ADDIS ABABA UNIVERSITY COLLEGE OF

NATURAL AND COMPUTATIONAL SCIENCES

DEPARTMENT OF CHEMISTRY

ANALYSIS AND DETERMINATION OF ACRYLAMIDE IN ROASTED POTATO CHIPS (POTATO CRISPS) FROM DIFFERENT POTATO CHIPS SELLING CENTERS IN ADDIS ABABA

BY: Getasew Yihune Arega

A Thesis Submitted to the College of Natural and Computational Sciences, Chemistry Department Presented in Partial Fulfillment of Requirements of the Degree of Masters of Chemistry.

Advisor: Dr. Estifanos Ele

Addis Ababa, Ethiopia

June, 2018

ADDIS ABABA UNIVERSITY COLLEGE OF

NATURAL AND COMPUTATIONAL SCIENCES

DEPARTMENT OF CHEMISTRY

ANALYSIS AND DETERMINATION OF ACRYLAMIDE IN ROASTED POTATO CHIPS (POTATO CRISPS) FROM DIFFERENT POTATO CHIPS SELLING CENTERS IN ADDIS ABABA

BY: Getasew Yihune Arega

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

DEPARTMENT OF CHEMISTRY

June, 2018

Approved by:	**Signature**
Dr. Estifanos Ele	_____
Advisor	
Dr. Weldegebriel Yohannes	_____
Examiner (1)	
Dr. Mekonnen Abebayehu	_____
Examiner (2)	

Declaration

I, under signed, declare that this MSC Thesis entitled **"**ANALYSIS AND DETERMINATION OF ACRYLAMIDE IN ROASTED POTATO CHIPS (POTATO CRISPS) FROM DIFFERENT POTATO CHIPS SELLING CENTERS IN ADDIS ABABA" is my original work and has not been presented for any degree in any other university before and that all sources of materials used for the study have been appropriately acknowledged.

Getasew Yihune Signature _____ Date _____

This work has been submitted for examination with my approval as a university advisor.

Advisor

Dr. Estifanos Ele Signature _____ Date _____

Place and date of submission: Addis Ababa University

Chemistry department

June, 2018

Table of Contents

List of figures

List of schemes

List of tables

Appendices

List of Abbreviations

AA	Acrylamide
Asp	Asparagine
2-BPA	2-Bromo propene amide
2,3-DBPA	2,3-Dibromo propane amide
EFSA	European Food Safety Authority
GC-MS	Gas chromatography mass spectrometry
HPLC	High pressure liquid chromatography
IPT	Inner proton transfer
IT	Interaction time
JECFA	Joint FAO/ WHO Expert Committee on Food Additives
Kg	Kilogram
LOD	Limit of detection
LOQ	Limit of Quantification
µg	Microgram
µL	Micro liter
µm	Micrometer
Mg/mL	Milligram per milliliter
$KBrO_3$	Potassium bromate
RT	Retention time
SBOPC	Brown roasted potato crisps prepared from mixture of fresh and reused palm oil
SFOPC	Light yellow roasted potato crisps prepared from fresh (not reused) palm oil
SD	Standard deviation
SPE	Solid phase extraction
SPME	Solid phase micro extraction
TEA	Triethylamine
WHO	World health organization

Acknowledgements

First of all I would like to thank Almighty God, the source of all knowledge and wisdom.

I am grateful to thank my advisor Dr. Estifanos Ele for his advice, and countless support during conducting this research work.

I would like to express my profound gratitude to Prof. Wendimagegn Mammo, for his encouragement and follow up in the research work.

I am thankful to members of chemistry department for their kind help and constructive criticism during the course of study for the accomplishment of the research work.

The department of chemistry at Addis Ababa University is gratefully acknowledged for providing the laboratory space and the necessary chemicals to conduct the research work.

I am also grateful to thank my family and friends to Ato Mubarek Hussien, Ato Melaku Assefa Ato Addisalem Abebe and Miss. Senit Dagne for their encouragement and support to this study.

Abstract

Acrylamide or 2-propene amide is an organic compound formed in French fries, potato crisps, bread and coffee when they are fried, roasted or baked at a higher temperature. It is formed from the reaction of reducing sugars such as glucose and fructose with amino acids (asparagine). Many animal studies have shown that acrylamide is a carcinogenic, neurotoxic and genotoxic compound. In this study, GCMS instrument was used to analyze the presence and amount of acrylamide in roasted potato chips. The GCMS method to analyze acrylamide requires bromination type of derivatization to minimize interferences. Roasted potato chips samples were collected from Kebena, Arat kilo, Amist kilo, and Sidist kilo in Addis Ababa in 2018. From each sites light yellow color roasted potato chips (SFOPC) prepared from fresh palm oil and dark brown color roasted potato chips (SBOPC) prepared from the mixture of fresh and reused palm oil were collected. In all samples of roasted potato chips significant amount of acrylamide was investigated by using GCMS. The amount of acrylamide extracted from dark brown color potato chips (SBOPC) prepared from the mixture of fresh and reused palm oil is much higher than light yellow color potato chips (SFOPC) prepared from fresh palm oil. The highest amount was detected in samples collected from Kebena S_5FOPC (light yellow potato chips) 10005µg/kg and S_5BOPC (dark brown potato chips) 13933µg/kg while the lowest amount was detected in samples collected from Arat kilo S_4FOPC (light yellow potato chips) 2511µg/kg and S_4BOPC (dark brown potato chips) 7453µg/kg, respectively. Generally, to investigate the presence of acrylamide in roasted potato chips in this study samples were collected, grounded, extracted, purified by Solid phase extraction (SPE), brominated and 1 µL sample solution of 2-bromopropene amide (2-BPA) were injected and analyzed by GCMS.

KEYWORDS:-Potato crisps, acrylamide, asparagine, Maillard reaction, gas chromatography−mass spectrometry.

1. Introduction

1.1. Background

Acrylamide is a chemical that can be formed in some foods during high-temperature cooking processes, such as frying, roasting, and baking. It is formed from the reaction between reducing sugars and amino acid asparagines that are naturally present in food[1,2]. It is a white non- volatile crystalline solid at room temperature, odorless, soluble in polar solvents like water, ethanol, methanol, acetone and acetonitrile. Also has a molecular weight of 71.08g/mol.

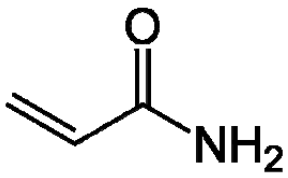


Figure 1. Chemical structure of acrylamide

Acrylamide (2-propenamide, C_3H_5NO) is a potential human carcinogen. Its potential health risks may extend to cause lung, breast, brain and thyroid cancers. Acrylamide induced significant characteristic neurotoxic symptoms in rats, such as decreases in grip strength and loco motor activity (paralysis). It can increase the risk of tumors of the mammary glands and also has other negative effects on public health, such as decreasing immune and blood systems[3-5].

Acrylamide does not only form in industrially manufactured foods, but also in foods prepared at homes. It can be found in potato crisps, French fries, and other roasted or fried potato foods, bread crust, crisp bread, roasted breakfast cereals, roasted coffee beans and ground coffee powder as a by-product of Maillard reactions. Factors affecting acrylamide formation are raw material variety, harvesting year, and storage conditions. In addition to these asparaginase and prolonged fermentation time (at least an hour) can reduce acrylamide formation in foods. Processing Conditions: heating temperature and time also affect amount of acrylamide content in roasted foods[6].

Gas chromatography (GC) has been used to quantify acrylamide in a variety of roasted food products. Quantitative measurement in food can be difficult as matrix-derived ions can interfere with acrylamide fragment ions of *m/z* 71, 55, and 41 when using this mode. Acrylamide often requires bromination type of derivatization to improve sensitivity and to minimize interferences during GCMS analysis[7].

1.2. Objectives of the study

1.2.1. General Objective of the study

The main objective of this study is to analyze and determine the presence and levels of acrylamide in roasted food products using GCMS.

1.2.2. Specific Objectives of the study

The specific objectives were to:-

- evaluate the presence of acrylamide in roasted potato chips (potato crisps) through GCMS analysis.
- determine acrylamide content in roasted potato chips (potato crisps) that is sold in Addis Ababa markets using external standard method.

1.3. Statement of the problem

Roasted food products such as potato chips, bread, coffee and cookies sometimes contain acrylamide[8]. Acrylamide is formed in food products due to overheating of those food products that contain asparagine and reducing sugars like glucose and fructose[1,2].

