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ARTICLE

Development of Two Certified Reference Materials for Acrylamide Determination in Foods

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Certified reference materials (CRMs) are a versatile tool for quality assurance in the chemical laboratory. In the case of acrylamide analysis, the availability of appropriate materials was rather limited. This lack of acrylamide matrix CRMs has now been overcome by the development of two European reference materials (ERM) for the determination of acrylamide in food (crispbread, ERM-BD272, and rusk, ERM-BD274). This article describes the preparation of the materials, provides the results of the homogeneity and stability studies, and presents and discusses the outcome of the certification studies. Expert laboratories from different European countries took part in the certification studies using various analytical methods. The acrylamide mass fractions were certified to 980 μg kg^{-1} for crispbread and 74 μg kg^{-1} for rusk.

KEYWORDS: Certified reference materials; ERM; acrylamide; crispbread; rusk; quality assurance; homogeneity; stability

INTRODUCTION

Food safety and quality are two of the most important factors determining the consumer acceptance and purchase dynamics of a product. Authenticity proof, detection of fraud, and determination of residues as well as contaminants are therefore in the focus of the current interest.

For implementation of food and feed legislation, there is a strong need for development and harmonization of analytical methods. The official food control laboratories have to use validated methods wherever possible, either by in-house validation of applied methods or by application of methods validated by collaborative trials. Commission Decision 2002/657/EC of August 2002 implementing Council Directive 96/23/EC establishes criteria and procedures for the validation of analytical methods to ensure the quality and comparability of analytical results generated by official laboratories. Method performance criteria for the determination of acrylamide were recently obtained from a method validation trial (*1*).

The use of reference materials (RMs) plays a key role in internal validation procedures aiming at a performance check of the methods. According to ISO Guide 30, a certified reference material (CRM) is defined as a RM, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes its traceability to an accurate realization of the unit in which the property values are expressed and for which each certified value is accompanied by an uncertainty at a stated level of confidence. They are useful tools in the verification of the accuracy of analytical measurements and are employed in analytical quality assurance, quality control schemes, laboratory accreditation, and in the establishment of traceability in the framework of internationally agreed standards (*2*,*3*). They can also be used for the process of measurement uncertainty estimation or the calibration of analytical instruments.

When Swedish scientists initially reported the occurrence of acrylamide in a range of baked and fried food products in 2002 (*4*, *5*), a worldwide surveillance of acrylamide in food was triggered, as the substance is classified as a probable human carcinogen by the International Agency for Research on Cancer (IARC). Recent studies confirmed that the main pathway of acrylamide formation in foods during thermal processing is linked to the Maillard reaction under particular involvement of the amino acid asparagine (*6*, *7*).

Since 2002, various analytical methods for the quantification of acrylamide in foodstuffs have been developed. They are primarily based on gas (*8*−*10*) or liquid chromatography (*11*−*14*). In recent years, immunoassays for acrylamide determination have been developed as well (*15*, *16*). Currently, two analytical methods are widely used for acrylamide determination: LC-MS/MS without derivatization and GC-MS after derivatization by bromination, employing [$^{13}C_3$]- or [$^{2}H_3$]-isotopic labeled acrylamide as internal standard (*17*−*19*). Both methods are suited to analyze acrylamide in the low μg kg^{-1}-range.

The increased interest in acrylamide determination and method development unveiled an urgent need for CRMs, which were not available in the commercial market at that time. To close this gap, two new reference materials were prepared and certified at the BAM Federal Institute for Materials Research and Testing. At the same time, a third commercially available matrix CRM for acrylamide determination was developed at IRMM (ERM-BD273, toasted bread) (*20*).

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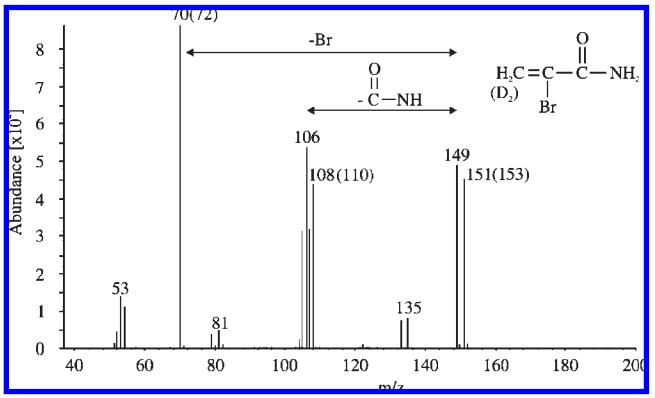

Figure 1. GC-MS (EI) mass spectrum of 2-bromopropenamide and the corresponding deuterated internal standard.

