Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 110 (2008) 504–511
FOOD CHEMISTRY
www.elsevier.com/locate/foodchem

Analytical Methods

Production of a certified reference material for the acrylamide content in toasted bread

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Received 13 September 2007; received in revised form 21 December 2007; accepted 10 February 2008

Abstract

The need for a certified matrix reference material (CRM) of acrylamide in a food type matrix was emphasized by the competent authorities as a tool to improve comparability, ensuring accuracy and traceability of analytical results. The institute for reference materials and measurements (IRMM) responded to the international request by producing a certified reference material, ERM-BD273, containing endogenous acrylamide in a toasted bread matrix. This work describes the production of the CRM, according to ISO Guides 34 and 35 [ISO Guide 34 (2000). *General requirements for the competence of reference materials producers*; ISO Guide 35 (2006). *Reference materials – General and statistical principles for certification*], which comprises the material processing, homogeneity and stability assessment, material characterisation and the acrylamide mass fraction value assignment in toasted bread. Heterogeneity of the material between the vials processed was determined by an in-house validated gas chromatographic methodology involving acrylamide derivatisation and mass spectrometric detection and found to be below 2%. Potential degradation during storage was also investigated and a shelf-life based on this value was established. A collaborative study for material characterisation involved sixteen laboratories applying different analytical methodologies including gas chromatography or high resolution liquid chromatography and isotopic dilution mass spectrometry. The certified value for acrylamide in ERM-BD273, traceable to the international system of units (SI), is (425 ± 29) ng g^{-1}.

Keywords: Acrylamide; Certified reference material; Food analysis; Chromatography; Isotope dilution mass spectrometry

1. Introduction

Acrylamide was classified as a probable carcinogenic compound for humans, group 2A, by the international agency for research on cancer (IARC) in 1994. The finding of high levels of acrylamide in starch-rich food processed at high temperatures, reported by the Swedish National Food Administration in 2002 (Tareke, Rydberg, Karlsson, Eriksson, & Törnkvist, 2002) was therefore taken as a concern by competent authorities worldwide. As a result, research programs were initiated with major findings being the elucidation on the generation of acrylamide by a Maillard reaction between an amino acid (asparagine) and reducing sugars at temperatures above 100 °C (Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002; Weisshaar & Gutsche, 2002). Despite the IARC classification, no regulation on the maximum levels of acrylamide in foodstuff has been elaborated since the compound cannot be categorised as a food contaminant *per se*. In fact the formation of acrylamide occurs while food is processed and/or cooked and therefore it is difficult to avoid and control, particularly in home made food. To minimise health risks to consumers, the food industry is focusing on the optimisation of processes to lower the acrylamide content in the final products. Meanwhile the evaluation and control of acrylamide levels by the international scientific community, in collaboration with food safety organisations and different food

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doi:10.1016/j.foodchem.2008.02.034

producers, resulted in the development of methods for the extraction and determination of acrylamide in foodstuff. However, comparability of the results and ensuring accuracy is not an easy task when analysing complex matrices. Therefore, competent authorities emphasized the need for certified matrix reference materials (CRMs) that could, in addition, support meteorological traceability of the results. As a response, and after the establishment of the European reference materials (ERM) initiative (Emons, Marriot, & Matschat, 2005), the institute for reference materials and measurements (IRMM) took on the task to produce ERM-BD273, a certified reference material containing endogenous acrylamide in a starch-rich food matrix processed at a high temperature, i.e. a toasted bread, following the principles of ISO Guides 34 and 35. In parallel to the development of ERM-BD273, the German Federal institute for materials research and testing (BAM) produced an additional bread-based material (ERM-BD272) containing a higher endogenous acrylamide level. Together these two materials cover a range of mass fractions fulfilling the needs of the analytical laboratories.

This communication describes the production of the ERM-BD273 toasted bread, including the material processing, the results of tests performed to assess its homogeneity and stability, and the strategy to assign reference acrylamide values in the material.

