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Development of a Stable Isotope Dilution Assay for the Quantitation of Glycidamide and Its Application to Foods and Model Systems

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On the basis of a stable isotope dilution assay and derivatization with 2-mercaptobenzoic acid, the presence of the carcinogenic glycidamide (**2**) in processed foods was verified for the first time. Using ^{13}C-labeled **2** as the internal standard and the formation of the thioether derivatives, a new stable isotope dilution assay for the quantitation of **2** was developed. Application of the method on several potato samples revealed amounts between 0.3 and 1.5 μg/kg depending on the processing conditions. In a model experiment, the formation of **2** by an epoxidation of the double bond in acrylamide, that is, by a reaction with linoleic acid hydroperoxides, was established. This result was in good agreement with data showing that French fries processed in sunflower oil, which is high in linoleic acid, contained more **2** as compared to fries prepared in coconut oil. The derivatization procedure allows the simultaneous quantitation of acrylamide and glycidamide in foods.

KEYWORDS: Acrylamide; glycidamide; lipid peroxidation; French fries; stable isotope dilution analysis

INTRODUCTION

Acrylamide (**1**), assumed to be involved in carcinogenesis, is by now a well-established constituent of many processed foods (*1*). Because the free amino acid asparagine, which occurs in many foods, has been characterized as the key precursor during thermal food processing (*2–4*), it is quite difficult to avoid its formation during food manufacturing. Nevertheless, various technological steps have been proposed for acrylamide mitigation (*5–14*).

In living organisms, **1** can be converted into its epoxide, assigned as glycidamide (**2**), which is thought to be considerably more toxic than **1** (*15–18*). However, because the epoxide is quite unstable, mainly studies on its quantitation as adducts with either hemoglobin (*19–21*), glutathione (*22*), or mercapturic acid (*23*) as well as with DNA (*25–27*) have been reported. However, up to now, no method for the quantitation of **2** has been reported and, also, no data on its occurrence in foods are available.

Hydroperoxides are known reactants forming epoxides by oxidation of a double bond in a given compound. This epoxidation, known as the Prileschajew reaction, has previously been shown by us to be involved in the formation of the intensely metallic smelling 4,5-epoxy-(*E*)-2-decenal from (*E*,*E*)-2,4-decadienal in the presence of 13-hydroperoxy-(*E*,*Z*)-9,11-octadecadienoate (*28*). As indicated in **Figure 1**, the same reaction might generate **2** from **1** in the presence of lipid hydroperoxides.

Thus, the aim of the present investigation was (i) to develop a stable isotope dilution analysis for the quantitation of **2** in combination with LC-MS-MS, (ii) to investigate the formation pathway of **2** in model systems, and (iii) to identify and quantify **2** in foods.

MATERIALS AND METHODS

Food Samples. Samples of potato chips and precooked French fries were purchased in a local supermarket. Precooked French fries (50 g) were heated at 180 °C in an oven for either 20 (light-colored) or 30 min (dark-colored). Additionally, frying of the precooked material was carried out either in coconut oil or in sunflower oil, respectively, at 180 °C for 5 (light-colored) or 8 min (dark-colored). Delios (mixture of saturated triglycerides) was obtained from Cognis (Illertissen, Germany).

Chemicals. [$^{13}C_3$]Acrylamide (99%) was obtained from CIL (Andover, MA), and acrylamide was from VWR International (Darmstadt, Germany). Glycidamide and [$^{13}C_3$]glycidamide (99%) were from Toronto Research Chemicals (North York, ON, Canada). 2-Mercaptobenzoic acid, linoleic acid, and soybean lipoxygenase type I were obtained from Sigma-Aldrich (Steinheim, Germany). All other reagents were of analytical grade.

CAUTION: Acrylamide and glycidamide as well as [$^{13}C_3$]acrylamide and [$^{13}C_3$]glycidamide are hazardous and must be carefully handled.

Synthesis of Linoleic Acid Hydroperoxides. Linoleic acid (200 mg) was reacted at pH 9.5 with soybean lipoxygenase type I (4 mg) for 60 min at room temperature as described previously (*30*). The amount of the hydroperoxides formed was calculated densitometrically at 234 nm after thin layer chromatography (*30*), leading to a conversion rate of 35%.

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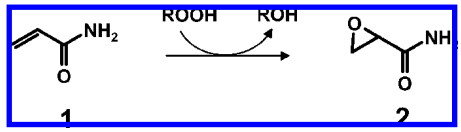

Figure 1. Possible pathway leading to the generation of glycidamide (**2**) by epoxidation of acrylamide (**1**).