Crispbread and rusk are food products commonly contaminated with acrylamide because of production conditions favorable for its formation. In 2007, the annual survey and exposition studies in Germany showed an increase of the acrylamide content for both food products. The tolerable maximum limits (signal values) could therefore not be reduced. This indicates a still unresolved problem and makes further efforts necessary. Particular care should be placed on rusk as a sensible infant food. Therefore, rusk and crispbread were selected for the preparation of reference materials in the frame of a European reference material (ERM) project.

This article presents the whole certification processes of ERM-BD272, Acrylamide in Crispbread, and ERM-BD274, Acrylamide in Rusk.

MATERIALS AND METHODS

Candidate Reference Materials and Sample Preparation. The matrices for the two reference materials are food products which tend to form acrylamide during their production process. Both materials, crispbread and rusk, were procured from food processing companies in Germany and were intended for human consumption. About 34 kg of crispbread material (BD272) was ground by a centrifugal mill (ZM 1000; Retsch GmbH, Haan, Germany) and sieved with a mesh size smaller than 0.5 mm. A 20 kg lot of rusk (BD274) was prepared in the same way. In order to avoid cross-contamination, the centrifugal mill was cleaned carefully after each usage. The sieved materials were homogenized by using a drum hoop mixer, followed by a second homogenization step and bottling using a version of the so-called cross-riffling procedure (*21*). A total of 500 units (BD272) and 200 units (BD274) were bottled in 125 mL amber glass bottles sealed with screw caps with PTFE inserts.

Secondary Matrix Characterization and Purity of Calibrant. Randomly selected bottles of each CRM were analyzed for their water content by coulometric Karl Fischer titration (KFT) on a 758 KFD Titrino (Metrohm AG, Herisau AR, Switzerland). The CHN-elemental constitution was determined using an Elemental Analyzer (Elementar, Hanau, Germany). The purity of the acrylamide (99+%, Merck, Darmstadt, Germany) used as a calibrant was confirmed by quantitative ^{1}H NMR analysis (Bruker DMX 400, 400.14 MHz; internal standard, benzoic acid) at the Federal Institute for Materials Research and Testing (BAM).

Analytical Method for Acrylamide Determination. In the present work, the analytical method employed for material characterization (homogeneity, stability, and certification studies) at BAM was based on GC-MS analysis (Finnigan Ultra GC with Trace DSQ) after bromination using deuterium-labeled [$^{2}H_3$]-acrylamide (99+%, Polymer Source Inc., Montreal, Canada) as an internal standard. Because of the instability of the derivatization product 2,3-dibromopropionamide, this compound is converted to the more stable 2-bromopropenamide by adding triethylamine prior to GC-MS analysis. The ions monitored for quantification were m/z 106 for 2-bromopropenamide and m/z 153 for 2-bromo(D_2)-propenamide (**Figure 1**). The calibration was done by linear regression analysis with six calibration levels, each measured in triplicate. The calibration solutions were prepared gravimetrically directly before the analysis.

Table 1. Analytical Methods Employed for the Certification of ERM-BD272 Acrylamide in Crispbread

analytical method	derivatization	no. of laboratories
GC-MS	bromination	5
GC-MS	without	3
HPLC-ESI MS/MS	2-mercapto-benzoic acid	1
HPLC-ESI-MS/MS	without	7

Certification Studies, Certified Values, and Uncertainties. Laboratories were selected based on documented experience and proficiency and invited to participate in the certification studies. A total of 16 (BD272) and 8 expert laboratories (BD274) took part in the interlaboratory comparison studies. Here, the laboratories are listed in alphabetical order: Chemisches and Veterinäruntersuchungsamt, Stuttgart, Germany; Chemisches and Veterinäruntersuchungsamt, Sigmaringen, Germany; Dublin Public Analyst Laboratory, Dublin, Ireland; Eurofins, Wiertz-Eggert-Jörissen, Hamburg, Germany; European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Geel, Belgium; Federal Institute for Materials Research and Testing, Berlin, Germany; General Chemical State Laboratory, Food Division and Division of Environment, Athens, Greece; German Research Centre of Food Chemistry, Garching (three different methods were applied, and each received a different laboratory code), Germany; Institut für Qualitätsförderung in der Süsswarenwirtschaft Köln, Germany; Kantonales Labor, Zürich, Switzerland; Lebensmittelchemisches Institut, Köln, Germany; Lebensmittelversuchsanstalt, Wien, Austria; National Food Administration, Uppsala, Sweden; Nestlé Research Center, Lausanne, Switzerland; VWA Keuringsdienst van Waren, Eindhoven, Netherlands.