2. Materials and methods

A commercially available heat-processed starch-rich food matrix, toasted bread, was selected as base-material for the preparation and the certification of the acrylamide mass fraction.

2.1. Processing of material

The base-material, toasted bread, was purchased off-the-shelf from a local grocery store and kept at room temperature prior to processing. The bread ingredients (wheat flour 98%, yeast, sugar, vegetable oil, iodine salt) and major proximates (per 100 g: Proteins 13.7 g, carbohydrates 71 g, fat 4.2 g, dietary fiber <4.1 and sodium 0.7 g) are provided as indicated in the commercial product label.

The bread slices, initially crushed using a PTFE pestle, were milled in an impact mill at a speed of 10,000 rpm, avoiding excessive heating of the mill and the material. Subsequently the powdered bread was sieved sequentially through a 500-μm and a 63-μm sieve. The resulting 63–500 μm fraction was homogenised in a turbula mixer prior to filling. Analysis of water content was performed by Karl Fischer titration (KFT) and particle size analysis (PSA) was performed by laser light diffraction for quality control of the bulk material processed. In total 37 kg of toasted bread were filled using an automatic filling machine loading approximately 30 g of material into 100 mL amber glass vials. The headspace above the material was evacuated and was filled with argon prior to capping and the vials were stored at a temperature of −20 °C.

2.2. Assessment of stability

Dispatching conditions for the CRM as well as long-term storage conditions have to be established based on the outcome of the short- and long-term stability studies, respectively. For both stability studies the isochronous test scheme as outlined by Lamberty, Schimmel, and Pauwels (1998) was applied, allowing the reduction of analytical data variation and the increase of significance of the result by measuring the samples under repeatability conditions. A pre-defined number of vials from the full production batch were selected by random stratified sampling. Three vials per temperature were stored at (−20, 4 and 18 °C) during three different periods of time (1, 2 or 4 weeks for the short-term and 8, 16 or 24 months for the long-term scheme). At the end of each period the vials were placed at the so-called reference temperature, −70 °C, where no material degradation was expected, until all the time periods were completed. In this way it was possible to analyse all the samples under repeatability conditions, including three additional vials (reference samples) that were kept at the reference temperature from the beginning of the study. In total, each stability study scheme comprised the analysis of 30 vials, in triplicate, by an IRMM in-house validated GC–MS methodology involving bromination and the use of an internal standard of isotopically labelled acrylamide. Briefly, 1 g methanol and 1 g of [$^{13}C_3$]-acrylamide standard (1 μg/g) were mixed with 2 g of the bread powder, followed by the addition of 20 mL of deionised water and amylase. The sample was then subjected to extraction under ultrasonication for 15 min at 50 °C. 100 μL acetic acid, 1 mL Carrez I and 1 mL Carrez II reagents were subsequently used for protein and sugar precipitation. The precipitate was eliminated by centrifugation at 4000 rpm for 5 min. Bromination by addition of 7 g KBr, 200 μL HBr (47%) and 2.5 mL saturated bromine water (for 2 h at 4 °C) was followed by addition of 200 μL $Na_2S_2O_3$ (1 N) and continued by extraction into 15 mL ethyl acetate. Finally the sample was reduced to 1 mL and 20 μL triethylamine were added prior to GC–MS analysis by means of a Thermo-Finnigan GC 2000 series coupled to a Trace quadrupole mass spectrometer (Thermo). Sample volumes of 1 μL were injected in splitless mode. A GC capillary column DB-Wax (30 m × 0.25 mm I.D., 0.25 μm film thickness) from J& W Scientific, USA, was used at an initial temperature of 80 °C for 1 min and raised at 8 °C/min to 200 °C. After 6 min the temperature was raised again at 40 °C/min to 250 °C and finally held isothermically for 3 min. The mass spectrometer was operated in electron impact ionisation mode (70 eV) and a temperature of 200 °C was applied to the ion source. Selected ions from the acrylamide, both labelled and non labelled 2-bromopropenamide derivatives formed, were monitored (m/z 154 and m/z 149, respectively) and quantification

was carried out by approximate matching calibration approach.