The major health risks of acrylamide in humans and animals are carcinogenicity, reproductive toxicity, and neurotoxicity [3-5].

In Addis Ababa and different other cites / towns, there is a culture of frying potato chips on road side for people to buy and eat. During frying, oil can be used over and over again. This might result in production of larger quantity of the acrylamide over time. Therefore, this study focuses on identification and quantification the level of acrylamide in potato chips sold in different parts of Addis Ababa.

2. Literature Review

2.1. Acrylamide

Acrylamide is a hydrophilic small molecule[9]. It is an odorless solid and its color is white. It is soluble in a number of polar solvents, *e.g*. acetone, acetonitrile, ethanol, methanol, water and susceptible to polymerization during heating. It boils at 225 °C and melts at 84-85^{o}C. It is regarded as a thermally unstable compound.

Acrylamide was discovered in foods in April 2002 by Ertrean Scientist Eden Tareken in Sweden when she found the chemical in starchy foods, such as potato chips (potato crisps), French fries (chips), and bread that had been heated higher than 120 ^{o}C. The production of acrylamide is the heating process way shown to be temperature dependent, not found in food that had been boiled, and in food that were not heated[8].

Scientists have discovered that acrylamide is forming in food as a result of a heat-induced reaction between two naturally occurring ingredients, the amino acid asparagine and reducing sugars[1,2]. Acrylamide has been added to the list of food born toxicants since the Swedish National Food Administration in 2002 found substantial amounts of acrylamide in several heat treated, carbohydrate-rich foods, such as potato chips and crisps, coffee, and bread[8]. After these findings, it has been clearly established that the major pathway for acrylamide formation in foods is a Maillard reaction with free asparagine as the main precursor[1,2]. Asparagine can thermally decompose by deamination and decarboxylation, but when a carbonyl source is present, the degradation of asparagine to acrylamide provides a much better explanation for the high concentration of acrylamide detected in foods rich in reducing sugars and free asparagine, such as fried potatoes and bakery products[1,10,11].

3-Aminopropionamide is an intermediate in Maillard reaction; it can also form by enzymatic decarboxylation of free asparagine and can yield acrylamide upon heating even in the absence of a carbonyl source[12]. Acrylamide formation occurs primarily at elevated cooking temperatures when frying or baking above 120 °C and in low moisture conditions[7].

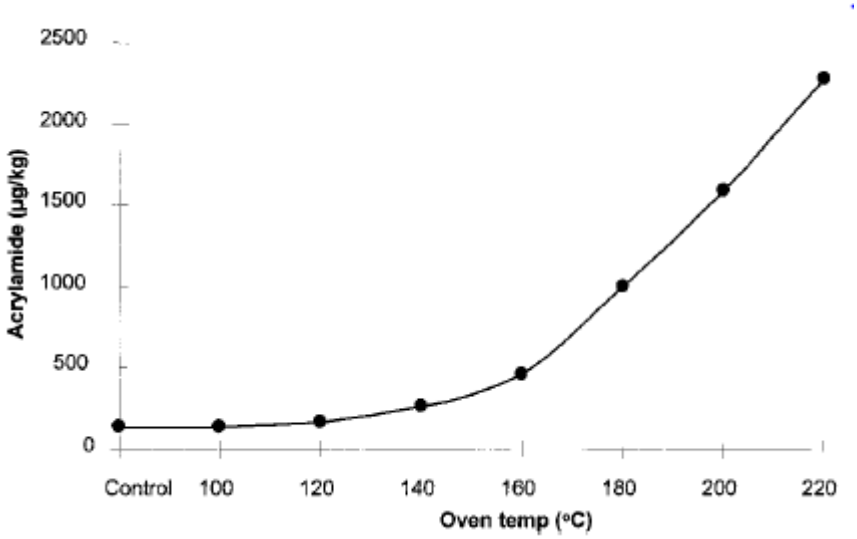


Figure 2. Influence of temperature on acrylamide concentration (µg/kg) in fried potatoes[13].

2.1.1. The toxicity of acrylamide

The major concern arising from heating processes come from the formation of hazardous compounds, the so-called food borne toxicants, i.e., compounds that are not naturally present in foods, but that may develop during heating or preservation and that reveal harmful effects, such as mutagenic, carcinogenic, and cytotoxic effects[14].

The Maillard reaction has recently been linked to the formation of various carcinogens and mutagens in a variety of thermally processed foods. Acrylamide, furan, and heterocyclic amines are among those toxicants. Recently acrylamide and furan have attracted a great interest because of their high toxicological potential and common occurrence in foods[1].

In 1994, the International Agency for Research on Cancer classified acrylamide as probably carcinogenic to humans, due to its neurotoxicity, carcinogenicity, and genotoxicity[4,5]. Acrylamide leads to DNA damage[4,15,16]. Workers who experience occupational exposure to acrylamide suffer from the damage of both peripheral and central nervous systems, since the neurotoxic effects of acrylamide are chronic[17]. Acrylamide is toxic to humans at various levels via inhalation, dermal absorption, and ingestion. Rodent studies have proven a high level exposure to be neurotoxic and even deadly[18]. The tolerable daily intake of acrylamide was 40 µg kg^{-1} per day for neurotoxicity, and 2.6 µg kg^{-1} per day for carcinogenicity[19].

2.1.2. The occurrence of acrylamide in foods

The Panel on Contaminants in the Food Chain (CONTAM Panel) evaluated a total of 43,419 analytical results from food commodities collected and analyzed since 2010 and reported by 24 European countries and six food associations. The data provided by the European countries and by food associations gave generally consistent and complementary information.

Acrylamide has been found in a wide variety of fried, baked or roasted foods; it is found in both foods processed by manufacturers and foods that are cooked in the home. Although the presence of acrylamide in food has been discovered comparatively recently, it has probably been present for many years, in staple foods forming a significant part of the human diet. Acrylamide has not been found in foods that are not fried or baked, such as boiled potatoes[20].

Acrylamide was found at the highest levels in "Coffee substitutes (dry)" (average levels of 1499 μg kg^{-1}) and "Coffee (dry)" (average levels of 522 μg kg^{-1}). However, due to dilution effects, lower levels are expected in "Coffee beverages" and "Coffee substitute's beverages" as consumed by the population. Acrylamide has been found primarily in plant-based foods. High levels were found in "potato crisps (potato chips) produce 1534μg/kg±42 acrylamide[21]. Lower acrylamide levels were found in "processed cereal-based baby foods" (average level of 73 μg kg^{-1}), "Soft bread" (average level of 42 μg kg^{-1}) and "Baby foods, other than cereal-based" (average level of 24 μg kg^{-1})[19]. The mean dietary exposure to acrylamide has been estimated by the Food Safety Authority of Ireland (FSAI) to be approximately 0.6 microgram (μg) per kg body weight per day for the Irish adult population, with highlevel consumers being exposed to approximately 1.75 μg/kg bw/day. These estimates of exposure of Irish consumers to acrylamide compare well to exposure estimates reported by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which ranged from 0.3 to 2.0 μg/kg bw/day for mean consumers and 0.6 to 3.5 μg/kg bw/day for high-level consumers.

The FSAI exposure analysis confirmed that consumption of potatoes and potato products provides the largest contribution to dietary intake of acrylamide followed by consumption of bread and biscuits, although coffee is also a contributor to exposure, as shown in the following Figure3.

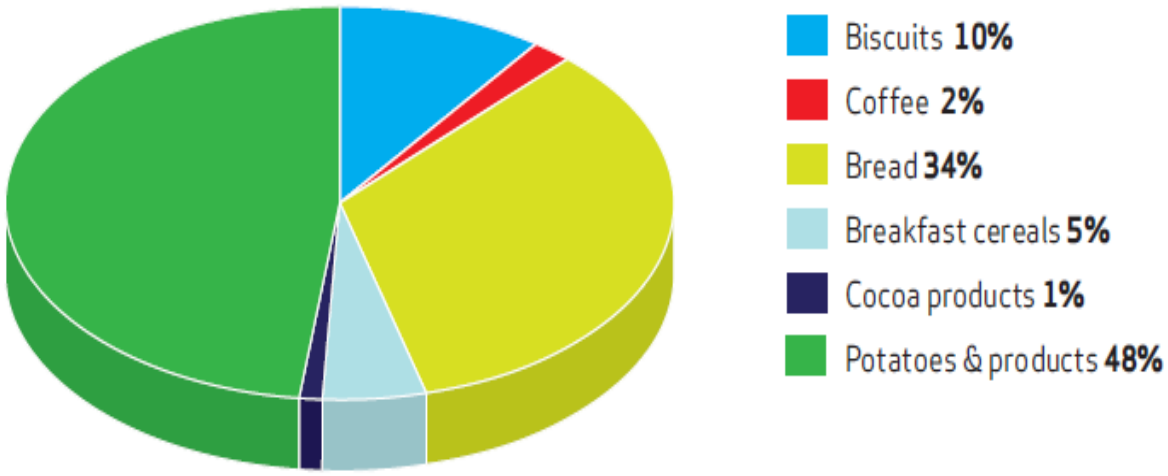


Figure 3. Contribution to acrylamide intake from selected food groups for the Irish adult population percentage (%) contribution to mean µg/kg bw AA exposure (total population)[22].

