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Determination of precursors of acrylamide formation in roasted maize

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ABSTRACT: Acrylamide, an organic compound with the formula CH_2=CHCONH$_2$, is a contaminant generated through high-temperature cooking processes as a result of Maillard reactions catalyzed by the presence of reducing sugars and free amino acids in starchy food compounds. Acrylamide and its major metabolite, glycidamide, have been considered probable human carcinogens. In this study, we report on the acrylamide content in roasted maize from some Kenyan markets. Raw maize was purchased from local markets and roasted under laboratory conditions. They were crushed and extracted using water and hexane in a ratio of 2:1. The extract was derivatized with potassium bromate and potassium bromide and further subjected to liquid–liquid extraction using ethyl acetate-hexane (4:1, v/v). The final bromoprop-2-enamide (BPA) analyte was analyzed using gas chromatography–flame ionization detector. Acrylamide was not detected in any of the samples (at limit of detection of 20 μg/kg), which was consistent with reports from other countries.

1 BACKGROUND

1.1 *Acrylamide*

Following the discovery of high concentrations of acrylamides by the Swedish National Food Administration (NFA) and researchers from Stockholm University in April 2002 in food rich in carbohydrates content, it has become a subject of public interest. Acrylamide was found to be carcinogenic in rodents and is classified as a probable human carcinogen (Swedish 2002; Vinci et al. 2012). Food such as French fries, potato crisps, and corn were reported to contain acrylamide when cooked at elevated temperatures (Boroushaki et al. 2010). However, there are no guidelines currently showing the permissible limits of acrylamide in processed food (Hariri et al. 2015). Acrylamide is an organic compound with a formula CH_2=CHCONH$_2$. It is a contaminant formed through the Maillard reaction when starchy foods with appreciable sugar content are heated to elevated temperatures (Gökmen & Şenyuva 2007; Lund & Ray 2017; Mottram et al. 2002). The formation of acrylamide has not been reported in boiled foods or foods that were not heat treated (Ahn et al. 2002). It has become evident that the formation of acrylamide cannot be stopped. However, there is a concerted effort to minimize its presence in human diets and this has called for accelerated research to reduce its formation in foodstuffs (Alam et al. 2018; Fu et al. 2018; Li et al. 2012; Ou et al. 2010; Zeng et al. 2009).

From available reports, the main concern about the possible health effects of acrylamide in food is its probable carcinogenic and genotoxic (DNA-damaging) effects, as evidenced by tumors in laboratory rats (Manière et al. 2005). Since it has been detected in food, detailed research has been done to evaluate

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whether acrylamide actually causes cancer in humans, but as yet there is sparse evidence that this is the case. However, acrylamide has been categorized by the International Agency for Research on Cancer (IARC) as a probable human carcinogen (Belkova et al. 2018; Gökmen & Palazoğlu 2008). The biological effect and risks associated with continued consumption of foods with acrylamide have been assessed by many international bodies including the European Food Safety Authority, the Food and Agriculture Organization of the United Nations (FAO), and the World Health Organization (WHO).

A Norwegian exposure assessment reported dietary acrylamide exposure with the mean and median exposure in adolescents and adults ranging between 0.3–0.5 μg/kg bodyweight per day. These estimates are in the same range as the mean daily exposures estimated by the European Food and Safety Authority (EFSA) for adolescents (0.4–0.9 μg/kg) body weight and adults (0.4–0.5 μg/kg body weight. Consumption patterns and dietary intake vary among people of different cultures and backgrounds (Normandin et al. 2013; Wyka et al. 2015).

Some foods analyzed for acrylamide, including infant powdered formula, coffee and chocolate powders, corn snacks, bakery products, and tuber-, meat-, and vegetable-based foods, showed that the levels of acrylamide present were variable among different foods and within different brands of the same food, as reported by European Union Authority (Authority 2012; Pacetti et al. 2015; Wilson et al. 2006). In a toxicological evaluation of acrylamide carried out by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in February 2005, it was noted that no data or limited information from Latin America and Africa were submitted. It was recommended that for useful assessment and mitigation of effects of acrylamide to reduce human exposure there was a need to have occurrence data on acrylamide in the foods consumed in developing countries (Arisseto et al. 2007).