The evaluation of the stability results at each temperature included a check for statistically significant outliers and linear regression analysis as a function of time, where slopes were tested for significance using *t*-tests. The uncertainty associated to the stability of the material was estimated as the uncertainty of the regression line with a slope of zero multiplied with the envisaged shelf life as described by Linsinger et al. (2001).

2.3. Homogeneity study

The homogeneity study assesses the distribution of the component to be certified in all the units bottled. That means that heterogeneity has to be quantified in terms of concentration of acrylamide, among randomly chosen vials. For that purpose homogeneity was investigated by checking the short-term stability data for samples stored at −20 °C, which corresponds to the storage temperature of the stock samples. The use of the short-term stability study data was appropriate for homogeneity evaluation since no sign of instability of the toasted bread with time was detected. Thus the data from the 12 vials tested in triplicate by GC–MS were evaluated. Care was taken that the analytical sequence did not correspond to the filling sequence of the vials, to be able to distinguish between analytical drift and a possible trend in the filling sequence.

The presence of outliers was tested by using the Grubbs procedure and regression analysis was performed to check any possible trend in the filling sequence. The distribution of results was checked using normal probability plots and histograms. Finally, analysis of variance (ANOVA) was performed to quantify the within-vial variation (s_{wb}; repeatability) and between vial variation (s_{bb}). The method repeatability sets a limit to the degree of heterogeneity that can be detected by a particular method with a given study set-up (number of repetitions per sample; number of samples). The maximum heterogeneity that could be hidden by method repeatability (u^*_{bb}) was calculated as described by Linsinger, Pauwels, van der Veen, Schimmel, and Lamberty (2005).

2.4. Characterisation

The aim of the characterisation is the assignment of a certified value of the target parameter. For this purpose, a collaborative study was organised including 16 selected laboratories with demonstrated expertise in the analysis of acrylamide in food matrices. Most laboratories were accredited to ISO 17025. Here, the laboratories are listed in alphabetical order to make the entries in Table 1 anonymous.

Bundesanstalt für Materialforschung und –prüfung, Berlin, Germany, DE.
Chemisches und Veterinäruntersuchungsamt, Stuttgart, Germany, DE.
Chemisches und Veterinäruntersuchungsamt, Sigmaringen, Germany, DE.
Dublin Public Analyst Laboratory, Dublin, Ireland, IE.
Eurofins, Wiertz-Eggert-Jörissen, Hamburg, Germany, DE.
European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Geel, Belgium, BE.
German Research Centre of Food Chemistry*, Garching, Germany, DE.
General Chemical State Laboratory, Food Division and Division of Environment, Athens, Greece, EL.
Kantonales Labor, Zürich, Switzerland, CH.
Lebensmittelversuchsanstalt, Wien, Austria, AT.
Lebensmittelchemisches Institut, Köln, Germany, DE.
National Food Administration, Uppsala, Sweden, SE.
Nestle Research Center, Lausanne, Switzerland, CH.
VWA Keuringsdienst van Waren, Eindhoven, Netherlands, NL.
(*Three different methods applied, each of them received a different laboratory code).

All laboratories applied in-house validated methodologies of their own choice, which included a mass spectrometric detection coupled to different separation techniques, either gas chromatography or high performance liquid chromatography. The methods are described in more detail in Table 1. Briefly, the acrylamide content of a certain portion of bread powder was extracted. Both the sample intake and the extraction solution composition employed varied from one laboratory to another, as well as the experimental conditions applied for the extraction. An isotopically labelled standard, either deuterated acrylamide or $^{13}C_3$ acrylamide was added prior to extraction and used for quantification purposes. A subsequent clean-up of the extract involved centrifugation that in some cases was followed by filtration, employing a variety of devices. From this point on the methodologies differed more. Methods involving GC–MS included either acrylamide bromination and subsequent extraction or simply evaporation prior to injection. A variety of GC columns having diverse dimensions and stationary phases were used. Different ions were employed by the different laboratories for both acrylamide mass spectrometric identification and quantification. Alternatively LC–MS/MS methodologies did not involve acrylamide derivatisation, lowering the complexity of the LC–MS/MS procedures in terms of sample preparation.