Table 1. Content of acrylamide in different food items according to JECFA (WHO) 2005 and Reported levels 2004

Food Item	JECFA (WHO) (µg/kg)	Reported levels[23] (µg/kg)	
		Minimum	Maximum
Breakfast cereals	366	22	1400
Bread toast (crisp bread)	446	30	1900
Potato crisps	752	170	2510
Potato chips (French fries)	334	59	1280
Potatoes boiled	-	4	50
Coffee (ground, roasted)	288	45	374
Coffee decaffeinated	34	-	-
Green tea (roasted)	306	142	567
Baby food (dry powder)	16	10	130
Baby food (biscuits)	181	18	650
Pizza	85	-	-
Fish and sea food	107	-	-
Meat and offal	325	-	-
Milk and milk products	147	-	-
Nuts and oil seeds	203	-	-
Coca products	23	-	-
Sugar and honey	133	-	-
Vegetables	193	-	-
Fruits dried and processed	49	-	-
Alcoholic beverages	99	-	-

2.1.3. Description of the Maillard Reaction

In 1912, the French chemist Louis Camille Maillard was the first scientist to study and describe changes resulting from thermal reactions between amino acids and sugars, also noting the variations in chemical and aroma formation as a function of the amino acids involved. It has been a major and even central challenge in food industry, since the Maillard reaction is related to aroma, taste, and color, particularly in traditional processes such as the roasting of coffee and cocoa beans, the baking of bread and cakes, the toasting of cereals and the cooking of meat[24].

The chemistry underlying the Maillard reaction is very complex. It encompasses not just a single reaction pathway, but a whole network of various reactions. It is the reaction taking place mostly between asparagines type of amino acid and reducing sugars like glucose and fructose through heating forming acrylamide.

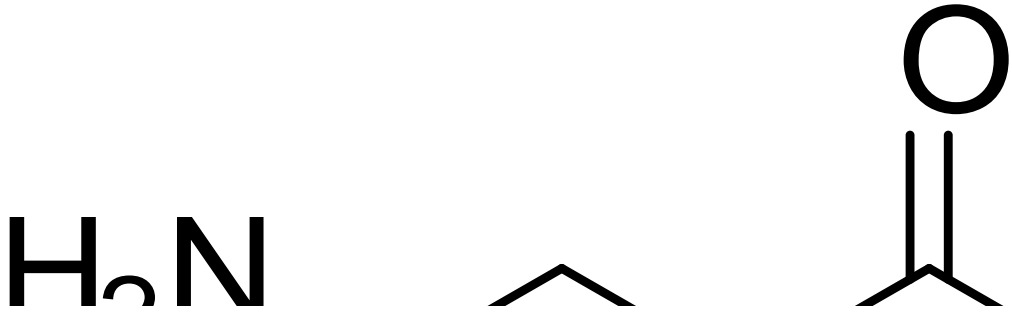


Figure 4. The Maillard reaction scheme adapted from Eriksson[25].

2.1.4. Methods for analytical detection of acrylamide

Acrylamide has been quantified in various foods by using chromatographic techniques, such as gas chromatography (GC) or liquid chromatography (LC) in combination with appropriately selective and specific detectors[7]. HPLC coupled with MS is the most preferred method for separation and quantification of acrylamide in foods, due to its sensitivity, selectivity, and versatility[26].

For the chromatographic separation of acrylamide, most researchers use reversed-phase chromatography. Quantitative analysis of acrylamide or its derivatives contained in foods were also performed by gas chromatography coupled with mass spectrometry. In the case of gas chromatography, derivatization of the analyte is required in order to increase volatility, selectivity, sensitivity and retention time. The most popular method of acrylamide derivatization is its bromination prior to the analysis[27]. This technique is highly selective and improves the precision, but it performs the difficult and time-consuming derivatization process[7]. Both GC based methods with and without derivatization was developed, showing a satisfactory agreement with LC-MS for the detection of acrylamide in various foods. A GC method coupled with MS following the derivatization was proposed for the detection of trace levels (<50 μg kg^{-1}) of acrylamide in cereal-based foods due to its high sensitivity (LOD 2 μg kg^{-1}) and great recoveries (93-104%)[28]. It is important to note, however, that the derivatization is not necessarily essential and some scientific groups have chosen to eliminate this lengthy procedure using a more polar GC phase, although this approach does not provide sufficiently low limit of detection in comparison to the derivatization techniques[29-31].

The main drawback of GC–MS without derivatization is the lack of characteristic ions in the mass spectrum of underivatized acrylamide and the interference caused by matrix decomposition. Due to the high background noise, a low limit of detection is impossible to obtain. The application of gas chromatography coupled to tandem mass spectrometry (GC–MS/MS) allows decreasing the interference, which results in a larger acrylamide peak area[7].

Extraction is a critical procedure in food pretreatment prior to the detection of acrylamide. The hydrophilic character of acrylamide allows for an application of water or organic solvents, such as methanol and acetonitrile, for its extraction from foods[32,33]. Water can minimize the co-extraction of hydrophobic compounds from foods, but other hydrophilic interferences still

remain that should be removed afterwards[27]. In comparison to water, organic solvents have the advantages of easier separation even without centrifugation and more convenient evaporation. The combination of water (salt solutions) and organic solvents improved the extraction efficiency in some studies[34,35].

Solid phase extraction (SPE) has been used extensively in purifying the extracts of food samples due to its simplicity, stability, suitability for automation, accuracy, precision, and the repeatability of instrumental analysis[27,36]. Depending on the selection of SPE cartridges used for purification of acrylamide from the extracts of food samples, two strategies have been developed. One strategy relies on acrylamide absorption from the complex extract by the cartridges through hydrogen bonding, π-π interaction, and cation exchange, followed by elution of acrylamide using other polar solvents. Some common cartridges are Oasis HLB, Oasis MCX, Isolute Multi-Mode, ENVI-Carb, and Isolute ENV+. The second strategy is applied to retain the interferences and collect the eluent containing acrylamide by using Oasis HLB cartridges coupled with Bond Elut-Accucat and a custom made SPE column filled with a mixture of C_{18}, SCX, and SAX sorbents. Among these cartridges, Oasis HLB and Isolute Multimode are the most preferred. Recently, novel filling materials such as carbon nanotubes, magnetic chitosan, and molecule imprinted polymers were used, resulting in an effective purification[27,36-38].

2.1.5. Mitigation strategies to reduce acrylamide content in foods

Several research projects have focused on ways to mitigate formation of acrylamide in foods. Temperature is a key factor that has been repeatedly shown to influence acrylamide levels. Microwaving and boiling generates minimal amounts, whereas harsher treatments such as frying and baking may lead to high levels[20].

The proposed techniques generally fall into one of several areas: reducing the availability of free asparagine or reducing sugars, modifying other ingredients, and changing the cooking time or temperature to reduce acrylamide content in food products.

Different techniques appear to be useful for certain types of products:, and reducing the time or temperature of cooking are frequently cited methods, while for cereal and potato products, selecting potatoes low in reducing sugars that are dry, matured, large in size and collected in dry seasons, controlling storage conditions of products, modifying the time or temperature of

cooking, avoiding the use of ammonium bicarbonate, and selecting materials low in asparagine may be more relevant to reduce acrylamide content in foods. Ammonium bicarbonate (NH_4HCO_3) can promote the production of acrylamide by increasing the formation of sugar fragments (glyoxal and methylglyoxal) that react rapidly with asparagine to furnish acrylamide in higher yield than the native reducing sugars under mild conditions[39-41]. Other suggested techniques for acrylamide mitigation include the use of minor ingredients (e.g., amino acids, calcium, and citric acid) that interfere with acrylamide formation, and the use of asparaginase to reduce asparagine levels prior to cooking by converting asparagine into aspartic acid and ammonia through hydrolysis[39-41].

