Effect of cooking medium (type of oil/fat) on concentration of acrylamide in *Solunum tuberosum* Products.

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DOI: 10.29322/IJSRP.15.04.2025.p16017
https://dx.doi.org/10.29322/IJSRP.15.04.2025.p16017

Paper Received Date: 17th March 2025
Paper Acceptance Date: 16th April 2025
Paper Publication Date: 21st April 2025

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ABSTRACT

Acrylamide is a neurotoxic compound classified as a probable human carcinogen and genotoxicant [1&2] Historically, acrylamide as a contaminant was only thought to be an issue in water and its potential exposure to humans and was not of serious concern[1]. However, the recent discovery of acrylamide formation in certain fried and baked foods and its rather high levels (concentrations ranging up to 10 mg/kg) found in highly consumed food products, such as potato chips and crisps [3], brought acrylamide to the forefront of interest among scientists, regulators, the industry, and consumer groups. This study therefore aimed at creating awareness of the presence of acrylamide in tuber products to both the consumer and producer through analysis of the levels and the factors that affect the levels. The study focused on effect of cooking media and additives in the levels of acrylamide in fried potato chips and crisps. In the analysis, five different types of cooking media (Oil/Fat) were used. Olive oil cooked chips and crisps were found to be having the highest amount of acrylamide 4.420 ± 0.01 mg/kg while cooking fat recorded the lowest amount of 3.213 ± 0.01 mg/kg. . Possibly, eating patterns should be devised to reduce consumption of these products in order to eliminate accumulation in the body.

Key words: Acrylamide, Carcinogen, Genotoxicant, acrolein, Maillard & Isotopomers

Introduction

Research has shown that cooking of starchy food could be an important source of acrylamide formation[4&5]. Recent estimates show that retail sales of potato chips, one of the most popular potato-based processed product, in the USA total around $ 6 billion/ year, representing about 33 % of the total snacks in the US market. All these factors might have contributed to increasing potato consumption in developing countries; it increased from 9 kg capita during the early sixties to 14 kg capita by the close of the last millennium. It still represents only a fraction of per capita consumption level of 63 kg capita in North America and 86 kg capita in Europe.

Fried potato products including crisps have been reported to contain high levels of acrylamide, a carcinogenic substance of great concern not only to consumers, but also to the authorities and the food industry [6&7]. Acrylamide has been classified and remains a suspected human carcinogen and a neurotoxicant that calls for a concentrated effort to minimize its presence in all human diets [7,8 & 9].

After ingestion, acrylamide is rapidly absorbed and distributed in animals and humans throughout the whole body. It can be found in many body parts including the thymus, liver, heart, brain, kidneys, human placenta and breast milk and thus can be transferred to the foetus or new born babies [10&11]. In experimental studies done in animals acrylamide has been shown to be genotoxic attributed to its main metabolite, glycidamide [12, 13 & 14] which is mutagenic[15,16&17]. In pregnant women, the foetus is also exposed to acrylamide through transplacental transfer [19 & 20] and to glycidamide after maternal metabolisation of acrylamide [20].

Potato as foodstuff is usually consumed in three major forms; fried chips, fried crisps or boiled. Crisps are fragile but firm slices that have been processed through deep frying and edible salt or acceptable food grade spices colour and flavour may have been added [21 & 22]. On the other hand, potato chips are long; thinly cut slices which have been deep fried [23 &24].

Despite the various calls by government bodies concerning acrylamide levels in food, it is important for the producers and consumers to be aware of the safety of the food in terms of levels and also know the variability of acrylamide in different conditions of either cooking, food processing or type of media influence on the levels. However, there has been scanty research done locally and there is very little or no information on African bodies on acrylamide management.

However, there has been scanty research done locally and there is very little or no information on African bodies on Acrylamide Management. There is little awareness of acrylamide formation, consumption and its effects onto the human body by both the producers and consumers. This study aimed at establishing the content of acrylamide in potato chips and crisps sold in a section of the Kenyan market in order to bring to books and to create awareness of any health risk to individuals and to the general public that may arise from uncontrolled consumption of potato chips and crisps.

Potato products are the easiest in processing and in consumption because they do not need much therefore, termed as fast foods. Despite the ease in production, little has been done on the effect of levels of acrylamide by use of different types of cooking media or heating media like firewood, gas, jiko and electricity in terms of varying temperature towards chips and crisps processing.