Different methods for extraction, purification, and derivatization were used by the participants. HPLC or GC separation techniques were applied by all participants. The method of choice for quantification is isotope dilution mass spectrometry using ^{13}C-labeled or deuterated acrylamide as internal standard. **Table 1** gives an overview of the analytical methods in the certification study of ERM-BD272. Except for the LC-MS after derivatization with 2-mercapto-benzoic acid, the same methods were applied in the certification study of BD274.

The assignment of a certified value for the acrylamide mass fraction, homogeneity evaluation of the batch, investigation of long- and short-term stability, and elaboration of an uncertainty statement were carried out in full compliance with the internationally accepted procedures laid down in ISO Guide 35 and GUM (*22*, *23*).

Traceability. The acrylamide mass fraction is, although not method-specific, clearly a parameter which is influenced by the method employed for its determination. Previous studies on crispbread material revealed a significant influence of the measurement technique and the composition of the extraction solvent on the analytical results (*24*).

The measurement step itself takes traceability from calibration using the pure substance (acrylamide, 99+%) and sample preparation steps from spiking using deuterated acrylamide. The overall recovery was estimated to be in the range of 95−105%. Remaining systematic between-method biases are sufficiently covered by the allowance made for the intercomparison contribution to the total uncertainty budget. The certified values are therefore traceable to the International System of Units (SI).

RESULTS AND DISCUSSION

The certification campaigns implied homogeneity evaluation of the packaged units (bottles), short- and long-term stability studies, the interlaboratory comparison for the assignment of the certified values, and a comprehensive uncertainty budget estimation also enabling statements of traceability.

Homogeneity Studies. Upon the basis of thorough batch homogenization and the results of preliminary studies, a satisfactory level of sample homogeneity was expected. For further quantitative demonstration, 10 units were selected randomly from the whole set of 500 crispbread bottles (12 units out of

Table 2. Data Evaluation of Homogeneity Studies by ANOVA (*F*-Test)

ERM	matrix	s_b^{2a} [μg^2 kg^{-2}]	s_w^{2b}[μg^2 kg^{-2}]	test criterion[c]	critical value $F(f_1, f_2, 5\%)$	u_{bb} [μg kg^{-1}]	$u_{bb\ rel}$ [%]
BD272	crispbread	747.64	446.03	1.68	2.21	8.68	0.80
BD274	rusk	4.574	2.348	1.948	2.067	0.746	1.35

[a] Variances between units. [b] Variances within units. [c] s_b^2/s_w^2.

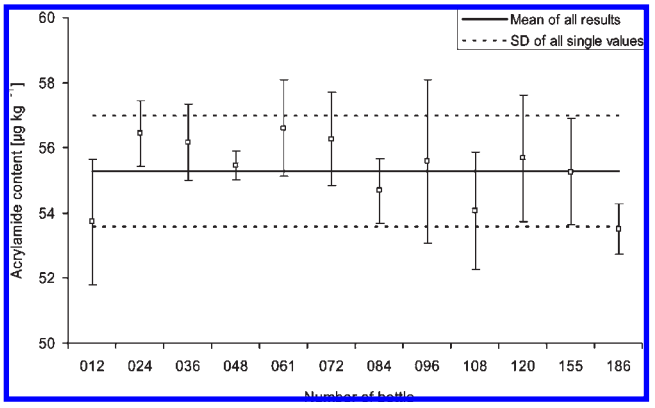

Figure 2. Homogeneity study BD274: 12 out of 200 bottles were randomly selected. Each unit was analyzed four times. Bottle mean values and the corresponding standard deviations are given.

