Full Length Research Paper

Analysis of acrylamide in traditional foodstuffs in Zimbabwe

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Accepted 14 December, 2011

We carried out a preliminary study on the occurrence of acrylamide in potentially high risk traditional foodstuffs consumed in Zimbabwe. Various studies have shown that acrylamide, a ‘probable human carcinogen’, is formed by high temperature thermal processing of carbohydrate rich foodstuffs (International Agency for Research on Cancer, 1994). Acrylamide concentrations in roasted maize, groundnuts, soy beans and boiled maize were determined using liquid chromatography tandem mass spectrometer (LC-MS/MS). The relative standard deviation of the analytical technique was 5% and the limit of detection was 0.02 µg/kg. Acrylamide was not detected in boiled maize. Roasted maize contained 450 µg/kg acrylamide. Roasted groundnuts had 140 µg/kg and finally, roasted soybeans with a concentration of 70 µg/kg acrylamide.

Key words: Acrylamide, liquid chromatography tandem mass spectrometer (LC-MS/MS), maize, groundnuts, soybeans, Zimbabwean traditional foods.

INTRODUCTION

Acrylamide (CH2=CH-CO-NH2; 2-propenamide) is a colorless and odorless crystalline solid that melts at 84.5°C (Friedman, 2003; Lineback et al., 2011). It is a highly polar organic compound and has an extremely high solubility in water (2 g/mL) (Becalski et al., 2003; Friedman, 2003; McCollister et al., 1964). Acrylamide is a low molecular weight dysfunctional monomer which contains a reactive electrophilic α, β-unsaturated carbonyl and an amide group (Besaratinia and Pfeifer, 2007; Klaunig, 2008; Tornqvist, 2005). The double bond can react with biological nucleophiles such as ‘amines, carboxylates, aryl and alkyl hydroxyls, imidazoles, and thiols’ (Shipp et al., 2006). Since sulfur has high nucleophilic activity, acrylamide covalently binds with thiols such as cysteine resulting in observed toxicity (Shipp et al., 2006). Acrylamide is known to have a wide range of genotoxic, carcinogenic and neurotoxic effect in mammals and has been classified by the International Agency for Research on Cancer (IARC) as a ‘probable human carcinogen’ and by the European Union’s Scientific Committee on Food (SCF) as a genotoxic carcinogen (FAO JECFA Monographs, 2011; Scientific Committee on Food, 2002; International Agency for Research on Cancer, 1994). In April 2002, researchers in Sweden from Stockholm University and the Swedish National Food Authority jointly announced the finding of high levels of acrylamide, predominantly in carbohydrate-rich foods (FAO/WHO, 2002; Mottram et al., 2002; Rosén and Hellenäs, 2002; Tareke et al., 2002).

As a result, the Joint Consultation on Health Implications of Acrylamide in Food held by World Health Organisation (WHO) and Food and Agriculture Organisation (FAO) recognized the presence of acrylamide in food as a cause of major concern in humans (FAO/WHO, 2002). It has been established by various studies that acrylamide is formed in carbohydrate-rich foods that undergo high temperature processing such as conventional fried potatoes and not boiling (Carrieri et al., 2010; Michalak et al., 2011; Yu Zhang et al., 2009). Despite the claim by El-Ghorab et al. (2006) that the Swedish report triggered a worldwide...
interest, developing countries seem to have been ignorant of the implications of acrylamide in food (El-Ghorab et al., 2006). There is no data on occurrence of acrylamide pertinent to countries and regions where the types of foods, methods of food preparation are different from those in Western nations. Lack of concise understanding of the mechanism of formation of acrylamide in foods does not allow speculations on the presence of acrylamide in foods that have not been analysed for acrylamide (Otles, 2004). The purpose of this study was to determine the occurrence of acrylamide in selected traditional foods in Zimbabwe using liquid chromatography tandem mass spectrometer (LC-MS/MS). The results of this study will add data on dietary exposure and risk assessment of acrylamide.

**MATERIALS AND METHODS**

Chemicals

$^{13}$C$_3$-acrylamide was purchased from Cambridge Isotope Laboratories (Andover, MA, USA) and methanol high performance liquid chromatography (HPLC) grade was purchased from Merck KGa, (Darmstadt, Germany). Acetonitrile was HPLC grade. Analytical grade water was purchased form (Millipore MilliQ treated).

Food samples

The analytical survey consisted of a sequence of traditional products, that is, boiled maize, roasted maize, roasted groundnuts and roasted soybeans. Traditional roasted foods (three samples of groundnuts and three samples of maize) were purchased from various traditional foods vendors in Harare, Zimbabwe on 27 July 2007. Maize grains were boiled at 100°C until grain husks began to break to obtain another sample. Three samples of soybean were prepared by roasting. The samples were sealed in polyethylene packages and shipped to Lantmännen Analyxen, Lidköping, Sweden (now Eurofins Food and Agro Sweden AB).

