Method Development and Risk Assessment of Acrylamide From Commonly Consumed Fried Foods from Southwestern Nigeria

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ABSTRACT

Acrylamide is a potential carcinogenic chemical that can be formed in some foods cooked at high temperature usually above 120°C. In Nigeria, fried foods are widely consumed as snacks. The research aims at developing a method for the analysis of acrylamide and thereafter determine the risk associated with the levels of acrylamide in some fried foods commonly consumed in Southwestern Nigeria. Selected food samples (Yam, Sweet potato, Plantain, Meat, and Irish potato) were purchased from a local marketin Lagos, Nigeria and fried in Soya oil at 120°C± 2°C. Samples were extracted with water, centrifuged and clean up was with Bond EluteC_{18} solid phase extraction. Internal standard calibration (50 - 500 µg/g, r^2 0.9948) using lamivudine was employed on an Agilent (1100 series) High performance Liquid Chromatograph coupled with Ultraviolet detector. Percentage recoveries for the internal standards were 81.95-91.77 % and percentage recoveries for samples were 92.66-99.67%. Differences were observed in the levels of acrylamide obtained with length of heating and temperature of frying. Food samples fried at 125°C for 20 minutes had higher levels of acrylamide compared to food samples fried at the same temperature for 10 minutes. Sweet potato gave the highest concentration of acrylamide (720 µg/g) after frying.

KEYWORDS; Acrylamide, Fried foods, Sweet Potato fries, Irish Potato fries, Fried plantain and fried yam.

Introduction

Acrylamide a toxic chemical also known as 2-Propenamide, discovered in food by researchers in 2002, has gained considerable attention in recent years as a possible carcinogen and neurotoxin (Giese, 2007). Prior to its discovery in food it was an industrial chemical used in the production of polyacrylamide used for water/waste water treatment and for paper processing (Wang et al., 2011,).

Concerns about acrylamide is from its widespread occurrence as a bye product during high temperature (above 120°C) cooking of food rich in carbohydrates i.e frying, baking and roasting (Ubaoji and Orji, 2016). Acrylamide in food is formed by several routes. The major route is from reaction between asparagines (amino group) and reducing sugars at elevated temperatures-the Maillard reaction (Golon et al., 2014). Other routes include the reaction of Acrolein and ammonia.

Bioassay studies of acrylamide in food and drinking water given to rats and mice reveal induction of excess cancer incidences at multiple sites (Klaunig, 2008). Altissimi et al. (2017) analyzed ten selected foods for acrylamide, and French fries (724 µg/kg) gave the highest mean concentration. Other pastries had concentration between 460 µg/kg and 230 µg/kg. At this concentration of acrylamide found, the average exposure to acrylamide was 0.452 µg/kg bw/day, the average intake at 50^{th} percentile was 0.350 µg/kg bw/day and the average intake at the 95^{th} Percentile was 1.539 µg/kg bw/day. These levels of exposures were within the range that indicated concern for health as defined by European Authority for Food Safety (EFSA).

Gas and liquid chromatography are usually used for acrylamide analysis. Acrylamide is highly reactive, does not fluoresce nor contain any strong chromophore (Eriksson, 2018) which makes it difficult to be detected. When water is used for extraction, other soluble compounds like sugars, proteins and organic acids co-extract with it (Fernandes and Soares, 2008). This is avoided by using a different organic solvent or using clean-up procedures like solid phase extraction.

In Nigeria, not much studies have been done on acrylamide in fried foods. Limited studies on acrylamide in fried plantain from Nigeria are available Omotosho et al. (2017). The aim of this research was to develop a method for the analysis of acrylamide and determine the risk associated with its levels in fried foods commonly eaten in Southwestern Nigeria.

Methodology

Sample preparation and determination of acrylamide in the fried foods was by High Performance Liquid Chromatography (HPLC) using the method of Krishna et al. (2014) with modification. Instead of Zidovudine as Internal standard, Lamivudine was used. Also, the HPLC conditions were modified to optimize the procedure.