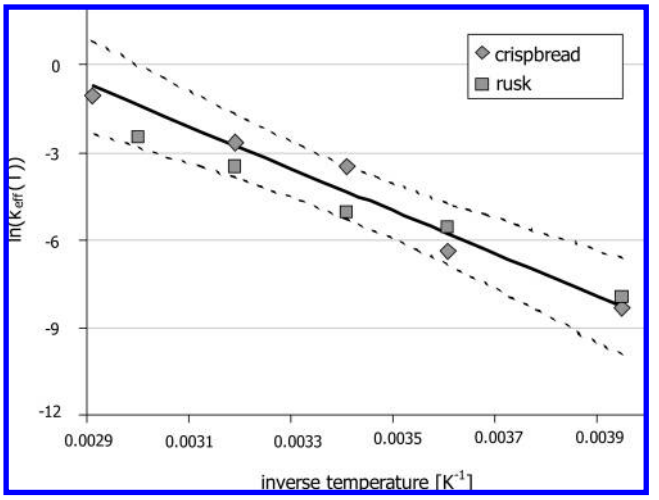

Figure 3. Effective reaction rate $k_{eff}(T)$ for acrylamide (in the crispbread and rusk matrices) depending on the inverse temperature. The straight line represents a linear regression over the crispbread data, and the dotted lines are the corresponding upper and lower confidence limits of the regression line.

200 rusk bottles) and analyzed four times each according to the analytical method described above. All units were extracted and processed once under repeatability conditions followed by a second set of extractions and processing in a randomized manner again and so on. Processed extracts were analyzed by GC-MS under repeatability conditions ensuring that all extracts were quantified against the same calibration after randomization. Results were obtained by the standard procedure (*22*) using the one-factorial analysis of variances (**Table 2**). The detailed homogeneity data for ERM-BD274 are displayed in **Figure 2**. Because the test criterion is smaller than the critical value, no significant inhomogeneity of the batch was detected. The estimated term $u_{bb,r}$ derived from the difference of the between- and within-groups variances was used to include an appropriate inhomogeneity contribution in the uncertainty budgets of the certified values.

Stability Studies. Preliminary studies showed a temperature-driven deterioration of acrylamide contents. To quantify these effects, selected units of the materials were submitted to accelerated aging at temperatures between 4 °C and 60/70 °C (rusk/crispbread) over periods of 1 week to 1.5 months (short-term study) and 1 month to 12 months (long-term study). All measurements were performed in the so-called isochronous scheme (*25*). After respective periods of time (short-term study, weekly; long-term study, after 1, 3, 6, and 12 months) individual units were stored at −20 °C where no degradation was to be expected. At the end of the stability study all units were analyzed for acrylamide content using the same method (GC-MS after bromination) under repeatability conditions.

For both materials, a non-negligible trend was observed for all temperatures above 4 °C. In order to obtain estimates for the thermal behavior of the samples especially at the storage temperature, an Arrhenius model was assumed for the dependence of the effective reaction rate $k_{eff}(T)$ on temperature. A plot of $k_{eff}(T)$ over the inverse temperature is given in **Figure 3**. For a complete description of the approach to stability assessment refer to ref *26*.

Obviously, the temperature dependence of the effective degradation rate can be approximated by a straight line. The estimated activation energy ΔE is (60.6 ± 0.4) kJ mol^{-1} for crispbread and (47.2 ± 0.5) kJ mol^{-1} for the rusk material. As can be seen from **Figure 3**, estimates for effective degradation rates in both matrices are comparable at all temperatures tested. In this sense, both matrices behave similarly and do not create additional degradation channels for the analyte under investigation. Nevertheless, one can observe a greater data variability for the results of the stability test on the crispbread matrix leading to a greater confidence interval of the regression line.

Table 3. Estimation of Shelf Life at Different Storage Temperatures Based upon Stability Testing and an Arrhenius Model

	calculated shelf life (years)	
storage temperature	ERM-BD272	ERM-BD274
at − 20 °C	3.24	15.10
at + 4 °C	0.50	2.36
at + 20 °C	0.13	0.78
at + 40 °C	0.02	0.22

