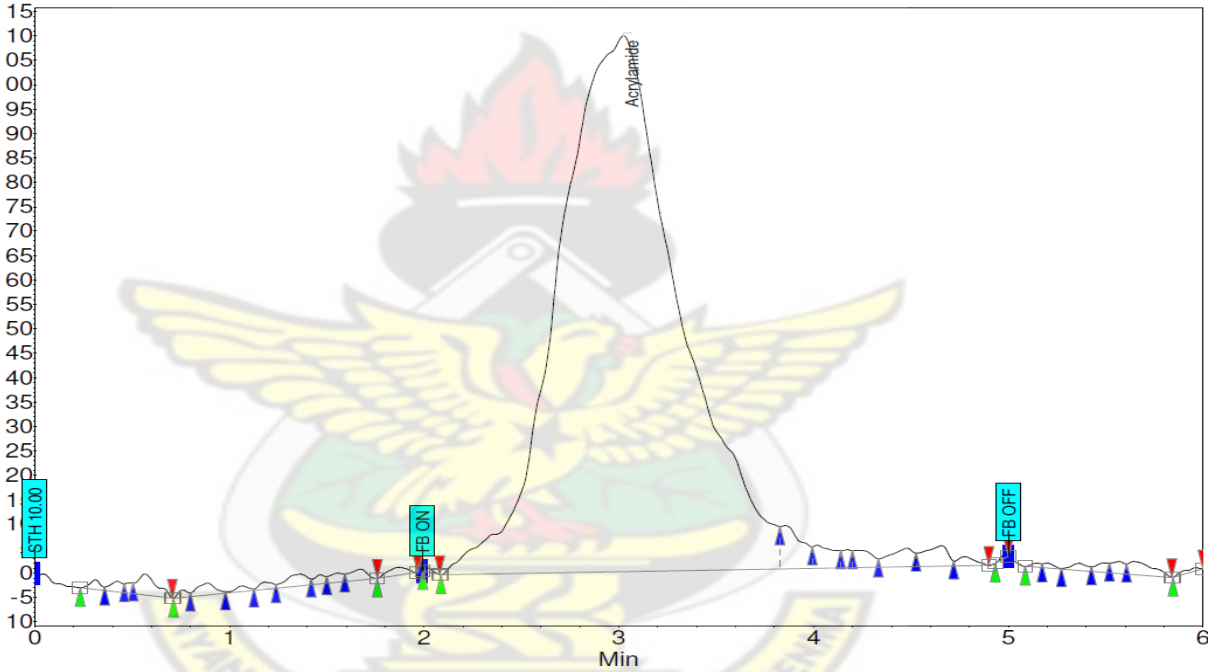
Equation for the standard curve: $y = bx + a$, where $a = -0.28848$, $b = 0.02179$, x = acrylamide concentration and y= Area for acrylamide concentration.

A2 Calibration table for Acrylamide Standards

Acrylamide standard	Area(mAU .Min)	Res (%)	Time(Min)
100ppm	1.89	0.89	3.11
200ppm	4.07	0.98	3.13
300ppm	6.25	4.92	3.09
400ppm	8.43	2.22	3.11
500ppm	10.61	3.74	3.10
600ppm	12.79	2.0	3.08

A3: Chromatogram of produced acrylamide for experimental runs

Run 1



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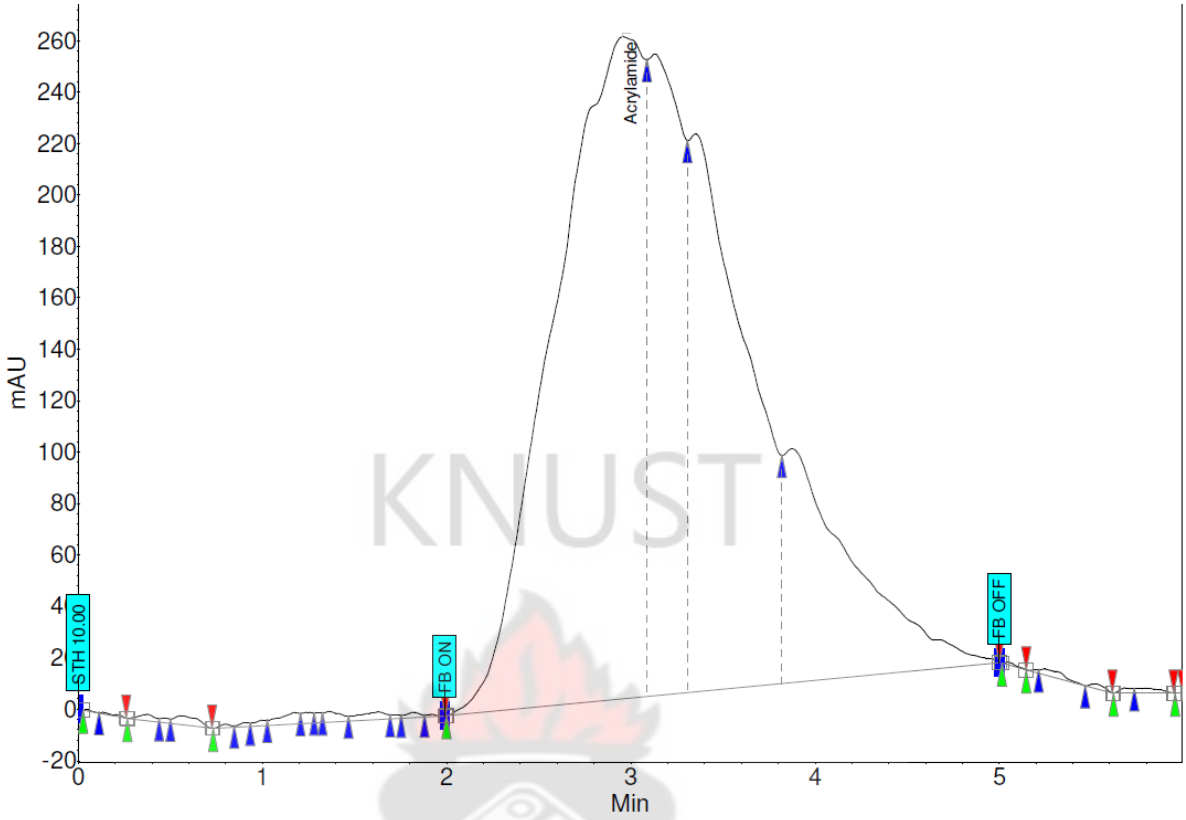

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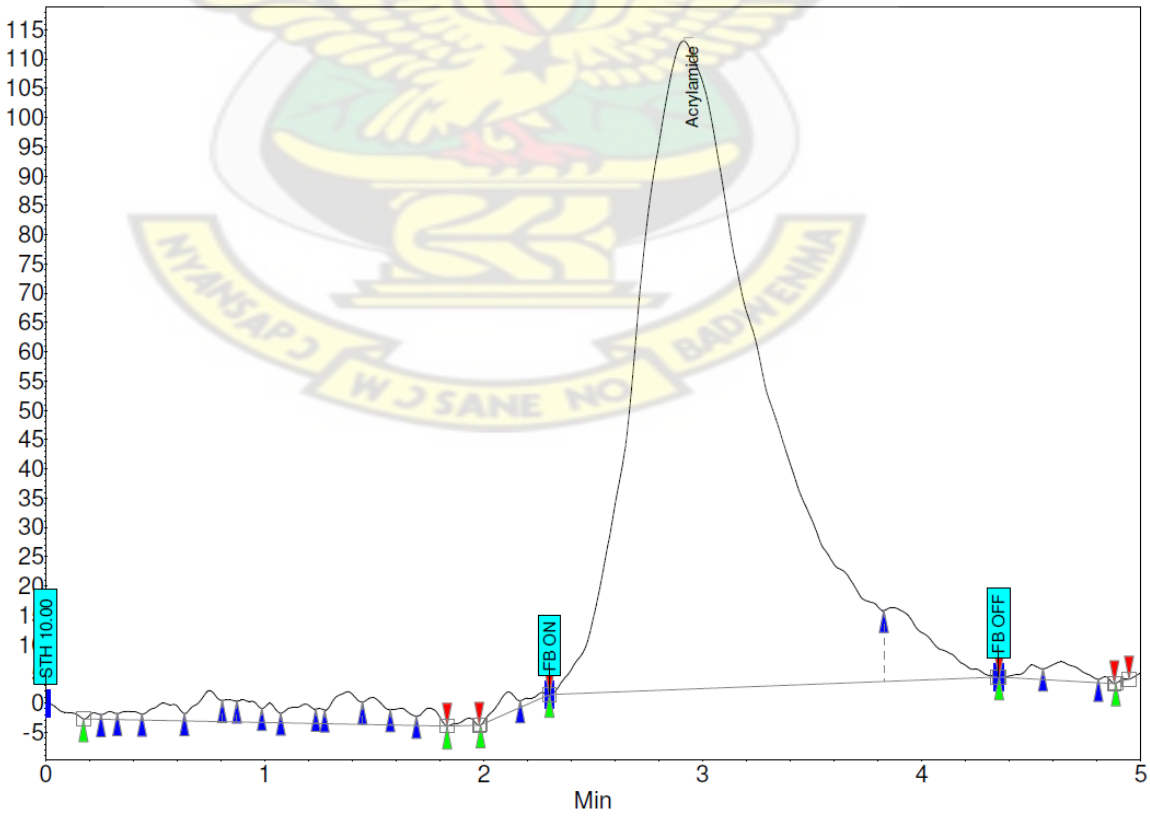

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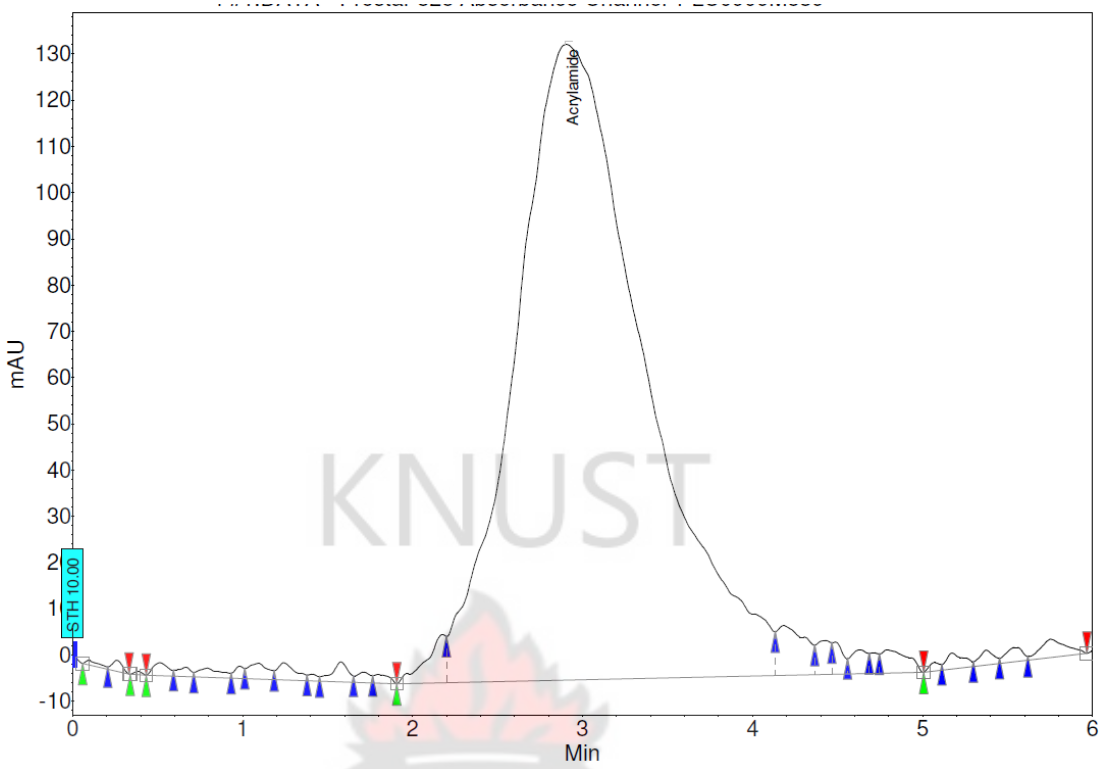