Experimental procedure

The samples were prepared and analysed for acrylamide using a method described by Tareke et al. (2002). The only modification was the use of a Micromass Quattro Premier LC-MSMS as described by Eriksson and Karlsson (2006). The samples were blended in a homogenizer and extracted using de-ionized water (100 ml). The internal standard, $^{13}$C$_3$-acrylamide (10 µg/ml, dissolved in HPLC grade water) was then added. The samples were centrifuged in a 12 Pyrex glass tube, and the supernatant was filtered using a 0.2 µm syringe to obtain 2 ml of filtrate. The filtrate was added on a Solid-phase extraction (SPE) column (Oasis HLB 0.2 g column) that had been activated by washing with methanol one column volume followed by five column volumes of HPLC grade water. The clean-up on the Oasis HLB 0.2 g column was followed by another clean-up step on an Isolute SPE column to obtain a laboratory sample ready for injection onto the LC-MS/MS. The analysis was carried out at ambient temperatures. The mobile phase was de-ionized at 0.2 ml/min. The runtime was 6.1 min and after the run the column was washed using 80% acetonitrile for 4 min. followed by column reconditioning for 10 min, using de-ionized water flowing at 0.2 ml/min. The solvent cycle was elution, washing and reconditioning before every injection of 20 µl test sample. The retention time of acrylamide was 3.20±0.3 min. The ions measured on LC-MS/MS at ESI+ mode in the present study were m/e 72 (MH+), 55 ([MH-NH3]+) and 27 ([C2H3]+). The transitions monitored were 72>55, 72>54 and 75>58, while the transition for the (13C3)-acrylamide internal standard was the latter one.

**RESULTS AND DISCUSSION**

Sample selection was based on two important aspects; (1) the potential risk of the foodstuffs and (2) whether any previous studies have been conducted. Roasted maize, groundnuts and soybeans are important aspects of the traditional diet in Zimbabwe. Moreover, these food samples are processed by roasting, a technique which have been shown to be responsible for the formation of acrylamide (Friedman, 2003). Except for soybeans, no data on occurrence of acrylamide is available on these samples. Table 1 shows the variation that occurred between the samples. The acrylamide contents in the selected traditional foods varied between the different types of samples. No acrylamide was found in boiled maize this agrees with previous studies which showed that acrylamide is not formed in carbohydrate rich foods prepared by boiling (Hoenicke et al., 2004; Törnqvist, 2005). Roasted maize had the highest amounts of acrylamide and roasted soybeans had the lowest average amount of acrylamide. This could be due to high concentrations of carbohydrates found in maize. Maize is the major source of carbohydrates in a traditional Zimbabwean diet. The high temperatures reached during roasting promoted the formation of acrylamide hence the high concentrations observed. Such a high level of acrylamide is of major concern considering the mutagenic

<table>
<thead>
<tr>
<th>Food</th>
<th>Number of samples</th>
<th>Minimum-maximum (µg/kg)</th>
<th>Average (µg/kg)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roasted maize</td>
<td>3</td>
<td>442.99 to 505.23</td>
<td>460</td>
<td>8.50</td>
</tr>
<tr>
<td>Roasted groundnuts</td>
<td>3</td>
<td>80.42 to 189.60</td>
<td>140</td>
<td>40.21</td>
</tr>
<tr>
<td>Roasted soybeans</td>
<td>3</td>
<td>52.07 to 94.49</td>
<td>70</td>
<td>29.25</td>
</tr>
<tr>
<td>Boiled maize</td>
<td>1</td>
<td>-</td>
<td>ND*</td>
<td>-</td>
</tr>
</tbody>
</table>

*ND- not detected.
The amounts of acrylamide quantified in the roasted soybeans (70 µg/kg in mean) were high compared to levels reported by Friedman in his review, 25 µg/kg (which he obtained from analysis of only one sample) (Friedman, 2003). However, even though the difference is more than 150%, the acrylamide contents in soybeans reported by this study are too small to cause a great change in dietary intake analysis. Moreover, soybeans are protein-rich and studies have shown that acrylamide is capable of forming protein adducts (Shipp et al., 2006). The relatively low amounts of acrylamide in soybeans are due to the low carbohydrate content in soybeans and that some of the acrylamide could have been lost by protein adduct formation. As shown in Figure 1, the soybean sample also had excessive baseline noise. This is the reasons why $^{13}$C$_3$-acrylamide was used as internal standard. It compensates very well for this kind of interferences. The variation in results between the different samples, which can be seen in Table 1, is what has been described by others (Friedman, 2003).

The amounts of acrylamide in the samples analysed in this study might have been underestimated. The reason for this was that the samples spent about four weeks in storage during their shipment. Recent studies have shown that, in some cases, acrylamide contents usually decrease with storage time. Such underestimation could have been eliminated significantly if the studies were carried out here in Zimbabwe, even though it would have required either development of a novel method for analysis of acrylamide or acquisition of new instruments such as LC-MS/Ms which are but very expensive.

The study showed that acrylamide was present in traditional foods found in Zimbabwe. Hence there is a need to carry out a total dietary survey in order to determine the extent of the risk associated with consumption of carbohydrate rich foods.
ACKNOWLEDGEMENTS

The authors would like to thank the following people for their insightful advice, CC Musuna, Tobacco Research Board and D Dube, National University of Science and Technology and Lantmannen Analycen AB, Sjöhagsgatan 3, SE-53140 Lidköping, Sweden, who performed the analysis of acrylamide.

REFERENCES