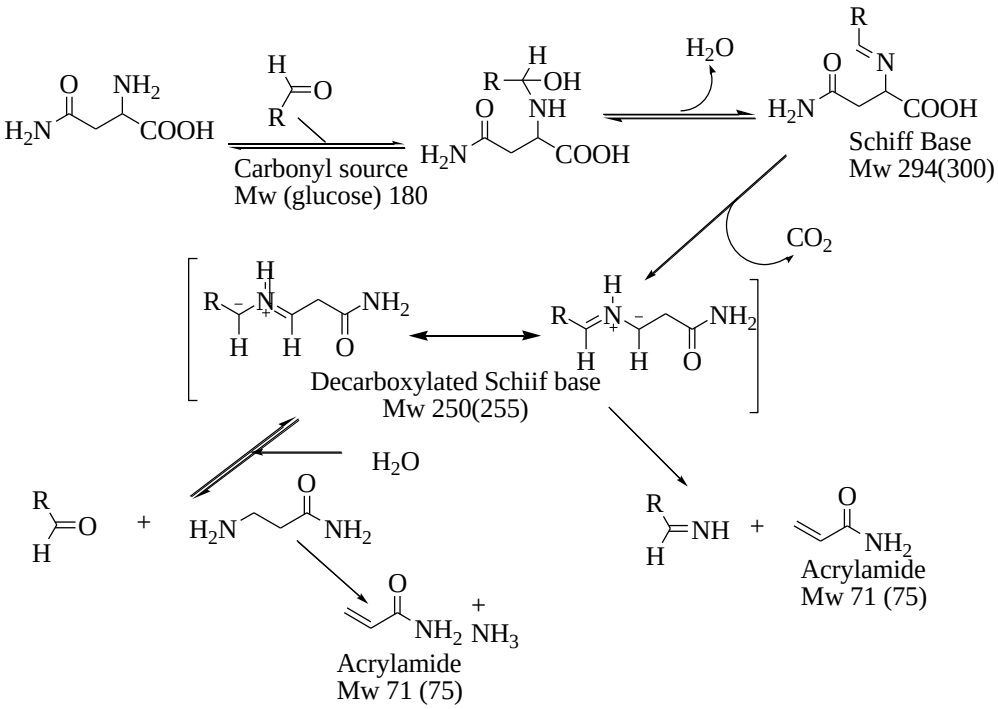
Chips and crisps are produced through use of oil in the form of deep frying though little research has been done on the effect of cooking media in deep frying in acrylamide formation. There are different types of cooking media produced by different companies, that is, cooking oil, cooking fat, vegetable oil and even most recently transformer oil. Therefore, there is need to a certain and clarify on the issue of cooking media towards acrylamide levels.

There is need for research on the effect of pre additives and post additives in crisps and chips processing as an effort to minimize the acrylamide in food. Further research should be done through the earlier mentioned gaps to advice the population of consumption patterns or dietary habits through calculation of per plate and per sachet consumption on a day. The levels of acrylamide can be affected if the major reactants in the Maillard reaction are altered therefore, there is need to do research on the primary effects of acrylamide formation by checking on the effect of maturity of the raw potatoes as part of the constituents of Maillard reaction.

Following a call by WHO/JECFA[25]on continued research concerning the high levels of acrylamide in starchy food, different regions in the world have been and are still doing more research to an extent of documenting various rules to govern their cooking some of them include; Europe with Acrylamide toolbox, USA_FDA Acrylamide information on diet, China with the Hapten synthesis, India with All food Processors Association. Therefore, with this in mind, there is need for establishing the content of acrylamide in potato chips and crisps sold in a section of the Kenyan market in order to bring to books and to create awareness of any health risk to individuals and to the general public that may arise from uncontrolled consumption of fried potato products.

Formation of Acrylamide

Acrylamide is metabolized in the body to glycidamide, a reactive compound formed through epoxidation of the double bond (Scheme 1)[4,26 & 27].



