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Research Article

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Quantification of acrylamide levels in selected food products sold in market outlets within Enugu metropolis, Enugu State, Nigeria

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Abstract Studies were carried out to evaluate the levels of acrylamide in ten processed food products (cake, fried plantain chips, fried potato chips, beans cake, chin-chin, puff-puff, cheese-box, butter pie, bread and pop corn) sold in market outlets within Enugu metropolis using Liquid Chromatography and Tandem Mass Spectrometric (LC-MS/MS) analytical technique. The mean values of acrylamide in the investigated cake, fried plantain chips, fried potato chips, beans cake, chin chin puff-puff, cheese box, butter pie , bread and pop corn food samples were 305.20±31.21, 329.07±16.22, 476.23±24.11, 186.32±8.40, 232.19±16.30, 352.19±30.77, 56.70±4.05,104.19±10.51, 163.32±21.06 and 266.49±20.84µg/kg respectively.

The food samples contained mean levels of acrylamide in the following decreasing order; fried potato chips>fried puff puff > fried plantain chips> cake>pop corn> chin chin>beans cake> bread> butter pie> cheese box.

The levels of acrylamide in the studied food samples were statistically significant at $p<0.05$.

The benchmark set for acrylamide was exceeded in the investigated fried potato chips, fried plantain chips, fried puff puff and cake samples. The investigated food samples with very high mean values of acrylamide were predominantly processed through frying.

Keywords Acrylamide, Frying, Baking, Maillard reaction, Reducing sugar, Asparagines, Fried potato chips, Fried plantain chips, Cake, Beans cake, Pop corn, Fried puff puff, Chin chin, Butter pie, Bread and Cheese box

Introduction

Food is important for an individual's well being as it provides him with energy and several essential nutrient needs [1]. Food habits are changing rapidly due to variations of life styles, economies and global culture. Fried as well as baked food products have earned a lot of acceptance among various age brackets, especially the younger generation because of its short time of preparation, ease of consumption and rich taste [2]. Any edible food product is a complex mixture of compounds, most of them are beneficial and essential to life while some are harmful components that may interact with each other or other food constituents. A good example of a harmful compound processed from baked, roasted or fried food products that may be toxic or non beneficial to humans is acrylamide.

In April 2002, the Swedish National Food Administration (SNFA) and the University of Stockholm together reported that carbohydrate-rich food products that underwent processing such as frying or baking at high temperatures may contain considerable levels of acrylamide [3].

Acrylamide is a chemical compound with chemical formular, C_3H_8NO and has a molecular weight of 71.08g/mol. It is a white odourless, crystalline solid that is soluble in ethanol, water, ether and chloroform [4].

According to [5], processed potatoes (potato chips), coffee, cereal based foods, french fries, crackers, toasts, bread, cookies, boxed breakfast, cereal, corn chips, bakery products and cocoa are the main sources of exposure to acrylamide in the diet.

The reaction that produces acrylamide is one of series of non-enzymatic reactions between sugars (reducing sugar) and amino groups, principally those of amino acids (asparagines), that have been given the umbrella name of the Maillard reaction. According to [6], this chemical reaction mainly occurs when foods are subjected to high temperatures as during frying, roasting or baking and in low moisture conditions. Acrylamide formation in food depends on food composition and processing conditions and significant quantities are produced during heat treatment above 120^oC. As temperature increases and moisture content gets lower, acrylamide production increases [7]. The darker in colour a food product is, the higher the acrylamide content.

In foods, acrylamide can be formed either by interaction of asparagines with reducing sugar or in the absence of sugar, that is when fats in foods are oxidized to 3-carbon molecule, which then reacts with asparagines to form acrylamide [8]. The major pathway for acrylamide formation in foods is known as the reducing sugar-asparagine route *via* Maillard reaction and this non-enzymatic reaction influences several aspects of food quality such as flavour, colour and aroma formation [9].

Ingredients plays an important role in acrylamide formation as different ingredients have various amounts of free asparagine and reducing sugar available for the formation reaction. [10] stated that wheat flour has a low content of asparagines but certain ingredients used in baking such as germ and bran contains significant amount of asparagines that encourages Maillard reaction and acrylamide formation during baking. The potential routes of human exposure to acrylamide are ingestion, dermal contact and inhalation [11]. The discovery of acrylamide in food products is a concern because according to [12], it is a well known industrial chemical, whose toxicological properties have been studied extensively. It is a potential human carcinogen and genotoxicant, based on high-dose animal studies, and a known human toxicant [13]. Owing to its low molecular weight and polarity, it is readily distributed and incorporated in mammals and is rapidly circulated via the blood stream and can be found in the liver, kidney, thymus, brain, heart and human breast milk [14].