Development of a Method for the Quantitation of Glycidamide. *Preparation of the Reference Derivatives.* Either **2** or [$^{13}C_3$]glycidamide ([$^{13}C_3$]-**2**) (150 nmol) was singly dissolved in water (20 mL). After the addition of 2-mercaptobenzoic acid in aqueous sodium hydroxide (1 mL; 154 mg in 10 mL of 0.5 mol/L NaOH), the solution was adjusted to pH 10.5 ± 0.3 by adding aqueous sodium hydroxide (1 mol/L). The reaction mixture was stirred in the dark at room temperature. Different reaction times (2, 4, 6, 12, and 24 h) were studied to evaluate the conversion rate. The excess of the reagent was removed by treatment with lead(II) acetate (2 mL of a saturated solution in water), and, after centrifugation at 2800*g* for 10 min (Beckman J2-HS, Munich, Germany), the supernatant was acidified to pH 1.5 using hydrochloric acid (5 mol/L). An aliquot (20 mL) of the solution was applied onto an Extrelut NT 20 column (VWR), and, after equilibration for 10 min, elution of **2** or [$^{13}C_3$]-**2** was performed with ethyl acetate (100 mL). The organic phase was dried over anhydrous sodium sulfate and, then, the solvent was removed under vacuum at about 20 kPa at 30 °C. The residue was taken up in acetonitrile/Millipore water (500 μL; 3:7, v/v; containing 0.1% of formic acid) and filtered (0.45 μm; Spartan13/0.45RC) (Schleicher & Schuell, Dassel, Germany), and an aliquot (10 μL) was used for LC-MS analysis. To obtain a calibration curve, defined mixtures of **2** and [$^{13}C_3$]-**2** were worked up as described above.

Liquid Chromatography−Tandem Mass Spectrometry (LC-MS-MS). Mass spectra were recorded by means of a triple-quadrupole tandem mass spectrometer (TSQ Quantum Discovery) (Thermo Electron, Dreieich, Germany) coupled to a Surveyor high-performance liquid chromatography system (Thermo Finnigan, Dreieich, Germany) equipped with a thermostated (20 °C) autosampler and a 150 × 2.0 mm i.d., 4 μm Synergi Polar RP 80 Å HPLC column (Phenomenex, Aschaffenburg, Germany) kept at 30 °C connected to a 4 × 2.0 mm i.d. Polar RP precolumn (Phenomenex). The solvent system was composed of formic acid in water (0.1%, by weight; A) and formic acid in acetonitrile (0.1%, by weight; B). A linear gradient was applied by increasing the concentration of B from 30 to 100% within 15 min. Separation was performed at a flow rate of 0.2 mL/min. The mass spectrometer was operated in the positive electrospray ionization mode (ESI^+) with a spray needle voltage of 4.5 kV and a spray current of 5 μA. The temperature of the capillary was 300 °C, and the capillary voltage was 35 V. The sheath and auxiliary gas (nitrogen) were adjusted to 40 and 5 arbitrary units, respectively. The collision cell was operated at a collision gas (argon) pressure of 0.13 Pa.

The derivatives of **2** and [$^{13}C_3$]-**2** were characterized by means of their molecular masses obtained in the full-scan mode. The following molecular ions were obtained as base peaks ($M^+ + 1$): *m/z* 242 for derivatized **2** and *m/z* 245 for derivatized [$^{13}C_3$]-**2**. These were then subjected to MS-MS (collision energy = 20 V). First, the most intense transitions of the precursor ions were determined (**2**: *m/z* 242 to 153, *m/z* 242 to 72, *m/z* 242 to 224; [$^{13}C_3$]-**2**: *m/z* 245 to 153, *m/z* 245 to 75, *m/z* 245 to 227), and, second, the yields of the product ions were optimized by performing a series of runs with different collision energies and flow rates of the sheath and auxiliary gas. The mass transitions *m/z* 242 to *m/z* 72 and *m/z* 242 to *m/z* 153 for **2** as well as *m/z* 245 to *m/z* 75 and *m/z* 245 to *m/z* 153 for [$^{13}C_3$]-**2** were selected for quantitation. On both mass filter quadrupoles, resolution settings were 0.7 full width at half-maximum, scan time for each transition and single reaction monitoring (SRM) was 0.50 s, scan width was 0.7 amu, and source CID was 14 V.

For calibration, five mixtures of **2** and [$^{13}C_3$]-**2** in defined concentrations (molar ratios 5:1 to 1:5) were analyzed, and the response factor was calculated from the results as described recently (*29*). Calibration was always performed prior to sample measurement.