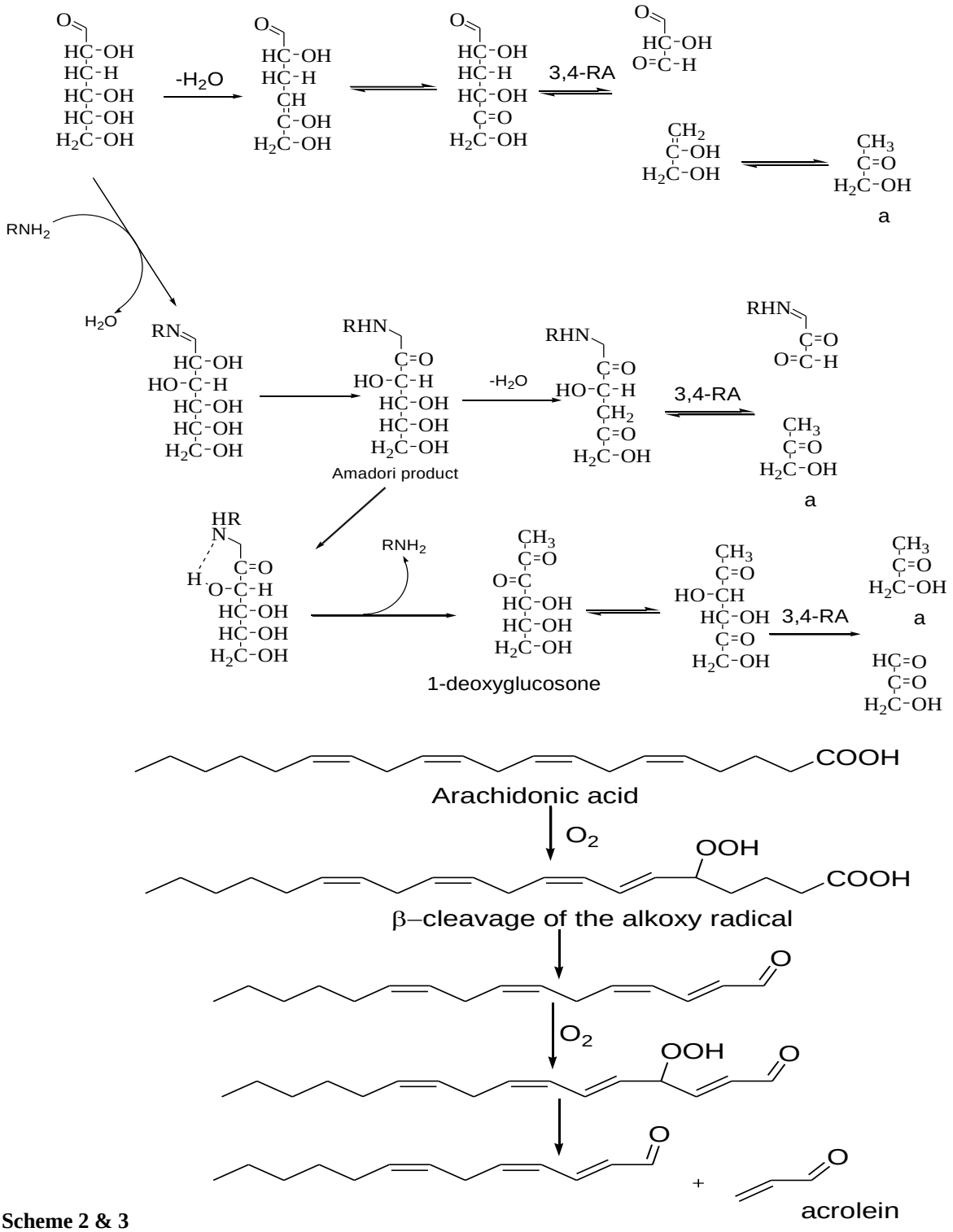
Scheme 1: Biosynthesis of Acrylamide

Acrolein (2-propenal, CH_2CHCHO) is a very probable precursor of acrylamide. Simple, fundamental chemical transformations (such as reaction with ammonia liberated from amino acids) can be converted to acrolein then to acrylamide. The production of acrylamide through the reaction of acrolein with ammonia can be anticipated. Alternative formation mechanisms of acrylamide do not necessarily involve acrolein.

The sources of acrolein that are most relevant to human exposure and toxicity can be grouped into dietary, endogenous, and environmental sources. The ubiquitous presence of acrolein is attributed to incomplete combustion of petrol, wood, and plastic, to smoking of tobacco products, frying of foods in oils, endogenous lipid peroxidation, and to endogenous polyamine metabolism [28 &29].

Heating or baking of carbohydrate-containing foods results in the formation of reactive carbohydrate intermediates that can undergo carbon-carbon cleavage or react with amino acid residues in proteins. For example, heating of glucose may result in loss of the hydroxyl group at position 4 through dehydration, which would yield the appropriate β-hydroxy ketone moiety for release of the acrolein precursor, hydroxy acetone, *via* retro aldol cleavage of the 3,4-bond.

In support of this fragmentation pathway, thermal degradation studies of the Amadori product resulting from the reaction of single ^{13}C-labeled glucose isotopomers with alanine yielded hydroxy acetone that contained carbon atoms 4, 5, and 6 of glucose[15 & 30]. A more prominent pathway produced hydroxy acetone from the C1-C3 fragment of glucose, which the authors rationalized by generation of 1-deoxyglucosone from the Amadori product followed by 3, 5-enolization and retro aldol cleavage of the 3, 4-bond. The next Scheme 2 & 3 shows formation of acrolein from glucose via hydroxyl acetone (a) RA, retro aldol-cleavage.

