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Acrylamide occurrence in *Keribo*: Ethiopian traditional fermented beverage

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ABSTRACT

Keribo is one of the most commonly used traditional beverages in both rural and urban areas of Ethiopia. However, the occurrence of some harmful compounds which could potentially be formed due to its processing methods has never been investigated. The aim of this study was to investigate the occurrence of acrylamide in *Keribo* and its association with processing conditions. Malted and unmalted barley roasted at three levels and also similar levels of sugar concentration were used in *Keribo* preparation. The barley flour to water ratio used during preparation was 1 kg: 10 L. A total of 18 *Keribo* samples were analyzed for their acrylamide contents using high performance liquid chromatography-diode array detector (HPLC-DAD). QuEChERS sample preparation procedure was used. In this study, there was a statistically significant variation ($P < 0.05$) in the acrylamide content of *Keribo* between malted and unmalted barely. The variation in acrylamide content between different levels of roasting and sugar concentrations was also statistically significant ($P < 0.05$). Statistically significant difference ($P < 0.05$) was observed for the three way interaction of malting, roasting and sugar level. The highest concentration of acrylamide (3440 μg/kg) was recorded from *Keribo* prepared from deep roasted unmalted barley with higher sugar concentration. The lowest concentration (1320 μg/kg) was obtained for light roasted unmalted barley with medium sugar concentration. It can be concluded that level of roasting has high implication on acrylamide concentration. Malted barley had a lower concentration of acrylamide and this warrants malting and light roasting of barely are crucially important to minimize the level of acrylamide concentration and reduce the potential health impacts.

1. Introduction

Acrylamide, monomers of polyacrylamides with various applications, is known as one of the food processing contaminants (Mesías & Morales, 2015). It is naturally formed when foods with high starch concentrate cooked at high temperatures and low moistures. Acrylamide can be formed in foods produced commercially, prepared in restaurants and in home produced foods such as fried potato products, roasted coffee beans and bakery products (Ciesarová, 2013). Acrylamide formation and degradation in foods depends on ingredients and its composition, usage of additives, processing conditions and storage duration (Petersen, 2015).

Acrylamide is one of the potential public health problems acquired from certain high starch foods. The chemical is reported to have adverse health effect like carcinogenicity, genotoxicity, neurotoxicity and reproductive toxicity. Its carcinogenicity and neurotoxicity properties has been experimentally demonstrated in animal studies (Efsa, 2015). Existing evidences from human studies on the impact of acrylamide in the diet is not yet conclusive; however, acrylamide in food is widely accepted by scientists as a potential cause of cancer in humans (Cancer, 2010; Claeys et al., 2016; Efsa, 2015).

Since discovery of acrylamide in heated foods by the Swedish scientists in 2002, several researches were carried out regarding its presence in various foods, detection methods, formation mechanism, exposure from diets, toxicology and metabolic consequences, mitigation techniques, and storage stability (Ciesarová, 2013; Claeys et al., 2016; Mesías & Morales, 2015; Michalak, Gujska, Czarnowska, & Nowak, 2014; Michalak, Gujska, & Kuncewicz, 2013; Mizukami, Yoshida, & Ono, 2016; Mustafa, 2008; Mustafa et al., 2009; Stadler, 2005; Taeymans et al., 2004; Ubaoji & Orji, 2016; Vineis,

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Considerable progress has been made on potential means of reducing the formation of acrylamide in certain food products specially in the EU. However, recently conducted case studies fail to demonstrate a decreasing trend in some food items (Claeys et al., 2016). Information on the occurrence of acrylamide in food consumed in developing countries is very scanty. But this information is crucially important to consider appropriate mitigation strategies to minimize acrylamide concentrations in food (JECFA, 2005; 2011).

Keribo is one of the traditional fermented beverages in Ethiopia, vastly popularized in the country in recent years. It is an indigenous traditional fermented beverage and being served on holidays, wedding ceremony and also used as sources of income of many households in Jimma Zone (Abawari, 2013). *Keribo* is prepared from over roasted barley grain and sugar as ingredients. It has different names in different areas such as *Keneto*, *Gasilo*, *Mawudad*, *Filiteri* (derived from filter), and Coca (named due to its similarities with Coca-Cola colour). Severe heat treatments during roasting and high starch content of barley are favourable conditions for the formation of acrylamide in *Keribo*. Despite its wide use and its risky preparation methods, there is no information on the acrylamide content of *Keribo* produced, marketed and consumed in Ethiopia. Therefore, the aim of this study was to determine the levels of acrylamide in *Keribo* and assesses the effects of processing conditions, roasting levels and amount of sugar on its concentration.

2. Materials and methods

2.1. Study site and sample collection

This study was conducted from January 2016 to March 2017. The ingredients for *Keribo* preparation such as barley, sugar and yeast were purchased from local market in Jimma town. The experiments were done in Jimma University College of Agriculture and Veterinary Medicine, Ethiopia except the acrylamide determination which was done at Bless Agri Food Laboratory PLC, Ethiopia.