According to [15], the half-life of acrylamide in the body ranges from 2 to 7 hours, which indicates the free excretion of this substance. Acrylamide in 1994 was classified by the International Agency for Research on Cancer as a possible carcinogenic compound for humans [16]. Acrylamide has been shown to be toxic to both the central and peripheral nervous system, and induces nerve terminal degeneration and has deleterious effect on the thalamus, hippocampus and celebral cortex [17]. Acrylamide poses an elevated health risk to children and the acrylamide in foods consumed by pregnant women has been shown to reduce birth weight and head circumference, key indicators of a child's future health [12].

[18] observed that acrylamide induces groups of white blood cells to produce free radicals which reduces their cellular glutathione concentration and hence consumption of acrylamide containing food products triggers inflammation and oxidative stress, which induces atherosclerosis.

Exposure to high levels of acrylamide in the workplace has been shown to cause neurological damage especially among workers using acrylamide polymers to clarify water in coal preparation plants [6]. [19] stated that longer exposure to acrylamide could result in more severe symptoms such as celebral dysfunction and neuropathy . Several approaches have been successful at preventing acrylamide formation by preventing the reactions responsible for generating it. For instance, lowering the pH of foods blocks the nucleophilic addition of asparagine with a carbonyl compound, thus preventing the formation of Schiff base, a critical intermediate in the formation of acrylamide [20]. Other mitigation approaches include addition of some nutrients or compounds such as proteins, amino acids like glycine, cysteine, organic acids, calcium ions, cyclodextrin, natural antioxidants (derived from vegetables and fruits consumption) and replacement of reducing sugar with sucrose. According to [1], blanching carbohydrate-rich foods such as potato, controlling the processing temperature and duration of the processing and genetic modification of the genes responsible for asparagines and reducing sugar synthesis helps to limit acrylamide formation in foods.

The investigated food samples are common ready-to-eat foods that are commonly consumed by all classes of people in rural, semi-urban and urban areas in Nigeria. The mode of processing adopted for the food products includes frying or baking, with the surface appearance of the food products ranging from light-golden to dark-brown colour. On daily basis people consume these food product(cake, fried plantain chips, fried potato chips, beans cake (akara), chin chin, fried puff puff, cheese box, butter pie, bread and pop corn) as snack or a food diet unmindful of possible exposure to a harmful food chemical product such as acrylamide. The health implications of constant and excessive exposure to this harmful food chemical product is already well documented in literature and therefore in that regard , evaluation of acrylamide levels in selected food products commonly consumed and sold in market outlets within Enudu metropolis , Enugu State, Nigeria, became imperative.

Materials and Methods

Five samples each of the processed food products(cake, fried plantain chips, fried potato chips, beans cake (akara), chin chin, fried puff puff, cheese box, butter pie, bread and pop corn) were purchased in market outlets within Enugu metropolis, Enugu State. The food samples were prepared, packaged and labeled and transported to the laboratory for analysis.

Reference standard of acrylamide, acetone, methanol, sodium chloride, magnesium sulphate , distilled water and disodium citrate were purchased and of analar grade.

The determination of acrylamide content in the ten food samples was carried out using LC-MS/MS as described by [21].

2g each of the food samples was crushed, homogenized and weighed into the centrifuge containing 400µL of the internal standard and 40ml of water was added. The centrifuge was shaken vigorously, vortexed for one hour and then stirred mechanically for one hour. After, the sample was centrifuged for 20min at 10^{o}C and then 10ml of the aqueous extract was transferred to a clean test tube and purified. The extraction of acrylamide from the food samples was performed with ultrapure water by liquid-liquid (LL) and or liquid-solid (LD) partitioning. The purified aliquot extract was analyzed by injection into the LC-MS.

To ensure the capability of the LC-MS/MS method for the analysis, limit of detection, limit of quantification, precision and accuracy were selected as defining variables to evaluate the performance characteristics. After spiking into the samples at varied concentrations (100, 150, 200, 250 and 300 µg/kg), the percentage of recovery was found in the range of 85-110% for all sample types. Similarly, for the precision analysis, five repeated injections for each sample was done and the range was found to be less than < 5%. The limit of detection and limit of quantification was measured on the basis of signal to noise ratio of 3:1 and 10:1 respectively by injection of series of dilute solutions of known concentration. Based on the ratio, the LOD and LOQ for acrylamide were 3.0µg/kg and 10.0µg/kg respectively.