By using these data and the assumed model, one can obtain an estimate when degradation will presumably force the acrylamide content to fall short of the lower expanded uncertainty limit of the certified value. In the sense of a worst-case estimation, these calculations are carried out for the effective reaction rates at the upper confidence limit of the line as shown in **Figure 3**. The results of the shelf life estimations are given in **Table 3**. The significant difference in the estimated shelf life of the two materials is a consequence of the above-mentioned worst-case estimation procedure and the larger scatter of the crispbread data. In order to ensure a shelf life of the order of five years, an additional allowance (u_{lts}: 36.73 μg kg^{-1}) was included in the uncertainty budget of BD272. The calculation of u_{lts} is based on the uncertainty of the insignificant slope of the regression line depicting the time dependence of the acrylamide content for samples stored at −20 °C. Shelf life at a storage temperature of −20 °C is quite considerable, exposure to room or even higher

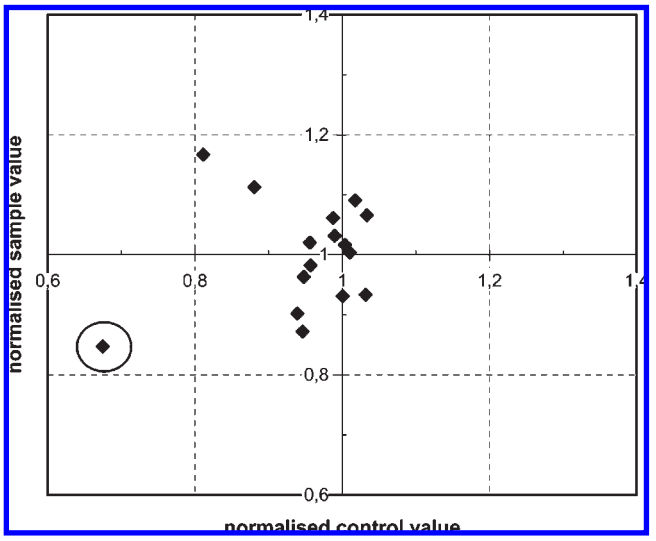

Figure 4. Plot of the mean values found by the laboratories for the sample BD272 (normalized by the mean of laboratory means) against the concentration of the control solution attained by the laboratory (normalized by the known concentration of acrylamide from preparation of the control solution).

temperatures over longer periods of time should, however, be avoided since it decreases the time of validity of BD272 and BD274 at the user's end.

The first estimation of stability will continuously be updated by further measurements of units stored at 4 and 20 °C over the period of availability of the material (post-certification monitoring).

Interlaboratory Comparison Studies (ILCs). The ILCs were performed to assign the certified values of BD272 and BD274. Three units of the candidate reference material were to be analyzed by each laboratory in triplicate on three consecutive days. All extracts were injected twice. In addition, each participant received a solution with an unknown concentration of acrylamide in water. This solution was measured every day. The results of the certification studies have been evaluated in accordance with ISO Guide 35 and the specific requirements of the ERM agreement.

Technical Evaluation. For each laboratory, the normalized mean value determined for the sample (normalized by the mean of laboratory means) was plotted against the recovery the laboratory attained for the control solution. **Figure 4** shows the corresponding plot for the BD272 study. As can be seen from the graph, 15 out of 16 laboratories group around the center. The laboratories show both positive and negative correlation between the sample and the control value. A clear trend cannot be observed. One single laboratory (encircled) is distant from the main group, showing an underestimation concerning the control and sample value. The data set of this laboratory was excluded from further processing because of technical reasons. For rusk, similar considerations did not lead to the exclusion of an outlier.

Statistical Evaluation. After the removal of one laboratory for technical reasons, the accepted data sets were further processed. In a first step, the data sets were investigated for significant influences of the laboratory itself and the investigated units using a two-way ANOVA test. As expected, the laboratory is a significant factor (between-laboratory variation is significantly larger than within-lab variation), while the unit investigated is insignificant, which is clearly in line with the results of the conducted homogeneity tests.

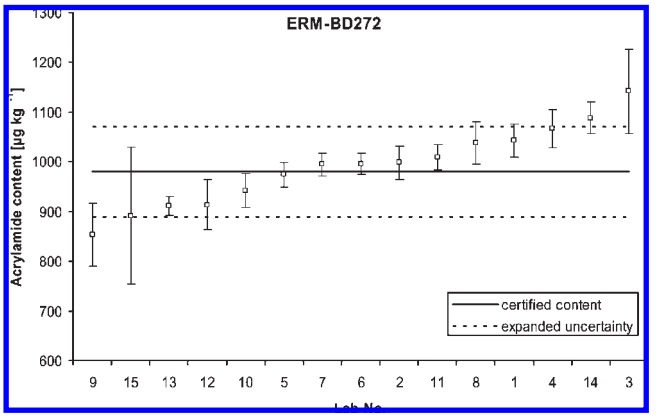

Figure 5. Accepted data sets of the ILC for certification of the acrylamide content in crispbread material (BD272).