Whenever one reactant (asparagine or glucose) is at a reduced concentration, there is a reduction in the formation of acrylamide. Reducing or eliminating a reactant (asparagine or reducing sugars) will alter the amount of acrylamide formed. Researchers have identified two possible ways to reduce the amount of reactant in potatoes. During storage the amount of sugar increases in potatoes. Using fresh potatoes rather than stored potatoes could result in less acrylamide being formed. Storing potatoes below 8-10ºC can promote the formation of reducing sugars. The presence of this reducing sugar together with asparagine may lead to acrylamide formation. The variety of potato also affects the amount of acrylamide formed because of the relative amounts of reducing sugar and asparagine. Particularly in the case of potatoes, soaking them in water and washing prior to baking or frying significantly reduces acrylamide levels, as it leaches away its precursors from surface. Further reduction has also been observed after the addition of salt, citric acid or amino acids (glycine, lysine, and cysteine) to the soaking solutions because can interfere formation of acrylamide[39-41].

The addition of amino acids such as glycine to dough used for making snacks also has a negative impact on acrylamide formation[42].

Putting potatoes in areas having ventilation is another ways to reduce amount of acrylamide by decreasing sugar levels, where as in areas having insufficient storage of ventilation leading to oxygen starvation, increasing both sugar levels and amount of acrylamide in potatoes. One of the most promising tools to control acrylamide content in heat-treated foods is the addition of the enzyme asparaginase. Asparaginase (L-asparagine amidohydrolase) is an enzyme promoting the hydrolysis of asparagine to aspartic acid and ammonia, thus lowering the content of acrylamide precursor asparagine. Asparaginase has been successfully applied on a lab scale both to potato products and cereal-based products resulting in acrylamide reduction by up to 85-90% and no effect on the taste and appearance of foods[42-44].

3. Experimental Part

3.1. Materials and Methodology

All samples analyzed for acrylamide contents were collected or purchased from road side venders around Arat Kilo, Amist Kilo, Sidist Kilo and Kebena in Addis Ababa, from October, 2017 – January, 2018. Samples were potato crisps prepared from fresh and reused palm oils. For determination of acrylamide, samples were homogenized and stored in refrigerator until analysis.

Figure 5. Potato Chips (Potato Crisps): A. Potato crisps prepared from fresh and reused palm oil
B. Potato crisps prepared from fresh palm oil

Table 2. Number of samples used for acrylamide analysis by GC/MS

Number	Product group	Number of samples
1.	Light yellow (golden) color roasted potato crisps prepared from fresh palm oil or SFOPC Samples	**12**
2.	Dark brown color roasted potato crisps prepared from mixture of fresh and reused palm oil or SBOPC Samples	**12**
Total		**24**

3.2. Chemicals

Analytical standards acrylamide (99%) were obtained from Sigma –Aldrich (St. Louis, Mo, USA). Deionized water was prepared by a Milli-Q (Millipore, Billerica, MA, USA) water purification system. Methanol, high performance liquid chromatography grade was purchased from Merck KGa, (Darmstadt, Germany). Sodium chloride and anhydrous magnesium sulfate were purchased from Scharlau (Barcelona, Spain). Hexane and acetone were obtained from Sigma-Aldrich (St. Louis, Mo, USA). Ethyl acetate, Potassium bromate ($KBrO_3$) was obtained from BDH Chemicals (England). Sodium thiosulfate, $Na_2S_2O_3$ was obtained from Scharlaus (Spain). Potassium bromide was obtained from Park Scintific limited (Northamptha, U.K, U.K). Sodium sulfate and Triethylamine $(CH_3CH_2)_3$ N were used.

3.3. Apparatus

Solid -phase extraction (SPE), C_{18} column or cartridges, syringes, 0.45 µm syringe filters. 50 and 100 mL flasks, filter papers, 250 mL separatory funnels, measuring cylinders, syringes, rotary evaporator, refrigerator, electrical balance, spatula, funnel, centrifuge, 50 mL centrifuge tubes, mortar and pistil were used.

3.4. Sample Preparation Procedure

The procedure used for acrylamide analysis was according to the method described by Zhiming Geng, Peng Wang and Aiming Liu (2011) with some modifications.

Generally the procedure involves homogenization, defatting, extraction, purification, bromination type of derivatiztion and analysis of acrylamide by using GCMS in roasted potato chips.

3.4.1. Derivatization or bromination of Acrylamide

100 mg of acrylamide was weighed and added to a 50 mL vial covered by Aluminum foil. 1 mL dilute H_2SO_4 (10%, v/v) was added into Aluminum foil covered vial. After shaking vigorously for 1 min the vial containing the mixture was placed in refrigerator (4 ^{o}c) for 15 min. 1 mL of 0.5 M $KBrO_3$ and 2.5g KBr powder were added to the reaction mixture. The vial was shaked by hand vigorously for 5 min. The reaction mixture was stored in refrigerator for 12 hrs at 4 ^{o}c.

5 mL of 0.5M $Na_2S_2O_3$ (sodium thiosulfate) solution was added to remove excess Br_2. After shaking for 1min the mixture was transferred to a 50 mL separatory funnel. 30 mL ethyl acetate-hexane (4:1, v/v) was added and shaked to extract 2, 3- dibromopropane amide (2, 3-DBPA) from aqueous layer. Then the upper organic layer was separated and dried over Na_2SO_4. The solution was filtered in to a flask. The separatory funnel and filter paper was rinsed by 15 mL ethyl acetate-hexane (4:1, v/v). The mixture was concentrated on a rotary evaporator.

Scheme 1. Brominating reagents for derivatization of acrylamide

The residue 2,3-DBPA was dissolved in 1 mL ethyl acetate followed by adding 100 µL of triethylamine base for dehydrobromination of 2, 3-DBPA to produce 2-bromopropene amide (2-BPA). The solution was stored for 12 hrs in refrigerator and filtered through 0.45 µm syringe filter in to HPLC vial for GCMS analysis.

Scheme 2. Bromination of acrylamide

3.4.2. Construction of calibration curve

In order to determine concentration of acrylamide in potato chips, calibration curve was constructed using peak area and concentration of 2-BPA. By taking 100 mg of acrylamide as standard 2- BPA was extracted by bromination reaction. The concentration of 2-BPA present in table 1 below in ppm (parts per million) helps to make the calibration curve and to determine the concentration of 2-BPA and amount of AA in µg/kg in potato chips was calculated based on dilution formula as follows.

To make 40 ppm of 5 mL solution of 2-BPA starting from by preparing 160 ppm of 5 mL solution of 2-BPA and methanol used as a solvent.

$$\text{ppm} = \frac{\text{mass of solute}}{\text{volume of solution}} \times 10^6$$

$$160\ \text{ppm} = \frac{\text{mass of } 2-\text{BPA}}{5\ \text{mL}} \times 10^6$$

$$\text{mass of } 2-\text{BPA} = \frac{160\ \text{ppm} \times 5\ \text{mL}}{10^6} = 0.0008\ \text{g}$$

By dissolving 0.0008 gm of 2-BPA with enough methanol until the volume of solution becomes 5 mL, 160 ppm solution of 2-BPA was prepared. From 160 ppm solution, 40 ppm of 5 mL solution of 2-BPA was prepared by adding enough methanol as a solvent.

$M_1V_1 = M_2V_2$ ---------- dilution formula

$160\ \text{ppm} \times V_1 = 40\ \text{ppm} \times 5\ \text{mL}$

$$V_1 = \frac{40 \text{ ppm} \times 5 \text{ mL}}{160 \text{ ppm}} = 1.25 \text{ mL}$$

Similarly the others concentration of 2-BPA in ppm were prepared by serial dilution formula.