Each laboratory analysed three ERM-BD273 vials randomly selected, each on a different day and in triplicate, as well as a "IRMM control sample" of undisclosed acrylamide concentration and an additional internal control sample, typically a standard solution employed by the laboratory as internal quality control measure for analogous type of determinations. Calibration solutions were not provided.

Table 1
Summary of the methodologies employed for the characterisation of ERM-BD273

Lab code	Extraction	Clean-up of extract	Derivatisation	Additional clean-up	Separation–detection chromatographic column	Ions monitored for quantification (m/z)
1	25 g Sample/75 g water, IS_1 (methacrylamide/d_3-acrylamide)	Centrifugation, addition of olive oil, evaporation		Acetonitrile/hexane extraction	GC–MS (Finnigan SSQ-Fisons MD 800)	Acrylamide: 72
	10 g of the mixture taken to the next step			1.5 ml of acetronitrile phase + IS_2 (butyric acid amide)	Precolumn: deactivated Carbowax 20 cm × 0.53 mm id	d_3-acrylamide: 75
					Carbowax 20 M, 10 m × 0.25 mm id × 0.4 μm	Butyric acid amide: 88
2	20 g Sample/200 ml water, IS (d_3-acrylamide)	Centrifugation, filtration of the supernatant	Bromination, extraction with ethyl acetate, evaporation		GC–MS (Varian CP 3800-Saturn MS 2200)	Acrylamide: 150
					BD-17 ms 30 m × 0.25 mm id × 0.25 μm	d_3-acrylamide: 155
3	2–4 g Sample/40 ml water, IS (d_3-acrylamide)	Centrifugation, filtration: Isolute multimode SPE, microdisk, ultrafilter			LC–MS/MS (Waters Alliance 2690-Micromass Quattro Ultima)	Acrylamide: 72 > 55
					Hypercarb 5 μm, 50 × 2.1 mm, Hypersil–Keystone	
4	10 g Sample/water/ acetonitrile, IS (d_3-acrylamide)	Centrifugation, extrelut cartridge, evaporation of solvent			GC–MS (Varian CP 3800-Saturn 2000)	Acrylamide: 72
					Free fatty acid phase-capillary column 30 m × 0.32 mm id, 0.25 μm	d_3-acrylamide: 75
5	25 g Sample/water/1-propanol, IS (d_3-acrylamide)	Centrifugation, addition of sunflower oil, extraction With acetonitrile, *n*-hexane			GC–MS (Varian CP 3800-Saturn 2000)	Acrylamide: 72
					Free fatty acid phase-capillary column 30 m × 0.32 mm id, 0.25 μm	d_3-acrylamide: 75
6	10 g Sample/water IS ($[^{13}C_3]$-acrylamide),	Centrifugation, filtration, *n*-hexane	2-Mercaptobenzoic acid, removal of excess by lead(II)acetate	Centrifuge, hydrochloric acid,	LC–MS/MS (Thermo-Finnigan LCQ)	Acrylamide: 226
				Extraction with Ethyl acetate + evaporation	Luna Phenyl–Hexyl 250 × 4.6 mm id, 5 μm	$^{13}C_3$-acrylamide: 229
7	2 g Sample/*n*-hexane. water/acetonitrile IS (d_3-acrylamide)	Centrifugation,			LC–MS/MS (Agilent 1100-Applied Biosystems API 2000)	Acrylamide: 72 > 55
					LiChosphere CN 250 × 4 mm id, 5 μm	d_3-acrylamide: 75 > 58
8	Sample/water/methanol	Centrifugation	Bromination, extraction with ethyl acetate	Silica gel, addition of triethyl amine	GC–MS (HP 5890-HP 5971)	Acrylamide: 106
	IS (d_3-acrylamide)				DB-5 ms 60 m × 0.25 mm; 0.25 μm	d_3-acrylamide: 153