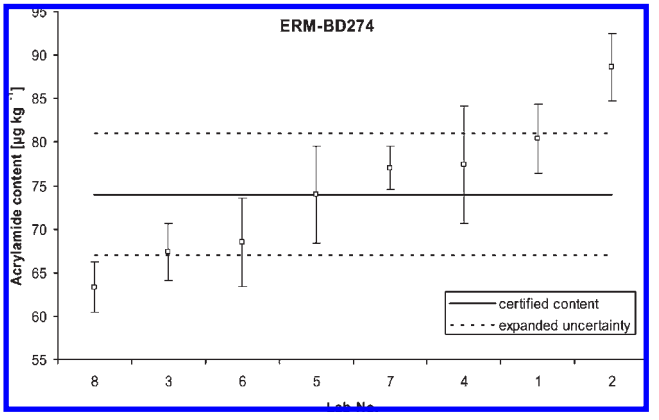

Figure 6. Accepted data sets of the ILC for certification of the acrylamide content in rusk material (BD274).

In a second step, we tested (separately by one-way ANOVA test on the data of each of the laboratories) whether significant differences were detected between the three units provided to each laboratory. Although this was not expected to occur, 4 out of the 15 laboratories detected virtual differences between their crispbread units (for rusk: 6 out of 8 laboratories). This can most probably be attributed to a considerably poor method repeatability, in some cases comparable to the between-laboratory variability.

Participants of the ILCs used different methods with different implementations. Obviously, there was no reason for assuming that the single values measured by the different laboratories would belong to a common population. Single measurement results could not be pooled, and therefore, the mean of laboratory means was considered to be an appropriate estimate for the certified value. This decision was justified by the outcome of the two-way ANOVA test described above as well as by further statistical analysis.

Statistical tests were carried out at significance levels α of 0.05 and 0.01. With the Cochran test, no observations were made at both significance levels. Dixon, Grubbs, and Nalimov tests did not indicate outliers (significance level of 0.01), but for the BD272 material, one laboratory was found to be a straggler in the Nalimov test. However, this was considered insufficient for exclusion, and the laboratory value was retained. The accepted data sets taken into account for calculation are shown in **Figures 5** and **6**.

On the basis of the available data, the hypothesis of normality of the data sets could not be rejected by either the Kolmogorov–Smirnov or the skewness and kurtosis tests. The results of the

Table 4. Statistical Evaluation of Accepted Data Sets from Interlaboratory Comparison Studies

	description	ERM-BD272	ERM-BD274
acrylamide content w_{char} [μg kg^{-1}]	unweighted mean of laboratory means, uncorrected for purity	988.46	74.58
uncertainty contribution from characterization u_{char} [μg kg^{-1}]	standard deviation of the mean of laboratory means	20.65	2.86
accepted data sets	after outlier exclusion	15	8
	Outlier Tests at an α of 0.01 (0.05)		
Dixon	outlying lab means?	no (no)	no (no)
Grubbs (single test)	outlying lab means?	no (no)	no (no)
Grubbs (double test)		no (no)	no (no)
Nalimov	outlying lab means?	no (yes(1))	no (no)
Cochran	outlying lab variances?	no (no)	no (no)
Kolmogorov−Smirnov (Lilliefors version) α = 0.01	normal distribution of data?	yes	yes
skewness/kurtosis α = 0.01	normal distribution of data?	yes	yes

Table 5. Certified Values for the Acrylamide Mass Fraction of ERM-BD272 and ERM-BD274

certified reference material		acrylamide mass fraction [μg kg^{-1}]		
ERM	matrix	certified value, corrected for purity	uncertainty of the certified value	expanded uncertainty of the certified value (k = 2)
BD272	crispbread	980	45	90
BD274	rusk	74	3.2	7

calculations and tests for data evaluation based upon the laboratory means are given in **Table 4**.

The means of the laboratory means of 988.46 μg kg^{-1} (BD272) and 74.58 μg kg^{-1} (BD274) were taken as the uncorrected (for purity) estimate for the value to be certified and the standard deviations of the means of laboratory means s_{char} as the uncertainty contribution from characterization by ILC (u_{char}).