Statistical Analysis: The obtained data were expressed as mean ± standard deviation and subjected to one way analysis of variance (ANOVA) at 5% confidence level using SPSS version 22.0.

Results and Discussion

Table 1: Mean levels of acrylamide in ten food samples sold in market outlets within Enugu metropolis, Enugu State

Sample	Mean acrylamide values (µg/kg)	F- test p value
Fried puff puff	352.16± 30.77	
Fried plantain chips	329.07±16.22	
Fried potato chips	476.23±24.11	
Beans cake (Akara)	186.32±8.40	
Chin chin	232.19±16.30	
Cake	303.20±31.21	0.01
Cheese box	56.70±4.05	
Butter pie	104.19±10.51	
Bread	163.32±21.06	
Pop corn	266.49±20.84	
Bench mark Level [22]	300	

Result of Table 1 shows that the mean acrylamide levels in the fried puff puff, fried plantain chips, fried potato chips, beans cake, chin chin, cake, cheese box, butter pie, bread and pop corn were 352.16±30.77, 329.07±16.22, 476.23±24.11, 186.32±8.40 232.19±16.30, 305.20±31.21, 56.70±4.05, 104.19±10.51, 163.32±21.06 and 266.49±20.84µg/kg respectively.

The processed food samples contained mean levels of acrylamide in the following decreasing order; fried potato chips>fried puff puff>fried plantain chips>cake>pop corn>chin chin>beans cake>bread>butter pie> cheese box as shown in Fig. 1.

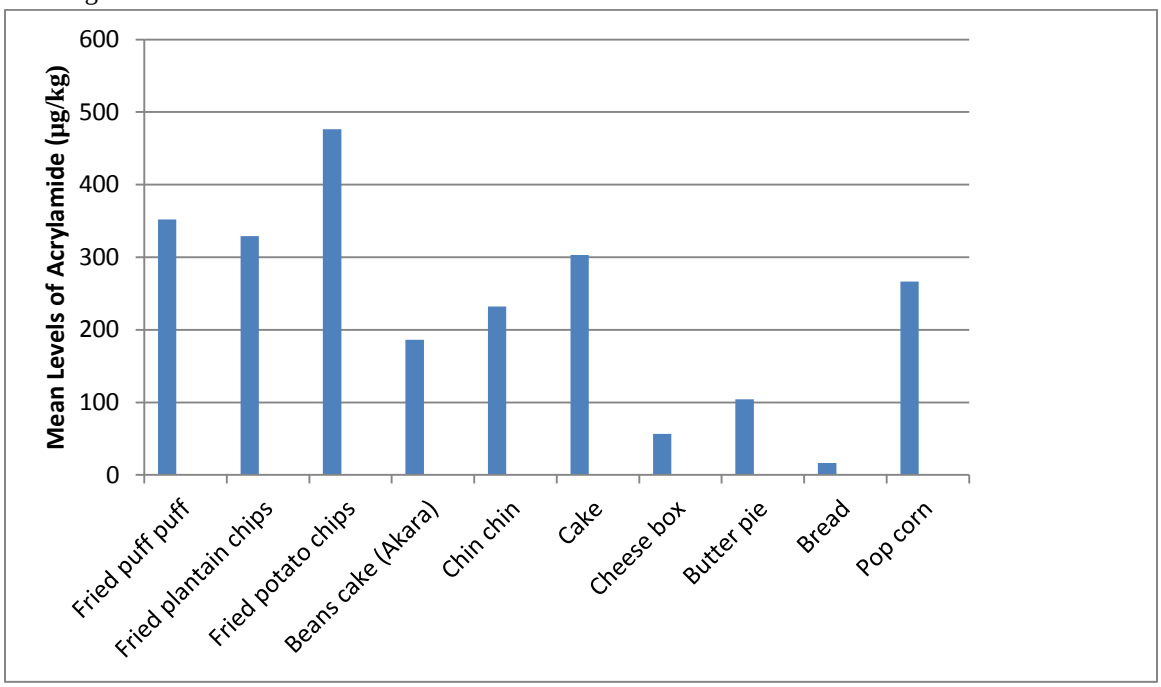

Figure 1: Bar chart representation of the mean levels of acrylamide in the processed food samples purchased in market outlets within Enugu metropolis, Enugu State

Result of Table 1 shows that acrylamide had the highest mean level in fried potato chips while the lowest value was gotten in cheese box samples. The reason for the very high mean value of acrylamide as observed in the fried potato samples could be as a result of the storage temperature of the tubers before the frying process, the stress the tubers underwent during transportation to the final destination, the frying temperature adopted by the potato chips processors.