9	2 g Sample/water/ methanol IS ([$^{13}C_3$]-acrylamide)	Centrifugation	Brominate, extract with ethyl acetate		GC–MS (Thermo Finnigan GC 2000-Trace MS)	Acrylamide: 149
					DB-Wax 30 m × 0.25 mm; 0.25 μm	$^{13}C_3$-acrylamide: 154
10	7 g Sample/water IS ([$^{13}C_3$]-acrylamide)	Centrifugation, filtration	Bromination	Extrelut filtration,	GC–MS (HP 5890-HP 5971)	Acrylamide: 152
				Extraction with ethyl acetate, evaporation	DB-17 30 m × 0.25 mm; 5 μm	$^{13}C_3$-acrylamide: 153
11	5 g Sample water/ methanol IS (d_3-acrylamide)	Centrifugation, filtration membrane filter			LC–MS/MS (Waters Alliance 2695-Micromass Quatro Ultima)	Acrylamide: 72 > 55
					Precolumn: RP-18 10 × 3.2 mm	d_3-acrylamide: 75 > 58
					Altima RP-18 150 × 3.2 mm i.d., 4 μm	
12	2 g Sample /water	Centrifugation, filtration membrane filter			LC–MS/MS (PE series 200-Applied Biosystems API 2000)	Acrylamide: 72 > 55
	IS (d_3-acrylamide)				Licrospher 100 CN 5 μm LichroART 250 × 4 mm id	d_3-acrylamide: 75 > 58
13	2 g Sample water/ dichloromethane IS (d_3-acrylamide)	Centrifugation, extraction with ethyl acetate		SPE Multimode cartridge	LC–MS/MS (Waters Alliance 2690-Micromass Quatro)	Acrylamide: 72 > 55
					Precolumn: Shodex RSpak DE-SG 10 × 4.6 mm Shodex RSpak DE-413L 250 × 4.6 mm id	d_3-acrylamide: 75 > 58
14	2 g Sample/water/*n*-hexane	Centrifugation	Bromination, extraction with ethyl acetate		GC–MS (Shimadzu GCMS-QP2010 GC–MS system)	Acrylamide: 150
	IS (d_3-acrylamide)				DB-5 ms 30 m × 0.25 mm; 0.25 μm, and deactivated fused silica guard column	d_3-acrylamide: 153
15	5 g Sample/i-hexane/*t*-butylmethylether water	Filtration, centrifugation		SPE	LC–MS/MS (Agilent 1100 series-Micromass Quatro Ultima)	Acrylamide: 72 > 55
	IS (d_3-acrylamide)			Multimode cartridge	Hypercarb 100 × 2 mm, 5 μm	d_3-acrylamide: 75 > 58
16	5 g Sample cyclohexane/ butylmethylether	Filtration, centrifugation		Freezing for further precipitation, centrifugation.	LC–MS/MS (Agilent 1100 series-Applied Biosystems API 2000)	Acrylamide: 72 > 55
	IS (d_3-acrylamide), ultrasonic extraction			Dilution of supernatant with water 1:4	Luna C18, 150 × 3 mm, 3 μm	d_3- acrylamide: 75 > 58

The IRMM control sample was carefully prepared on gravimetrical basis and contained either 1011.4 ng/g of acrylamide in MilliQ water or 1082.1 ng/g of acrylamide in ethylacetate. This blind sample served as a tool to evaluate the quality of the results. If the reported acrylamide levels in the control samples deviated by more than 10% from the concentration provided by gravimetrical preparation, the characterisation data for ERM-BD273 were also rejected for the laboratory in question.

3. Results and discussion

3.1. Quality control during processing

Results obtained by in-house validated PSA based on laser diffraction (not shown) proved the absence of particles larger than 500 μm in the bulk powder material, whereas about 12% (in mass) of finer particles below 63 μm were detected. The presence of bread particles detected on the low end size range, may originate from slight milling effects occurring during the final homogenisation step prior to bottling. Micrographs (not shown) confirmed the particle size measured by PSA and illustrated the round shape of the particles. A water content of 30 g/kg was found in the bulk powder material by in-house validated KFT. Any potential variation between vials introduced during the filling sequence is covered by the assessment of homogeneity.