The mechanism of formation of acrylamide in starchy foods is illustrated in Figure 1 (Krishnakumar & Visvanathan 2014).

Over the last few years various studies have reported the formation of acrylamide in foods to be assisted by precursors such as reducing sugars: fructose and glucose (Elmore et al. 2005; Mesias et al. 2018). Various methods have been employed for analysis, including high-performance liquid chromatography (HPLC-DAD) coupled to ultraviolet–visible (UV) detection (at 195 nm) with the limit of detection (LOD) of 10 μg/L in aqueous matrices (Ghiasvand & Hajipour 2016), and gas chromatography—an electron capture technique on the basis of the bromination of the acrylamide double bond has been developed (Zhang et al. 2006).

The aim of our study was to evaluate the levels of precursors of acrylamide in roasted green maize using gas chromatography-flame ionization detection using a new modified method (Geng et al. 2011) and monitoring precursors (glucose and fructose) using polarimetry. The new method introduced refluxing in

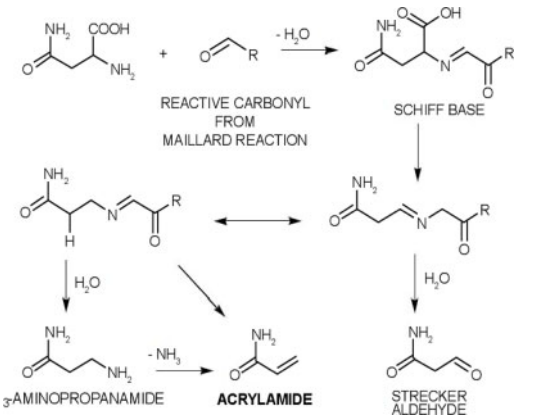

Figure 1. Proposed mechanism for the formation of acrylamide in heat-treated foods. Source: (Krishnakumar & Visvanathan 2014).

place of ultrasonic shakers in the process of extraction. Therefore the simplicity of this method makes it possible to investigate many samples in any laboratory setup.

2 MATERIALS AND METHODS

2.1 *Chemicals*

Acrylamide (99%), potassium bromate ($KBrO_3$), and potassium bromide (KBr), were purchased from Sigma Aldrich; n-hexane and ethyl acetate were redistilled before use; all the other reagents used were of analytical grade.

2.2 *Equipment/apparatus*

Experiments were done with a Varian 3400 CX chromatograph equipped with a flame ionization detector and a splitless injector. Separations were conducted on a 5, and 30 m × 0.25mi.d PTE capillary column.

2.3 *Sampling procedures and roasting of maize*

For the purpose of this study, 24 raw maize samples were sampled and bought from the local market and transported to the laboratory where roasting was immediately done while they were still fresh. Both raw and roasted maize were crushed, homogenized, and samples analyzed separately.

2.4 *Preparation of roasted maize*

The roasting of maize was done using a laboratory procedure similar to the setups for roasting maize found in homes and on the streets in towns and cities, as illustrated in Figure 2. Raw maize samples collected from the local market were subjected to high-temperature roasting, and then cooled, stored in

polyethylene bags, and frozen in the refrigerator to enable the preparation of analysis.

Figure 2. Roasting of maize.

2.5 *Sample preparation*

Maize samples were crushed using a pestle and mortar to obtain a uniform mixture. The extraction of the analyte followed a modified protocol developed by Geng and others (Geng et al. 2011). A measured 5.0 g of homogenized maize sample was weighed and placed in a 100 mL round bottom flask, extracted using 70 mL of distilled water, and refluxed for 50 minutes at 80°C. The defatting process was accomplished using hexane to allow for fatty components to remain in organic phase by adding redistilled hexane (20 mL), shaking, and allowing the mixture to settle. The fatty components remained in the organic layer. Ten milliliters of the lower aqueous layer and 0.6 mL sulfuric acid (10 %v/v) were sequentially added into brown glass tubes. The tubes were then placed into refrigerating cabins for precooling (4°C, 15 min). 0.1 mL of 0.1 M of derivatization reactants, including potassium bromate ($KBrO_3$) and 2.5 g of potassium bromide (KBr) powder, were added to the precooled solution. The tubes were briefly shaken with a vortex mixer and the reaction mixture was allowed to stand for 45 min at 4°C. The derivatization reaction was finalized by adding 1 mL of 0.1 mol/L sodium thiosulfate solution. The mixture was transferred to a 100 mL separatory funnel and extracted with 15 mL of ethyl acetate-hexane (4:1, v/v). The organic phase was filtered into a 100-mL round bottomed flask using a filter paper size (whatman 70 mm Cat No 1004-070) covered with 2 g of calcinated sodium sulfate. The separating funnel and the filter were rinsed twice with 5 mL aliquots of ethyl acetate-hexane (4:1,v/v). Pooled fractions were evaporated to dryness on a rotary evaporator (40, 140 mbar).The residue was then dissolved in 5 mL of hexane prior to analysis by GC-FID (Zhang et al. 2006).