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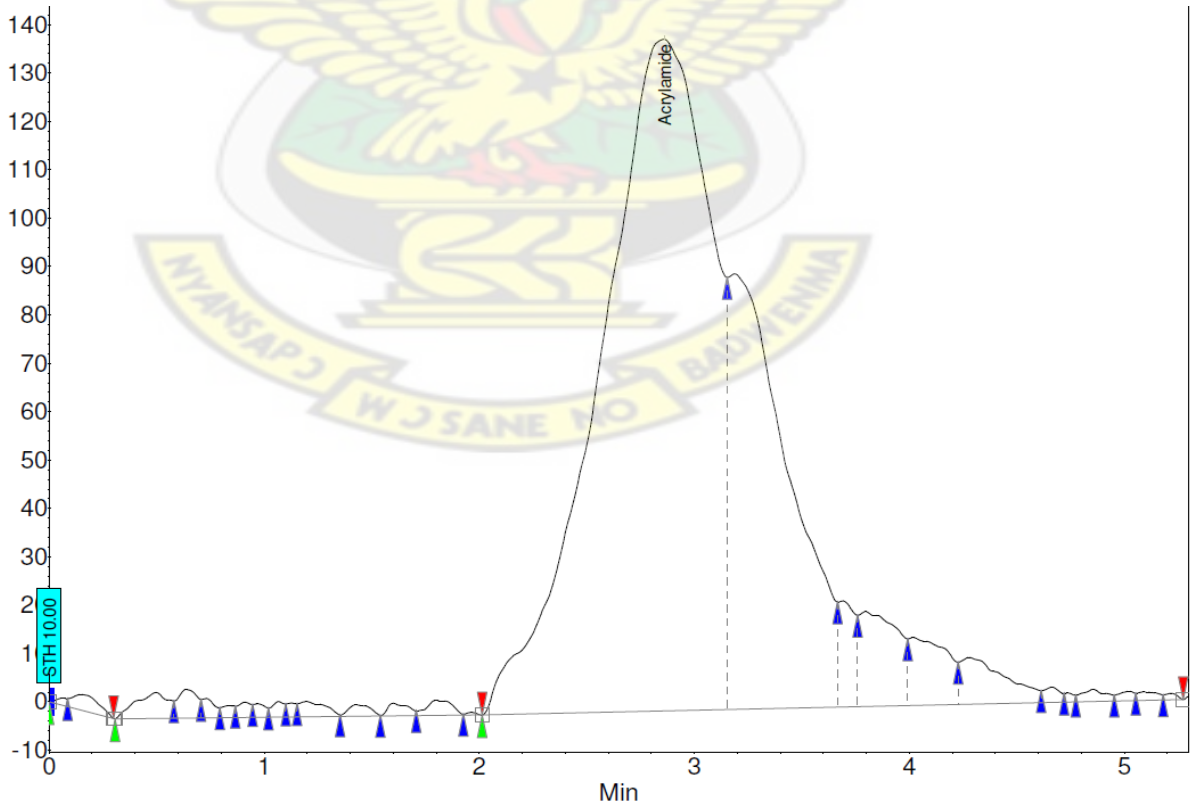

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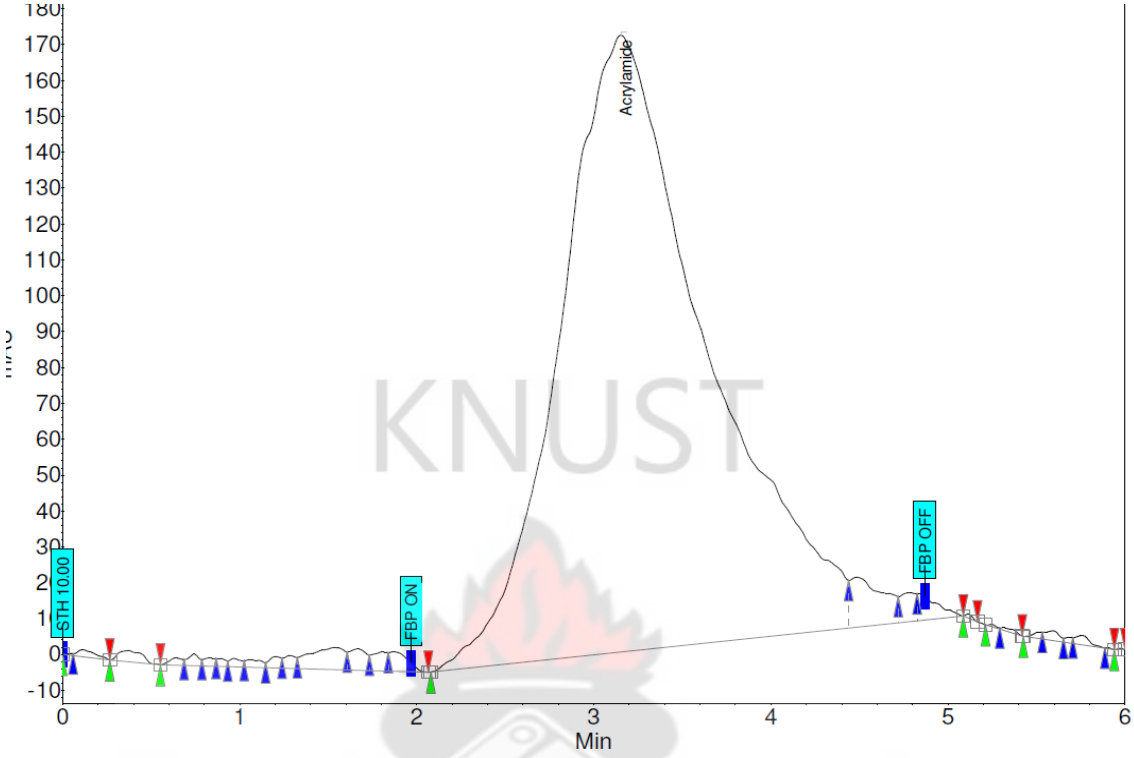

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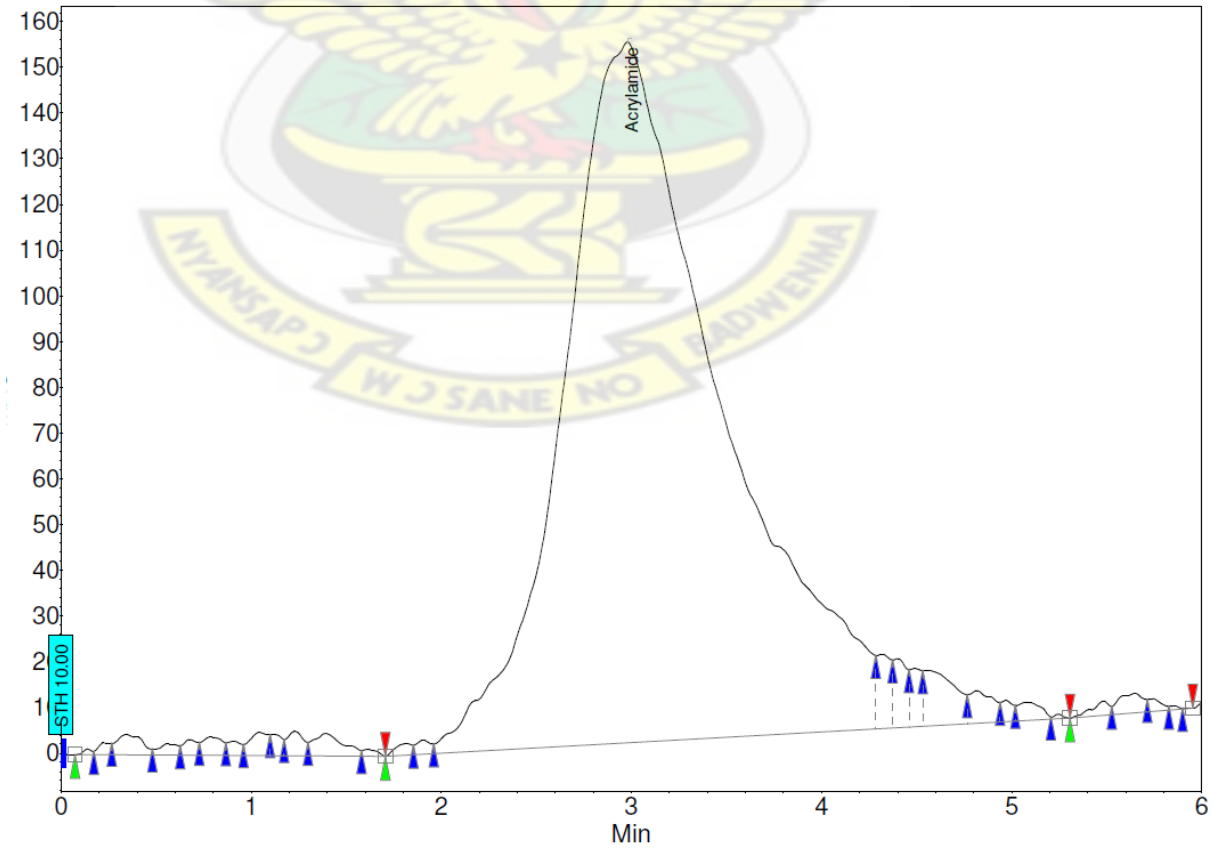

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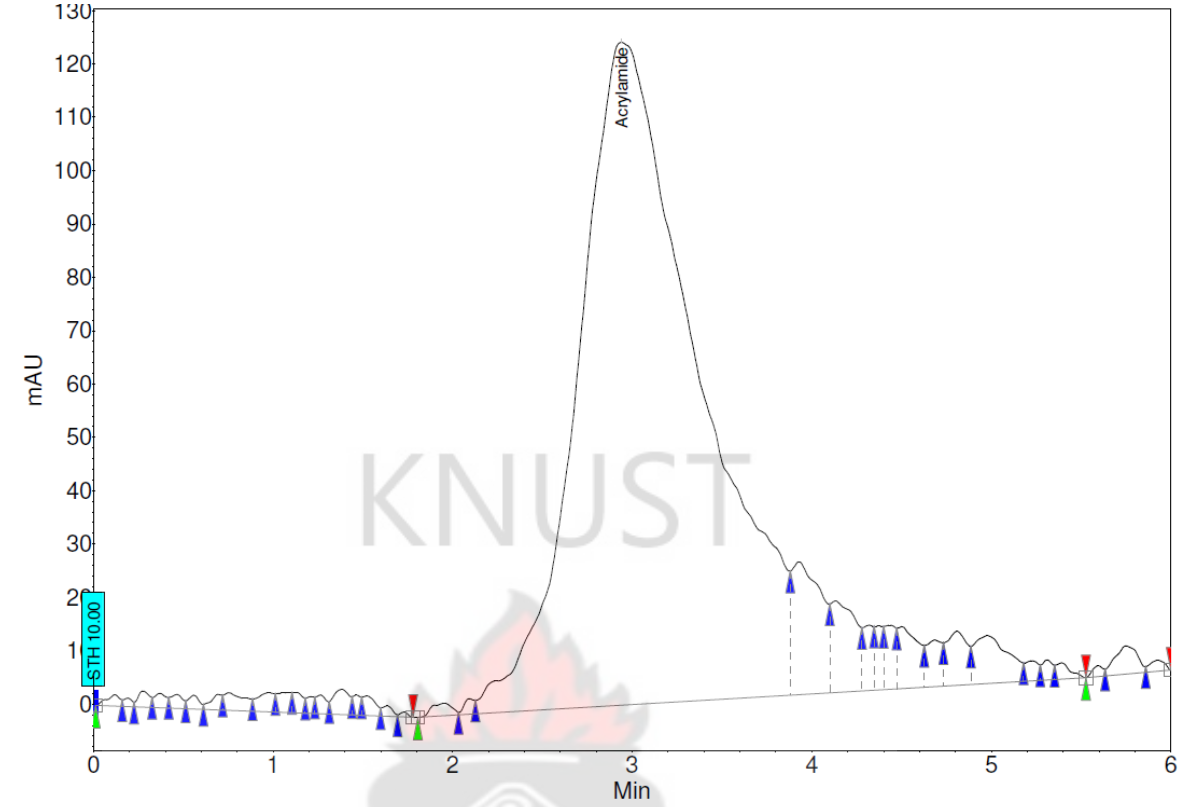
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Min
Acrylamide
STH 10.00

