Analysis of Tuber Maturity on Levels of Acrylamide in Selected *Solanum Tuberosum* Products (Chips and Crisps).

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Abstract- Studies indicate that cooking starchy foods under high temperatures produces acrylamide in those foods. Consequently, intensive investigations have been undertaken, on the potential health risk of this contaminant in the human diet. Fried products including potato chips and crisps have been reported to contain high levels of acrylamide. The study proposed to determine the effect of potato maturity on the levels of acrylamide in its fried products (chips and crisps). Stratified sampling technique used in harvesting of S*olanum tuberosum* tubers in an identified area within two seasons of two months for the first batch and four months for the second batch this were prepared to standard and cooked to standard cooking procedure. The chips and crisps were analytically prepared before they were analysed. potato chips and crisps recorded (0.692mg/kg) and (2.91 mg/kg) respectively as lower values compared to premature potato chips and crisps that found (2.91 mg/kg) and (68.731 mg/kg) as the highest values.

Index Terms- *Solunum tuberosum*, Acrylamide, toxicology, toxicology, Amino acids.

I. Introduction

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 [1] On the other hand, potato chips which are locally referred to as chips are long; thinly cut slices which have been deep fried [2;3].

Potato chips and crisps are among the starchy foods produced from the tuberous crop *Solanum tuberosum*. They have a high content of carbohydrates and vitamin C and thus, forming an important component of a balanced diet. Worldwide, it is the fourth most widely grown food crop after rice, wheat and maize [4]. In Kenya, potato is the second most valuable cash and food crop after the cereal grains [5].

Following this discovery intensive investigations have been undertaken, involving the analysis, occurrence, chemistry, toxicology and potential health risk of this contaminant in the human diet [6]. In addition, the discovery of acrylamide in some cooked starchy foods in 2002 prompted concerns about the carcinogenicity of those foods [7]. Despite this, little is known to the local population of the levels of acrylamide in chips and crisps, this applies to both consumers and producers.

Acrylamide is a small and simple molecule that could be formed in heated foods via several different mechanisms, which may involve reactions of carbohydrates, proteins and amino acids, lipids and possibly other minor food components. It is a chemical substance formed by a reaction between amino acids and sugars(disaccharides) typically occurs when foods with high starch content such as potatoes, root vegetables and bread, are cooked at high temperatures (over 120°C) in a process of frying, roasting or baking that is capable of forming the Maillard reaction.

Maillard reaction as a non-enzymatic reaction between reducing sugars and amino acids in temperatures exceeding 120ºC [8-10 & 11]. 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.

Instruments and reagents

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).

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 $^2H_3$ -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.

Analysis

Potatoes were planted in a 50 feet by 100 feet plot of land, stratified sampling was used in which a total of five samples were collected in triplicate at five different sampling points within the plot in the first two months of planting harvested and cooked in accordance to the standard size of both chips and crisps, this was also repeated at the fourth month where this potatoes were termed to be mature. This were cooked to standard cooking temperature and later on prepared and analysed using an HPLC for quantification of the amount of acrylamide present.

II. RESULTS AND DISCUSSION

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.

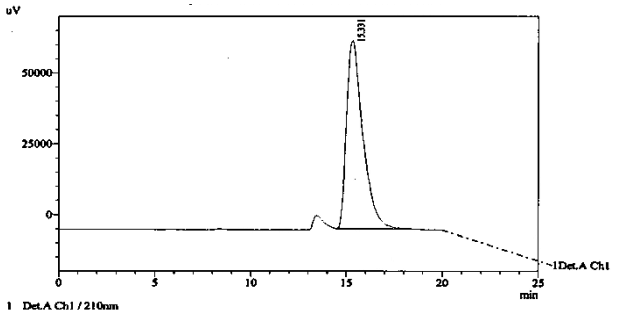

Figure1: 20ppm standard

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. The next table represents data values of chips samples from mature potatoes. Table 1 represents mature potatoes (Chips) acrylamide levels.

Table 1 Mature potatoes (Chips)

ID	(P 1)	(P2)	(P 3)	A.P.A	C.(mg/kg)
M1X	27393	27050	27706	27383	0.849
M2X	25171	25955	26293	25806	0.800
M3X	26764	26141	27314	26740	0.829
M4X	25268	24148	24855	24757	0.768
M5X	22049	22634	22277	22320	0.692

In the set of data M1X (0.849 mg/kg) recorded the highest compared to M4X (0.768 mg/kg) this variation in concentration could be due to difference in reducing sugar levels in the potato samples despite their similar age since they develop differently. In mature potato chips, the range was found to be 0.157 while, the standard mean of the concentration was found to be 0.7876 mg/kg and the standard deviation calculated to be 0.0551. Table 2 represents pre-mature potatoes (Chips) acrylamide levels.