2.6 *Determination of moisture content*

The moisture content of the green raw maize and the roasted maize was determined following the method described in the Association of Official Analytical Chemists (AOAC) 922.6. Maize was crushed using a pestle and mortar until it was a uniform mixture, from which 5.0 g of the sample was heated at 105 ± 2°C in an oven (DSO-500D) for 5 hrs. It was then cooled in a desiccator and weighed. The process of heating and cooling was repeated until a constant mass of the dried maize sample was obtained. The determination was run in duplicate.

2.7 *Determination of glucose and fructose content*

Raw green and roasted green maize samples were crushed separately and homogenized. To this paste in 100 mL round bottomed flask was added 10 mL 0.1% hydrochloric acid solution. The mixture was thoroughly mixed on a mechanical shaker for 20 minutes and then refluxed for 2 hours. It was cooled, filtered, and analyzed for glucose and fructose using a polarimeter (ADP 600 Series).

2.8 *Bromination of calibration standards*

Acrylamide stock solution (1000 mg L^{-1}) was prepared by dissolving 0.1000 g acrylamide solid in acetonitrile in a 100 mL volumetric flask from which working standards in the range of 5–200 μL of acrylamide were prepared and kept in brown glass vials, followed by consecutive addition of distilled water and 0.6 mL sulfuric acid, respectively. Bromination of the standards was done in the same manner as the sample bromination was done. The final solutions were subjected to SPE cleanup procedures and kept in vials under refrigeration at +4°C as preparations for analysis by GC-FID were made.

2.9 *Gas chromatograph conditions*

Acrylamide standards were analyzed on a GC-FID 3400 CX Varian model, Supelcowax capillary 60 m length, 0.25 rom i.d., 0.25 /m film thickness, column used. The injector temperature was maintained at 260°C and the nitrogen carrier gas linear velocity was maintained at 62 cm/sec at 100°C, the oven temperature was held at 100°C for 0.5 min before it was allowed to increase at a rate of 15°C/min to attain a final temperature of 200°C.

3 RESULTS

In this study, foods were sampled in the Njoro area of Kenya. From the results it was observed that the moisture content of raw maize had a mean of 44% whereas the moisture content of roasted maize had a mean of 35% moisture content, indicating a loss of water during the roasting process at elevated temperatures.

Both the raw and roasted samples were extracted with water then analyzed by a polarimeter and the specific angle of rotation was recorded. Acid hydrolysis

is done with (0.1 % HCl) and then the analyte was subjected to polarimetry, and the specific angle of rotation was also recorded.

3.1 *Quantification of acrylamide*

Working standard solutions for the standard curve as well as the sample recovered in hexane were analyzed using the GC-FID chromatographic system.

4 DISCUSSION

A study of the levels of precursors responsible for the formation acrylamide (AA) in roasted maize at high temperature is reported. Although the amounts of acrylamide in the roasted maize samples were not quantified, the glucose–fructose ratios explain sufficiently the overall trend of acrylamide content in the samples. It was observed that the moisture content of raw maize was higher when compared to that of roasted maize, indicating a loss of water when samples were roasted at elevated temperature. However, the loss of moisture content after roasting was not significantly high. This implies that the moisture content in roasted maize is still high enough to suppress the formation of acrylamide, which accounts for the low limit of quantification (LOQ) factor observed in this study. This finding is in agreement with observations made by Elmore and Zhang that formation of acrylamide is related to moisture content and it only forms when it falls below 5% for some foods (Elmore et al. 2005; Zhang et al. 2009). The specific angle of rotation recorded from water-based extracts obtained when raw and roasted maize samples were extracted with water proved to be levorotatory, as shown in Figure 3.