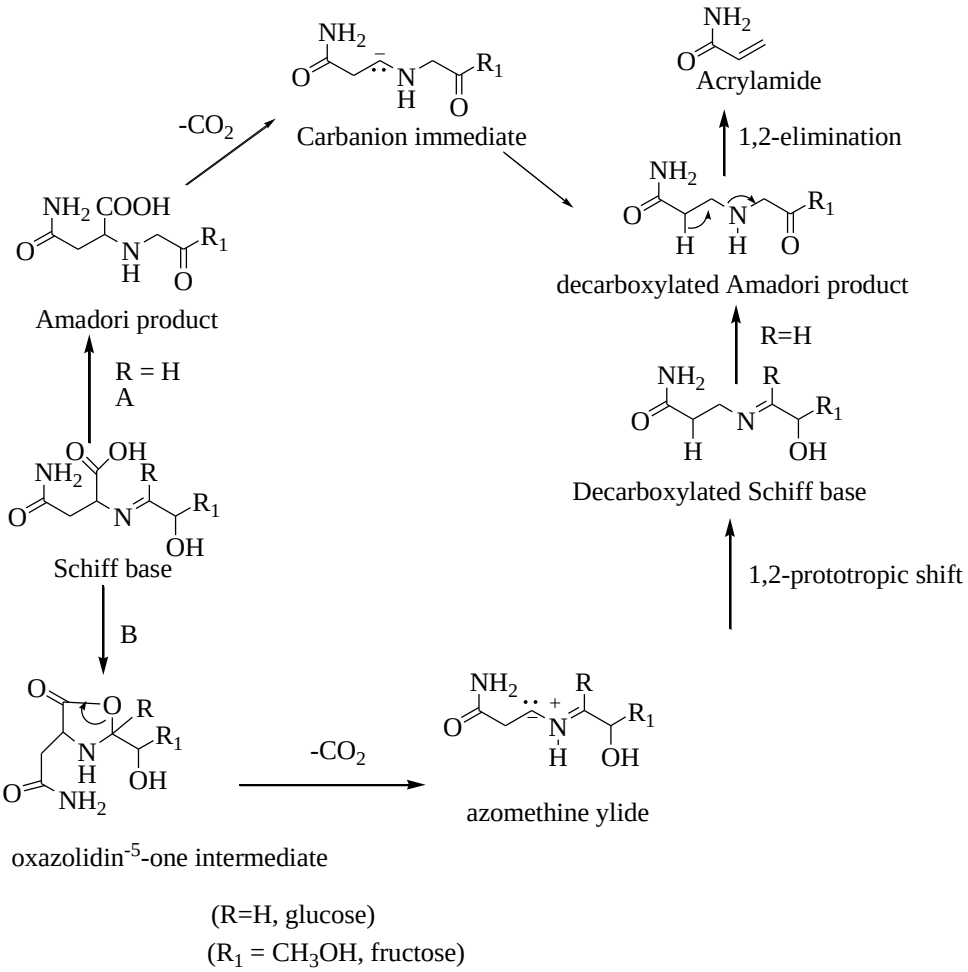


Scheme 2 & 3

Formation of acrolein from glucose via hydroxyl acetone (a) RA, retro aldol-cleavage

Acrolein arises from degradation of amino acids and proteins degradation of carbohydrates and the MR between amino acids or proteins and carbohydrates. Many possible routes for the formation of this three-carbon aldehyde, taking the starting point from many different sugars or amino acids may be proposed. Its formation from methionine by the Strecker degradation in the frame of the MR is one example. Alanine, with its three-carbon skeleton, has also been suggested as a possible source. However, reactions of longer carbon chains are common and well known, so at present there is no basis to give priority to any specific reaction routes [15 & 30].

Since sugar dehydration and Maillard reactions occur simultaneously in thermally processed foods, the contribution of HMF and other reactive carbonyls for example from sugar dehydration should be considered in the AA risk assessment. Foods that contain relatively high amounts of HMF include dark roasts coffees and malt. The finer detail of the mechanism of AA formation is evidently complex and still under discussion. The relative amounts of AA and 3-APA formed may depend not only in the nature of the carbonyl compound that reacts with Asn but also on conditions of temperature, time, water content, pH and the physical state of the reactants [28 & 31]. The next structure represents the mechanism of formation of acrylamide through thermally induced decarboxylation of Amandori products.



Scheme 4: Mechanism of formation of acrylamide through thermally induced decarboxylation of Amandori products

Studies of the thermal degradation of 3-APA to AA under aqueous or low water conditions at temperatures between 100°C and 180°C have demonstrated that it is a very effective precursor of AA in heated foods [32 & 33]. Granvogl [32] found that heating of 3-APA in aqueous model systems always generated more AA than in the same reaction using Asn the highest yields were circa 28 mol% in the presence of carbohydrates (170°C aqueous buffer) and about 63 mol% in the absence of carbohydrates under the same conditions.

Heating of propanoic acid amides with an amino or hydroxyl group in the α-position, for example, 2-aminopropionamide were ineffective in generating AA indicating that elimination of the amino group occurred only at the β-position. Zamora [34] studied the deamination reaction in model systems of 3-APA and 3-(alkylamino) propionamides (benzyl, phenylethyl, butyl, and octyl). The formation of AA occurred at almost neutral or basic pH values and yields appeared not only to depend on the type of aminopropionamide involved, but also on the water activity.

The occurrence of 3-(alkylamino) propionamides in foods is not known. On the other hand, the role of carbonyl compounds in the AA produced, appeared to have less impact than either the type of amine or the water activity. Cai [35] found that chlorogenic acid, present at high concentrations in some raw foods, could influence AA formation. Factors that appeared to favour increased AA formation from Asn/glucose model systems in the presence of chlorogenic acid included: increased formation of HMF, which acts as a more efficient precursor than glucose to form acrylamide; decreased activation energy for conversion of 3-APA to AA (from 173.2 to 136.6

kJ/mol), and hence increased rate of deamination; a high redox potential during the Maillard reaction which may prevent the destruction of AA by free radicals. In addition to the non-enzymatic (Maillard) decarboxylation reactions of Asn, Granvogl[32] proposed a biochemical pathway to 3-APA involving decarboxylases present in raw materials. The next scheme represents biochemical pathway for the conversion of Asn into 3-APA.

The application of food processing practices to generate MRPs can improve the oxidative stability of food products, and preserve food from oxidation and microorganism contamination as well [36]. Maillard reaction is responsible for many flavors and colours in foods, such as coffee roasting, browning of various meats when seared or grilled, browning and umami taste in fried onions. It contributes to the darkened crust of baked foods, the golden-brown colour of potato chips and other crisps, of malted barley as found in malt beer and whiskey, and the colour and taste of dried and condensed milk, confection milk toffee, chocolate, roasted peanuts and black garlic.

Acrylamide (AA) formation depends on the system used in terms of constituents, such as oil oxidation and type, and processing conditions. Findings from model potato system suggested that lipid oxidation products do not affect AA formation [37]. However, studies carried out on baked cookies concluded that lipid oxidation products should be considered as an important factor in AA formation during baking of fat-rich products but only after prolonged heating time [38].

Similarly, in fat-rich model systems, an increase in AA formation paralleling oil oxidation was observed, particularly in sugar-free systems [39]. As far as the oil type is concerned, the use of olive oil resulted in some- what higher AA contents than frying in corn oil [4]. In contrast, the use of palm and vegetable oil does not significantly influence AA formation neither in fried potato crisps [40] nor in artificial potato products heated in a closed stainless steel tubular reactor [41].