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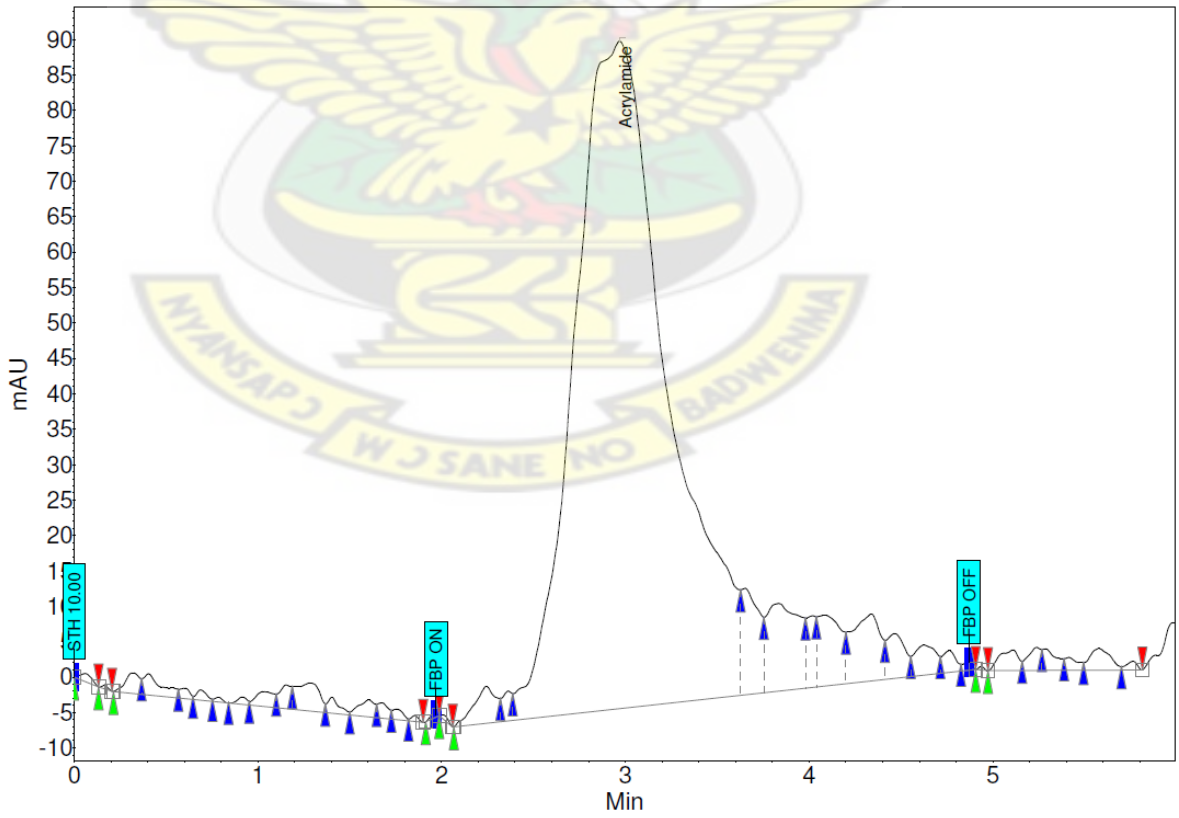
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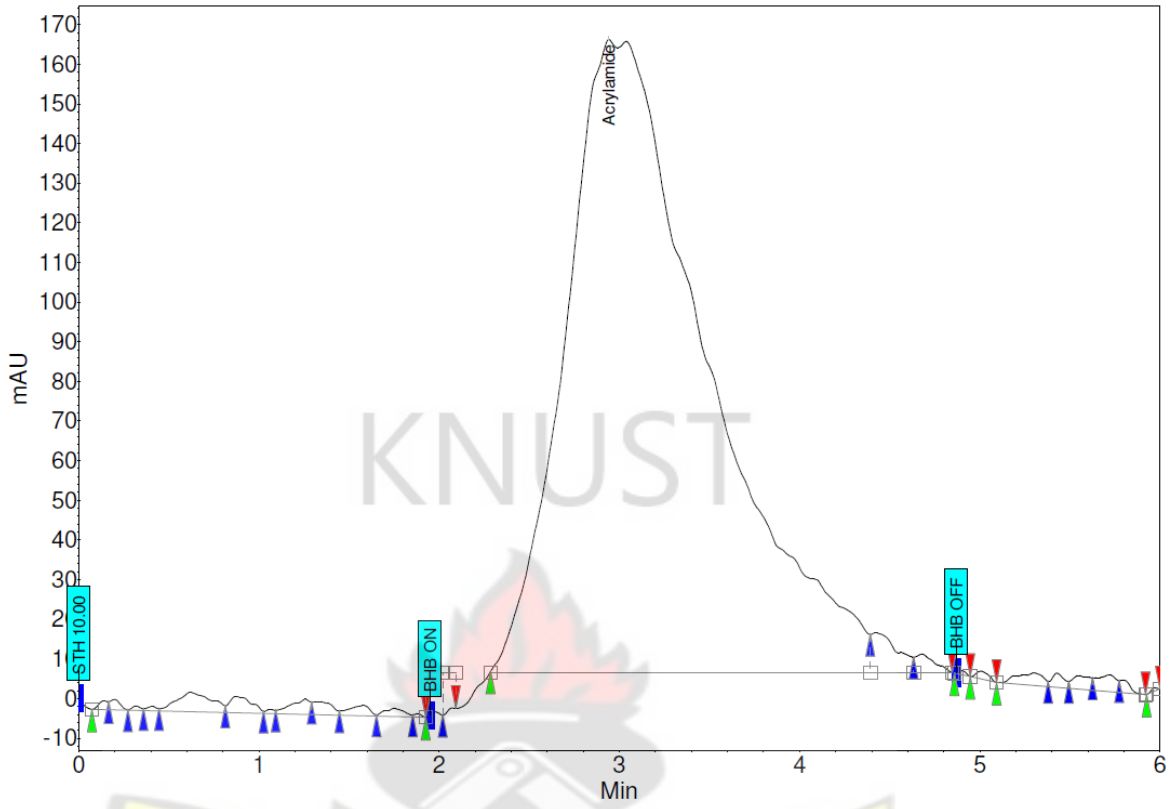
Min
Acrylamide
STH 10.00
FBP ON
FBP OFF

Run 10



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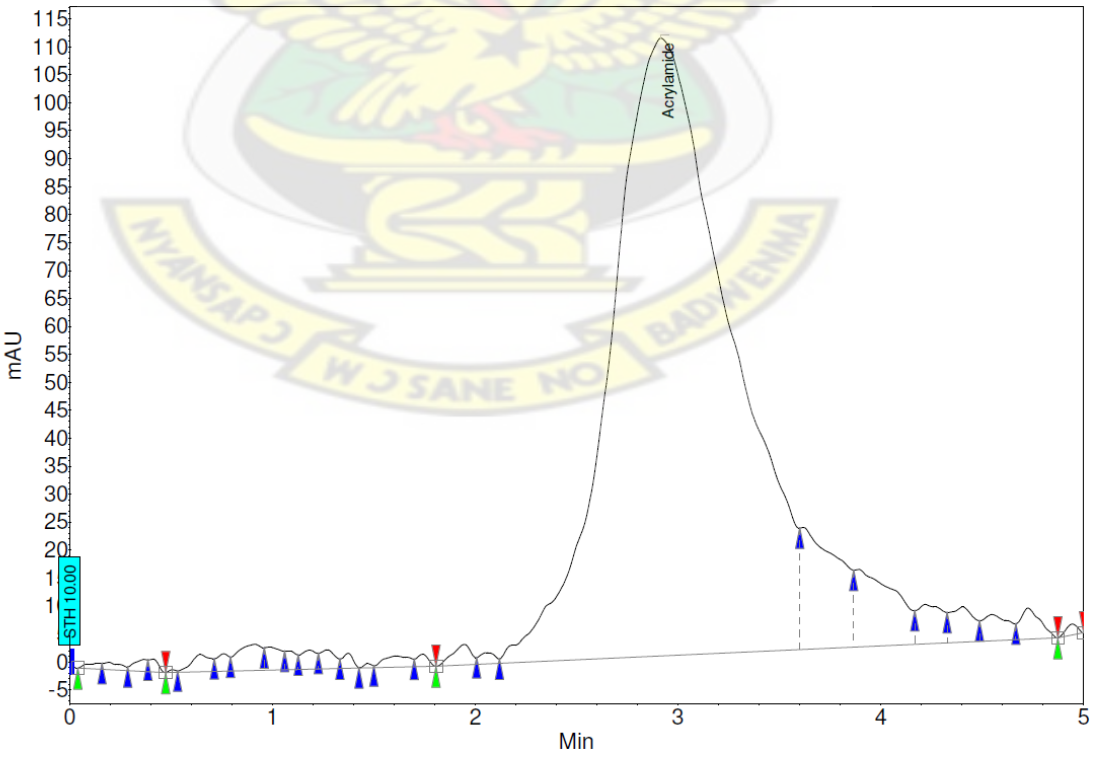

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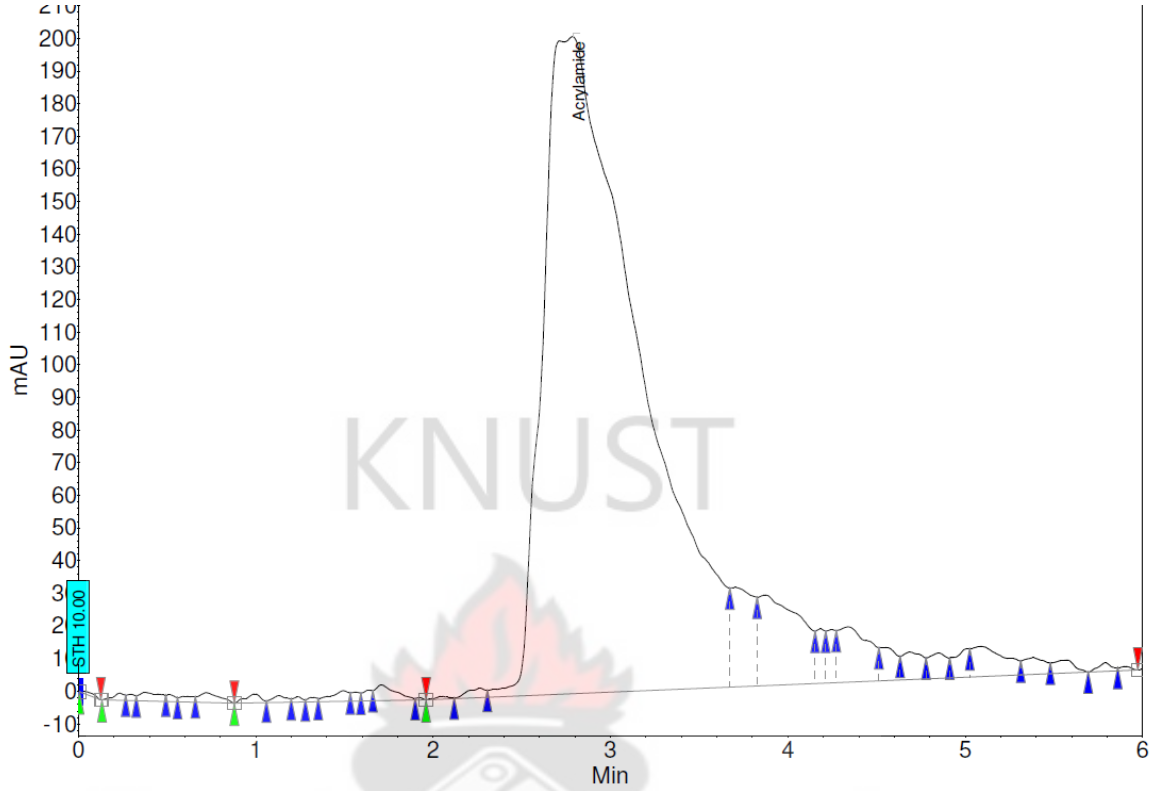