Table 2 Pre-mature potatoes (Chips)

ID	(P1)	(P2)	(P3)	A.P.A	C.(Mg /kg)
PM1X	60524 2	60679 7	60777 6	60660 5	1.881
PM2X	60335 7	60407 1	60457 9	60400 2	1.873
PM3X	60484 8	60420 8	60549 9	60485 2	1.875
PM4X	605100	60632 7	60742 5	60628 4	1.880
PM5X	62016 9	62076 4	62093 6	62062 3	1.924

In the set of data, sample PM5X recorded the highest (1.924 mg/kg) While, sample PM4X recorded 1.880 mg/kg which was the lowest this could be due to variation in maturity of the potatoes despite their age since they could have developed at a different rate. The range for this set of results was found to be 0.044 while

standard mean was found to be 1.8866 Mg/kg and standard deviation was found to be 0.018937 for the given data.

Similar studies indicate high levels of sugars in pre mature potatoes therefore, leading to high acrylamide levels. In these cases, sugars have not been converted to starch yet; small and immature tubers tend to have a higher level of sugars [12]. Consequently this present research indicates high levels (1.924 mg/kg) of acrylamide in chips cooked from pre mature potatoes compared to chips cooked from mature potatoes that recorded (0.849 mg/kg) as the highest concentration in mature potato chips. In the present research acrylamide levels were found to be ranging between 1.880-1.924 mg/kg compared to the concentration of mature potatoes that had levels ranging between 0.692- 0.849 mg/kg, this could be in relation to high sugar levels in premature potatoes which is in line with [13] that argued that sugars correlate well with levels of acrylamide, whereas asparagine levels alone were not strong predictors of potential levels of acrylamide in fried products their research was based on three samples that contained relatively low amounts (2-3 mg/fresh weight) of total free amino acids.

These potentials correlated well with the product of the concentrations of reducing sugars and asparagine. Glucose and fructose were found to determine acrylamide formation. The cultivars showed large differences in their potential of acrylamide formation which was primarily related to their sugar contents. Agricultural practice neither influenced sugars and free asparagine nor the potential of acrylamide formation. It is concluded that acrylamide contents in potato products can be substantially reduced primarily by selecting cultivars with low concentrations of reducing sugars. Table 3 represents mature potatoes crisps acrylamide levels.

Table 3 Mature potatoes (Crisps)

ID	(p_1)	(P_2)	(P_3)	A.P.A	C.(mg/kg)
PM1X	89743	89871	90671	**90095**	2.7935
PM2X	86269	86911	87200	86793	2.6691
PM3X	883211	88749	89376	88815	2.7538
PM4X	853222	85837	86429	85863	2.6622
PM5X	93034	93717	94437	93729	2.9062

In the results sample PM5X recorded the highest result of 2.9062 (mg/kg) compared to PM4X (2.6622 mg/kg) this could be due to potato development during growth or due to temperature variation during the cooking despite similar temperatures. The range of the given set of data was calculated to be 0.244 while, the standard mean was calculated to be 2.75696 and standard deviation calculated to be 0.089794.

Similar results were reported by [14] that reported low levels of acrylamide due to low asparagine levels in potatoes due to maturity. In the same study, they found a linear relationship between asparagine level and acrylamide content in potato crisps whereas there was an increase in asparagine the acrylamide level increased and vice versa.

Similar results were reported upon working on different potato varieties with different harvest times[15] where low levels of acrylamide in potato crisps from mature potato varieties were recorded. However, higher levels of acrylamide were recorded in pre mature crisp samples compared to crisp samples for mature[16]. Potatoes in this study this could be due to different storage times of the potatoes after harvest therefore, a change in acrylamide levels.

Table 4 represents acrylamide levels in pre-mature potatoes crisps.

Table 4 Pre-mature potatoes (Crisps)

ID	(P1)	(P2)	(P3)	A.P.A.	C.(mg/kg)
PM1X	212512 6	212647 1	212916 6	212692 1	65.951
PM2X	218153 7	218307 7	218915 9	218459 1	67.743
PM3X	209713 8	209846 7	209621 2	209727 2	65.035
PM4X	221214 5	221851 2	221962 7	221676 1	68.731
PM5X	204323 3	205430 3	204767 0	204840 2	63.513

The results recorded the highest value in sample PM4X (68.731mg/kg) compared to PM5X (63.513mg/kg) this could be due to temperature variation despite the cooking conditions being the same. This difference could be caused by difference in size of the slice while chopping the crisps. The range of the given set was calculated to be 5.218 while, the standard mean was calculated to be 66.1946 and standard mean calculated to be 1.8861.

Similar studies indicate several factors affecting the acrylamide formation in heated potato products including the level of precursors and their ratios in the raw potato tubers, temperature and time of the processing, surface to volume ratio of the food, pH and water activity [17]. From this, maturity of potatoes has an influence in levels of reducing sugars therefore, leading to varied levels of acrylamide in the samples.

Acrylamide formation is mainly depending on free asparagines and reducing sugars, the limiting factor being the sugar as asparagines are usually more abundant in potatoes than reducing sugars. The molar ratio of reducing sugars to asparagines content is greater than two, meaning that there is an abundance of reducing sugars than the asparagines content might be the limiting factor for acrylamide formation. This is a better indication of the right time of harvesting at the point of chemical maturity to reduce the potential of high acrylamide percentage formation during processing [18].

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