Certified Values and Uncertainties. The estimates of the certified value derived from the data evaluation of the ILC (w_{char}) have to be corrected for the purity of the calibration standard (f_{pur}) used in all of the experiments according to eq 1.

$$w_{cert} = w_{char} \cdot f_{pur} \quad (1)$$

The corresponding combined uncertainty u_c was appropriately combined from the uncertainty of characterization u_{char}, the contribution from a possibly undetected inhomogeneity u_{bb}, and the uncertainty of the purity correction u_{pur} according to eq 2, where the index r refers to the relative uncertainties.

$$u_{c,r}^2 = u_{char,r}^2 + u_{bb,r}^2 + u_{pur,r}^2 \quad (2)$$

The purity of the calibration standard and its corresponding uncertainty were taken from the certificate of acrylamide as f_{pur} = 0.995 and u_{pur} = 0.0029 (assuming a rectangular distribution). A level of confidence of about 95% is obtained by expansion of the combined uncertainty with a coverage factor of k = 2, as defined in ref *23*. The certified values for both ERM as well as the combined and expanded uncertainties are summarized in **Table 5**. The values are rounded according to the recommendations of GUM (*23*) and given with respect to raw sample mass. The water content of the reference materials was seen to remain stable.

In summary, this article describes the development and certification of two new reference materials for acrylamide determination in foods intended for quality assurance purposes. The development of new methods, especially LC-MS/MS techniques and immunoassays, are a direct follow up of the unbroken interest in reduction and control of acrylamide in foods. Considering this situation, the availability of reliable reference materials is urgently needed. Therefore, the presented certified reference materials provide the basis for further improvements in quality assurance and control. Their importance is not only useful in existing laboratory internal quality control but also in facilitating the validation of new analytical methods.

ABBREVIATIONS USED

ANOVA, analysis of variances; CRM, certified reference material; ERM, European reference material; ILC, interlaboratory comparison study; IRMM, Institute for Reference Materials and Measurements.

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LITERATURE CITED

(1) Wenzl, T.; Karasek, L.; Rosén, J.; Hellenaes, K.-E.; Crews, C.; Castle, L.; Anklam, E. Collaborative trial validation study of two methods, one based on high performance liquid chromatography−tandem mass spectrometry and on gas chromatography−mass spectrometry for the determination of acrylamide in bakery and potato products. *J. Chromatogr., A* **2006**, *1132*, 211–218.

(2) Emons, H.; Linsinger, T. P. J.; Gawlik, B. M. Reference materials: terminology and use. Can't one see the forest for the trees? *Trends Anal. Chem.* **2004**, *23*, 442–449.

(3) Amigo, J. M.; Arana, G.; Etxebarria, N.; Fernández, L. A. Emerging needs for sustained production of laboratory reference materials. *Trends Anal. Chem.* **2004**, *23*, 80–85.

(4) Tareke, E.; Rydberg, P.; Karlsson, P.; Eriksson, S.; Törnqvist, M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J. Agric. Food Chem.* **2002**, *50*, 4998–5006.

(5) Rosén, J.; Hellenäs, K. E. Analysis of acrylamide in cooked foods by liquid chromatography tandem mass spectrometry. *Analyst* **2002**, *127*, 880–882.

(6) Gökmen, V.; Palazoğlu, T. K. Acrylamide Formation in Foods during Thermal Processing with a Focus on Frying. *Food Bioprocess Technol.* **2008**, *1*, 35–42.

(7) Friedman, M.; Levin, C. E. Review of methods for the reduction of dietary content and toxicity of acrylamide. *J. Agric. Food Chem.* **2008**, *56*, 6113–6140.

(8) Pittet, A.; Périsset, A.; Oberson, J.-M. Trace level determination of acrylamide in cereal-based foods by gas chromatography−mass spectrometry. *J. Chromatogr., A* **2004**, *1035*, 123–130.

(9) Dunovská, L.; Čajka, T.; Hajšlová, J.; Holadová, K. Direct determination of acrylamide in food by gas chromatography−high-resolution time-of-flight mass spectrometry. *Anal. Chim. Acta* **2006**, *578*, 234–240.