Research design

The study adopted an experimental design which involves quantitative data collection methods through laboratory analysis. The samples were prepared before analysis to avoid contamination and ease the analytical procedure on the instrument.

Instrumental and Apparatus

A 4-Digit Laboratory Weighing Balance Electronic Analytical Scale Four Decimal, model FA2004B from China was used for weighing. Detection and quantitation of acrylamide in chips and crisps were determined using High performance liquid chromatography (HPLC). Agilent 1100 (Waldbronn, Germany) HPLC system consisting of a quaternary pump with vacuum degasser, a DAD was used. Chromatographic separations were performed on an ODS-3 C^{18} column (250 mm × 4.6 mm, Intersil, Japan).

Sample concentrations were conducted on RE-2000 rotary evaporator (Shanghai Yarong Biochemical Apparatus Company, limited, Shanghai, China) and the solution thoroughly mixed using a Vortex mixer (Shanghai Qite Analytical Apparatus Company, limited, Shanghai, China). This was done before analysis.

A HL-2070 multi-function food processor (Shanghai Herine Electric Appliance Company limited, Shanghai, China) was used to pulverize and homogenize samples. Sample extractions were performed using HS2060A ultrasonic shaker (Kunshan Ultrasonic Instrument Company limited, Kunshan, Jiangsu, China).

Centrifugal separation was carried out by using A Refrigerated Centrifuge (Biofuge stratos, Germany). While, the residue was conditioned through a carboPrep™ 200 SPE made in Germany, Filtered using 0.2 µm micro-filters both made in the USA. A 60 ml separatory funnels, 50-ml round-bottom flasks, brown glass tubes, autosampler vials, a stainless steel pan with 6 litre capacity, 24.3 cm inner diameter and 0.5 cm thickness stainless steel lid with hermetic property and an electrical heater (model thermal, HK3-2, Germany) while, Accu-BOND Si (6 ml, 500 mg) solid-phase extraction (SPE) cartridges were supplied by Agilent Technologies (Santa Clara, CA) were used.

Reagents, Chemicals and reference materials

All solvents and chemicals used in the analysis procedure were of analytical grade. They were purchased from Merck (Darmstadt, Germany). Analytical water grade was used since the instruments were highly sensitive and to avoid breakdown after calibration for the HPLC instrument, analytical grade of HPLC water was used.

Acrylamide (standard) (99%) and $^{2}H_{3}$ -labeled acrylamide (isotopic purity 98%) was purchased from Sigma-Aldrich (St. Louis, MO) and Cambridge Isotope Laboratories (Andover, MA), respectively. Methanol (HPLC-grade) was supplied by Merck (Darmstadt, Germany). Water was purified with a Milli-Q system (Millipore, Bedford).

Standards and reagents stock solution of acrylamide (1 mg/ml) and $^{2}H_{3}$ –labeled acrylamide (0.1 mg/ml) were prepared by dissolving suitable amount of the compounds in water. These solutions were then appropriately diluted with water to prepare working standards at 10 and 4 µg/ml, respectively. All stock solutions and working standards were kept at 4°C for a month.

Sample preparation

Potato chips and crisps samples were prepared for acrylamide analysis by weighing 1 g of crushed potato chips/crisps and mixing with 10 ml methanol on a wrist action shaker for 20 minutes to enable the sample to fully soak in the extract solution. The samples were refrigerated for 2 minutes for easier extraction of the oily top layer to avoid interferences. The supernatant was filtered through a 0.20 μm nylon syringe (silica based) filter and the filtrate discarded and the residue stored for further cleanup and analysis.

The residue was conditioned through a carboPrep™ 200 SPE (silica based) tube using, 6 ml of the sample in 2 ml acetone and 2 ml methanol. The sample solution was allowed to pass through the tube by gravity and 0.5-1.0 ml water was run through the tube to wash the sample. A vacuum pump was used to dry excess water from tube for 1 minute followed by elution with 2 ml of acetone through gravity therefore ready for analysis in the instrument. Many sample extracts can be analysed directly, however, sample cleanup and solvent pre-concentration was essential.

Effect of cooking media on Acrylamide levels

Five different types of cooking media (Fats/oils) were randomly chosen and purchased from the local retailers potato samples were randomly collected and prepared as per the standard size of both chips and crisps. They were further cooked in the various cooking media earlier identified. Cooking was done and later on prepared and analysed using an HPLC for quantification of the amount of acrylamide present. The cooking media were identified as shown in Table 1.

Table 1. Types of cooking medium

S/No.	Medium	Chips (Identification)	Crisps (Identification)
01	Olive oil	CO4	CCO4
02	Corn oil	CO1	CCO1
03	Castor oil	CO2	CCO2
04	Vegetable oil	CO5	CCO5
05	Cooking fat	CO3	CCO3

RESULTS AND DISCUSSION

Introduction and preparation for analysis

The samples were obtained as described earlier in the regions identified, labelled and analysed based on the methods discussed earlier. Analysis was done based on the objectives described earlier. Blank solutions (blank chromatograms) that were done every after change of the blank solutions that were used in sample preparations, different dilutions of the standards varying from 30 ppm, 20 ppm, 10 ppm, 5 ppm, 2.5 ppm and 1 ppm. Increase in dilution led to a better peak area. A dilution of 20 ppm was found appropriate for use while preparing standards. Figure 1 represents 20ppm dilution standard.