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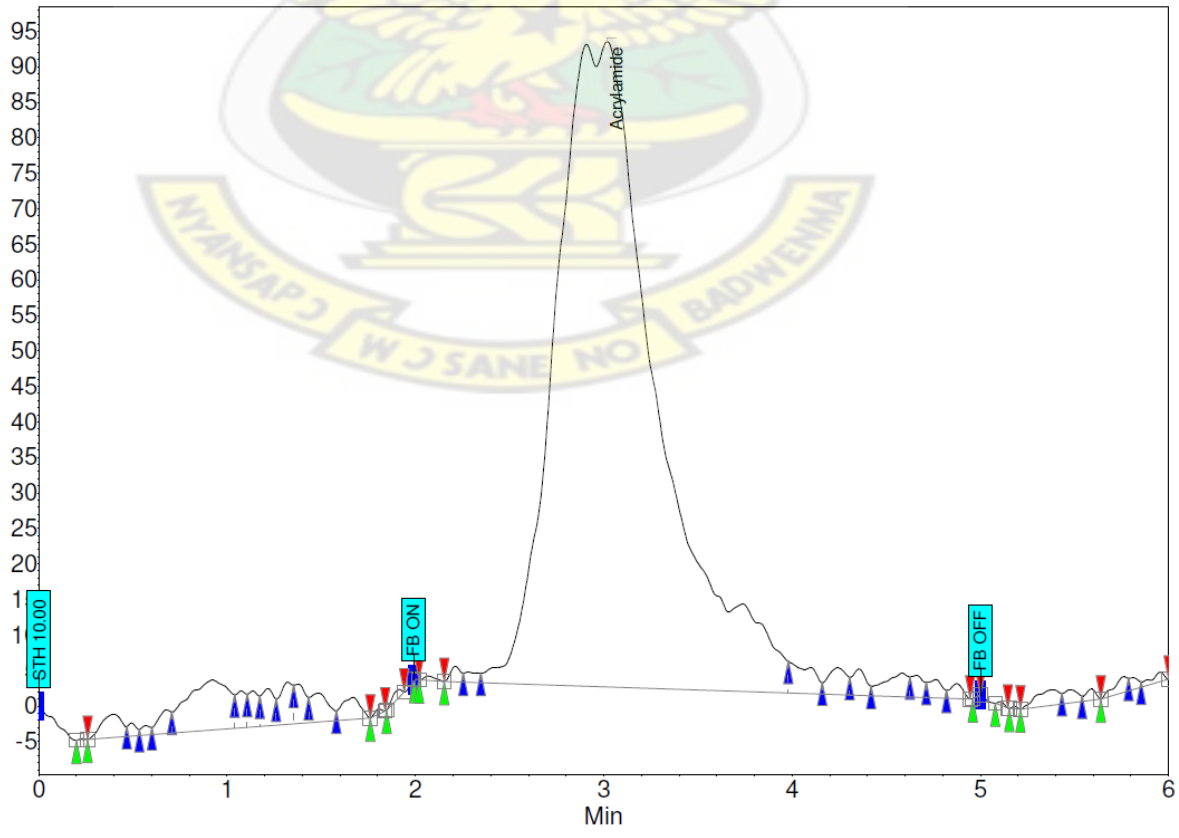

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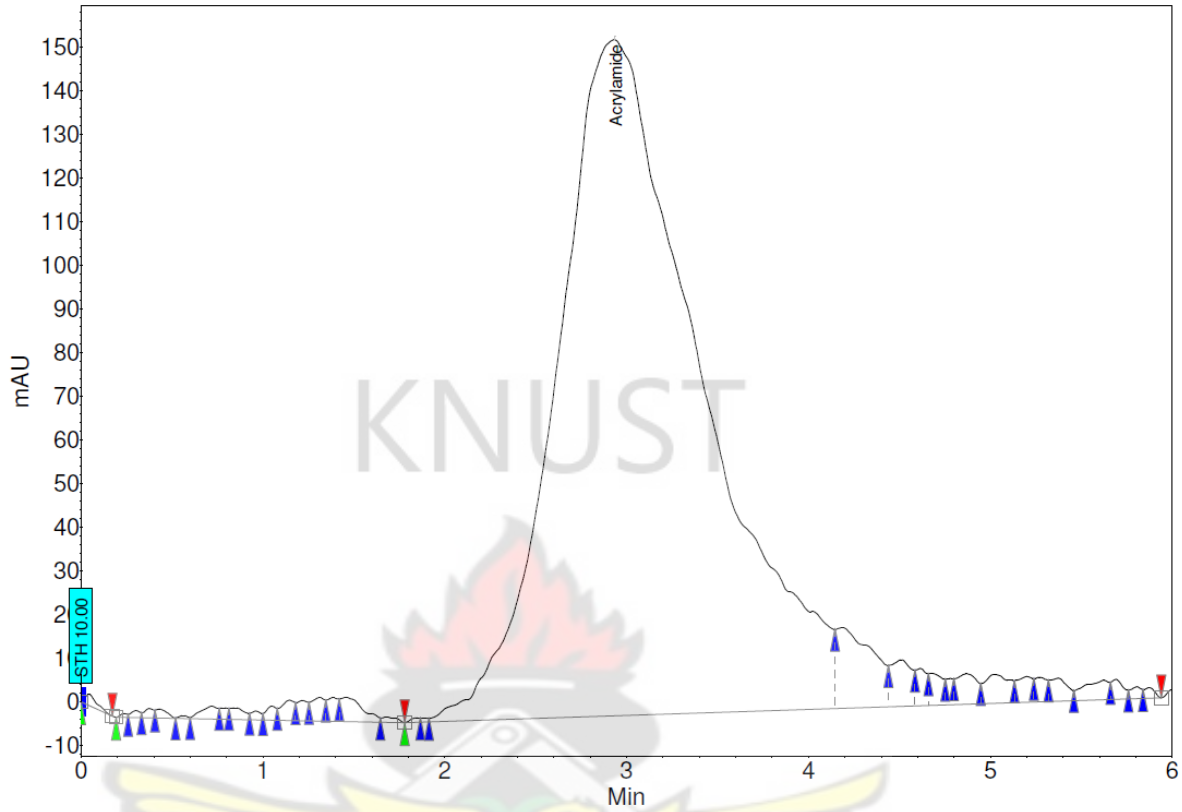
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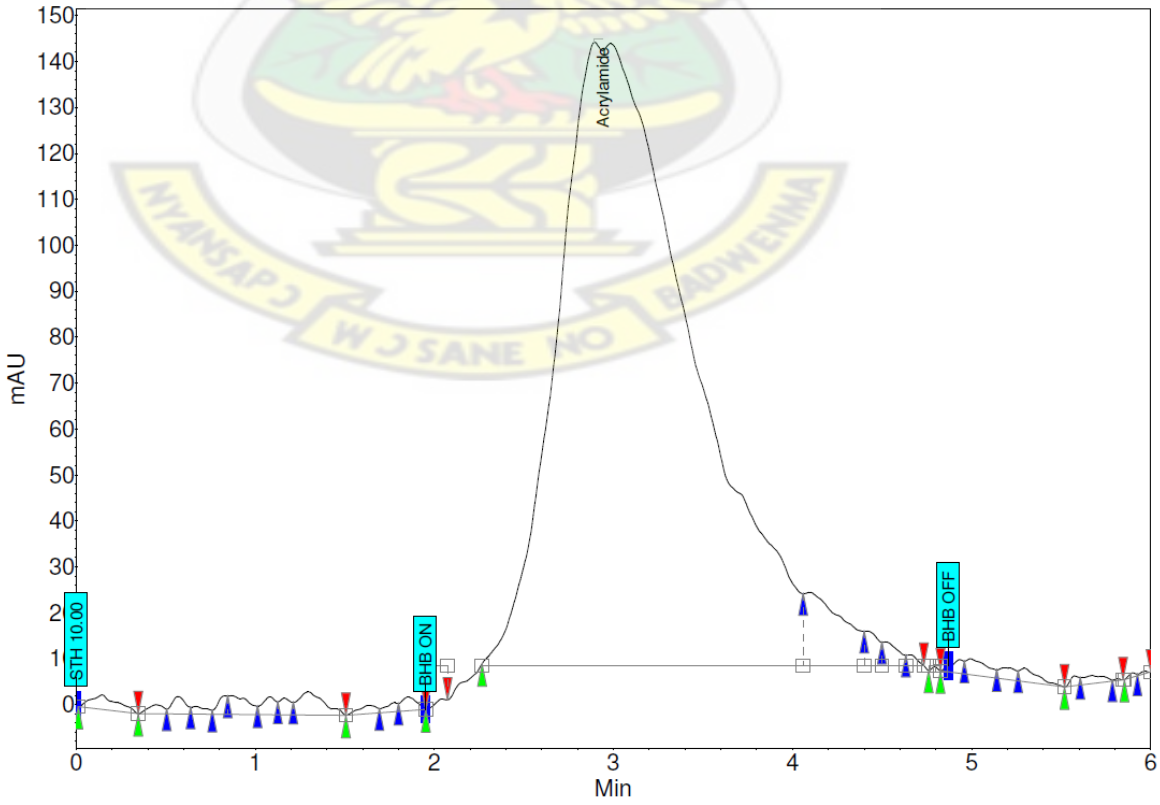
STH 10.00
mAU
Min

Run 15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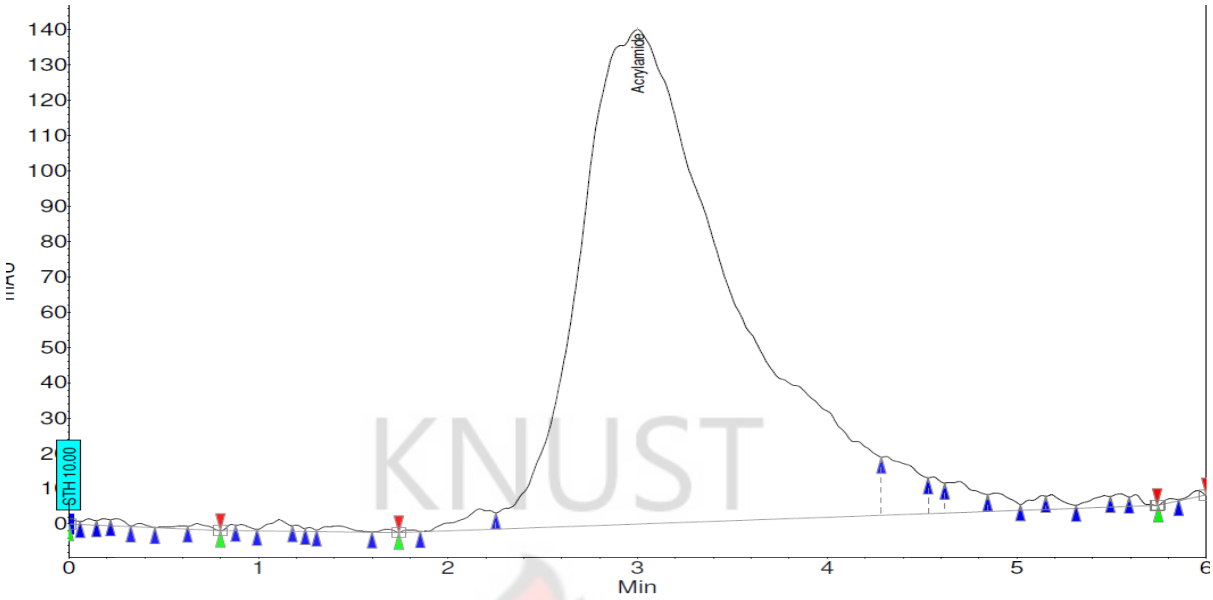
BHB ON
BHB OFF
mAU
Min

Run 16



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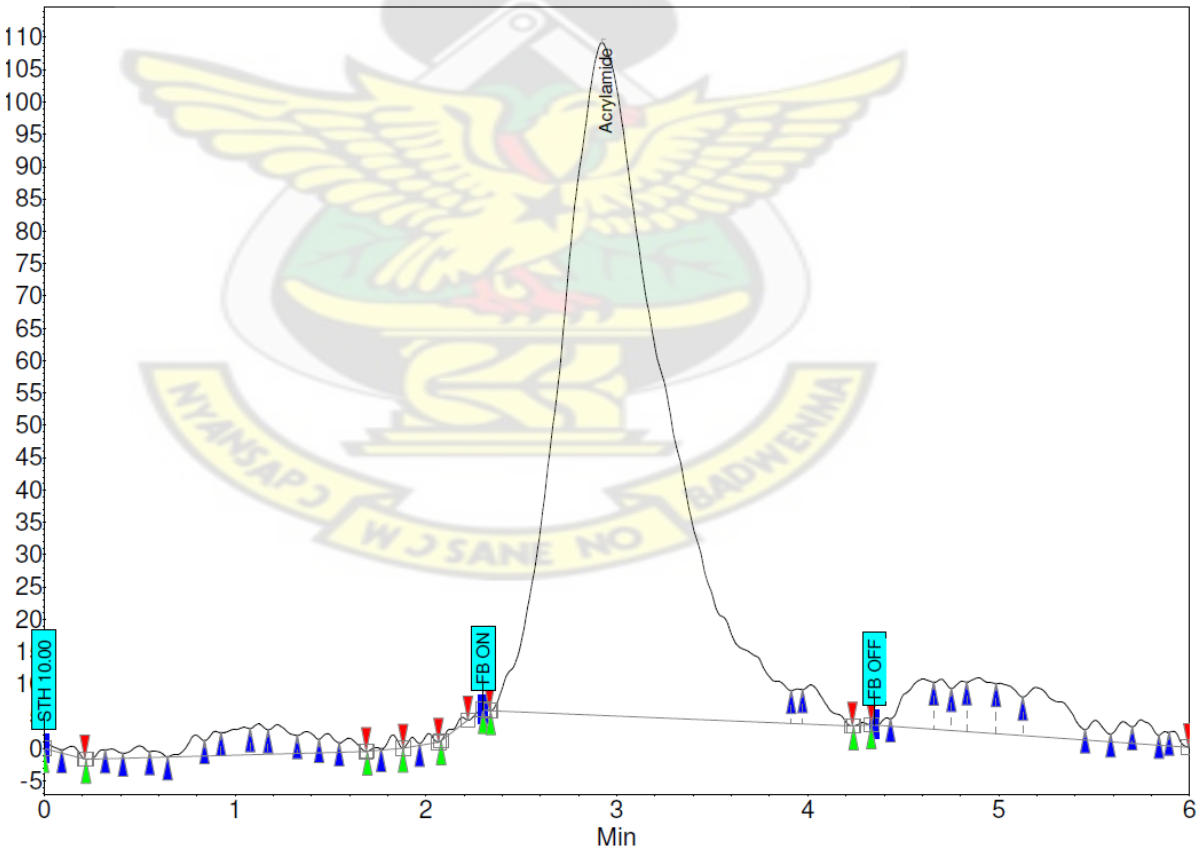