(10) Zhu, Y. H.; Li, G. R.; Duan, Y. P.; Chen, S. Q.; Zhang, C.; Li, Y. F. Application of the standard addition method for the determination of

acrylamide in heat-processed starchy foods by gas chromatography with electron capture detector. *Food Chem.* **2008**, *109* (4), 899–908.
(11) Hoenicke, K.; Gatermann, R.; Harder, W.; Hartig, L. Analysis of acrylamide in different foodstuffs using liquid chromatography-tandem mass spectrometry and gas chromatography-tandem mass spectrometry. *Anal. Chim. Acta* **2004**, *520*, 207–215.
(12) Riediker, S.; Stadler, R. H. Analysis of acrylamide in food by isotope-dilution liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *J. Chromatogr., A* **2003**, *1020*, 121–130.
(13) Jezussek, M.; Schieberle, P. A New LC/MS-method for the quantitation of acrylamide based on a stable isotope dilution assay and derivatisation with 2-mercaptobenzoic acid. Comparison with two GC/MS methods. *J. Agric. Food Chem.* **2003**, *51*, 7866–7871.
(14) Zhang, Y.; Jiao, J. J.; Cai, Z. X.; Zhang, Y.; Ren, Y. P. An improved method validation for rapid determination of acrylamide in foods by ultra-performance liquid chromatography combined with tandem mass spectrometry. *J. Chromatogr., A* **2007**, *1142* (2), 194–198.
(15) Zhou, S.; Zhang, C.; Wang, D.; Zhao, M. P. Antigen synthetic strategy and immunoassay development for detection of acrylamide in foods. *Analyst* **2008**, *133* (7), 903–909.
(16) Preston, A.; Fodey, T.; Elliott, C. Development of a high-throughput enzyme-linked immunosorbent assay for the routine detection of the carcinogen acrylamide in food, via rapid derivatisation pre-analysis. *Anal. Chim. Acta* **2008**, *608* (2), 178–185.
(17) Wenzl, T.; de la Calle, B.; Anklam, E. Analytical methods for the determination of acrylamide in food products: a review. *Food Addit. Contam.* **2003**, *20* (10), 885–902.
(18) Zhang, Yu; Zhang, G.; Zhang, Yi. Occurrence and analytical methods of acrylamide in heat-treated foods. Review and recent developments. *J. Chromatogr., A* **2005**, *1075*, 1–21.
(19) Taeymans, D.; Wood, J.; Ashby, P.; Blank, I.; Studer, A.; Stadler, R. H.; Gond′E, P.; van Eijck, P.; Lalljie, S.; Lingnert, H.; Lindblom, M.; Matissek, R.; Müller, D.; Tallmadge, D.; O'Brien, J.; Thompson, S.; Silvani, D.; Whitmore, T. A Review of acrylamide: An industry perspective on research, analysis, formation, and control. *Crit. Rev. Food Sci. Nutr.* **2004**, *44*, 323–347.
(20) Dabrio, M.; Sejeroe-Olsen, B.; Musser, S.; Emteborg, H.; Ulberth, F.; Emons, H. Production of a certified reference material for the acrylamide content in toasted bread. *Food Chem.* **2008**, *110* (2), 504–511.
(21) Veen, A. M. H.; van der; Nater, D. A. G. Sample preparation from bulk samples: an overview. *Fuel Process. Technol.* **1993**, *36*, 1–7.
(22) ISO Guide 35 Reference materials: General and statistical principles for certification; ISO/REMCO, **2006**.
(23) *Guide to the Expression of Uncertainty in Measurement* (*GUM*); International Organization for Standardization (ISO): Geneva, Switzerland, 1995.
(24) Wenzl, T.; de la Calle, B.; Gatermann, R.; Hoenicke, K.; Ulberth, F.; Anklam, E. Evaluation of the results from an inter-laboratory comparison study of the determination of acrylamide in crispbread and butter cookies. *Anal. Bioanal. Chem.* **2004**, *379*, 449–457.
(25) Lamberty, A.; Schimmel, H.; Pauwels, J. The study of the stability of reference materials by isochronous measurements. *Fresenius' J. Anal. Chem.* **1998**, *360*, 359–361.
(26) Bremser, W.; Becker, R.; Kipphardt, H.; Lehnik-Habrink, P.; Panne, U.; Töpfer, A. Stability testing in an integrated scheme. *Accredit. Qual. Assur.* **2006**, *11*, 489–495.

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