High performance Liquid Chromatographic (HPLC) method development of Acrylamide

Three methods were tried: (i) using a 50 μg/ml calibration standard, quantitative separation of acrylamide was performed in the isocratic elution mode with HPLC Agilent Technologies Chemstation LC System (1100 series) equipped with a Ultra Violet detector using acetonitrile and water (20:80) w/w. The separation was on a C_{18} column at temperature of 40 ^{0}C and 225nm wavelength. (ii) second method was similar to the first but the mobile phase was acetonitrile and acidified water (0.1% formic acid used) 20:80 and the wavelength reduced to 220 nm. (iii) the third method was similar to the second but the mobile phase was acetonitrile and acidified water (0.1% formic acid) 30:70, pH of the mobile phase adjusted to 3.5 with orthophosphoric acid and at 225 nm wavelength. Calibration and analysis of acrylamide in food samples were by the third method.

Sampling and Sample preparation

Yam, sweet potato, plantain, Irish potato and meat (cow beef) were from a local market in the Lagos. Samples were washed dried, peeled and cut into equal sizes (9 x 9mm). The meat was boiled and cut into a similar size. Food samples were each shared into two portions. The first portion was deep fried for 10 minutes while the second portion was deep fried for 20 minutes at 150^{o}C ± 5^{0}C in Soya oil, unused oil was analysed for acrylamide. After frying the samples were homogenized and stored at -20^{o}C prior to analysis.

Extraction

5g of food samples were spiked with 100 μL (10 μg/ml) of Lamivudine as internal standard. Samples were shaken on a vortex for 10 minutes and 10 mL of water added. The mixture was vortexed for further 10 minutes and centrifuged for 20 min. Centrifugation was repeated for samples where supernatant remained turbid. Supernatant was decanted and cleaned up with a BondElut C_{18} solid phase extraction (SPE) cartridge and eluent collected and injected for analysis.

Calibration, Quantification and Quality control

Standards of acrylamide (0μg/ml to 25 μg/ml) were prepared and lamivudine added as internal standard to check matrix effects. Recovery studies were done by spiking 1ml of acrylamide (1ug/ml) into samples prior to extraction. Internal standard recovery was by comparing peak area of internal standard in samples with average peak area of internal standard in calibration standards.

Risk assessment of acrylamide in fried foods

Estimated daily intake (EDI) values for acrylamide in Nigerian fried foods by an adult and children(2-5 years) were calculated using the equation:

EDI (µg/kg body weight) = (Mean concentration of acrylamide (µg/kg) X Amount of fried snack consumed per day (kg/day))/ the average weight of an individual (Daniali et al., 2010). 60 kg was used as the average body weight of a Nigerian adult and 16.7 g for a child (aged 2-5 years) Oyeyiola et al. (2017). Fried snack consumed per day (g/day) was taken as 0.08 kg/day as in Iwegbue et al. (2013). For meat a value of 0.180kg/day Ekhator et al. (2017) was used.

Results and Discussion

Method development

The first HPLC analysis gave unresolved peaks between the acrylamide standard and internal standard (Figure 1).

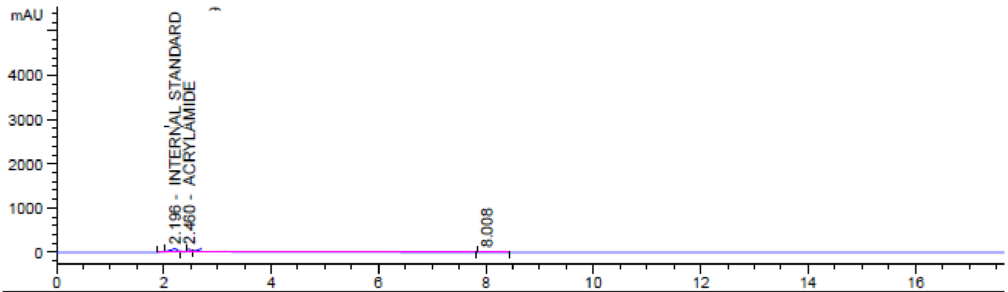

Figure 1: Chromatogram showing unresolved peaks of acrylamide and internal standard from method 1

The second method gave no significant difference between the retention times of acrylamide standard and internal standard (Figure 2).