Table 3. Calibration data

NO	Sample Name	Concentration of 2-BPA Solution	Peak area of 2-BPA
1.	0.5	0.5 ppm	45901.85
2.	1	1 ppm	58361.55
3.	5	5 ppm	292146.61
4.	10	10 ppm	593735.76
5.	20	20 ppm	1331691.05
6.	40	40 ppm	2782987.9

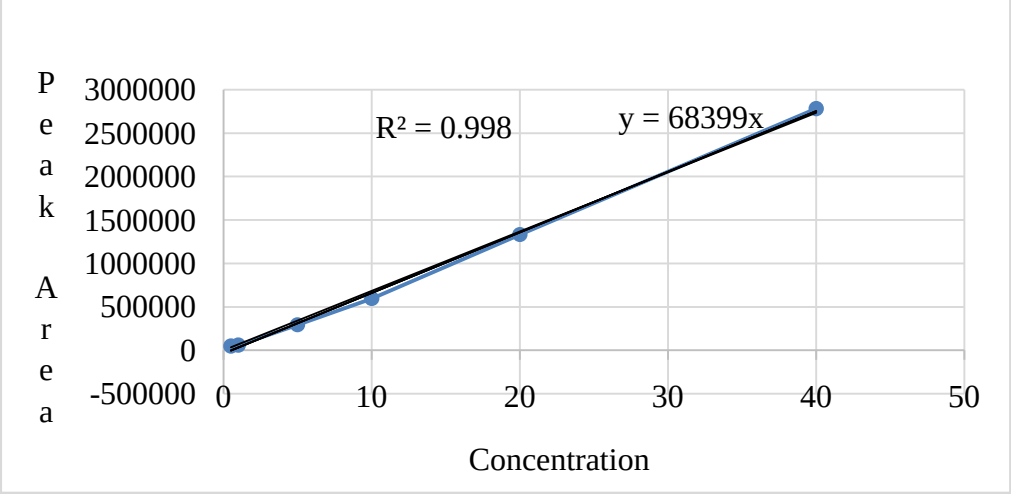


Figure 6. Calibration curve for 2-bromopropene amide (2-BPA)

3.4.3. Preparation of potato chips for analysis

Potato crisps were collected from different sites in Addis Ababa. Potato crisps (chips) were ground by using mortar and pistil. 5 grams from each ground sample of potato chips was weighed and 2.5 g ground was added in a 50 mL centrifuge tubes. 35 mL de-ionized H_2O, 0.5 g NaCl and 4 g $MgSO_4$ were added and shaked vigorously for 5 min. The reaction mixture was centrifuged at 1500 rpm for 30 min to get clear supernatant and to eliminate the solid part. The clear supernatant was transferred to a 50 mL separatory funnel and 20 mL hexane was added to extract acrylamide. The lower aqueous layer was collected in to a 50 mL vial covered by Aluminum foil. 1 mL dilute H_2SO_4 (10%, v/v) was added in to the vial. After shaking vigorously for 1min the vial was placed in refrigerator (4 ^oC) for 15 min. 1 mL of 0.5 M $KBrO_3$ and 2.5 g KBr powder were added to 5 g potato chips sample solution. The vial was shaked by hand vigorously for 5 minutes. The reaction mixture was allowed to stand for 12 hrs at 4 ^oC in refrigerator. 5 mL of 0.5 M $Na_2S_2O_3$ (sodium thiosulfate) solution was added to remove excess solution Br_2. After shaking for 1min the mixture was transferred to a separatory funnel. 30 mL ethyl acetate-hexane (4:1, v/v) was added and shaked to extract 2, 3-DBPA. The upper organic layer was filtered and dried over Na_2SO_4. The solution was filtered in to a flask. The separatory funnel and filter paper was rinsed by 15 mL ethyl acetate-hexane (4:1, v/v). Then the organic layer was concentrated on a rotary evaporator. The residue in a round bottom flask was dissolved in 5 mL hexane followed by a SPE clean up step. Then SPE column were conditioned with 5 mL acetone followed by 5 mL hexane. The sample mixture was transferred to the SPE column and eluted with 5 mL of acetone using a vacuum system. The eluent was collected in a 50 mL round bottom flask and evaporated to dryness on a rotary evaporator. The residue 2, 3-DBPA was dissolved in 1 mL ethyl acetate followed by adding 100 µL of triethylamine base. The solution was transferred from the round bottom flask in to a vial and stored for 12 hrs in refrigerator. The solution was filtered through 0.45 µm filter and analyzed by GCMS.

3.5. Methodology

3.5.1. GCMS analysis

GC-MS analysis was conducted using an Agilent technology 7820A GC and 5977E MSD systems which is equipped with auto sampler. Chromatographic separations were carried out using DB-1701 column with 30 m length, 0.25 mm internal diameter and 0.25 µm column phase thickness. Injection mode was split- less, helium was a carrier gas and 1 µL volume of the sample was injected to the inlet heated to 275 ^{o}C. Initial oven temperature was 60 ^{o}C with 2min hold then heated to 220 ^{0}C with ramp 20 ^{o}C/min and 3 ^{o}C/min to 240 ^{o}C. The samples were run on GCMS and their results were expressed as mean of concentration values± standard deviation (M± STD). Each sample was run in triplicate.

Figure 7. GCMS System

4. Results and Discussion

In this study, roasted potatoes were observed to produce acrylamide after frying and extraction. For potatoes chips, the detected level of acrylamide was significant amount. Higher levels of acrylamide were detected for fried potatoes at higher temperature when 1 µL sample solution of light yellow potato chips prepared from fresh palm oil and dark brown potato chips sample solution prepared from mixture of fresh and reused palm oil were separately injected to GCMS. The potato sample was derivatized in order to increase selectivity, sensitivity, volatility and to minimize interferences during analysis by GCMS. Attempt that was made to analyze acrylamide as it is by HPLC and GCMS was not as fruitful as analyzing the derivatized acrylamide by the GCMS. To get better results 5 g of potato chips from each sample was homogenized, extracted and analyzed. In the GCMS chromatogram and spectra, peak corresponding 2-bromopropene amide and mass fragments were clearly detected. Finally the concentration of acrylamide was deduced from the concentration of 2-BPA. In order to see the peaks without any doubt, ion extraction methods were used.

Equation of the calibration curve prepared from concentration of 2-BPA in ppm was put as follows.

$$Y = 69813X$$

Where, Y = Average peak area of 2-BPA obtained from triplicate samples of potato chips.

X = Average concentration of 2- BPA in ppm from triplicate samples.

69813 = Slope

The concentration of concentration of 2-BPA in ppm from potato chips was calculated as follows.

$$X = \frac{Y}{68913}$$

Mass of 2-BPA extracted from 1 µL (10^{-3} mL) solution of potato chips injected to GCMS calculated as

$$\text{mass of } 2-\text{BPA in 1 µL solution} = \frac{\text{ppm} \times \text{volume of solution injected}}{10^6}$$

$$\text{mass of } 2-\text{BPA in 1 µL solution} = \frac{\text{ppm} \times 10^{-3}\ \text{mL}}{10^6}$$

$$\text{mass of } 2-\text{BPA in 5 g potato chips or 1 mL solution} = \frac{\text{ppm} \times 1000\ \mu\text{L}}{10^6}$$

The mass of 2- BPA that was expressed by gram in 5 g potato chips or 1 ml solution can be converted in to microgram by dividing 10^{-6}.

$$\text{mass of } 2-\text{BPA in 1 Kg potato chips } = 200 \times \mu\text{g of } 2-\text{BPA in 5 g potato chips}$$

The mass of acrylamide (AA) in 1 kg of potato chips (µg of AA/kg) can be determined from µg of 2-BPA/kg by stoichiometric calculation as follows.

$$mass\ of\ AA\ in \frac{\mu g}{Kg} = \frac{mass\ of\ 2-BPA\ in\ 1\ Kg\ potato\ chips \times 71.08\ g/mol}{149.98\ g/mol}$$

Where, 71.08 g/mole = molar mass of acrylamide

149.98 g/mole = molar mass of 2- BPA

The following table shows the amount of acrylamide and 2-BPA in µg/kg detected in samples collected from different sites in Addis Ababa calculated based on the above formulas.

