

Analytical Methods

Determination of acrylamide in roasted chestnuts and chestnut-based foods by isotope dilution HPLC-MS/MS

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ABSTRACT

A collaboratively trial tested isotope dilution liquid chromatographic method with positive electrospray ionisation tandem mass spectrometry for the analysis of acrylamide in bakery ware and potato products has been extended to the determination of acrylamide in roasted chestnuts and chestnut-based foods. As chestnuts have a similar composition to potatoes, considerable amounts of acrylamide can be expected, especially in roasted chestnut products. This paper presents the concentrations of acrylamide in 31 different chestnut samples (fresh, roasted, flour, cooked, glazed) that were collected in nine European countries during 2005/2006. The influence of the roasting time on the acrylamide content was also experimentally investigated. A test portion was extracted after homogenisation with water and isotopically labelled acrylamide was added. The extract was centrifuged and the supernatant was cleaned-up in two consecutive solid phase extraction steps. The final extract was analysed by high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). An HPLC column based on graphitised carbon was applied for chromatographic separation. Acrylamide concentrations in purchased roasted chestnuts were in the range of <math><8\text{--}1278\ \mu\text{g}/\text{kg}</math> whereas only low amounts (<math><4\text{--}159\ \mu\text{g}/\text{kg}</math>) were found in chestnut products. However, the median acrylamide content of the commercial roasted chestnut samples was 90 $\mu\text{g}/\text{kg}$. The influence of the roasting time on the acrylamide content in roasted chestnuts was evaluated too. As with roasted and fried potato products, the roasting time has a significant influence on the acrylamide formation. Therefore, the consumers might be exposed to significant amounts of acrylamide by eating roasted chestnuts, especially when a batch remains in the roasting vessel for too long time.

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1. Introduction

Acrylamide (2-propenamide) has been classified as a probable human carcinogen by the International Agency for Research on Cancer (IARC, 1994) and is known as neurotoxin WHO (World Health Organisation, 2002). Since 2002 there is much concern regarding acrylamide contents in food products (JECFA, 2005), especially in fried and baked products containing carbohydrates and amino acids, especially asparagine (Ahn et al., 2002; Riediker & Stadler, 2003; Rosén & Hellenaes, 2002; Senyuva & Gökmen, 2005; Wenzl, Beatriz De La Calle, & Anklam, 2003). Chestnuts have a similar composition to potatoes, and are either consumed in their roasted or cooked form. Considerable amounts of acrylamide can be expected, especially in roasted chestnut products.

The nutritional composition of different chestnut varieties has been studied by a number of working groups (e.g. Breisch, 1995; Miguez De La Montana, Miguez Bernardez, & Garcia Queijeiro, 2004): The moisture content in chestnuts ranges between 49%

and 60%, the starch content lies between 56% and 82%, sucrose between 10% and 30%, the protein content between 3% and 9% and lipid content was found between 1.5% and 6% based on dry matter.

Chestnuts are a quintessential seasonal food, the peak season being in Europe from September through January, and are usually served roasted or incorporated into many recipes, mainly in their cooked form, although the consumption of roasted chestnuts is low compared to bakery ware and potato products. Depending on the conditions during processing, particularly during roasting, formation of acrylamide can be assumed and the latter may even be present in high amounts.

The aim of this study was the determination of the acrylamide content in roasted chestnuts and chestnut-based foods from different sources, as well as the evaluation of the influence of roasting time at a certain temperature on the final acrylamide content of the product. However, this work does not claim to carry out a full survey on the acrylamide content of the roasted chestnuts in European countries.

The isotope dilution HPLC-MS/MS method applied for the analysis of acrylamide in roasted chestnuts and chestnut-based foods was developed by Rosén and Hellenaes (2002); Rosén, Nyman,

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and Hellenaes (2007) and successfully validated in a collaborative study on bakery ware and potato products (Wenzl et al., 2006). The scope of the method was extended in this work to chestnuts and chestnut-based foods. Method performance parameters were evaluated for these commodities as well.

