Comparison of Two Methods for Acrylamide Determination and Dietary Intake of Acrylamide from Potato Crisps in Slovakia

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Abstract: Two GC/MS methods for acrylamide determination in potato crisps were used. By the method without derivatisation the presence of acrylamide was confirmed. The quantities of acrylamide were compared by the bromination method with $^{13}C_3$-acrylamide and D_3-acrylamide as internal standards. A suitable agreement between the results obtained from two independent laboratories was achieved; the difference was less than 5%. Using average level of acrylamide in crisps 986.5 μg/kg and mean consumption data on potato crisps in Slovakia it was calculated that consumers are exposed to 8.5 μg acrylamide daily from its which means 0.12 μg/kg body weight/day. This amount contributes to 20–40% of daily acrylamide intake from food.

Keywords: acrylamide; acrylamide determination; dietary intake; potato crisps; GC/MS

INTRODUCTION

Since April 2002 when acrylamide findings in some heat-treated foods became known [1] a lot of laboratories have created various analytical methods for its determination. The methods are usually based on GC/MS and LC/MS/MS methodologies and they are validated only for a limited range of matrices [2]. Due to the small molecular weight of acrylamide (71 g/mol) GC/MS has been historically used for the detection [1]. Bromination of acrylamide has the advantage that a more volatile compound is produced and the selectivity of determination is increased. However, derivatisation of acrylamide with bromine is laborious and time consuming, therefore a direct method of acrylamide determination after extraction and clean-up is preferred now. It was found that the use of dry *n*-propanol as extractant decreased acrylamide recovery drastically [2]. De-fatting can be accomplished by extraction of the sample residue containing the analyte with hexane or n-hexane/acetonitrile mixture to eliminate the influence of high fat content of sample on the analysis.

Acrylamide in food is largely derived from heat-induced reactions between the amino group of the free amino acid asparagine and the carbonyl group of a reducing sugar such as glucose during baking and frying [3]. Widely consumed processed foods with high levels of acrylamide include French fries, potato chips, tortilla chips, bread crust, crispbread, and various baked goods and cereal formulations. However, the wide variations in levels of acrylamide in different food categories as well as in different brands of the same food category (e.g. French fries, potato chips – crisps) appear to results not only from the amounts of the precursors present but also from variations in processing conditions (e.g. temperature, time, nature of frying oil, nature of food matrix).

In Central Europe fried, roasted and baked potato products belong to main sources of acrylamide intake combining high acrylamide concentration with high consumption. Based on the available data average intakes for the general population were estimated to be in the range of 0.3 to 0.8 μg of acrylamide per kilogram of body weight per day [4]. According to the survey of NFA the percentage contribution of crisps results in 10% [5].

In our study we compare two methods for qualitative and quantitative acrylamide determination and the use of different internal standards for

relevant measurement of acrylamide in potato crisps. Based on average level of acrylamide in crisps and the mean consumption of crisps in Slovakia we estimated the acrylamide human intake from crisps.

EXPERIMENTAL

Food samples. Sample of potato crisps was purchased in a local supermarket.

Chemicals. Acrylamide (99.9%), propionamide (98%) and methacrylamide (99%) were obtained from Merck, Darmstadt, Germany, $^{13}C_3$- and D_3-acrylamide (98%) were obtained from CIL Andover, MA, USA. All other chemicals of analytical grade were obtained from Lachema Brno, Czech Republic.

Sample preparation for GC/MS without derivatisation. Ten g of potato crisps was homogenised by ultra-turrax blender, 50 ml of ethanol was added and acrylamide was extracted at 70°C for 30 min in ultrasonic bath. After clarifying with Carrez solutions, filtration and centrifugation the obtained extract was de-fatted with hexane and evaporated to the volume of 1 ml. The internal standards propionamide and methacrylamide were added before extraction.

Sample preparation for GC/MS with derivatisation. Five grams of potato crisps was homogenised by ultra-turrax blender; sample was double extracted with hot water and swelled in ultrasonic bath. The obtained extract was clarified with Carrez solutions, double de-fatted with hexane and derivatised by bromine (1 h, 0°C). The excess of bromine was decomposed with sodium thiosulphate and sample was cleaned-up by SPE to ethyl acetate. The volume of obtained eluate was made-up to 1 ml by rotary evaporator. The internal standard $^{13}C_3$-acrylamide and D_3-acrylamide, respectively, was added before water extraction.