Quantitation of Glycidamide in Model Systems. *Model Systems.* Delios (10 mL) and 2-propanol (2 mL) were mixed with linoleic acid hydroperoxides (70 mg). After 10 min of stirring, 120 mg of **1** was added. After another 5 min of stirring, the mixture was heated for 45 min at 80 °C in closed glass vessels in an oven. The experiment was repeated at 140 and 200 °C, respectively.

In a next series of experiments, 60, 120, or 600 mg of **1** was added to the mixture containing Delios, 2-propanol, and the linoleic acid hydroperoxides. After 5 min of stirring, the mixtures were heated at 160 °C for 45 min. In control experiments, either the hydroperoxides, **1**, or 2-propanol was omitted in the base mixture to prove if these compounds are required for the generation of **2**.

Quantitation of Glycidamide. After cooling of the samples of the model system described above to room temperature, the internal standard [$^{13}C_3$]-**2** (1.25 μg in 1 mL of ethanol) was added to all samples and, after 10 min of stirring, the samples were extracted with distilled water (2 × 10 mL). The water phase was defatted three times by extraction with *n*-hexane (total volume = 60 mL). After the addition of a solution of 2-mercaptobenzoic acid in aqueous sodium hydroxide (1 mL; 154 mg in 10 mL of 0.5 mol/L NaOH) for derivatization (pH was increased to about 10), the reaction mixture was stirred in the dark for 12 h. The excess reagent was removed by treatment with a saturated solution of lead(II) acetate (2 mL). After centrifugation at 2300*g* for 10 min, the supernatant was acidified to pH 1.5 using hydrochloric acid (5 mol/L). Then, an aliquot of the solution (15 mL) was applied onto an Extrelut NT 20 column and, after an equilibration time of 10 min, elution was performed with ethyl acetate (100 mL). After drying over anhydrous sodium sulfate, the solvent was removed at about 20 kPa and 30 °C. The residue was dissolved in acetonitrile/Millipore water (250 μL; 3:7, v/v; containing 0.1% of formic acid). An aliquot of this solution (200 μL) was injected onto a 250 × 8.0 mm i.d., 5 μm Nucleosil RP 100 Å HPLC column (Macherey-Nagel, Düren, Germany) coupled to a 4 × 2.0 mm i.d. C_{18} precolumn (Phenomenex). The sample was separated at a flow rate of 1.6 mL/min. The solvent system was composed of formic acid in water (0.1%, v/v; A) and formic acid in acetonitrile (0.1%, v/v; B). A linear gradient was applied by increasing the concentration of B from 20 to 30% within 15 min and then to 100% within 3 min. Using these parameters, derivatized **2** and [$^{13}C_3$]-**2** were isolated by manually collecting a fraction between 11.0 and 13.5 min. The solvent was evaporated using a vacuum centrifuge (RC 1022) (Jouan, Unterhaching, Germany), and the residue was dissolved in acetonitrile/water (150 μL; 3:7, v/v; containing 0.1% of formic acid). For LC-MS-MS measurements (conditions see above), an aliquot of this solution (10 μL) was used.

Determination of the Structures of 3 and 4 by Nuclear Magnetic Resonance Spectroscopy (NMR). The thioethers **3** and **4** (**Figure 2**) were isolated in a semipreparative scale (10−30 mg) by reversed-phase HPLC as described above, the solvent was removed at about 20 kPa and 30 °C, and the residue was dissolved in dimethyl-d_6 sulfoxide (DMSO-d_6). 1D- and 2D-NMR experiments were performed on a Bruker Avance-500 (Bruker, Rheinstetten, Germany). Chemical shifts were determined using tetramethylsilane (TMS) as the internal standard in the proton dimension and from the carbon signal of DMSO-d_6 (39.9 ppm) in the carbon dimension.

NMR data of compound **3** (second fraction of HPLC) (the arbitrary numbering of the carbon atoms refers to structure **3** given in **Figure 2**): ^{1}H NMR (500 MHz; DMSO-d_6; COSY) δ 3.59 [dd, 1H, *J* = 5.1, 10.9 Hz, H−C(9a)], 3.78 [dd, 1H, *J* = 8.2, 10.9 Hz, H−C(9b)], 3.89 [dd, 1H, *J* = 5.1, 8.2 Hz, H−C(H−C(8)], 7.22 [pt, 1H, *J* = 7.4 Hz, H−C(4)], 7.47 [pt, 1H, *J* = 7.62 Hz, H−C(5)], 7.51 [d, 1H, *J* = 7.7 Hz, H−C(6)], 7.79 [d, 1H, *J* = 7.3 Hz, H−C(3)]; ^{13}C NMR (125 MHz; DMSO-d_6; HMQC, HMBC, DEPT-135) δ 51.7 [CH, C(8)], 62.2 [CH_2, C(9)], 125.2 [CH, C(4)], 127.4 [CH, C(6)], 130.9 [CH, C(3)] 131.4 [C, C(2)], 132.0 [CH, C(5)], 138.8 [C, C(7)], 168.5 [C, C(1)], 172.1 [CH, C(10)].