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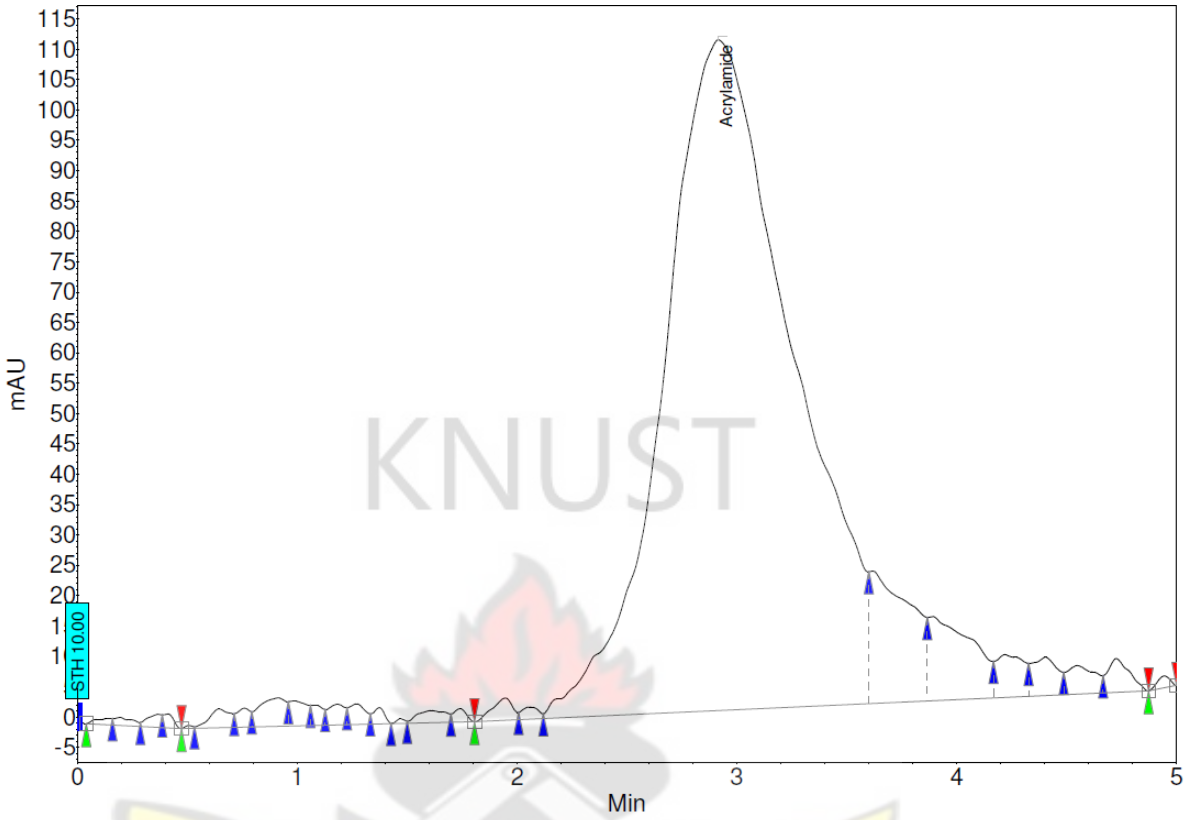

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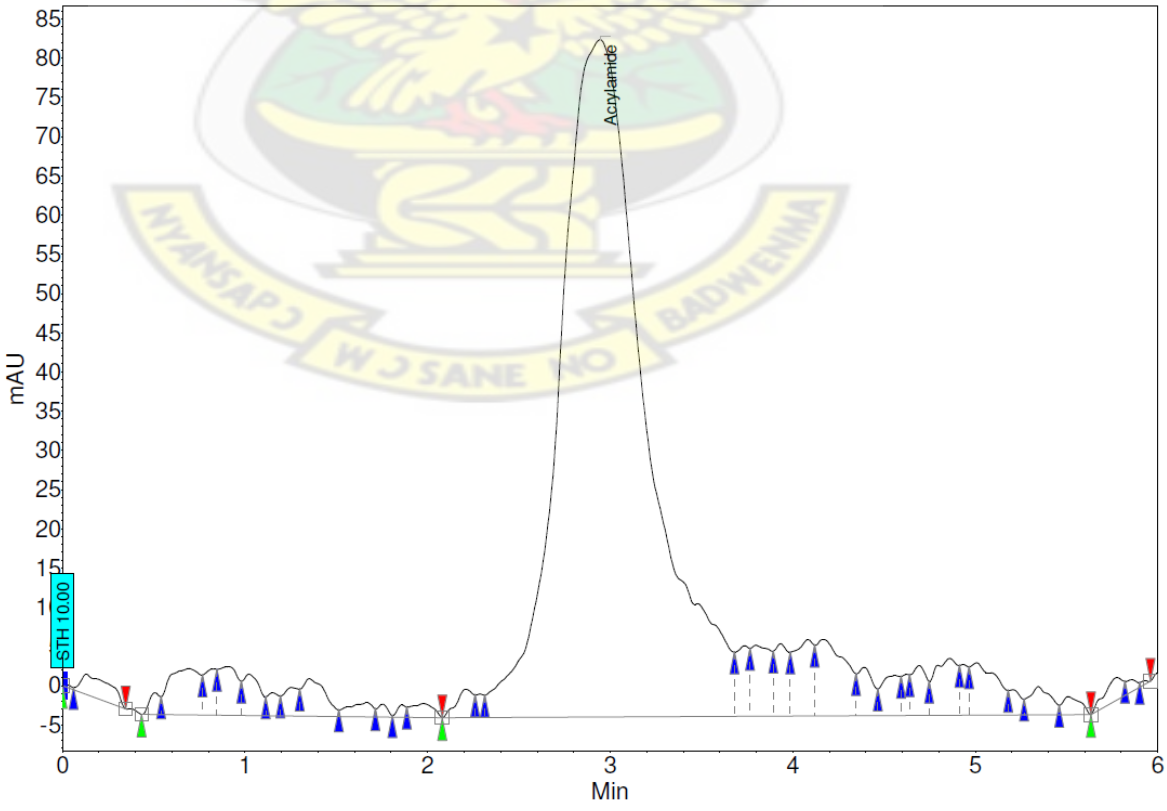

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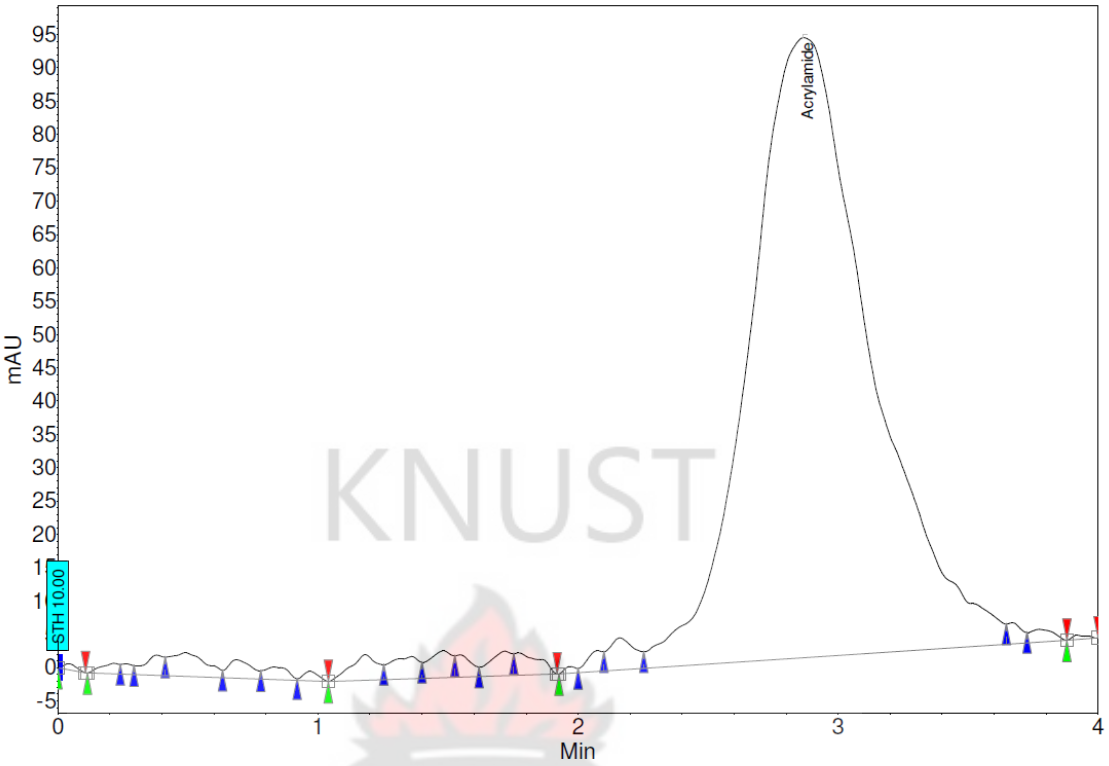

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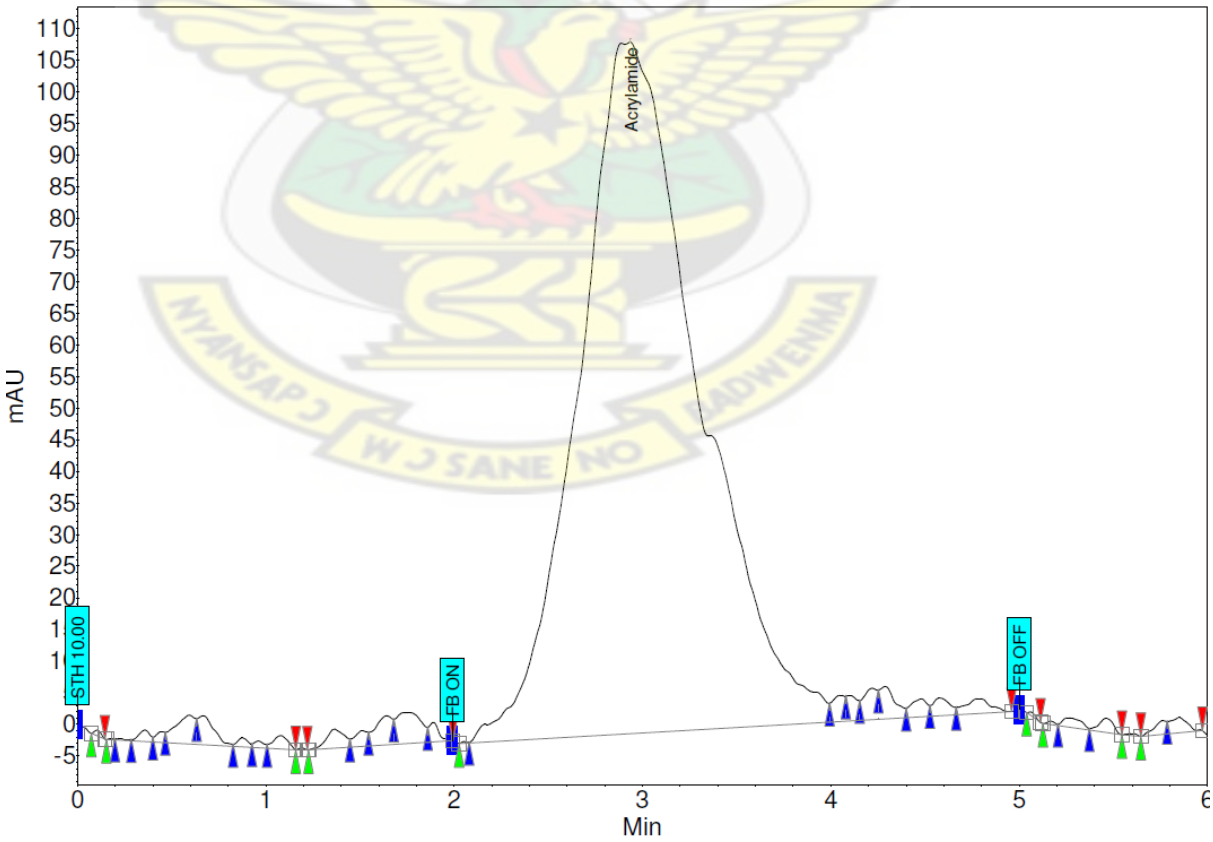