Table 4. Concentrations of acrylamide detected in potato chips (potato crisps) in µg/kg

NO	Sample Name	Sample Collected site	Number of Sample	Mean Conc. ±SD (ppm)	2-BPA Produced (µg/kg)	AA Produced (µg/kg)	AA levels reported[23] maximum (µg/kg)
1	S_4FOPC 1-3	4 Kilo	3	26.49±5.30	5299	2511	2510
2	S_4BOPC 1-3	4 Kilo	3	78.63±10.08	15726	7453	
3	S_5FOPC 1-3	Kebena	3	105.55±24.72	21111	10005	
4	S_5BOPC 1-3	Kebena	3	147.00±3.26	29400	13933	
5	S_6FOPC 1-3	6 Kilo	3	98.03±4.13	19607	9292	
6	S_6BOPC 1-3	6 Kilo	3	118.95±0.14	23790	11275	
7	S_7FOPC 1-3	5 Kilo	3	30.46±0.44	6093	2887	
8	S_7BOPC 1-3	5 Kilo	3	75.88±3.45	15178	7193	

Data in the table above shows different concentrations of 2-BPA and acrylamide in potatoes samples that were collected from different sites of Addis Ababa. The light yellow (golden) color potato chips (S_4FOPC 1-3 to S_7FOPC 1-3) contain lower amount of acrylamide than dark brown color potato chips (S_4BOPC 1-3 to S_7BOPC 1-3) which could be due to the amount of heat used and/or the time used for frying. The highest amount of acrylamide was detected in samples collected from Kebena while the lowest amount was detected in samples collected from Arat kilo. The amount of acrylamide produced from roasted potato chips, in all samples, were higher than the amount reported from WHO. The variation in concentration of acrylamide in samples collected from different sites could be due to

1. variation in nature (type) of potato used.
2. extent of heat applied during frying.
3. time taken to fry the chips which is possibly different from person to person.
4. thickness of the slices of the potato which may affect penetration of oil during frying.

The WHO recommendation of daily intake of AA per weight is 0.3 to 2.0 µg/kg. The level measured in potato chips purchased from street side in Addis Ababa city is between 2511 to 13933 µg/kg. The amount of acrylamide is 837 to 6966.5 time higher which potentially dangerous to human health. This data implies that one should be aware of the amount of chips that should be consumed on daily bases. Data obtained in this research is based on oil fried chips. In order to have broadened view of the situation, different types of chips need to be considered.

5. Conclusion

Acrylamide is formed in cooked starchy foods mainly by the reaction of the amino acid asparagine with reducing sugars such as glucose and fructose as part of the Maillard reaction. Potato chips (potato crisps) are among the food products that can contain high levels of acrylamide. For potatoes chips, the detected level of acrylamide was significant. Higher levels of acrylamide were detected for fried potatoes at higher temperature when 1 µL sample solution of light yellow potato chips prepared from fresh palm oil and dark brown potato chips sample solution prepared from mixture of fresh and reused palm oil were separately injected to GCMS. Potatoes samples that were collected from different sites of Addis Ababa contain different amount of acrylamide in most potato chips. The light yellow (golden) color potato chips (S_4FOPC 1-3 to S_7FOPC 1-3) contain lower amount of acrylamide than dark brown color potato chips (S_4BOPC1-3 to S_7BOPC 1-3) which could be due to the amount of heat used and/or the time used for frying. The potato sample was under go brominated type of derivatization to increase sensitivity and to minimize interferences during analysis of acrylamide by GCMS.

6. Recommendations

Based on the study of findings, in order to minimize the problem of roasted food products due to the presence of acrylamide the following are recommended.

- A large consumption of fried potato chips (potato crisps) should be avoided.
- Foods should not be over cooked or over fried at high temperatures above 120 ^{o}C.

7. References

1. Mottram, D. S.; Wedzicha, B.L.; Dodson, A. T. Acrylamide is formed in the Maillard reaction. *Nature.* **2002**, *419*, 448-449.
2. Stadler, R. H.; Blank, I.; Varga, N.; Robert, F.; Hau, J., Guy, P. A., Robert, M. C. Riediker, S. Acrylamide from Maillard reaction products. *Nature.* **2002**, *419*, 449-450.
3. Lyon, F. IARC monographs on the evaluation of carcinogenic risks to humans. *Some Ind. Chem.* **1994**, *60*, 389-433.
4. Erkekoglu, P.; Baydar, T. Toxicity of acrylamide and evaluation of its exposure in baby foods. *Nutr. Res. Rev.* **2010**, *23*, 323-333.
5. Hogervorst, J. G. The carcinogenicity of dietary acrylamide intake: a comparative discussion of epidemiological and experimental animal research. *Crit. Rev. Toxicol.* **2010**, *40*, 485-512.
6. Khalaf, H. H. A., Sharoba, A. M., El-Desouky, A. I., El-Bassiony, K. R. A., & Afifi, S. A. M. Effect of some pre-treatments on acrylamide concentration in potato chips. *Annals of Agric. Sci., Moshtohor*. **2015,** *53*, 211–220
7. Oracz, J.; Nebesny, E.; Zyzelewicz, D. New trends in quntification of acrylamide in food products. *Talanta.* **2011**, *86*, 23-34.
8. Tareke, E.; Rydbeg, P.; Karlsson, P.; Erikssin, S.; Tornqvist, M. Analysis of acrylamide, a carcinogen, formed in heated foodstuffs. *J. Agric. Food Chem.* **2002**, *50* 4998-5006.
9. WHO; Acrylamide Health and Safety Guide; *IPCS International Programme on Chemical Safety In Health and Safety Guide* **1991**, *45*.
10. Yaylayan, V. A.; Wnorowski, A; Perez Locas, C. Why asparagine needs carbohydrates to generate acrylamide. *J. Agric. Food Chem.* **2003**, *51*, 1753-1757.
11. Weisshaar, R.; Gutsche, B. Formation of acrylamide in heated potato products-model experiments pointing to asparagine as precursor. *Deutsche Lebensmittel-Randschau* **2002**, *98*, 397- 400.
12. Granvogl, M.; Jezussek, M.; Koehler, P.;Schieberle, P. Quantification of 3- Amino propionamide in potatoes: a minor but potent precursor in acrylamide formation. *J.*

Agric. Food Chem. **2004**, *52*, 4751-4757.

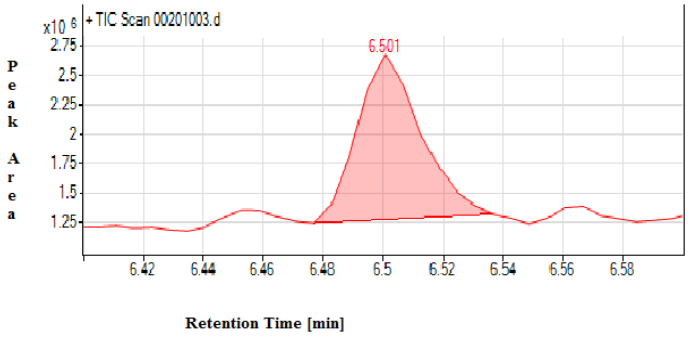
13. Tareke, E.; Rydberg, P.; Karlsson, P.; Eriksson, S.; Törnqvist, M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J. Agric. Food Chem.* **2002**, *50*, 4998-5006.
14. Van Boekel, M.; Fogliano, V.; Pellegrini, N.; Stanton, C.; Scholz, G.; Lalljie, S.; Somoza, V.; Knorr, D.; Jasti, P. R.; Eisenbrand, G. A review on the beneficial aspects of food processing. *Mol. Nutr. Food Res.* **2010**, *54*, 1215-1247.
15. Fazendeiro, M. S. DNA damage induced by acrylamide: Role of genetic polymorphisms in DNA damage levels. **2013**, 35-47.
16. Watzek, N. B. N 7-glycidamide-guanine DNA adduct formation by orally ingested Acrylmide in rats: a doseeresponse study encompassing human diet-related exposure levels. *Chem. Res. Toxic* **2012**, *25*, 381-390.
17. Pennisi, M. M. Neurotoxicity of acrylamide in exposed workers. *Int. J. Environ. Res. Public Health.* **2013**, *10*, 3843-3854.
18. Friedman, M. Chemistry, Biochemistry, and Safety of Acrylamide. A Review. *J. Agric. Food Chem.* **2003**, *51*, 4504-4526.
19. Tardiff, R. G. Estimation of safe dietary intake levels of acrylamide for humans. *Food Chem. Toxic* **2012**, *48*, 658-667.
20. Matthaus, B.; Haase, N.; Vosmann, K. Factors affecting the concentration of acrylamide during deep-fat frying of potatoes. *Eur. J. Lipid Sci. Tech.* **2004,** *106*, 793-801.
21. Zhiming G., Peng W., Aiming L. Determination of Acrylamide in Starch- Based Foods by HPLC with Pre-column Ultraviolet Derivatization. *J. Chromatogr. Sci*., **2011**, *49*, 818-824.
22. Food safety authority of Ireland. Acrylamide in food. Toxicology fact sheet series food. **2009**, *1*, 1-7.
23. Joint FAO (WHO) food standards committee. Discussion pape on acrylamide. Netherland: **2004**, *15*, 3.
24. Martins, S.; Jongen, W.; Van B. A review of Maillard reaction in food and implication to kinetic modelling. *Trends in Food Sci. Tech.* **2001**, *11*, 364-373.
25. Eriksson, S., Karlsson, P. Some analytical factors affecting measured levels of acrylamide in food products. *Chemistry and safety of acrylamide in food*. **2005**, New York: Springe, Boston, MA, 285–291.