NMR data of compound **4** (first fraction of HPLC) (the arbitrary numbering of the carbon atoms refers to structure **4** given in **Figure 2**): ^{1}H NMR (500 MHz; DMSO-d_6; COSY) δ 2.98 [dd, 1H, *J* = 8.2, 12.9 Hz, H−C(8a)], 3.30 [dd, 1H, *J* = 3.6, 12.9 Hz, H−C(8b)], 4.04 [dd, 1H, *J* = 3.6, 8.2 Hz, H−C(H−C(9)], 7.18−7.22 [m, 1H, *J* = 2.0, 7.7 Hz, H−C(4)], 7.45−7.50 [m, 2H, H−C(5), H−C(6)], 7.82 [d, 1H, *J* = 7.7 Hz, H−C(3)]; ^{13}C NMR (125 MHz; DMSO-d_6; HMQC, HMBC, DEPT-135) δ 37.4 [CH_2, C(8)], 70.2 [CH, C(9)], 124.6 [CH, C(4)], 126.8 [CH, C(6)], 131.0 [CH, C(3)], 131.5 [C, C(2)], 132.4 [CH, C(5)], 140.8 [C, C(7)], 168.3 [C, C(1)], 175.0 [CH, C(10)].

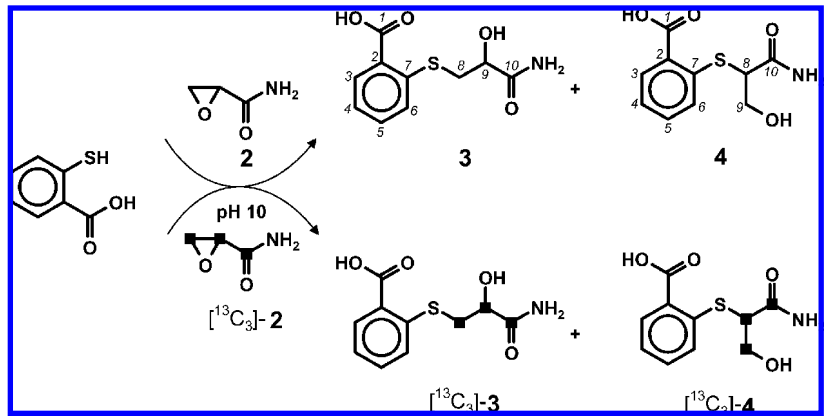

Figure 2. Conversion of glycidamide (**2**) and [$^{13}C_3$]glycidamide ([$^{13}C_3$]-**2**) into the thioethers **3**, **4**, [$^{13}C_3$]-**3**, and [$^{13}C_3$]-**4**, respectively, prior to LC-MS-MS analysis.

Quantitation of Acrylamide and Glycidamide in Foods. After the addition of the internal standards [0.1−0.5 μg of [$^{13}C_3$]-**2** and 5−10 μg of [$^{13}C_3$]acrylamide ([$^{13}C_3$]-**1**) in ethanol depending on the amounts of **1** and **2** determined in a preliminary experiment] and water (250 mL), the sample [50 g; powdered after freezing in liquid nitrogen by means of a commercial blender (Moulinex, Radolfzell, Germany)] was homogenized and extracted using an Ultra-Turrax (Jahnke & Kunkel, Oberstaufen, Germany) for 5 min followed by an ultrasonic treatment for 5 min. The homogenate was centrifuged at 4800*g* for 10 min at 10 °C (centrifuge GR 412) (Jouan), the supernatant was centrifuged twice at 25000*g* for 10 min at 10 °C (Beckman), and the solution was subsequently defatted twice by extraction with *n*-hexane (total volume = 60 mL). If necessary, filtering by means of a glass filter (pore size 4) was used.