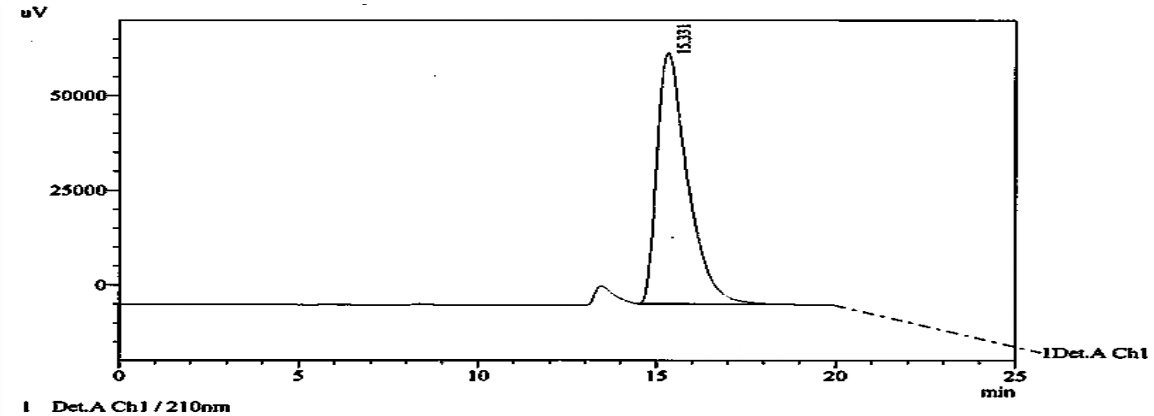


Figure 1 20ppm standard

Further, application of the results gave a graph with the best of fit which resulted in a quadratic equation that gave a guideline towards determination of individual concentrations of the samples that were analysed.

Quality control measures

Limits of Detection and Quantification (LOD & LOQ)

The samples purged with helium appeared to be having a negative peak. Based on these results, the peak appeared due to a difference in the amount of dissolved oxygen in the mobile phase and sample solution. This was observed and Figures 2 and 3 respectively, illustrating chromatograms of limits of detection.

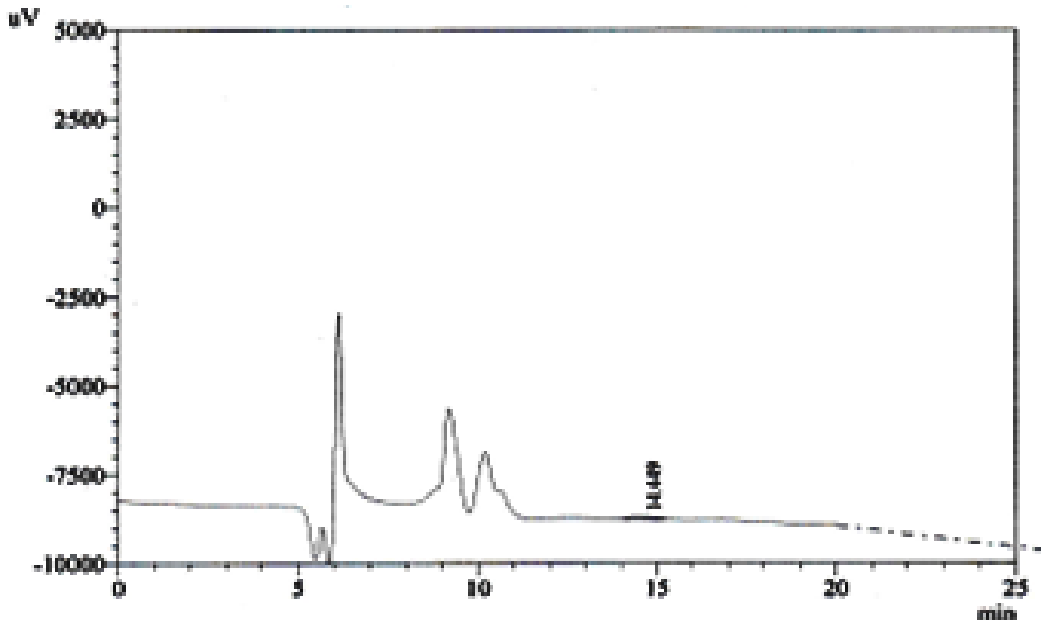


Figure 3 Limits of detection.

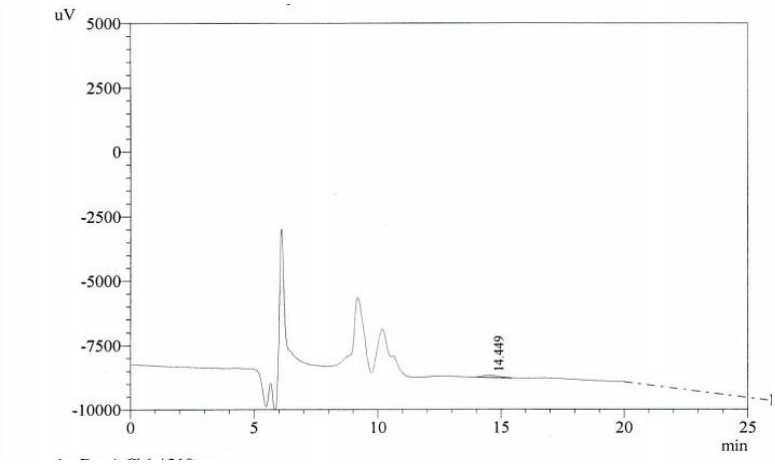


Figure 2 Limits of Quantification

The difference in peak areas in LOD could be attributed to the calibration and difference in size of the volumetric flask since many dilutions were prepared separately before analysis and this could be accounted for by the differences in cooking and processing parameters, methods, type and quality of raw materials, as well as formulations.