3.2. Stability studies

The analytical data produced for each temperature and time scheme (data not shown) were evaluated and the slopes were calculated to determine any possible influence of the time of temperature for a certain storage time on the acrylamide mass fraction. Table 2 shows the slopes and the statistical parameters associated to the stability study, both for short-term (dispatching conditions) and long-term storage.

The evaluation of the results indicated stability of the ERM-BD273 material under the initially established storage conditions. Particularly for the assessment of the short-term stability, none of the storage temperatures investigated showed a statistically significant trend ($P = 0.05$) during the short-term stability study (Table 2) since neither of the slopes of the regression lines were statistically significantly different from zero. Thus, taking into account that the method was precise enough to allow meaningful evaluation (RSD = 2.5%), and that the material was stable under all conditions tested, it was decided that a temperature of 18 °C should not be exceeded during CRM dispatch.

The assessment of potential degradation during long-term storage for samples subjected to different temperatures during a 24-months period (−20, 4 and 18 °C) indicated degradation of the material only at a temperature of 18 °C, showing a statistically significant slope. No such effects were visible at −20 °C, the initially chosen temperature for long-term storage of ERM-BD273 material, and consequently a shelf-life of four years could be set resulting in a relative uncertainty contribution of 2.5%.

Table 3
One-way ANOVA results from homogeneity study

Source of variation	Sum of squares (SS)	df	Mean of squares (MS)
Between groups	3264	11	297
Within groups	3019	24	126
Total	6283	35	

3.3. Homogeneity assessment

The analytical data (not shown) assessment revealed the absence of outliers. Moreover, the normal and unimodal distribution of the results made it possible to analyse the variances by ANOVA (Table 3). Standard deviations within-vials (s_{wb}) resulting from a method repeatability of 2.5%, and between vials ($s_{bb} = 1.71\%$), related to the analysis of 12 bottles in triplicate, were calculated as described by van der Veen, Linsinger, and Pauwels (2001).

Potential variation of the acrylamide mass fraction between different vials of the same batch of CRM is described by u_{bb} (the uncertainty estimated for between vial homogeneity). Usually u_{bb} is given by the larger potential heterogeneity estimated for the material, either u^*_{bb} or s_{bb}, as described earlier in the text. For u^*_{bb} the estimated value, 0.79%, is lower than s_{bb}, 1.71%. Therefore, the latter was assigned as u_{bb}. This contribution, lower than the a priori

Table 2
Linear regression and statistical parameters associated to stability evaluation

	Short-term stability (1 month)			Long-term stability (24 months)		
Storage temperature:	−20 °C	4 °C	18 °C	−20 °C	4 °C	18 °C
Statistical parameters						
Slope (b)	−2.1	0.5	−2.7	0.2	−0.1	−1.1
Std. error slope (s_b)	1.5	1.6	1.6	0.2	0.2	0.2
Degrees of freedom (df)	34	34	34	10	10	10
$\lvert b\rvert/s_b$	1.4	0.3	1.7	1.0	0.5	5.5
$t_{(0.05;30)}$	2.042	2.042	2.042	2.228	2.228	2.228
Statistical significance ($P = 0.05$)	No	No	No	No	No	Yes

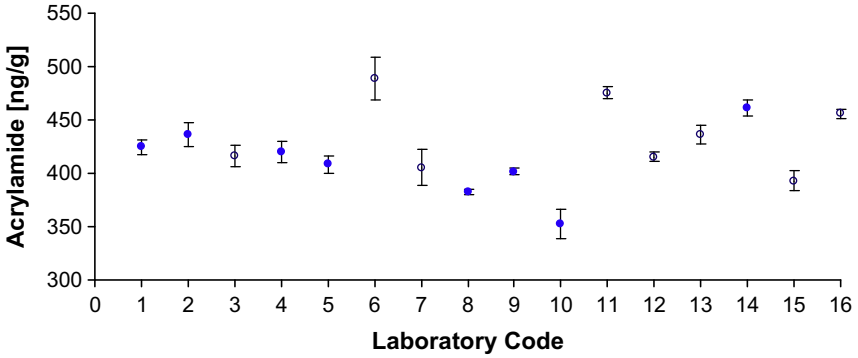

Fig. 1. Acrylamide mass fraction (ng/g), mean values ± standard deviations of ERM-BD273 data sets reported by all laboratories participating in the collaborative study by using GC–MS (●) or LC–MS (○).