2. Materials and methods

2.1. Standards and solvents (Chemicals and consumables)

Acrylamide (CAS 79-06-1) with purity $\geq 99.9\%$ was purchased from Sigma–Aldrich and deuterium-labelled (D_3 -acrylamide, deuterium content $>98\%$) was purchased from Polymer Source Inc. (Dorval, Quebec, Canada). Stock solutions were prepared by dilution in water and stored at $4\text{ }^\circ\text{C}$. The standards for instrument calibration contained 5, 10, 50, 100, 500, 1000, 2000 and 5000 ng/mL acrylamide, and 400 ng/mL of D_3 -acrylamide. All organic solvents were of HPLC grade quality. Isolute Multimode[®] (6 mL, 500 mg) and Isolute ENV+[®] (6 mL, 500 mg) solid phase extraction cartridges were purchased from International Sorbent Technology (Mid Glamorgan, UK).

2.2. Samples

The roasted chestnut samples were purchased from street vendors in Austria, Czech Republic, France, Germany, Poland and Spain during winter 2005/2006. The fresh nuts for the investigation of the influence of the roasting time on the acrylamide content were purchased in retail outlets in Belgium (French origin), Germany (French origin) and Poland (Spanish origin). Chestnut-based food products - puree, flour, pasta, and sweet chestnuts (glazed) were purchased from local markets in Hungary, Germany, Italy, and Spain.

As chestnuts have high moisture content which can cause them to spoil quickly, care must be given to proper handling and storage. Fresh, as well as roasted chestnuts were kept at $-20\text{ }^\circ\text{C}$, whereas chestnut-based food products were refrigerated.

The influence of the roasting time on the acrylamide content was investigated experimentally by roasting fresh chestnuts in a household oven. Only firm and unblemished chestnuts were selected for these experiments. On the flat side of each nut an “X” shaped cut was made in order to allow steam to escape. They were processed at $200\text{ }^\circ\text{C}$ and samples were taken every 5 min. The outer shell and the thin, papery skin inside were peeled away prior to grinding and homogenisation, which was carried out under liquid nitrogen.

2.3. Sample preparation and extraction

Portions of 2.0 g of homogenised sample were placed into 50 ml Falcon tubes and were extracted after addition of 400 μl of internal standard solution (absolute amount of isotopically labelled acrylamide was about 0.8 μg) with 40 ml of water by shaking intensively for 15–30 sec on a vortex shaker. Afterwards they were put for 60 min on a horizontal shaker adjusted to maximum sample-extractant agitation speed e.g. 100 rpm and centrifuged in a cooled centrifuge ($10\text{ }^\circ\text{C}$, 4000 rpm, 20 min).

2.4. Clean-up

An aliquot (10 mL) of the supernatant was passed through the preconditioned Isolute Multimode[®] solid phase extraction cartridge placed on a vacuum manifold. The eluate from the Multimode column was collected and then loaded on a preconditioned Isolute ENV+[®] solid phase extraction column. The eluate was dis-

carded and acrylamide was eluted with 3 mL of 60% methanol, which was evaporated from the extract, never exceeding $40\text{ }^\circ\text{C}$, by a gentle stream of nitrogen. The block temperature of the evaporator was set to maximum $40\text{ }^\circ\text{C}$. The final volume of the extract was approximately 500 μL .