26. Tekkeli, S.; Onal, C.; Onal, A. A review of current methods for the determination of acrylamide in food products. *Food Anal. Methods.* **2012**, *5*, 29-39.
27. Zhang, Y.; Zhang, G.; Zhang, Y. Occurrence and analytical methods of acrylamide in heat-treated foods: Review and recent developments. *J. Chromatogr. A.* **2005**, *1075*, 1-21.
28. Pittet, A.; Perisset, A. O. Trace level determination of acrylamide in cereal-based foods by gas chromtography-mass spectrometry. *J. Chromatogr. A.* **2004**, *1035*, 123-130.
29. Soare, C.; Cunha, S.; Fernandes, J. Determination of acrylamide in coffee and coffee products by GC-MS using an improved SPE clean-up. *Food Addit. Contam*. **2006**, *23*, 1276-1282.
30. Wenzl, T.; Karasek, L.; Rosen, J.; Hellenaes, K.; Crews, C. C.; Anklam, E. Collaborative trial validation study of two methods, one based in high performance liquid chromatography-tandem mass spectrometry and on gas chromatography-mass spectrometry for the determination of acrylamide in bakery and potato products. *J. Chromatogr. A.* **2006**, *1132*, 211- 218.
31. Becalski, A. S. Furan precursors in food: a model study and development of a simple headspace method for determination of furan. *J. AOAC. Int.* **2005**, *88*, 102-106.
32. Rosen, J.; Hellenas, K. Analysis of acrylamide in cooked foods by liquid chromtography tandem mass spectrometry. *Analyst*. **2002**, *127*, 880-882.
33. Tateo, F.; Bononi, M. A GC-MS method for the routine determination of acrylamide in food. *Ital. J. Food Sci.* **2003**, *15*, 149-151.
34. Zhang, Y. J. An improved method validation for rapid determiantion of acrylamide in foods by Utra-performance liquid chromatography combined with tandem mass spectrometry. *J. Chromatogr. A.* **2007**, *1142*, 194-198.
35. Yamazaki, K. A method for the determination of acrylamide in a broad variety of processed foods by GC-MS using xanthydrol derivatization. *Food Addit. Contam.* **2012**, *29*, 705-715.
36. Xu, L. Z. Determination of trace acrylamide in potato chips and bread based on SPE and HPLC. *J. Chromatogr. A.* **2012**, *75*, 269-274.
37. Bortolomeazzi, R.; Munari, M.; Anese, M. V. Rapid mixed mode solid phase extraction method for the determination of acrylamide in roasted coffee by HPLC-MS/MS. *Food Chem.* **2012,** *135*, 2687-2693.

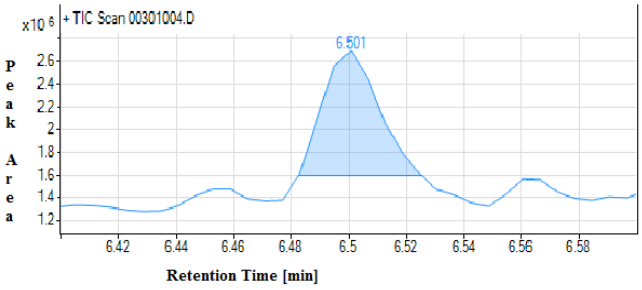
38. Xu, L. Q. Preparation of a hydrophilic molecularly imprinted polymer and its application in solid- phase extraction to determine of trace acrylamide in foods coupled with high-performance liquid chromatography. *Food Anal. Methods.* **2013**, *6*, 838-844.
39. Pedreschi, F.; Kaack, K.; Gramby, K. Reduction of Acrylamide formation in patato slices during frying. *Lwt-Food Sci Technol.* **2004**, *37*, 679-685.
40. Ishihara, K.; Matsunaga, A. N.; Koga, H. Examination of conditions inhiniting the formation of acrylamide in the model system of fried potato. *Biosci. Biotechnol., Biochem.* **2006**, *70*, 1616-16211.
41. Low, M.; Koutsidis, G.; Parker, J.; Elmore, J.; Dodson, A.; Mottram, D. Effect of cotric acid and glycine additional on acrylamide nad flavour in a potato model system. *J. Agric. Food Chem.* **2006**, *54*, 5976-5983.
42. Capuano, E.; Ferrigno, A.; Acampa, I.; Serpen, A.; Açar, Ö.; Gökmen, V.; Fogliano, V. Effect of flour type on Maillard reaction and acrylamide formation during toasting of bread crisp. *Food Res. Int.* **2009**, *42*, 1295-1302.
43. Capuano, E.; Ferrigno, A.; Acampa, I.; Ait-Ameur, L.; Fogliano, V. Characterization of Maillard reaction in bread crisps. *Eur. Food Res. Technol.* **2008**, *228*, 311-319.
44. Vass, M.; Amrein, T.; Schonbachler, B.; Escher, F.; Amadò, R. Ways to reduce acrylamide formation in cracker products. *Czech. J. Food Sci.* **2004**, *22*, 19-21.

8. **Appendices**

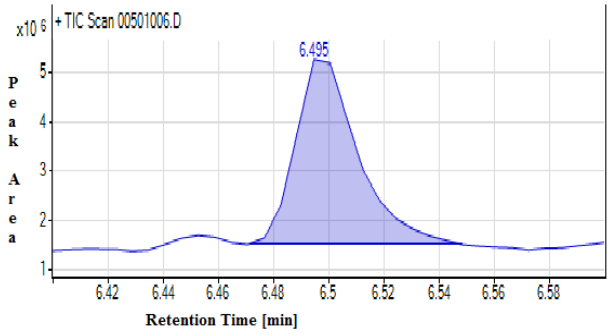
Appendix 1. GCMS Chromatogram of 2-BPA for S_4FOPC-1 and 2



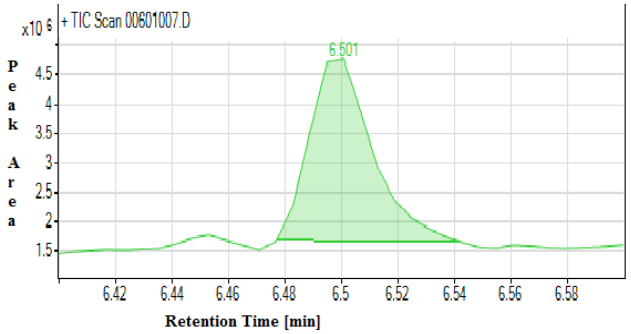
Appendix 2. GCMS Chromatogram of 2-BPA for S_4FOPC-3



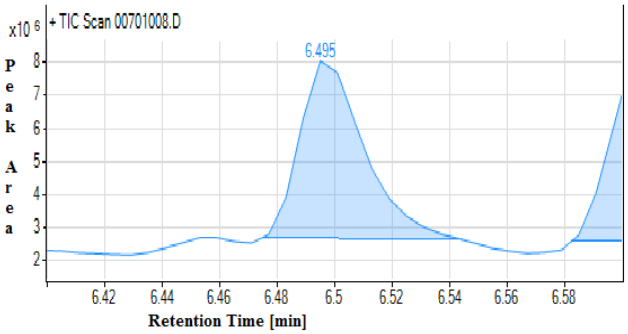
Appendix 3. GCMS Chromatogram of 2- BPA for S_4BOPC-1 and 2



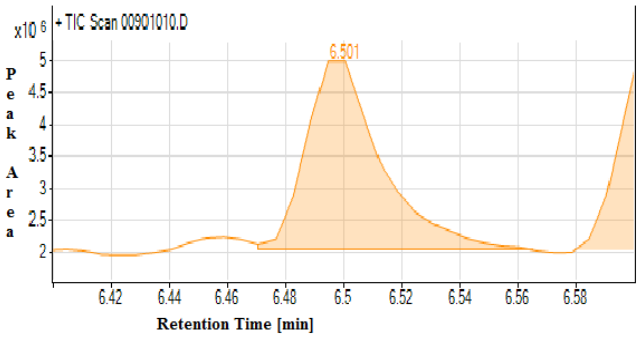
Appendix 4. GCMS Chromatogram of 2-BPA for S_4BOPC-3