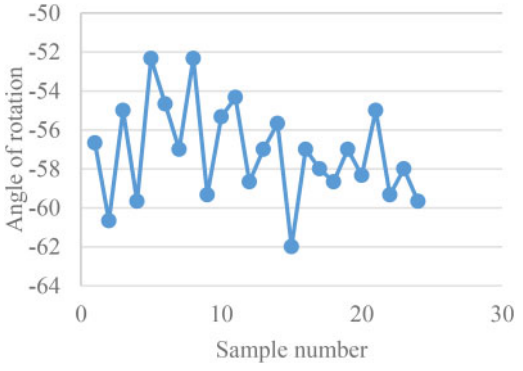

Figure 3. Angle of rotation of water-based extracts obtained from raw maize samples.

The angle of rotation for sucrose in maize or any other starchy food is expected to be dextrorotatory, but observations indicating the angle of rotation to be levorotatory in nature is due to the presence of a mixture of glucose and fructose in a sample. Indeed levorotatory fructose has a greater molar rotation than the dextrorotatory glucose (Panpae et al. 2008).

From our study, and as shown in Figure 4, it is clear that the angles of rotation measured from water-based extracts obtained from roasted maize samples were lower than those observed in raw maize samples—an indication of decreased amounts of fructose in roasted maize, probably due to the Maillard reaction.

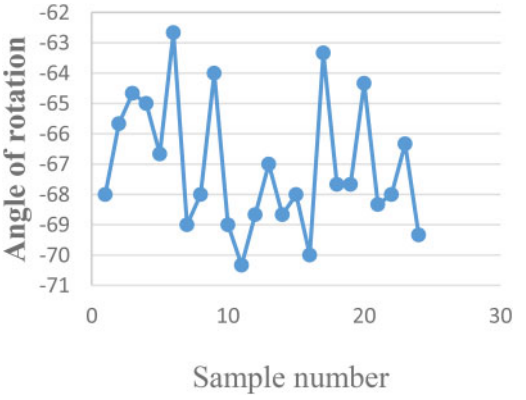

Figure 4. Angle of rotation of water-based extracts obtained from roasted maize samples.

Acid hydrolysis of the sample extracts resulted in a decrease in sucrose content due to its inversion to glucose with subsequent formation of a glucose–fructose mixture. It may be concluded that the more the glucose is formed through inversion process, the more dextrorotatory behavior the extract exhibits, as shown in Figure 5.

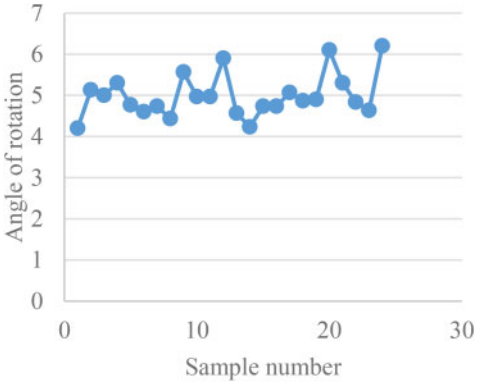

Figure 5. Angle of rotation of raw maize acid hydrolyzed extracts.

The angle of rotation of roasted maize is reduced when maize is roasted suggesting the depletion of glucose occurs when raw maize is roasted, as observed in Figure 6.

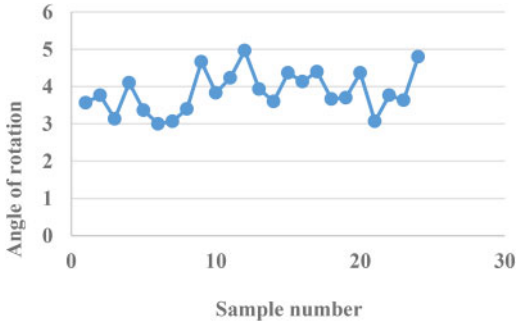

Figure 6. Angle of rotation of roasted maize acid hydrolyzed.

The ratio of fructose to glucose in raw maize samples is higher when compared to that in the roasted maize samples (Figure 7). There is a loss of the amount of glucose/fructose when roasting is done, probably due to the formation of acrylamide; this loss is related to the observed difference in angle of rotation. Reducing sugars such as glucose and fructose are the major contributors to acrylamides (Pedreschi et al. 2014).