2.5. HPLC-MS/MS analysis

The HPLC-MS/MS analyses of roasted chestnuts were performed with an Agilent 1100 HPLC system consisting of a binary pump, an autosampler and a temperature controlled column compartment, coupled to a Waters Micromass Quattro Ultima PT tandem mass spectrometer. The analytical separation was performed on a Thermo Hypercarb (Thermo Electron Corporation, Waltham, MA, USA) graphitised carbon HPLC column. Separation was performed isocratically with a mobile phase consisting of 0.2 mL of formic acid in 1.0 L of MilliQ water. The flow rate was 0.4 mL/min. The injection volume was 10 μL . The HPLC-MS/MS was operated in positive electrospray and selected reaction monitoring mode. Nitrogen was used as the cone (300 L/h) and desolvation (600 L/h) gas. The source temperature was maintained at $125\text{ }^\circ\text{C}$ and desolvation temperature was $400\text{ }^\circ\text{C}$. The capillary voltage was kept at 2.2 kV, cone voltage at 35 V. The collision energy was optimised for the following transitions: $m/z\ 72 > 55$, $72 > 54$, $72 > 44$ and $75 > 58$. Dwell time for each transition and inter-channel delay were optimised to reach high sensitivity, absence of crosstalk and a sufficient number of data points per peak. The transitions $m/z\ 72 > 55$ and $75 > 58$ of acrylamide and D_3 -acrylamide, respectively were used for quantitation, and $m/z\ 72 > 55$, $72 > 54$ and $72 > 44$ were used for confirmation of the peak identity. The ion intensity ratio of these transitions was calculated and compared to the ratios calculated for calibration standard. The maximum tolerable deviations of the ion intensity ratios were set in accordance with Commission Decision, 2002/657/EC.

Each sample was injected twice. The calibration standard solutions were injected before, between and after the sets of sample extracts. A run time of 10 min was used for samples, in order to allow matrix components to elute from the column. The column was rinsed with 100% methanol at 0.2 mL/min in between the set of samples and after the end of the sequence run. The response of the HPLC-MS/MS may vary from day to day and the HPLC column may deteriorate. Therefore, the system suitability was checked prior to each analysis by the injection of a standard solution containing 50 ng/mL acrylamide and 400 ng/mL of D_3 -acrylamide.

2.6. Analytical quality assurance

For the assessment of the stability of the analysis procedure, a candidate reference material (crisp bread, ERM[®]-BD272 from the Federal Institute for Materials Research and Testing, Berlin, Germany) with a preliminary certified value of 980 $\mu\text{g}/\text{kg}$ was analysed together with the investigated samples within each sequence of 10 samples. No statistically significant difference from the certified value was found. The analysis procedure was also successfully applied in a proficiency test on the analysis of acrylamide in crisp bread (z-score 0.8).

3. Results and discussion

3.1. Performance of method

Altogether 22 samples of roasted chestnuts and nine chestnut-based foods from eight different European countries were investigated for their acrylamide content. With respect to the possible matrix interferences, quantitation of results was based on an

Table 1
Acrylamide content in roasted chestnuts of different origin.

Origin	Acrylamide ($\mu\text{g}/\text{kg}$)	Number of samples/site
Austria – Graz	46–203	9
Vienna	<LOQ–74	3
Czech Republic – Prague	88–315	2
France – Provence	828	1
Germany – Aachen	23–265	4
Cologne	32–100	2
Spain – Segovia	1278	1

isotope dilution method. A series of chestnut samples were spiked with D_3 -acrylamide. Sufficient retention of acrylamide ($t_R = 2.05 \pm 0.06$ min) was achieved using a Thermo Hypercarb graphitised carbon HPLC column (5 cm, 2.1 mm, 5 μm). The limit of quantitation (LOQ), based on the transition m/z 72 > 55, was 9 $\mu\text{g}/\text{kg}$ for roasted chestnuts and 4 $\mu\text{g}/\text{kg}$ for chestnut puree samples.

The analysed chestnut samples were mainly roasted over charcoal fire, and were sold from street vendors. Sampling was done at different locations in the respective cities, which implies different vendors and therefore roasting procedures (temperature and time). Roasted chestnuts are frequently sold in portions of 250 g corresponding to about 10–12 roasted chestnuts per portion. From each portion, only intact and impeccable chestnuts were selected for analysis.

Quite a large variability of the acrylamide content in the roasted chestnut samples was found, which is comparable to many other types of food products that are listed in the EU database on acrylamide levels in food (European Commission, 2006). The highest content of commercial roasted chestnuts was determined in one sample coming from Spain (1278 $\mu\text{g}/\text{kg}$). However, this seems to be an extreme value. The median acrylamide content of the com-

Table 2
Acrylamide content in chestnut-based products of different origin (one sample of each).