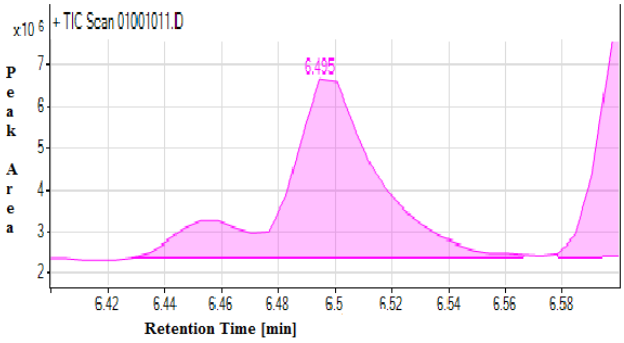
Appendix 5. GCMS Chromatogram of 2-BPA for S_5FOPC-1 and 2



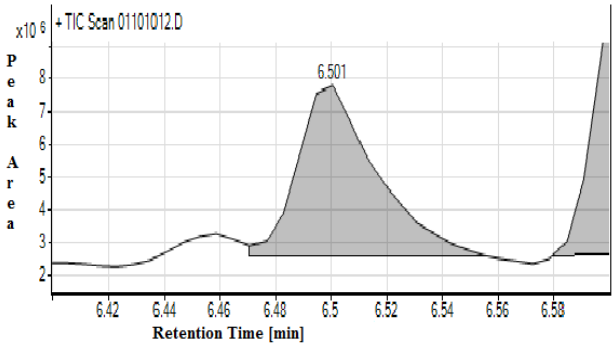
Appendix 6. GCMS Chromatogram of 2-BPA for S_5Fopc-3



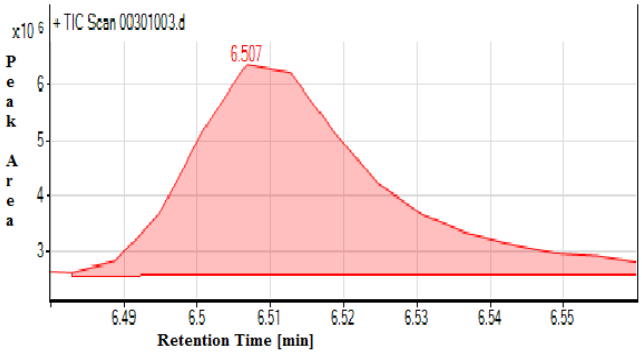
Appendix 7. GCMS Chromatogram of 2-BPA for S_5BOPC-1



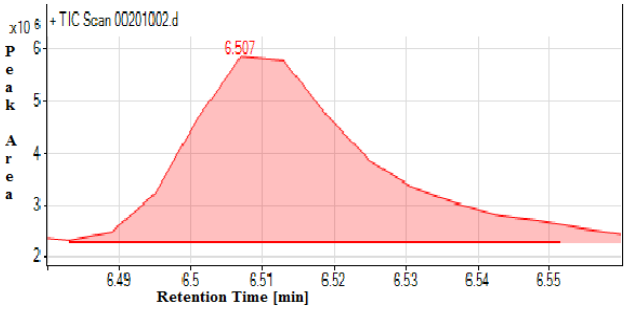
Appendix 8. GCMS Chromatogram of 2-BPA for S_5BOPC-2 and 3



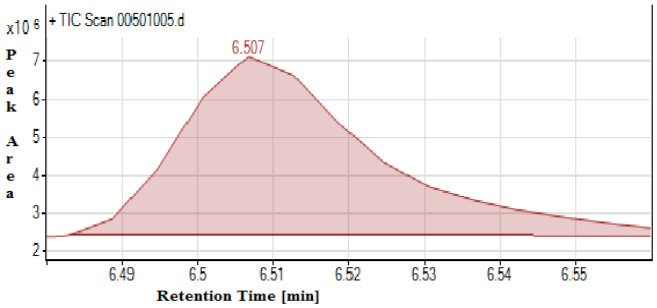
Appendix 9. GCMS Chromatogram of 2-BPA for S_6FOPC-1 and 3



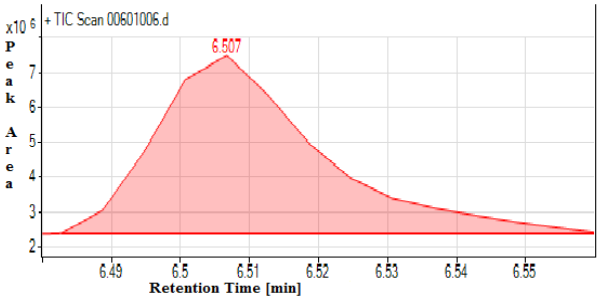
Appendix 10. GCMS Chromatogram of 2-BPA for S_6FOPC-2



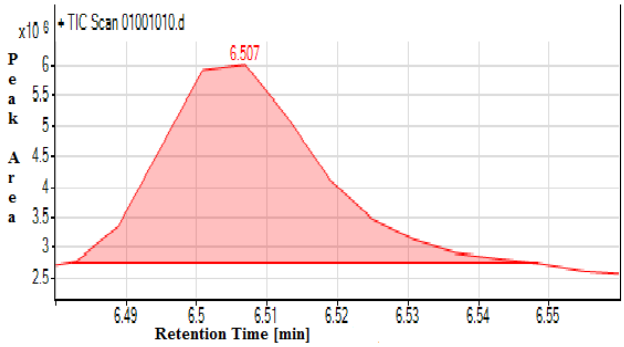
Appendix 11. GCMS Chromatogram of 2-BPA for S_6BOPC-1 and 2



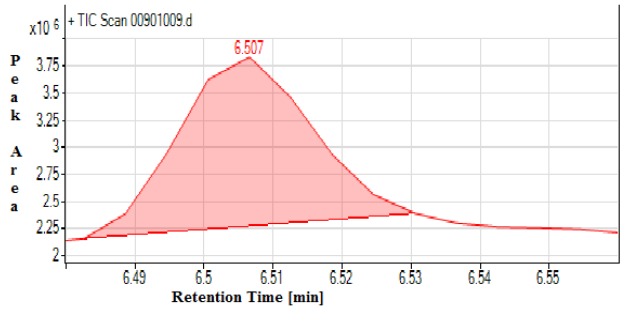
Appendix 12. GCMS Chromatogram of 2-BPA for S_6BOPC-3



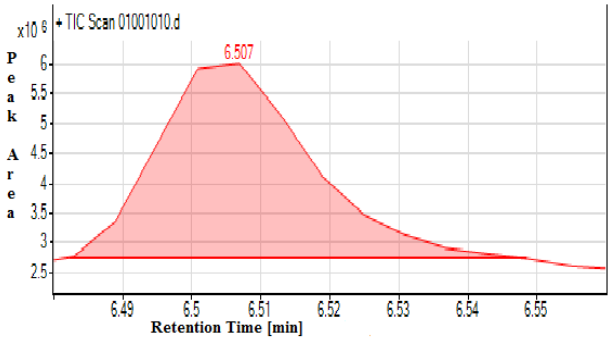
Appendix 13. GCMS Chromatogram of 2-BPA for S_7FOPC-1 and 2



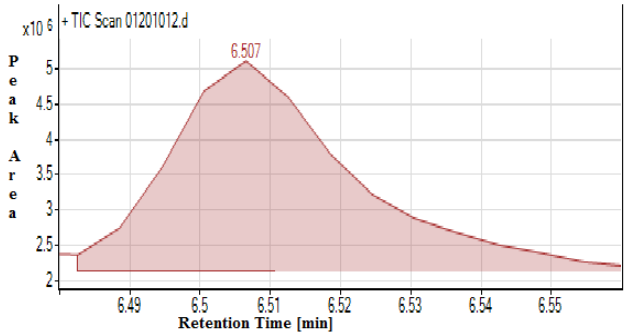
Appendix 14. GCMS Chromatogram of 2-BPA for S_7FOPC-3



Appendix 15. GCMS Chromatogram of 2-BPA for S_7BOPC-1



Appendix 16. GCMS Chromatogram of 2-BPA for S_7BOPC-2 and 3



Appendix 17. Some of the apparatus used in the study

Centrifuge with centrifuge tubes

Mortar and pistil

Measuring cylinder, Flasks, Funnel, SPE, Rota evaporator, vials and Centrifuge tubes