After the addition of 2-mercaptobenzoic acid in aqueous sodium hydroxide (1 mL; 154 mg in 10 mL of 0.5 mol/L NaOH) for derivatization, the extract was adjusted to pH 10.5 ± 0.3 by adding aqueous sodium hydroxide (1 mol/L). Then, the reaction mixture was stirred for 12 h in the dark at room temperature. The excess of the reagent was removed by treatment with lead(II) acetate (2 mL of a saturated solution in water), and, after centrifugation at 2800*g* for 10 min, the supernatant was acidified to pH 1.5 using hydrochloric acid (5 mol/L).

Two aliquots (each 15 mL) of the solution obtained above were applied onto two Extrelut NT 20 columns, and, after equilibration for 10 min, derivatized **1** and **2** as well as the derivatized labeled internal standards [$^{13}C_3$]-**1** and [$^{13}C_3$]-**2** were eluted with ethyl acetate (100 mL for each column). The organic phases were combined and dried over anhydrous sodium sulfate. Then, further workup was done as described above for the model studies. For LC-MS-MS measurements, an aliquot of this solution (10 μL) was used. For quantitation of **1**, the mass spectrometer was operated in the SRM mode and mass transitions *m/z* 226 to 191 (for **1**) and *m/z* 229 to 194 (for [$^{13}C_3$]-**1**), respectively, were recorded (*31*).

RESULTS AND DISCUSSION

Method Development for Quantitation of Glycidamide. On the basis of a recently published method for the selective quantitation of **1** involving a derivatization step with 2-mercaptobenzoic acid (*31*), a new stable isotope dilution assay for the quantitation of **2** using [$^{13}C_3$]-**2** as the internal standard was developed. The method should also allow the parallel quantitation of **1** and **2** in food samples.

First, the derivatives were synthesized by reacting **2** as well as [$^{13}C_3$]-**2** singly with 2-mercaptobenzoic acid to obtain stable derivatives according to the reaction scheme illustrated in **Figure 2**. In comparison to the derivatization of **1** (50% conversion after 3 h), **2** reacted more slowly. After 2 h, only 6% of **2** had been converted into the respective derivative, but the yields increased to 30 and 70% after 4 and 6 h, respectively. After 12 h, **2** was no longer detectable and, thus, this reaction time was used in the derivatization procedure.

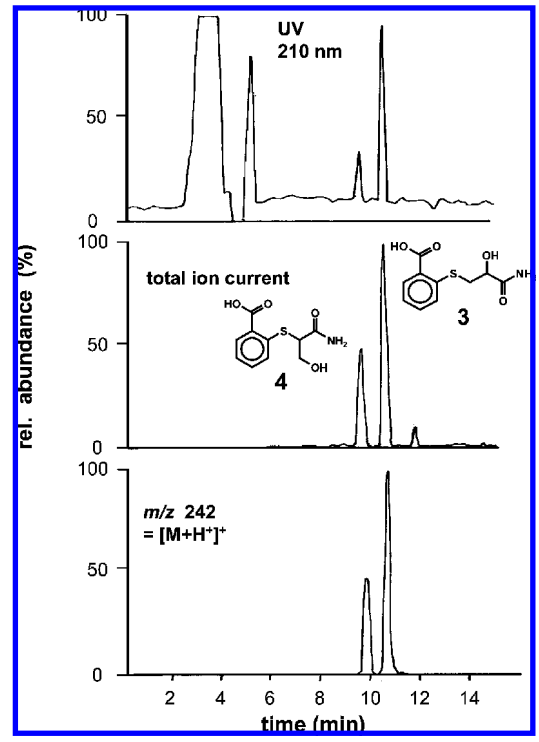

Figure 3. LC-MS chromatogram of the thioethers **3** and **4**. Retention times differ from ones in **Figures 6** and **8** because a different LC-MS instrument (LCQ Classic, Thermo Finnigan) was used.

Derivatization of **2** might result in two different reaction products depending on which carbon atom is attacked by the SH group, namely, 2-[(3-amino-2-hydroxy-3-oxopropyl)thio]benzoic acid (**3**) and 2-{[2-amino-1-(hydroxymethyl)-2-oxoethyl]thio}benzoic acid (**4**). To assign the correct structures, the compounds formed in the reaction of **2** and 2-mercaptobenzoic acid were separated by HPLC and analyzed by 1D- and 2D-NMR experiments. In particular, the two double doublets and the characteristic chemical shifts at the H-atoms at C(8) of **3** and at C(9) of **4**, respectively, confirmed the structures of the two isomers shown in **Figure 2**. LC-MS runs confirmed the formation of both products in the reaction of **2** with 2-mercaptobenzoic acid in a ratio of 3:1 (**Figure 3**). The formation of both products was in agreement with previous studies (*32*) also using the nucleophilic opening of epoxides. The corresponding labeled compounds ([$^{13}C_3$]-**3** and [$^{13}C_3$]-**4**) were formed from [$^{13}C_3$]-**2**. For LC-MS-MS measurements,