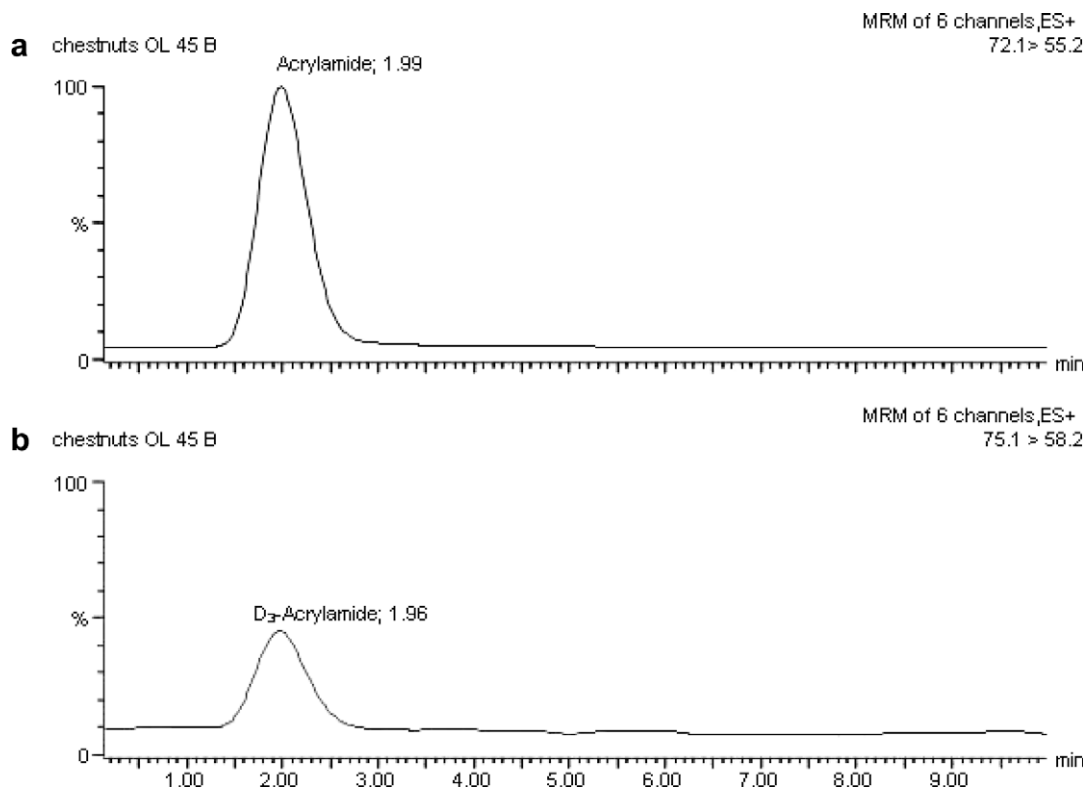
Product	Origin	Acrylamide ($\mu\text{g}/\text{kg}$)
Cooked chestnuts A	France	36
Cooked chestnuts B	France	38
Chestnut puree	Hungary	<LOQ
Chestnut puree	Italy	4–5 (2 samples)
Chestnut puree	France	9
Chestnut flour	Italy	159
Chestnut pasta	Italy	4
Sweet chestnuts	Italy	12

mercial roasted chestnut samples was determined to be about 90 $\mu\text{g}/\text{kg}$. The data for the acrylamide content in the various roasted chestnuts are listed in Table 1. Examples of chromatograms are shown in Fig. 1.

The acrylamide content of other kinds of chestnut based food (mainly cooked products) was also investigated. These were chestnut puree, flour and glazed chestnuts as listed in Table 2. As expected, the acrylamide content of these samples was much lower than that of roasted chestnuts, being also the case for potato products. The acrylamide content was found to be below or close to the LOQ (4 $\mu\text{g}/\text{kg}$) for the majority of samples. Therefore, these food items are of minor relevance with regards to the acrylamide intake of humans via food, due to their low content and probable low quantities consumed.

3.2. Influence of roasting time at household heating conditions

Time and temperature are important parameters in the formation of acrylamide in heat-treated food (Bräthen & Knutsen, 2005; Taubert, Harlfinger, Henkes, Berkels, & Schomig, 2004).