2.2. Sample preparation and pre-treatments prior to Keribo preparation

Barley grain was initially cleaned; the broken kernels, chaff and other impurities were removed. One portion of cleaned barley grain was malted to study the effect of malting on acrylamide content of *Karibo* while the other part was roasted without malting. The cleaned grain was malted for three days and dried to moisture of 11–12% under sun drying. Both the cleaned malted and unmalted barley grain were roasted at light, medium and deep stage of roasting with coffee roaster and grinded with laboratory grinder.

2.3. Production of Karibo

The roasted barley flour (both from malted and unmalted) was added to water in the proportion of 860:10, 820:10 and 760:10 for light, medium and deep roasting respectively and boiled at 80 °C for 10 min. Then the boiled water-barley flour mixture was filtered using wire mesh sieve. Three levels of sugar (0.5, 1 and 1.5 Kg/10 L of filtrate) were added to *Keribo*. Then it was allowed to cool and kept overnight. After 24 h, yeast (5 g) was added to each treatment and the containers were closed with lids and left to ferment overnight and started to be served.

2.4. Acrylamide determination

The acrylamide content of the *Keribo* (liquid samples) was determined using HPLC-DAAD using QuEChERS extraction methods. About 1 g of sample was taken in 50 ml centrifuge tube, 5 ml of hexane was added and vortexed. 10 ml of water, 10 ml ACN was added and shaken vigorously. The total components were centrifuged for 5 min at 5000 rpm and the hexane layer was discarded. About 1 ml of ACN layer was transferred to microcentrifuge vial packed with PSA and 150 mg of $MgSO_4$, and vortexed for 30 Sec. and centrifuged for 1 min at 500 rpm. The 500 μl of extract was taken to auto-sampler and analyzed by LC/MS/MS.

The HPLC column of reverse phased C18, with length and diameter of 2.1 × 150 mm, 3 μm, the column temperatures were maintained at 30 °C, the mobile phase was used in isocatic mode with 2.5% Methanol and 97.5% of 0.1% formic acid with flow rate of 0.2 ml/min. The run time of 7 min was used. The mass detector was used (positive electrospray ionization mode with jet stream technology). C3-acrylamide was added as the internal standard for the preparation. Both numerical values and chromatograms for all analyzed sample were generated by the system. The chromatogram for selected samples, blank and acrylamide sample standard are illusterated (Figs. 1–4) as an addition information.

2.5. Data analysis

The data were analyzed using Minitab version 16 Software and Analysis of Variance (ANOVA) was used to investigate the significance differences in acrylamide contents of *Keribo* samples. Mean separation was conducted using Tukey's test at $\alpha = 0.05$ level of significance (Table 3).

3. Results and discussions

In the present study the effects of processing conditions such as roasting, malting and level of sugar addition on the formation of acrylamide in *Keribo* were investigated. As described in Table 1, the initial weight of the sample after roasting was reduced from 1000 to 774, 738, and 684 for light, medium and deep roasting malted Barley grain respectively. Similarly, for unmalted barley the initial weight was reduced from 1000 to 860, 820 and 760 for light, medium and deep roasting respectively.

The data of the acrylamide content of *Keribo* sample prepared from malted and unmalted barley roasted at different stages and with level of added sugar is presented in Table 2. Significant ($P < 0.05$) differences were observed for three way interaction of malting, roasting and sugar levels. Significantly higher acrylamide content (3440 μg/kg) was obtained from *Keribo* sample prepared from deep roasted unmalted barley at higher level of added sugar (1.5 kg/10 L) while the lowest value (1320 μg/kg) was recorded for light roasted unmalted sample. This might be due to the higher temperature and time of deep roasting of barely grain. Acrylamide results from various food products were reported around the world. Although roasted barley is boiled to extract the juices prior to fermentation, it is not a factor for acrylamide formation in *Keribo* as sufficient water is added during boiling (see Tables 3 and 4).

Studies on acrylamide content of alcoholic or non-alcoholic fermented beverages are very limited although acrylamide related studies have been conducted and findings are being reported on different food products in many countries around the world. Only few surveys are available on acrylamide in alcoholic beverages (Lachenmeier, Przybylski, & Rehm, 2012). Mo, He, Xu, Huang and Ren et al. (2014) reported a ranges of acrylamide levels in various fermented food products such Curry products (32–155 μg/kg), Rice wine (5–22 μg/kg) and Flavoring of instant noodle (5–281 μg/kg), Tomato sauce (5–7.4 μg/kg), Salad dressing (5–11 μg/kg), Soy sauce (5–1152 μg/kg) and Chinese yellow rice wine (8.3 μg/kg). According to Dupire (2003) the acrylamide