Percentage recovery

This was done in terms of dilutions and the 100% dilution was made up of 20 ppm and out of it, diluted to; 80%, 50%, 30%, 10% and 5%. This means if 20 ppm of a sample solution was used then the output was 100% recovery but if it was a dilution of the 20 ppm it took another percentage recovery depending on the dilution that is 10 ppm represents 50% recovery. Percentage recovery values against concentrations are given in Table 4.1.

Table 2 Percentage (%) recovery values verses concentrations

S/no.	% recovery	Peak area	AA. Conc. (mg/kg)	S.D
01	5	102706	5.8780	0.096
02	10	201457	11.5296	0.415
03	30	574234	32.8641	0.259
04	50	1164766	66.6610	0.079
05	80	1513534	86.6213	0.005
06	100	2282495	130.6300	0.9296

Figure 4 shows an increase in percentage recovery due to increase in concentration. Similarly the percentage recovery decreased with the decrease in concentration.

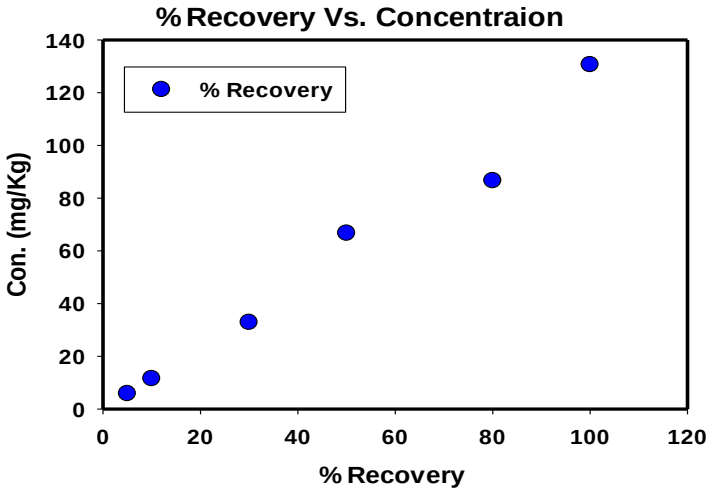


Figure 4: Concentration verses percentage recovery

Results

Effect of cooking medium

Table 3 represents acrylamide levels in cooked potato chips and crisps in different types of cooking medium.

Table 3 Acrylamide levels in different types of oils for chips

Oil type	Peak 1	Peak 2	Peak 3	Mean Peak	Conc. mg/kg
Corn oil	74304	74322	74190	74272	4.251
Castor oil	65897	66678	68128	66901	3.829
Cooking fat	56798	55475	56129	56134	3.213
Olive oil	77190	76897	77585	77224	4.420
Vegetable oil	61245	59896	61289	60810	3.480

Figure 5 represents acrylamide levels in different types of cooking medium in chips.

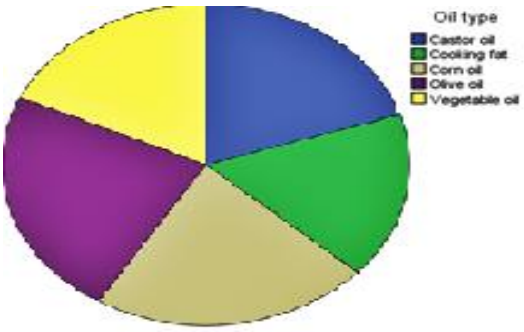


Figure 5 Acrylamide levels in chips in different media

From the figure, there is little difference in acrylamide levels between the different types of oils. However, olive oil seemed to have had a little high (4.420 mg/kg) amount compared with that of cooking fat (3.213 mg/kg) which had the lowest amount among the five

samples of cooking media in chips. This could be due to the effect of temperature such that olive oil absorbs high temperature within a short time compared with cooking fat which takes time to first dissociate into liquid then gain heat to cook. Statistical analysis of the data on different cooking medium was done and tabulated in Table 4.

Table 4 Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Peak 1	5	56798.0	77190.0	67086.800	8595.9915
Peak 2	5	55475.00	76897.00	66653.6000	9142.73555
Peak 3	5	56129.00	77585.00	67464.2000	8870.27991
Grand mean				**156228.5**	

From the descriptive data statistics Figure 6 represents analysis in chips cooking media.