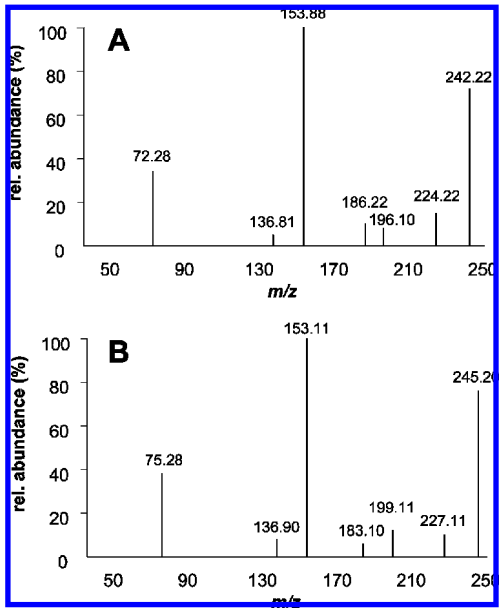

Figure 4. ESI$^+$ mass spectra of the thioethers **3** and **4** (**A**) as well as [$^{13}C_3$]-**3** and [$^{13}C_3$]-**4** (**B**).

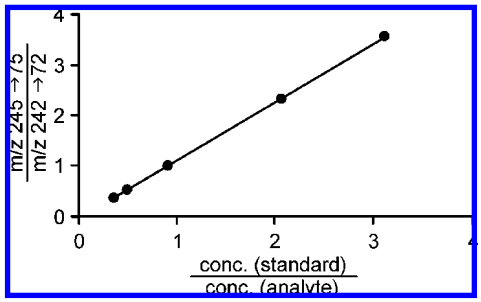

Figure 5. Standard curve used in the determination of the response factor for quantitation of **2**.

unlabeled and labeled derivatives were collected from several HPLC runs and subjected to off-line ESI$^+$ mass spectrometry providing clear base peaks at *m/z* 242 for **3** and **4** as well as at *m/z* 245 for [$^{13}C_3$]-**3** and [$^{13}C_3$]-**4** (data not shown).

To prevent matrix interferences, and for increased selectivity, tandem mass spectrometry was used for unequivocal quantitation. By fragmentation of the protonated molecule ions, MS-MS spectra of the unlabeled and labeled thioethers were obtained (**Figure 4**). From each fragment spectrum, the most abundant ion transitions were selected and used for quantitation by LC-MS-MS in the SRM mode. Ion transitions *m/z* 242 to 72 and *m/z* 242 to 153 (**3** and **4**) as well as *m/z* 245 to 75 and *m/z* 245 to 153 ([$^{13}C_3$]-**3** and [$^{13}C_3$]-**4**), respectively, were selected for quantitation. To increase the sensitivity, the collision energy during LC-MS-MS measurement was systematically changed until maximum intensities of the precursor and product ions were obtained. Considering these data, five calibration mixtures containing different concentrations of both the analyte and the labeled internal standard were analyzed. On the basis of the calibration curve obtained (**Figure 5**), an MS response factor of 0.97 was calculated. A typical LC-MS-MS chromatogram obtained for a calibration mixture is shown in **Figure 6**.

The limit of quantitation (LoQ) of **2** was estimated to be 0.001 μg/kg on the basis of a correlation between the intensity of the respective ions and the background noise.

Model Studies on the Epoxidation of Acrylamide. First, linoleic acid hydroperoxides were synthesized. The presence of the hydroperoxides was verified by means of thin layer

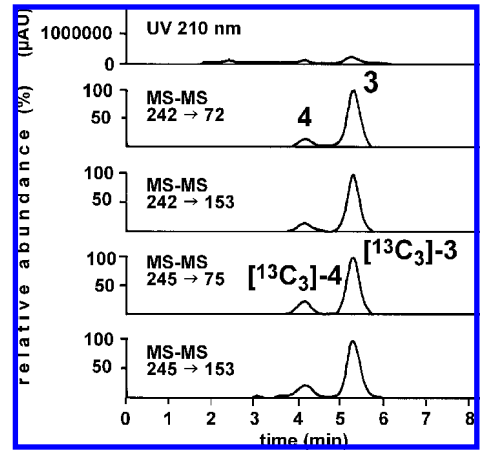

Figure 6. LC-MS-MS chromatogram obtained for a calibration mixture of **3** and **4** as well as [$^{13}C_3$]-**3** and [$^{13}C_3$]-**4**.