**Fig. 1.** Example of HPLC-MS/MS chromatograms: (a) acrylamide in roasted chestnuts (828 $\mu\text{g}/\text{kg}$), (b) D_3 -acrylamide internal standard added to the same chestnut sample.

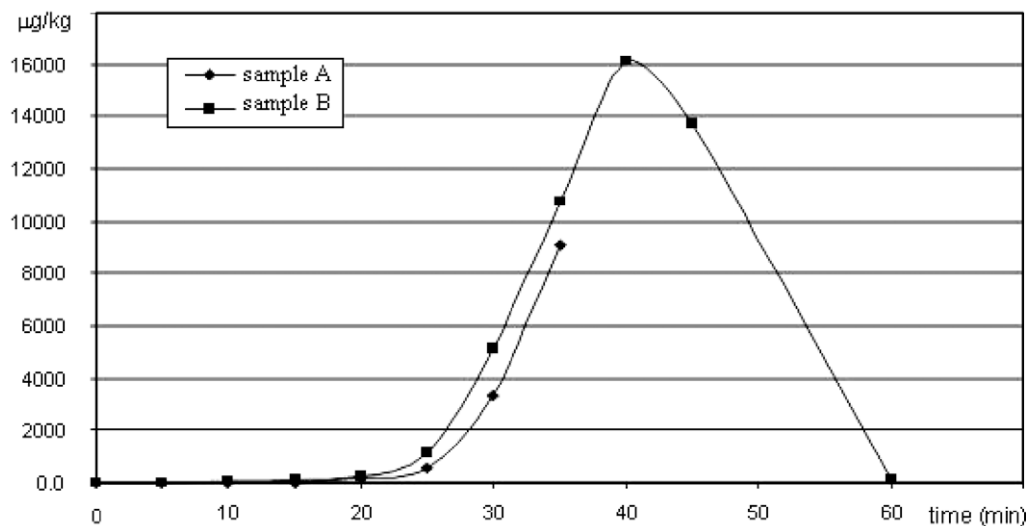


Fig. 2. Influence of the roasting time on the acrylamide content in roasted chestnuts during roasting in the oven at 200 °C.

The influence of the roasting time on the acrylamide content in roasted chestnuts is shown in Fig. 2. Two batches of fresh chestnuts purchased in two countries were roasted in two different ovens (household and laboratory) at nominal temperatures of 200 °C, and samples were taken every 5 min. In the first experimental set up (sample A), sampling was stopped after 35 min, when the colour of chestnuts in the oven was getting deeply dark and the product would not have been eaten anymore as such. The acrylamide content was at that time around 9000 µg/kg. From the analysis and evaluation of results of that experiment, it became clear that a further increase of the acrylamide content could be expected when the roasting process continues. Hence the experiment was repeated (sample B) with a prolonged roasting time of up to 60 min. After 40 min the acrylamide content decreased, confirming the findings of Eriksson, Rydberg, Tareke, Karlsson, and Ehrenberg (2003) that the acrylamide contents of different kind of foodstuffs decrease at prolonged processing.

In conclusion, these results indicate that the acrylamide level in roasted chestnuts could be very high, especially when a batch remains in the roasting vessel on street markets for quite a long time, or when chestnuts are roasted in the household, not keeping an exact baking time or applying too high temperatures. However, all but one of the 22 investigated samples from street vendors contained acrylamide at levels below 1000 µg/kg.

4. Conclusions

The scope of the by collaborative trial tested isotope dilution high performance liquid chromatographic method with positive electropray ionisation tandem mass spectrometry was successfully extended to the determination of the acrylamide content in roasted chestnuts and chestnut-based foods. The method was in-house validated for acrylamide analysis in various chestnut products. The influence of the roasting time on the acrylamide content was also experimentally investigated. Although acrylamide contents in roasted chestnuts could be quite high, it is unlikely that this kind of food has significant impact on the total acrylamide intake of humans via food. Chestnut-based products did not contain high levels of acrylamide and the impact of these products on the acrylamide intake of humans is considered insignificant.

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