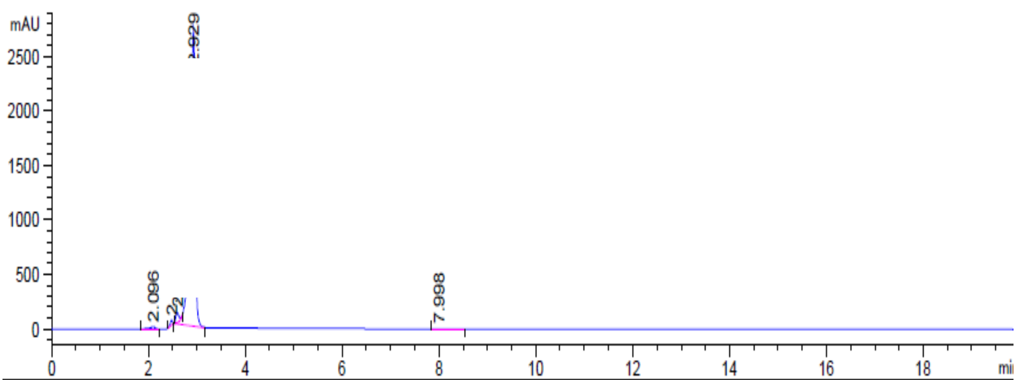

Figure 2: Chromatogram showing unresolved peaks of acrylamide and internal standard from method 2

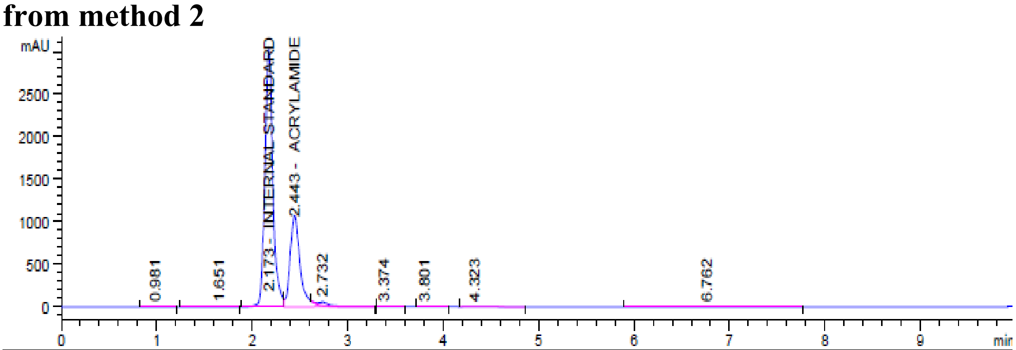

Figure3: Chromatogram showing resolved peaks of internal standard and acrylamide solution after optimization in method 3.

Third method produced well-resolved peaks with internal standard and acrylamide standard solution giving retention times of 2.199 minutes and 2.440 minutes respectively (Figure 3). In a study of acrylamide by Singh et al. (2010), 30:70 v/v of acetonitrile to water was used though the pH of water was not stated. Method 3 was adopted for calibration and analysis of all the samples in this study. Calibration curve gave a straight line, good coefficient (R^2), 0.99487 for the concentration range (0–25 µg/ml). Recovery of internal standard from samples was 75% to 85% and recovery for acrylamide was 92.66 and 99.89 %.

Levels of acrylamide in fried food samples

The levels of acrylamide in sweet potato fries, Irish potatoes fries, fried plantain, fried yam and fried meat were 606.67 – 720 µg/g, 360-633.33 µg/g, 460 – 500 µg/g, ≤4.00- 166.7 µg/g and ≤4.00 - ≤4.00 µg/g respectively. Sweet potato fried for 20 minutes gave the highest level of acrylamide (720 µg/g) while yam fried for 10 minutes, fried meat, used and unused oil gave the lowest levels of acrylamide ≤4.00 µg/g (Table 1). No acrylamide was detected in the oil samples. Percentage recoveries from samples were high (92.66 and 99.87%).