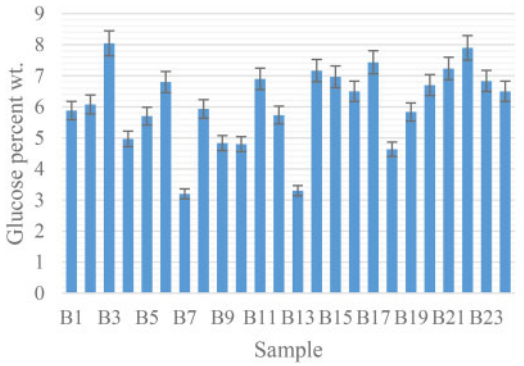

Figure 7. Glucose expressed percent weight in roasted samples.

The decrease in fructose/glucose ratio in raw maize compared to roasted maize could probably explain their role in the formation of acrylamides. However, in our study, acrylamide levels were below LOQ, which is consistent with other studies involving maize-based products. In a study involving quantification of acrylamides in food products done in Brazil, it was found that the concentration of acrylamide in the samples ranged from <20 to 2528 mg kg^{-1}, with a considerable difference between individual foodstuffs within the same class. However acrylamide in maize-related products determined using LC-MS/MS showed levels below LOQ (Arisseto et al. 2007). In Colombia, bakery products made from corn flour and investigated using chromatography with mass spectrometry (GC/MS) for acrylamides showed a lower acrylamide content (< 75 µg kg^{-1}) in comparison with similar bakery products made of wheat flour (Pacetti et al. 2015). It was also observed that the acrylamide content of white corn flour (WCF) extrudates studied using liquid chromatography–tandem mass spectrometry (LC-MS/MS) (Masatcioglu et al. 2014) was below the limit of detection (LOD) value and this was attributed to the fact that the levels of glucose/fructose present in the sample could not aid its formation. However quantification of acrylamide has been reported in deep-fried potatoes samples collected and analyzed in Nairobi, Kenya (Ogolla et al. 2015). This is consistent with the reports published by other authors (Boroushaki et al. 2010; Hariri et al. 2015). French fries and other potato-based products usually contain a high level of acrylamide compared to roasted maize which is below LOQ. This is due to the fact that potatoes have a higher content of reducing sugars and asparagine that aids the Maillard reaction (Hariri et al. 2015).

5 CONCLUSIONS AND RECOMMENDATIONS

The main factors responsible for the formation of acrylamide in cereal products and starchy foods are reducing sugars (mainly glucose and fructose) and free asparagines (amino acids), respectively. In this current study glucose and fructose were monitored by polarimetry, however, the amount of acrylamide in raw and roasted maize sold in some Kenyan markets was low and could not be quantified. Different maize varieties need to be studied, taking into account seasonal and regional variability for high-temperature processes such as roasting, frying, and baking. There is therefore a need for further research on a wider range of foodstuffs and a larger number of samples, including other carbohydrate-rich foods, such as sweet potatoes, cassava, banana products, and other home-cooked foods. Sample preparation included water extraction under reflux conditions prior to defatting with n-hexane, derivatization with $KBrO_3$ and KBr, and liquid–liquid with hexane and ethyl acetate (4:1); further optimization of these processes should be performed in order to satisfy the preparation of a large number of samples and applied to different types of starchy foods for the determination of acrylamide content.

The relationship between the loss of content of reducing sugars and asparagine and the role they play in acrylamides formation in roasted maize should be explored further. It has been found that the use of inorganic fertilizers has an effect on acrylamide formation. A case study showed that decreasing nitrogen fertilization caused increases in the reducing sugar concentration from 60% up to 100% in potato tubers for all varieties reported. There was a high correlation between the reducing sugar content and the generation of acrylamide during frying. This resulted in a parallel increase in the acrylamide concentration of the French fries (De Wilde et al. 2006; Stockmann et al. 2018).

It is expected that these results will contribute to data accumulation for worldwide health risk assessment and be helpful in establishing approaches to lower acrylamide formation during cooking processes.

CONFLICT OF INTEREST

The authors declare that they have no potential conflict of interest in relation to the study in this paper.

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