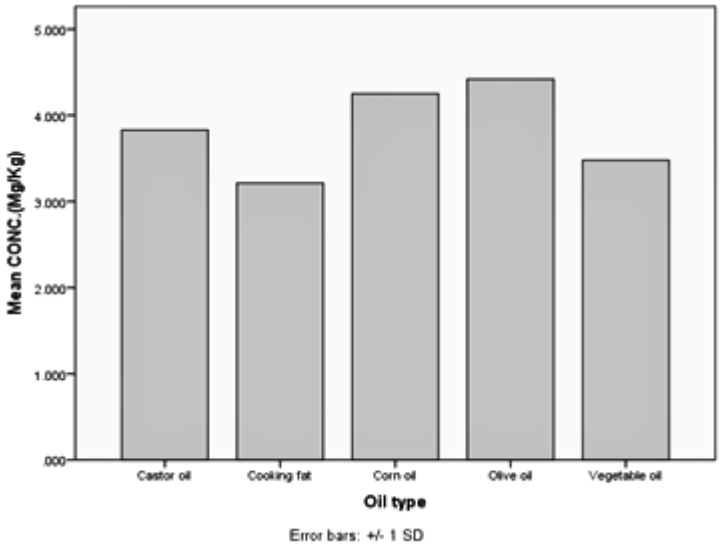


Figure 6 Analysis in chips cooking media

From the analysis of chips there seemed to be little or no effect since this were within the error of +/- 1 and statistically they were termed to be equal. Therefore, giving a conclusion of no effect of cooking media on levels of acrylamide. Table 5 represents acrylamide levels in different types of oils for Crisps.

Table 5 Acrylamide levels in different types of oils for Crisps

Oil type	Peak 1	Peak 2	Peak 3	Mean Peak	Conc. mg/l
Corn oil	1041279	1041465	1041408	1041384	59.600
Castor oil	963797	963986	962420	963401	55.137
Cooking fat	845849	846653	845999	846167	48.427
Olive oil	929798	928443	928162	928801	53.156
Vegetable oil	882676	882197	881811	882228	50.491

Figure 7 represents acrylamide levels in different types of cooking media in crisps.

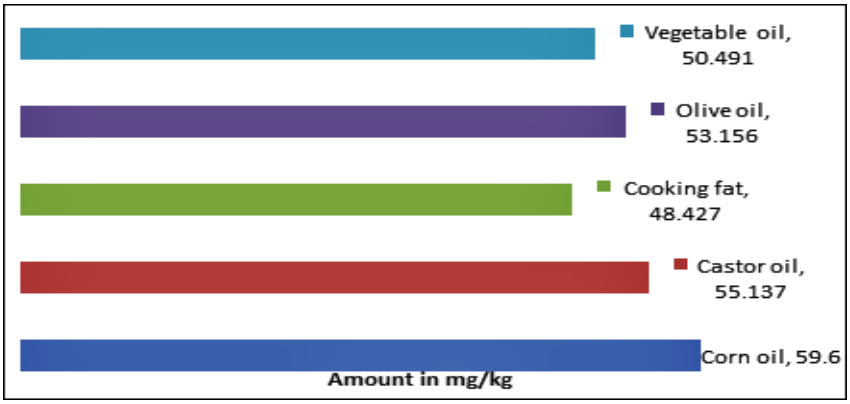


Figure 7 **Acrylamide levels in crisps in different cooking media**

From the results of figure 7, it is evident that there was a slight difference in levels of acrylamide irrespective of the type of cooking media though there was a clear difference in cooking fat that seemed to have lower levels compared to the other cooking mediums in both chips and crisps. Although, the used oil was highly thermoxidized, the contribution of oil oxidation products to the overall formation of AA appeared to be similar in all types of media.

Cooking fat was found to be having low levels of acrylamide though the difference between all types of oils was small. This high level could be due to less absorptivity of cooking fat compared to the other four cooking media. The high level of acrylamide found in olive oil in this study could be due to the high absorptivity of the olive oil compared to the rest therefore causing a high level of reducing sugars that lead to high level of acrylamide in the Maillard reaction. Theo [42], observed that soybean oil generated the greatest quantity of acrylamide (2,600 ± 440 µg/kg) with Lady Rosetta variety and corn oil the least (1,920 ± 320 µg/kg), though, the overall difference between the two types of oil was small.

From literature, acrylamide forms due to the Maillard reaction on reducing sugars, amino acids, asparagine and temperature therefore, results in this study confirm this since there was no significant difference between the types of oils used. According to Theo *et al.* (2016), it was found that there was no significant effect of the type of frying oil on the levels of acrylamide which is also clear in the results in this study.

Similar studies by [43], postulated that palm oil exhibited much higher acrylamide formation, compared to the other deep-frying oils which are also similar to the results in this research where vegetable oil is seen to be having slightly higher acrylamide levels both in chips and crisps while these results slightly differ with those of Matthäus [44] and Williams, [40] that have no difference at all.

In another study it was found that olive oil resulted in higher formation of acrylamide compared to corn oil [4]. This is in line with the results from this research that gave 4.420 mg/kg values in olive oil in chips compared to that of corn oil in chips that had 4.251 mg/kg which was slightly higher. Therefore, from the present research, as far as the impact of oil type on AA formation in concerned, it was shown that the nature of oils (cooking media) present (mono and polyunsaturated) does not influence AA formation in our potato system.

Table 6 represents descriptive statistics on the data of effect of cooking media on acrylamide levels in crisps.

Table 6 Descriptive Statistics

Minimum	Maximum	Mean	Std. Deviation
845849.0	1041279.00	932679.80	75515.4603
846653.00	1041465.00	932548.80	75467.35384
846653.00	1041465.00	932548.80	75467.35384

Average mean = 2176078.2

Similar results were reported by Pedreschi [45] that reported low levels of acrylamide due to use of different cooking oils. In the same study, they found a linear relationship between the type of cooking oil and acrylamide content in potato crisps whereas there was an increase in asparagine the acrylamide level increased and vice versa.

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