Acrylamide was not detected in yam fried for 10 minutes and in oil (unused and used oil). Our findings on acrylamide in frying oils were similar to the findings of Totani et al. (2007) and Pule and Torto (2012). The highest level of acrylamide was found in sweet potatoes fried for 20 minutes. Of the foods fried for 10 minutes, the sweet potatoes fries had the highest concentration of acrylamide. Lower concentration of acrylamide was found in yam compare to the Irish potatoes fried for both 10 and 20 minutes. This may be due to the differences in their sugar contents. The sugar content of sweet potatoes, Irish potatoes and yam are 4.18 g/100g, 0.89 g/100 g and 0.50g/100g respectively (USDA, 2016b, USDA, 2016c, USDA, 2016d).

Table1: Concentration of acrylamide in fried food samples

Sample type	Concentration (µg/kg) confirm units.		Spiked Level (µg/kg)	% Recoveries in samples	Limit of Detection for method (µg/kg)
	10 Minutes fried	20 Minutes			
French fries	360.0	633.3	20	98.27	2
Sweat Potato chips	606.3	720.0	2	99.67	2
Yam	≤4.00	166.7		92.66	2
Plantain	460.0	500.0		99.98	2
Meat	≤4.00	≤4.00		98.67	2
Unused oil	≤4.00	≤4.00		99.89	2
Unsed oil	≤4.00	≤4.00		98.45	2

Plantain has higher sugar content 15g/100g (USDA, 2016a) compared to sweet potatoes 4.18g/100g but had a lower level of acrylamide. This may be because they belong to different groups of food. Plantain is a fruit while sweet potatoes is a root food so mechanisms in these foods may be different. Acrylamide was not found in fried meat. This may be because meat is a protein and does not contain reducing sugars. Our result is in contrast to Yong-Hong et al. (2012) where the level of acrylamide in processed and cooked meat were 49.06 ug/kg and 78.57 ug/kg respectively. Foods fried for 20 minutes in this study had more acrylamide than those fried for10 minutes (Table1). Similar trends were observed with other studies. Matthäus et al. (2004) Mulla et al. (2017). The result of acrylamide in Irish potato fries and fried plantain gave the same range as other studies (Table 2).

Table 2: Levels of acrylamide in various samples from other studies

Sample	**Method**	**Acrylamide Concentration**	**Place of study**	**Reference**
Very green unripe Plantain Light green unripe Plantain Not really ripe Ripe Plantain Very Ripe Plantain	HPLC-UV	49.8±24 ug/kg 185.6 ± 3.5 240.2 ± 3.6 **315.6 ± 24.1** 2062.0 ± 26.9 µg/kg	India	Shamla and Nisha (2017)
Sweet plantain chips	GC- MS	162± 1.2-479± 1.1 µg/kg	Malysia	Daniali et al. (2010)
Plantain Fried for 6mins	LC-MS/MS	280.00 ± 2.64 µg/kg	India	Mulla et al. (2017)
Plantain fried for 20mins	HPLC-UV	100± 4.67 µg/kg	Nigeria	Omotosho et al. (2017)
Sweet Potatoes Fries		452.0 ng/g	USA	Truong et al. (2014)
Potatoes Chips (Irish Potatoes, Irish Potatoes, French fries)) 3.5 mins	LC-MS/MS	10-1800ng/g	Canada	Becalski et al. (2004)
Potatoes Chips (Irish Potatoes, French fries)	HPLC-UV	3267±142 µg/kg	Mexico	Sanchez-oter et al. (2017)
Potatoes Chips (Irish Potatoes, French fries)	HPLC-DAD	940± 35 µg/kg	China	Liu et al. (2014)
Processed Meat	LC-MS/MS and GC	2.31- 49.06 µg/kg	China	Chen et al. (2012)
Ripe Fried Plantain		9033 µg/kg	Nigeria	Azeke and Chukwuedo (2011)

Risk Assessment of Acrylamide

Estimation of Dietary Intake

Estimated daily intake (EDI) values for acrylamide in Nigerian staple foods by an adult and children (2-5 years) were calculated using the equation.

Table 3: Estimated Dietary intake of Acrylamide from fried foods

Samples	**EDI Adult** (µg/kg body weight)	**EDI Children** (µg/kg body weight)
French fries	0.6622	2.379162
Sweat Potato chips	0.8842	3.176766
Yam	0.111133	0.399281
Plantain	0.64	2.299401

Conclusion

Reference

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