GC/MS without derivatisation conditions. HP 5890 series II gas chromatograph coupled with MS detector HP 5971A with electron ionisation (70 eV) was used. The ZB FFAP column 30 m × 0.25 mm × 0.25 μm was used with following temperature program: 60°C, 1 min, 10°C/min, 230°C. GC was operated at constant pressure 70 kPa with helium as a carrier gas. Two μl of sample were injected at 250°C by splitless technique. Mass spectra were scanned in the range 29–200 amu at 1 scan/sec. Obtained mass spectra were compared with NIST library mass spectra.

First GC/MS with derivatisation conditions (FRI Bratislava). HP 5890 series II coupled with MSD

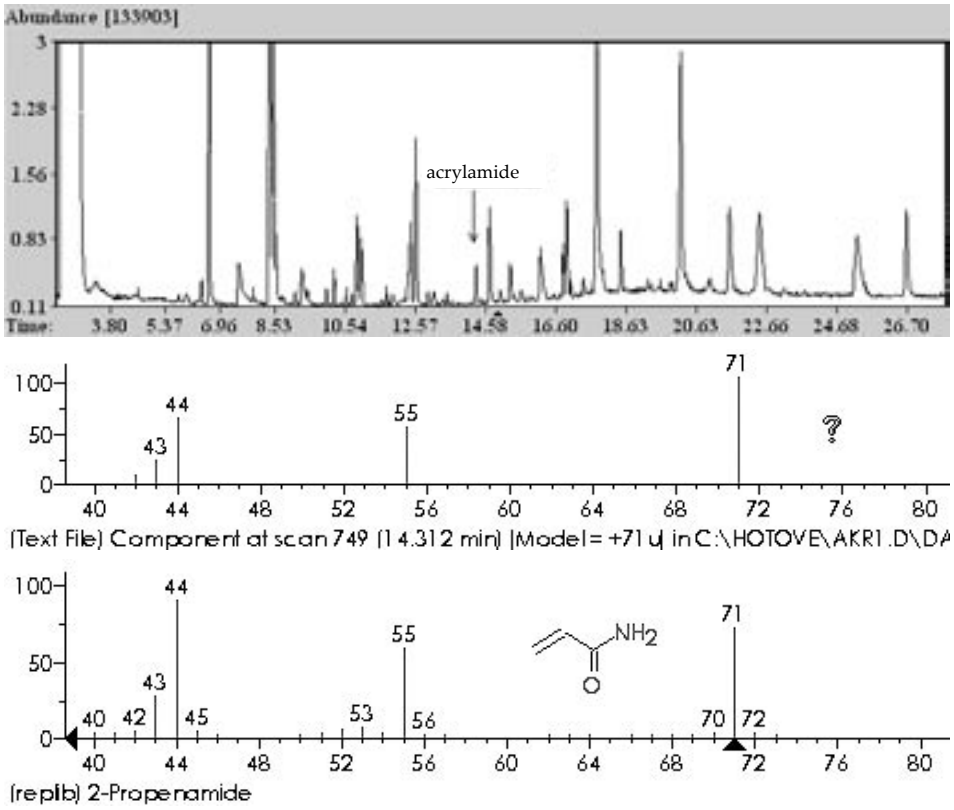

Figure 1. GC/MS analysis of potato crisps without derivatisation: TIC, recorded mass spectrum and mass spectrum of acrylamide from the NIST library

HP 5971A with electron ionisation (70 eV) was used. The DB5 column 30 m × 0.25 mm × 0.5 μm was used with following temperature program: 60°C, 1 min, 10°C/min, 240°C, 5 min. GC was operated at constant pressure (70 kPa) with helium as a carrier gas. One μl of sample was injected at 190°C by splitless technique. SIM chromatograms of *m/z* 150 for 2,3-dibromopropionamide and *m/z* 155 for D_3-2,3-dibromopropionamide recorded in low resolution mode at dwell time 50 msec and EM voltage 1300 were evaluated.

Second GC/MS with derivatisation conditions (IPH Karviná). GC/MS Polaris Q (Thermo Finnigan) with HP-5MS (30 m × 0.25 mm × 0.25 μm) column was used at the constant flow of helium 1 ml/min. The temperature program was following: 55°C, 1 min, 10°C/min, 270°C, 15 min. The detector in both MS and MS/MS mode with electron ionisation (70 eV) at 200°C was used for scanning of mass spectra *m/z* 150 + 152 and *m/z* 133 + 135 in ratio 1:1 for 2,3-dibromopropionamide and *m/z* 153 + 155 and *m/z* 136 + 138 in ratio 1:1 for $^{13}C_3$-2,3-dibromopropionamide.