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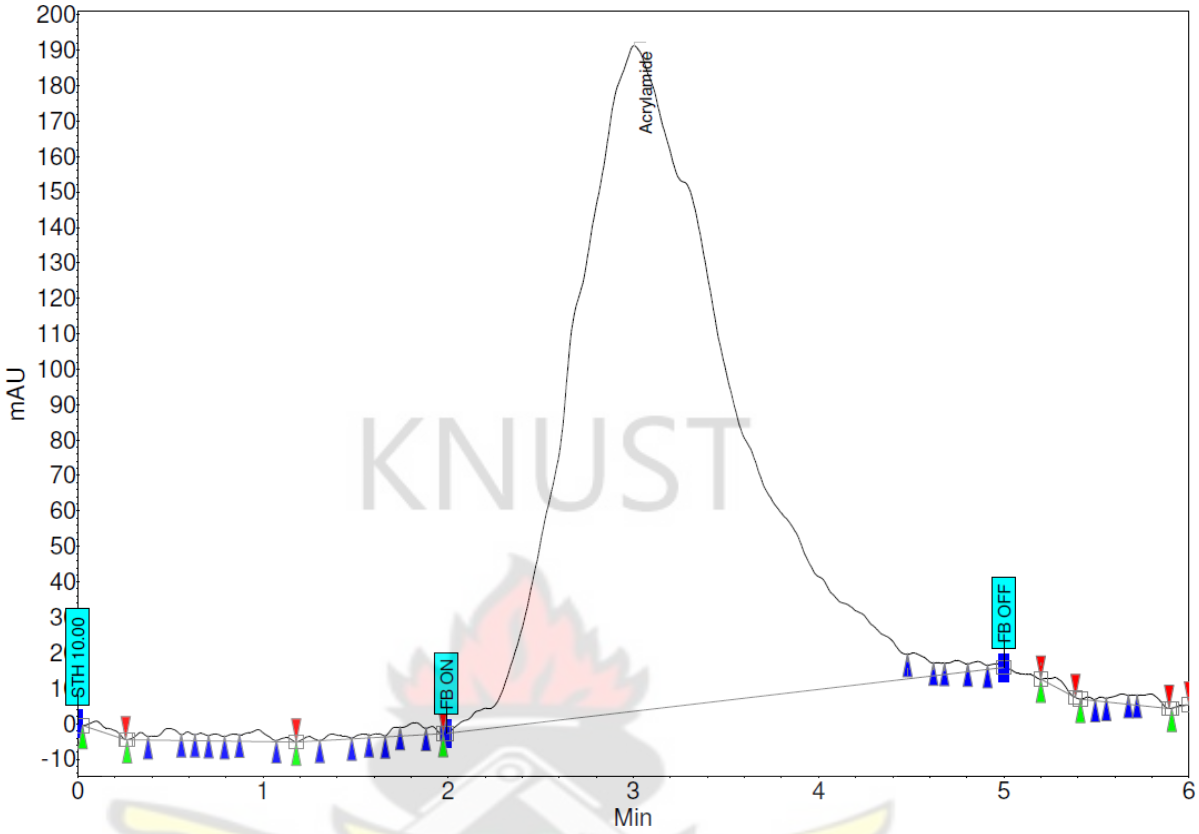
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Min
Acrylamide
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FB ON
FB OFF

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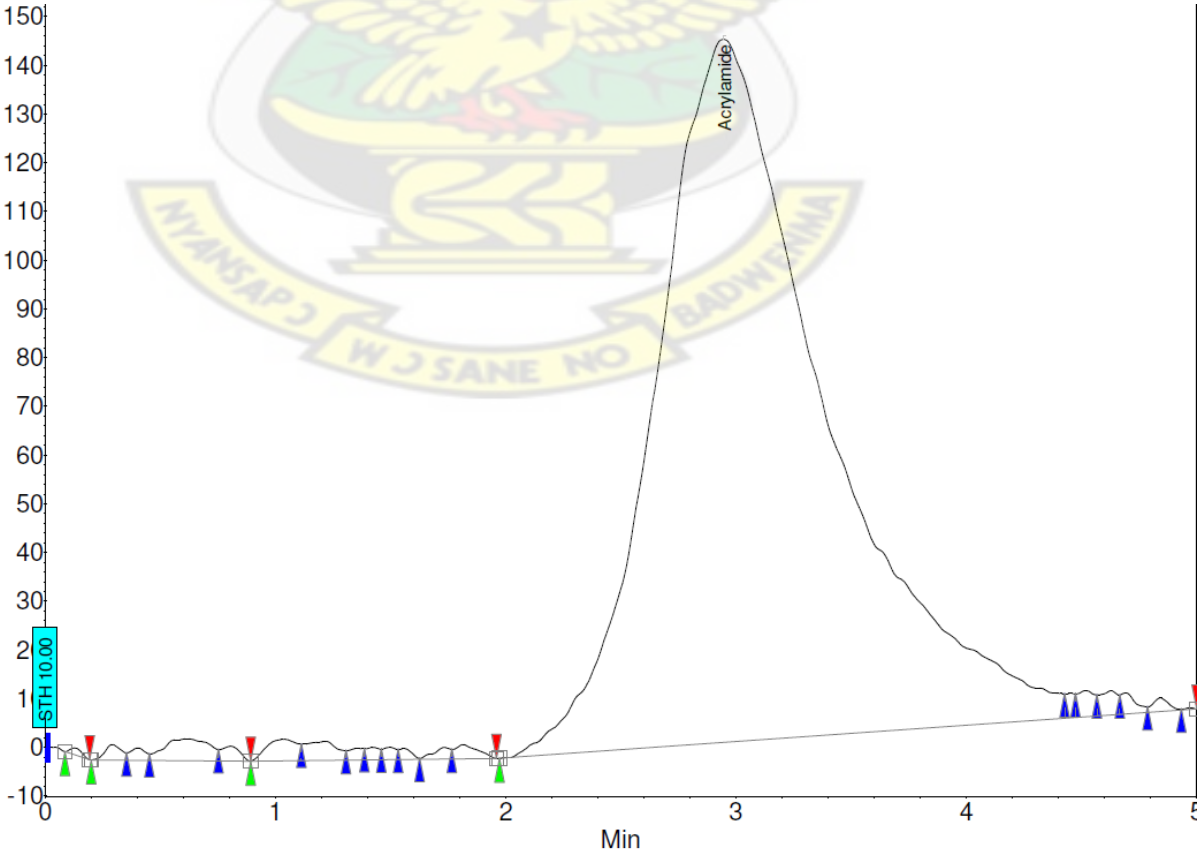
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Acrylamide
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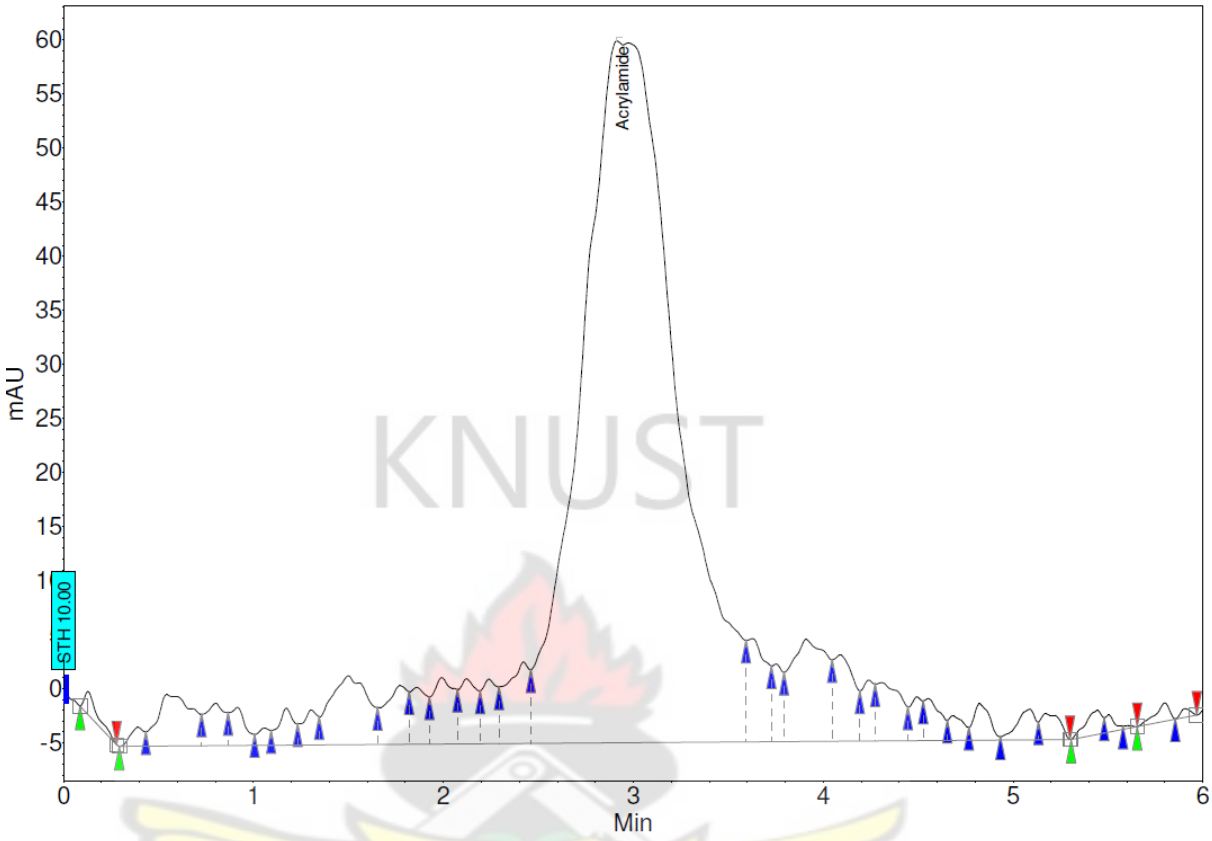

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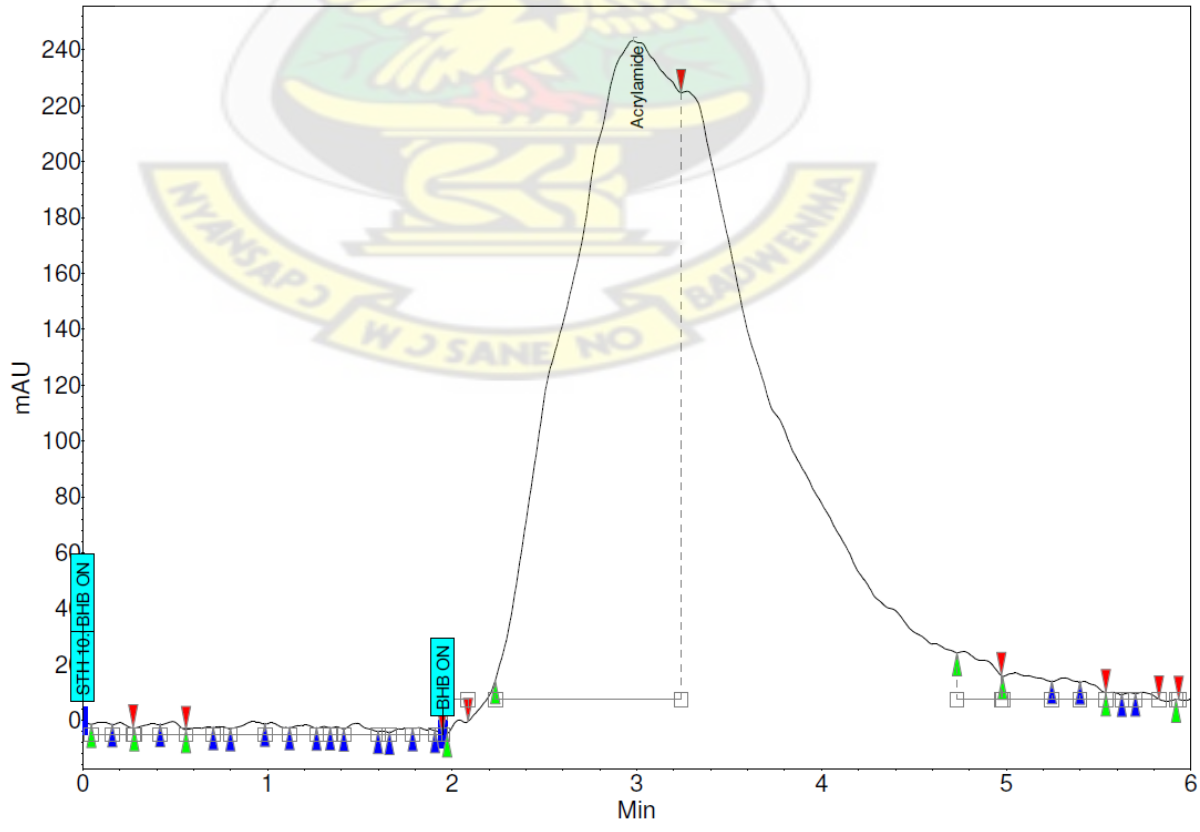