When potato tubers are stored at below 10^{o}C, or grown in an infertile land that lacks adequate nitrogen supply, has injuries as a result of stress during transportation among other post harvest processes, increased reducing sugar level are usually observed in such tubers as reported by [23,24]. Therefore when tubers with high levels of reducing sugar is fried at very high temperatures usually above 120^{o}C , the resulting Maillard reaction causes the browning of the finished product, an evidence of increased presence of acrylamide in such a product. So, most tubers used for potato chips making in our environments are usually stressed, not fully mature and usually fried at very temperature and all of this could have given rise to the increased acrylamide formation in the chips sold within the metropolis. Because the fried potato chips are used as snack or food diet and are consumed more especially by children because of its rich taste, the risk of undue exposure to this harmful by this age group is usually very high and therefore a health concern.

Cake, fried plantain chips and fried puff puff food samples also recorded high acrylamide levels because of reasons ranging from high processing temperature; addition of ingredients during the processing that raises the levels of reducing sugar and asparagine, chief limiting factors for Maillard reaction, and the duration of the processing. Hence the varied levels of free amino acids (especially asparagines) and reducing sugar in the raw food materials , type and quantity of ingredients added in the production of each of the investigated food samples, processing method

adopted, processing temperature and duration of the processing could have significantly contributed to the varied levels of acrylamide gotten in the studied food samples. Because the colour appearance of any fried, baked or roasted food product plays a critical role in determining the levels of acrylamide in such foods, the levels of acrylamide in processed food samples such as cheese box and butter pie were comparatively low.

The mean levels of acrylamide in the investigated processed food samples sold within Enugu metropolis were statistically significant. The investigated cake, fried plabtain chips, fried potato chips and cake samples had mean levels of acrylamide above the benchmark limit of 300µg/kg set by [22]. It is pertinent to note that the food samples with very high mean values of acrylamide were predominantly fried. The health implications of undue exposure to acrylamide especially via foods should be of a serious health concern, especially considering the already established clinical studies.

According to [12,19], prolonged exposure to acrylamide especially at very high doses could result in more severe symptoms such as celebral dysfunction, neuropathy, numbness of hand, ataxia, reduced birth weight and head circumference and skeletal muscle weakness. [13] stated that acrylamide is a human carcinogen and genotoxicant, based on dose animal studies. [2] reported lower mean levels of 151.13±7.44 ,80.59±5.66 and 40.29±1.82µg/kg for fried carbohydrate rich, protein rich and baked food products respectively in Bangladesh, than what was reported in this study for the investigated fried and baked food samples sold within Enugu metropolis. Similarly, [26], obtained lower mean values of 134.64±2.81 and 14.82±.91µg/kg for potato chips and bread respectively consumed daily in Romania, than what was reported in the studied food samples sold within Enugu metropolis. The reason that could be adduced from the low levels of acrylamide in the investigated food samples in Bangladesh and Romania respectively compared to the high mean values of acrylamide in investigated food samples sold within Enugu metropolis could have varied from the different agronomic environments where the raw food materials were grown and harvested, different ingredient application and possibly the different regulations on ingredient and sugar application in fried or baked or roasted food products and the different processing methods adopted during the processing of the products in the countries reviewed .

Conclusion

Acrylamide, a known food toxicant was found at varying levels in ten food products which included cake, fried plantain chips, fried potato chips, beans cake (akara), chin chin, fried puff puff , cheese box, butter pie, bread and pop corn consumed daily by inhabitants living in Enugu metropolis, Enugu State. The mean levels of acrylamide in the cake, fried plantain chips, fried potato chips and fried puff puff samples were above the benchmark limit set by European Union for the food toxicant in baked, fried or roasted food products. The investigated food samples with comparatively very high levels of acrylamide were predominantly fried. Because these four food samples with excessive levels of acrylamide form part of a daily food diet or snack consumed by the inhabitants living within Enugu metropolis, the health implications of consistent exposure to acrylamide through consumptions of the food products by the people is therefore of a serious public health concern.

Processing conditions that raises the levels of acrylamide in the investigated food samples and indeed every food product either fried, baked or roasted should be advocated against and in that wise, ideal processing conditions that minimizes acrylamide levels in food products should be encouraged by relevant food regulatory authorities. It is advised that relevant food regulatory authorities in the State and indeed Nigeria, should periodically evaluate the acrylamide levels in food products baked, roasted or fried for consumption by the Nigerian public, so as to ensure that such food products do not pose health risk to the consumers due to high dose of acrylamide exposure.

Conflict of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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