Consumption data. Monthly consumption data were obtained from the representative food survey on 4150 consumers in Slovakia which was provided by Median SK in 2001 and 2002.

RESULTS AND DISCUSSION

For confirmation of presence of acrylamide in crisps sample we used the GC/MS method without derivatisation with both methacrylamide and propionamide as internal standards in full scan mode. Drawing a comparison between obtained mass spectrum and NIST library mass spectrum of acrylamide we confirmed a present acryamide in the crisps sample (Figure 1). However, this way of analysis is available only for qualitative determination for its less sensitivity.

For quantification of acrylamide amount we used GC/MS bromination method whereas MS detector was running in SIM mode. The method development was carried out at two different laboratories: FRI Bratislava and IPH Karviná. The concentrations of acrylamide were calculated from the ratio of selected ions areas with D_3-acrylamide and $^{13}C_3$-acrylamide, respectively, as internal standards. The linearity of calibration curve was achieved between 10 ng/ml and 1000 ng/ml with the regression coefficient 0.9990. By this method we found out that the amount of acrylamide in analysed crisps sample was 963 μg/kg measured in FRI laboratory and 1010 μg/kg in IPH laboratory, respectively (Figure 2). These results were in sufficient agreement 95%.

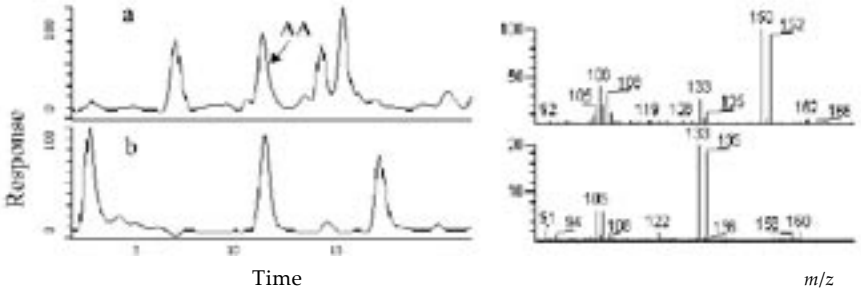

Figure 2. GC/MS chromatogram of crisps after derivatisation: a – Full scan chromatogram with separated ions 150 + 152 and parent spectrum; b – GC-MS/MS chromatogram with separated ions 133 + 135 and typical daughter acrylamide spectrum

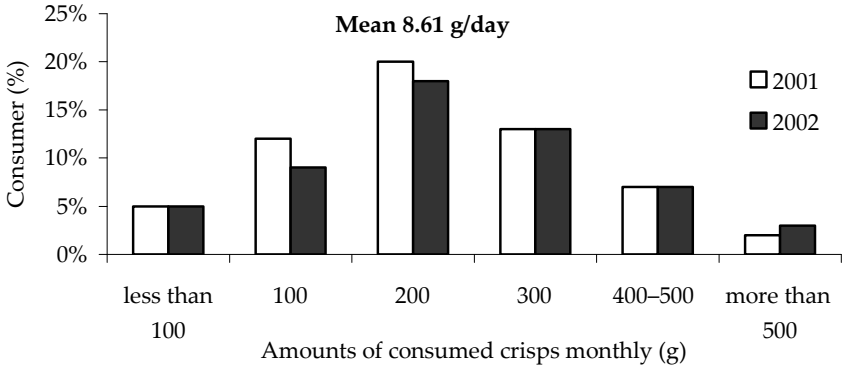

Figure 3. Distribution of crisps consumption in Slovakia from representative survey on 4150 consumers in 2001 and 2002

The dietary intake of acrylamide from crisps consumption data on 4150 Slovaks in 2001 and 2002 was estimated. On these surveys more than 54% respondents quoted of crisps consumption, whereas the highest crisps intake was for girls at 14–15 years. The mean consumption of crisps was 8.6 g per day (Figure 3) which corresponds to 0.12 μg of acrylamide per kg of body weight daily approximately considering the average of adult body weight 70 kg. These results were similar to published data [4, 5]. Three percents from population which consumed daily more than 20 g of crisps was exposed to more than 16 μg of acrylamide corresponding to 0.23 μg/kg body weight per day.

CONCLUSIONS

Determination of acrylamide by GC/MS bromination method offers the results with high accuracy and sensitivity. Comparable results were achieved from parallel measurements of the identical crisps samples in two different laboratories with 95% agreement. The acrylamide intake from crisps was estimated to 0.12 μg/kg body weight per day.

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