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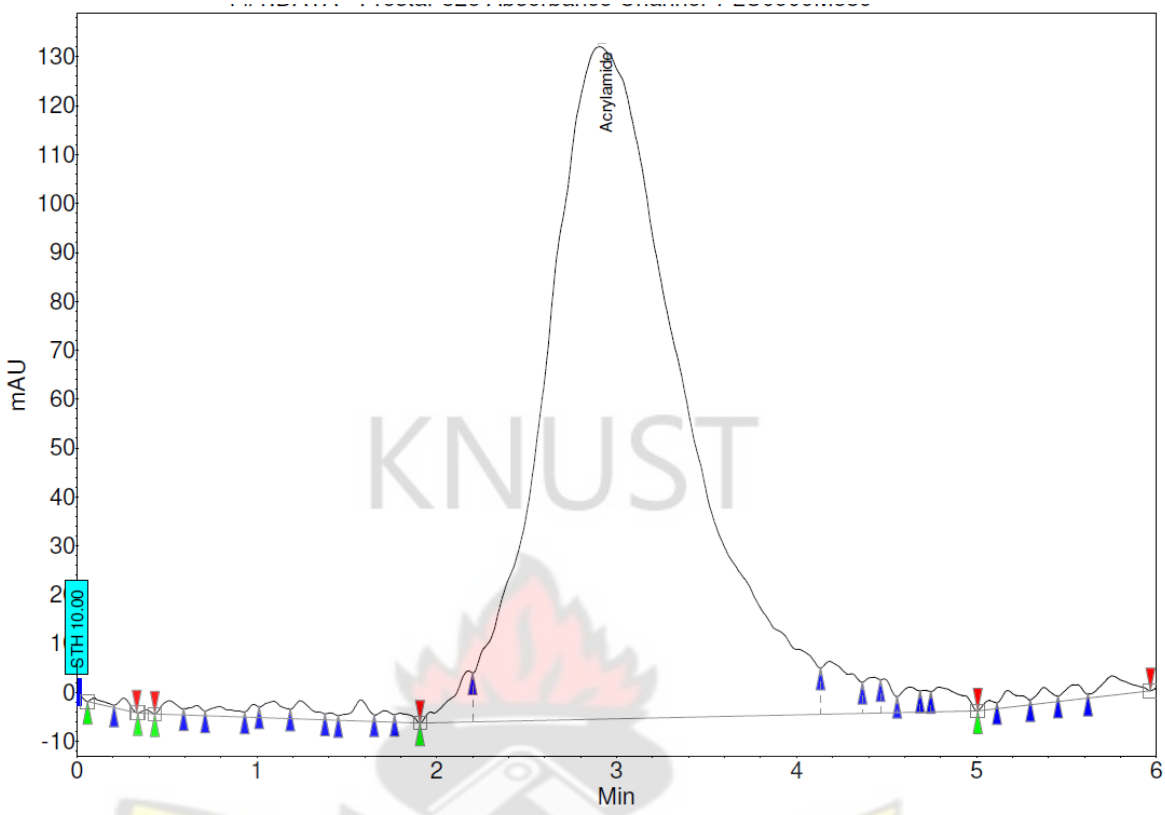
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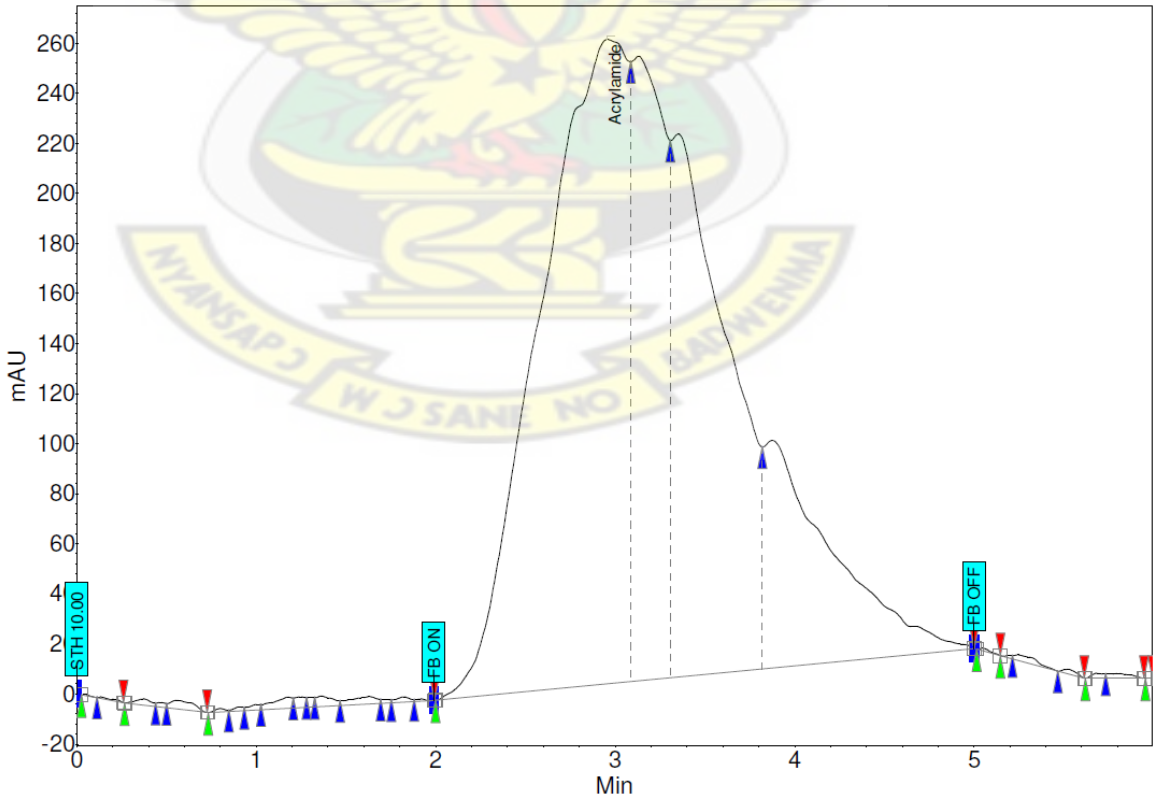
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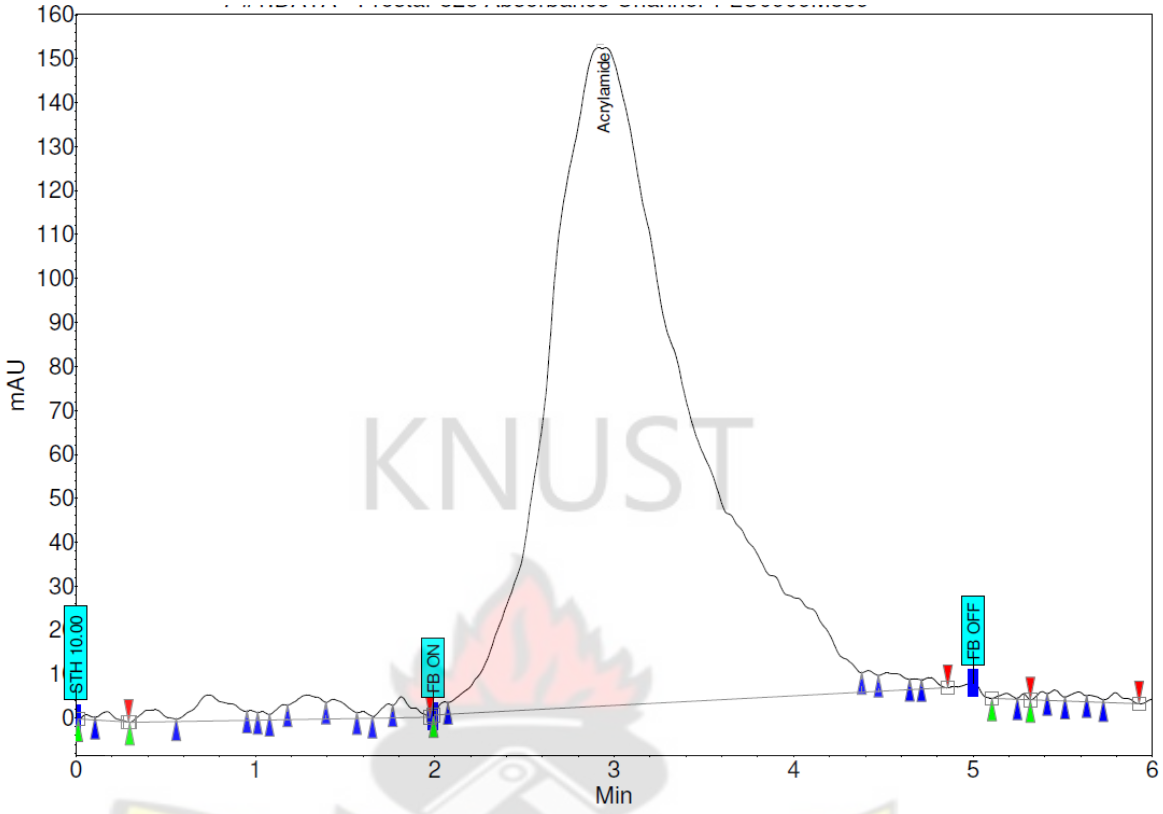
Min

Run 30



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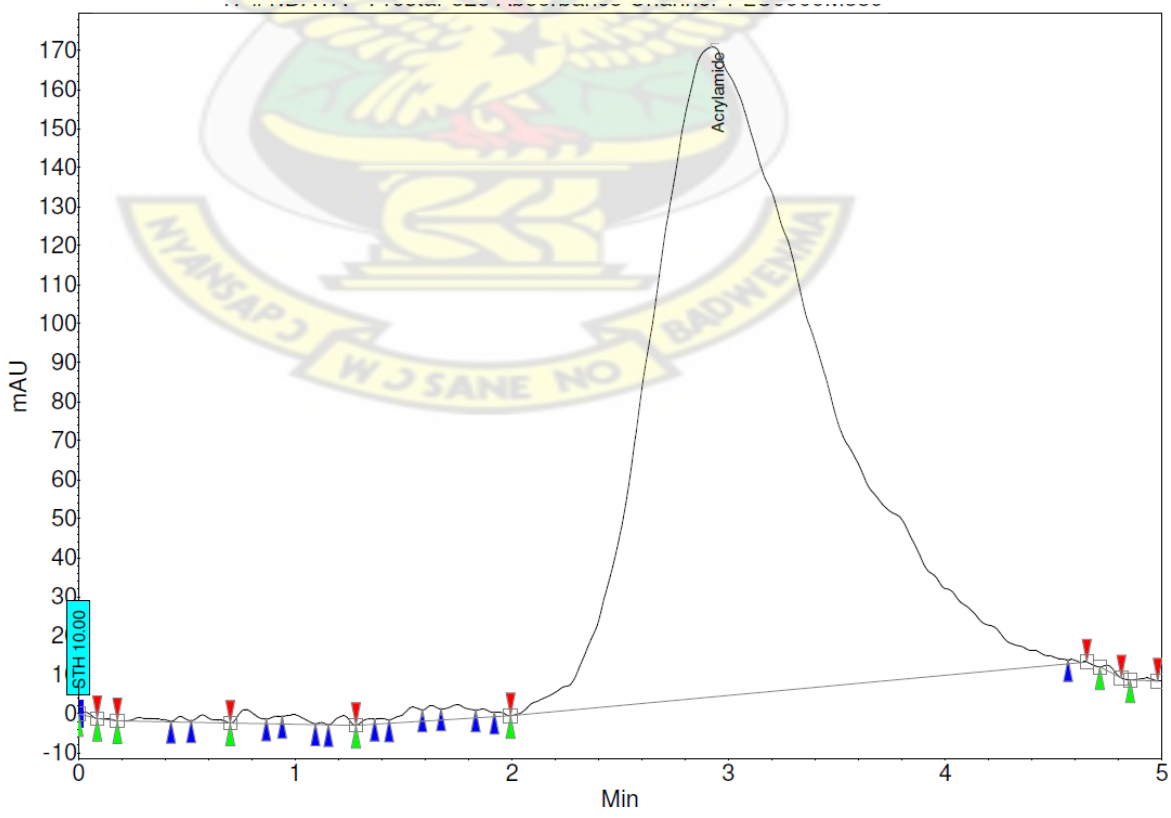