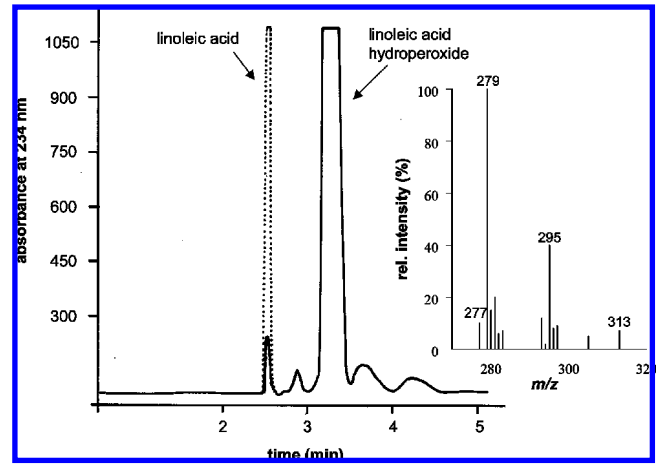

Figure 7. HPLC-UV chromatogram of the product mixture after incubation of linoleic acid with soybean lipoxygenase type I (continuous line) as well as APCI$^+$ mass spectrum of the monohydroperoxide. HPLC-UV chromatogram of linoleic acid before incubation is given for comparison (dotted line).

chromatography including a specific detection reagent for hydroperoxides (*N*,*N*-dimethyl-*p*-phenylenediammonium dichloride), HPLC with UV detection, as well as mass spectrometry (LC-MS; APCI$^+$) (**Figure 7**). On the basis of a photometric quantitation (*30*), 35% of the fatty acid was converted into hydroperoxides.

Defined mixtures of **1** and linoleic acid hydroperoxides were then reacted at elevated temperatures, and the amounts of **2** formed were quantified. In **Figure 8**, mass chromatograms obtained for derivatized **1** and **2** as well as the respective labeled internal standards are displayed. Due to the fact that the two isomeric thioethers were chromatographically separated on the SPE column and because the main product was eluted first, the minor product was not used for quantitation. However, the same results were obtained if the sum of both thioethers obtained from **2** as well as from [$^{13}C_3$]-**2** was used for quantitation.

These data clearly showed the formation of **2** by epoxidation of **1**. Depending on the reaction temperature (heating time = 45 min), different thermoconversion rates were found (**Table 1**). Using 120 mg of **1** provided about 6 μg of **2** at 80 °C (**Table 1**, expt 1) and about 10 μg of **2** at 120 °C (expt 2), whereas a further increase of the temperature (200 °C; expt 3) led to a decrease of **2** comparable to the amounts generated at 80 °C. Obviously, the epoxide formed is unstable at higher temperatures.

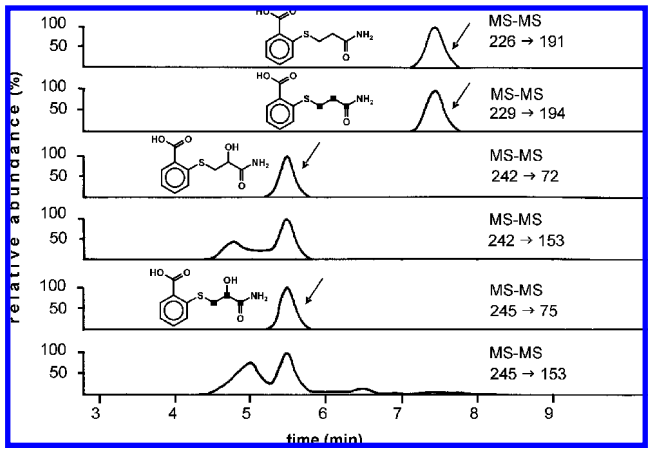

Figure 8. LC-MS-MS chromatogram obtained from a model experiment showing the thermoconversion of **1** into **2** in the presence of linoleic acid hydroperoxides. Traces 1 and 2 from top were used for quantitation of **1**; traces 3 and 5 were used for quantitation of **2**; traces 4 and 6 are additional mass transitions to confirm the presence of **2**.

Table 1. Conversion of Acrylamide (**1**) into Glycidamide (**2**) As Affected by the Reaction Temperature and the Initial Amount of **1**

	reaction conditions[a]		
expt	amount of **1** (mg)	temperature (°C)	amount of **2**[b] (μg)
1	120	80	6.1
2	120	140	9.3
3	120	200	6.0
4	60	160	5.6
5	120	160	12.1
6	600	160	29.2

[a] Reaction was performed in the presence of linoleic acid hydroperoxides in a lipid matrix consisting of saturated triglycerides for 45 min in a closed glass vessel. [b] Mean values of triplicates. Coefficient of variation was below 5%.