established criterion for acceptance (2%) was taken into consideration in the estimation of the total uncertainty of the certified value.

3.4. Characterisation

The average acrylamide mass fraction values reported for ERM-BD273 by all the laboratories participating in the collaborative study are given in Fig. 1.

Results (not shown) reported for the "IRMM control sample", a blind sample to the participants, were used as a filter, establishing the rejection of data sets deviating by more than 10% from the concentration provided by gravimetrical preparation. Laboratories 6, 7, 8, 10 and 11 exceeded this acceptance criterion taking into account within-laboratory repeatability data, and consequently their reported values for ERM-BD273 were not considered for the value assignment of the reference material. The other eleven data sets were further scrutinized for the presence of outliers, with none detected. The laboratory means were normally distributed, according to the skewness and Kurtosis tests performed at significance levels of $P = 0.05$ and $P = 0.01$. No grouping of the results by analytical technique employed could be observed. The mean of the laboratory means of acrylamide in toasted bread obtained from the eleven laboratories was 425 ng/g and this value was defined as the certified mass fraction of acrylamide in the material. The standard error of the average, 6.42 ng/g (1.5% expressed as a RSD), was taken as u_{char}, the relative contribution of the characterisation to the total uncertainty.

3.5. Uncertainty estimation

The uncertainty associated to the certified value (Linsinger et al., 2001) was estimated by combining uncertainty contributions arising from the characterisation (u_{char} 1.5%), the homogeneity (u_{bb} of 1.7%) and stability during storage (u_{lts} of 2.5%), as

$$U_{CRM} = k \cdot \sqrt{u_{char}^2 + u_{bb}^2 + u_{lts}^2}$$

The expanded uncertainty U_{CRM} of the certified quantity value was calculated as 6.7% (29 ng/g), by using a coverage factor of 2, covering approximately a confidence level of 95%.

3.6. Certified value and meteorological traceability

The acrylamide mass fraction obtained as the mean of means from all accepted sets of data, 425 ng/g, was assigned as certified value in the toasted bread reference material ERM-BD273, with an associated expanded uncertainty of 29 ng/g ($k = 2$). The certified value is traceable to the international system of units (SI) as it is derived from results obtained by validated methods including a diversity of methodologies for sample preparation, different separation techniques and detection and quantification by isotope dilution mass spectrometry.

4. Conclusion

A reference material has been produced and certified for the acrylamide mass fraction in a toasted bread matrix in compliance with ISO Guides 34 and 35. The material is intended as a quality control tool for laboratories to demonstrate the accuracy of results obtained by their method during method validation and analysis of acrylamide in relevant food matrices.

Acknowledgements

The authors would like to thank other IRMM staff involved in the material processing, A. Oostra, J. Charoud-Got and the scientists from other Research Centres participating in the material characterisation, M. Biedermann, J. Buhlert, M. Granvogl, C. Griffin, T. Goldmann, J. Jansen, K. Hoenicke, Y. Panagiotopoulou, A. Perco, M. Raters, J. Rosén, A. Töpfer, R. Weisshaar.

In addition the authors would like to thank T. Linsinger and A. Held, from IRMM, and P. Gowik (Federal Office of Consumer Protection and Food Safety, DE), E. Nordkvist (National Veterinary Institute, SE) and J. de Boer

(Institute for Environmental Studies, Vrije Universiteit Amsterdam, NL), as experts of the Certification Advisory Panel for organic molecules, for participating in the reviewing of this project.

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