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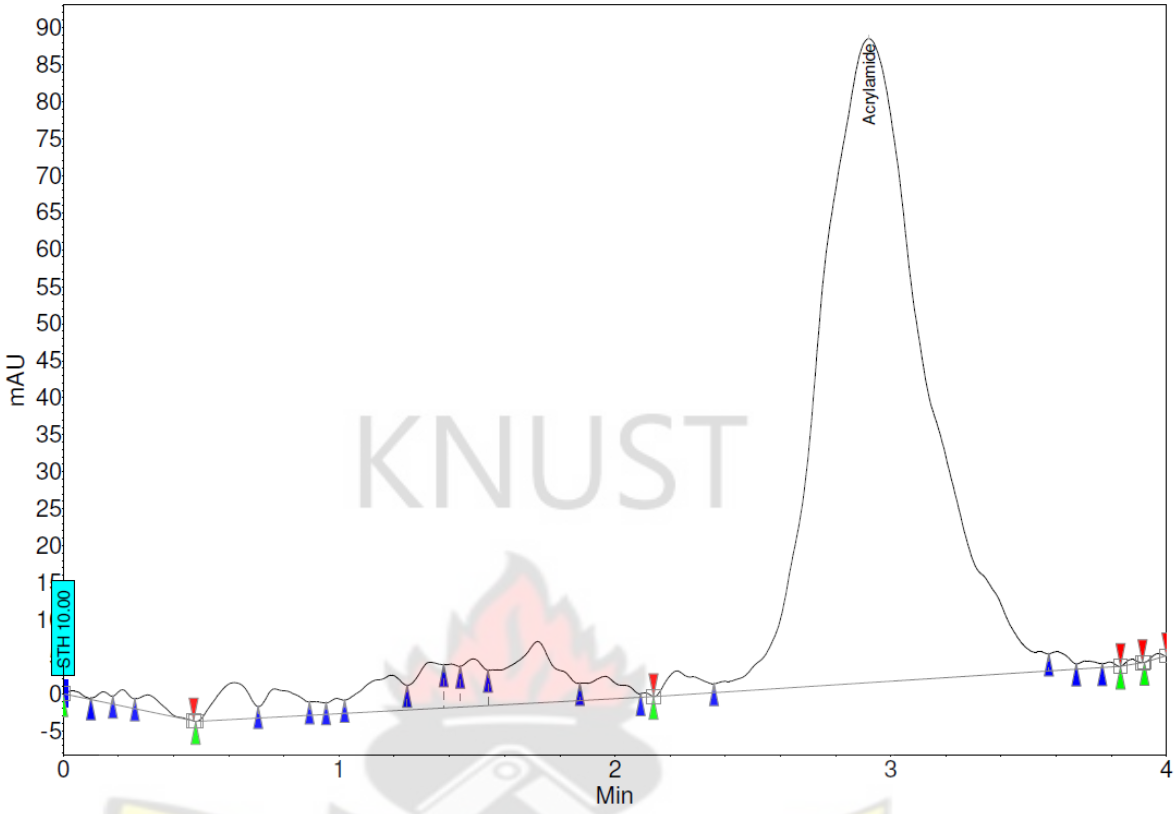
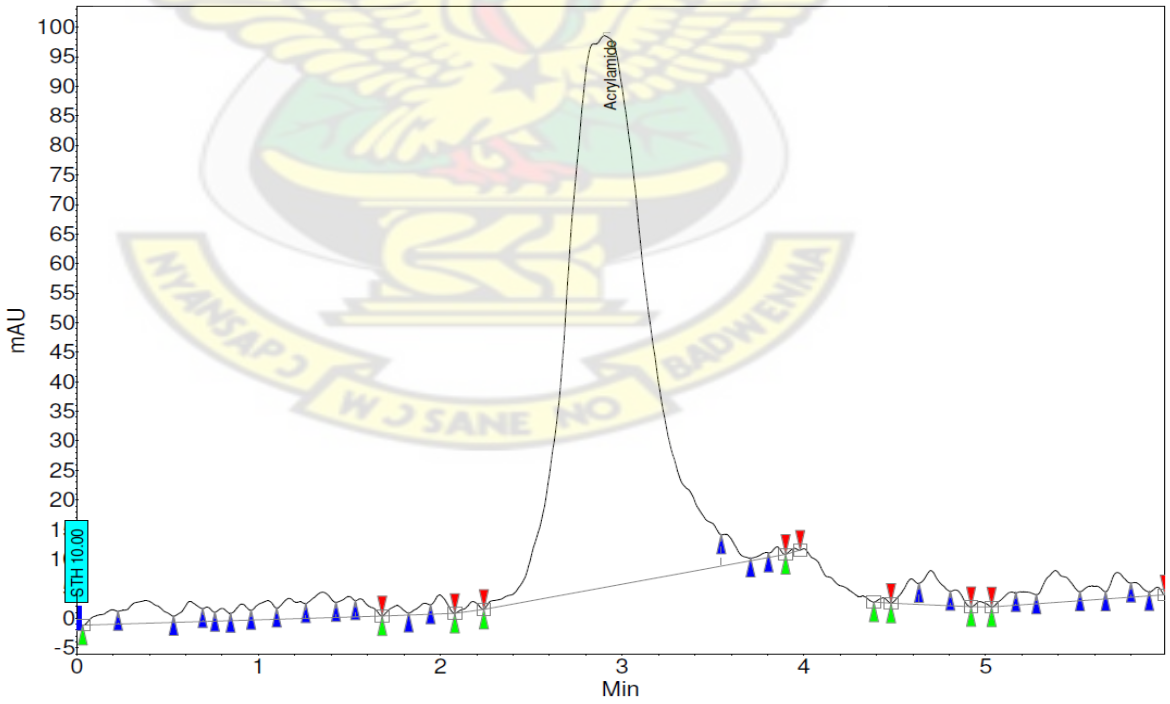


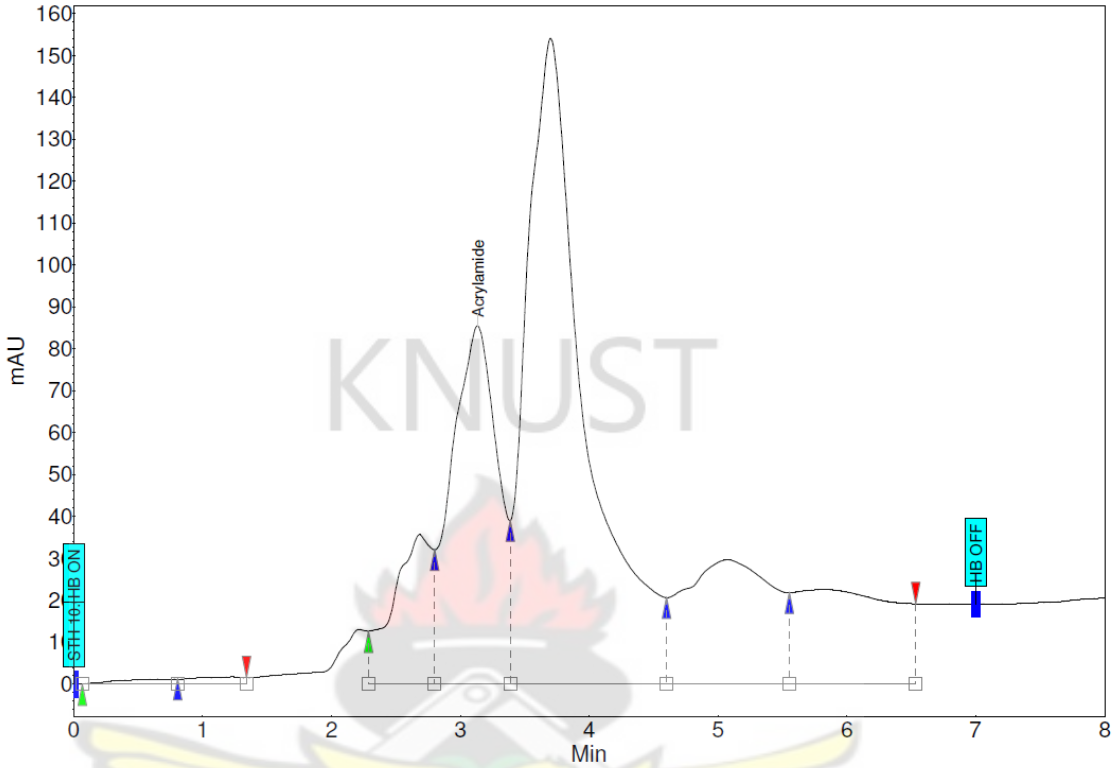
Run 34



A4:Peak results for experimental runs

Experimental runs	Retention time (Min)	Acrylamide concentration (g/Kg)	Height of peak (mAU)	Area of peak (mAU.Min)	Area (%)
1	3.03	3.615	109.7	78.5	91.52
2	2.95	6.61	257.5	143.7	44.95
3	2.91	3.35	110.7	72.7	89.05
4	2.9	5.522	137.4	119.7	91.29
5	2.86	3.784	138.8	82.2	63.91
6	3.15	7.516	172	163.5	93.57
7	2.98	6.707	153.3	145.9	91.03
8	2.9	4.42	124.2	96	81.11
9	2.97	2.559	94.2	55.5	78.81
10	2.94	6.677	159.7	145.2	93.66
11	2.91	3.42	110.5	74.4	82.87
12	2.79	5.775	201.2	125.6	81.32
13	3.02	2.612	90.9	56.6	85.93
14	2.93	6.483	155	141	89.57
15	2.89	5.367	135.6	116.7	90.51
16	3	5.902	140	128.3	91.69
17	2.92	2.955	104	64.1	83.3
18	2.91	3.426	110.5	74.4	82.87
19	2.95	2.159	86.3	46.8	72.36
20	2.87	2.296	93	49.7	90.51
22P	2.93	3.685	109.3	80	92.03
23	3.01	8.368	187.7	182.1	96.75
24	2.95	5.601	144.3	121.8	95.27
25	2.91	1.735	64.9	37.5	68.99
27	2.98	7.228	235.7	158.5	91.72
28	2.9	5.509	137.4	119.7	91.28
29	2.95	6.607	257.5	143.7	44.95
30	2.91	6.147	149.8	133.7	94.69
31	2.92	7.038	166.6	153.1	98.74
32	2.92	1.758	87	38	83.74
34	2.9	2.189	93.3	47.4	84.18

A4: Chromatogram for produced acrylamide in *Cajanu cajan* processed with optimum condition



A5: Peak results for produced acrylamide in *Pigeon Pea* processed with optimum condition

Time (min)	Acrylamide Concentration (g/kg)	Height of peak (mAU)	Area of Peak (mAU.Min)	Area (%)
3.13	1.758	85.5	35.3	20.079

B1: ANOVA table for preliminary tests to select treatment condition

File Version	9.0.0.7		
Study Type	Response Surface	**Runs**	34
Design Type	Box-Behnken	**Blocks**	No Blocks
Design Model	Reduced Cubic	**Build Time (ms)**	360.00

Factor	Name	Units	Type	Subtype	Minimum	Maximum
A	Mass of add	g	Numeric	Continuous	0.10	1.00
B	Roasting time	min	Numeric	Continuous	10.00	60.00
C	Roasting temp	oC	Numeric	Continuous	80.00	120.00
D	soaking solv		Categoric	Nominal	H3PO4	Citrate

Response	Name	Units	Obs	Analysis	Minimum	Maximum
R1	Acrylamide	g/kg	31	Polynomial	1.735	8.368

B2 Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS
Linear	1.75	0.3079	0.2015	0.0410	110.34
2FI	1.79	0.4411	0.1616	-0.2412	142.81
Quadratic	1.75	0.5465	0.1998	-0.3161	151.42
Cubic	1.97	0.7315	-0.0070		

Focus on the model maximizing the "Adjusted R-Squared"and the "Predicted R-Squared".

B3 Statistics of the Model Regression Parameter for response surface linear model for produced Acrylamide.

R R-Squared	Adj R-Squared	Pred R-Squared	Adeq Precision
0.3079	0.2015	0.0410	6.189