In a next series of experiments, the formation of **2** at a fixed temperature (160 °C), but from different amounts of **1**, was investigated. The results (expts 4−6) clearly showed that the formation of **2** increased with the amounts of **1** available in the reaction system. In a control experiment the hydroperoxides were omitted in the model mixture, resulting in no detectable amounts of **2**. The same result was obtained in the absence of **1**. The presence of 2-propanol had no influence on the amounts of **2** formed.

Food Samples. As compared to the recently published method for the determination of **1** in food (*31*), the sensitivity of the quantitation of **1** after derivatization with 2-mercaptobenzoic acid was further increased in the present study. By replacing the dichloromethane extraction with a solid phase extraction (SPE; Extrelut column), the yield of the derivative was increased by a factor of 2.5. After solvent evaporation, the eluate of the SPE could directly be used for the determination of **1** by means of LC-MS-MS.

For the quantitation of **2**, a further cleanup step was, however, required due to the much lower concentration as compared to **1**. Therefore, the SPE eluate was respectively injected onto an HPLC column, the relevant peaks were manually collected, combined, and, finally, subjected to LC-MS-MS measurement.

To check whether the newly developed method could be applied to real food samples, the amounts of **1** and **2** were analyzed in commercial potato chips as well as in French fries (**Table 2**). Besides being identified for the very first time in a food sample, **2** showed concentrations of 1.5 μg/kg in chips and 0.3−0.6 μg/kg in French fries, depending on the heating time (5 and 8 min, respectively). In potato chips, the amount of **2** was 0.5% of **1**, whereas this proportion was only 0.2% in French fries without showing a clear dependence on heating time (**Table 2**, expts 1, 4, and 7).

Table 2. Concentrations of Acrylamide (**1**) and Glycidamide (**2**) in Potato Chips and Precooked French Fries As Affected by the Heating Conditions

expt	sample	concn of **1**[a] (μg/kg)	concn of **2**[a] (μg/kg)
1	potato chips[b]	302.7	1.51
2	precooked French fries (light-colored)[c,f]	na[h]	0.002
3	precooked French fries (light-colored)[d,f]	na	0.21
4	precooked French fries (light-colored)[e,f]	200.5	0.29
5	precooked French fries (dark-colored)[c,g]	na	0.02
6	precooked French fries (dark-colored)[d,g]	na	0.41
7	precooked French fries (dark-colored)[e,g]	350.1	0.63

[a] Mean values of triplicates. Coefficient of variation below 5% (**1**) and below 10% (**2**), respectively. [b] Commercial sample. [c] Commercial precooked French fries (50 g) were prepared in coconut oil. [d] Commercial precooked French fries (50 g) were prepared in sunflower oil. [e] Commercial precooked French fries (50 g) were prepared in an oven. [f] Light-colored: heating was performed for 5 min at 180 °C. [g] Dark-colored: heating was performed for 8 min at 180 °C. [h] Not analyzed.

On the basis of the initial thoughts that hydroperoxides can promote the conversion of **1** into **2**, French fries were prepared in coconut oil containing mainly saturated fatty acids and in sunflower oil with a high percentage of unsaturated triglycerides. As already shown for the model systems, the results supported the assumption of an influence of hydroperoxides on the formation of **2**. Whereas French fries prepared in coconut oil contained **2** in amounts of only 0.002 μg/kg (light-colored) or 0.02 μg/kg (dark-colored) (**Table 2**, expts 2 and 5), respectively, the same batch fried in sunflower oil revealed concentrations of 0.2 and 0.4 μg/kg (expts 3 and 6), respectively. Furthermore, during the frying process in coconut oil, the fat seemed to "protect" **1** by avoiding the formation of hydroperoxides and, thus, the formation of **2**. This assumption was corroborated by the data obtained for precooked French fries, which were dry-heated in an oven. Under these conditions (expts 4 and 7) much higher concentrations of **2** in comparison to the fries processed in coconut oil were formed. The amounts of **2** by heating the fries in air were even higher than in sunflower oil (cf. expts 3 and 4 as well as expts 6 and 7).

Although only a small number of samples has been analyzed so far, **2** was unequivocally identified for the first time in food, which is of considerable importance due to the fact that **2** was shown to exhibit a much higher toxicity in cell assays than **1** (*33*).

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