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Development of Two Stable Isotope Dilution Assays for the Quantitation of Acrolein in Heat-Processed Fats

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Supporting Information

ABSTRACT: Two stable isotope dilution assays were developed for the quantitation of acrolein in fats and oils using [$^{13}C_3$]-acrolein as the internal standard. First, a direct GC-MS headspace method, followed by an indirect GC-MS method using derivatization with pentafluorophenyl hydrazine, was established. Analysis of six different types of oils varying in their pattern of fatty acids showed significant differences in the amounts of acrolein formed after heating at various temperatures and for various times. For example, after 24 h at 140 °C, coconut oil contained 6.7 mg/kg, whereas linseed oil was highest with 242.3 mg/kg. A comparison of the results showed that the extent of acrolein formation seemed to be correlated with the amount of linolenic acid in the oils. Although the acrolein concentrations were lowered in all six oils after frying of potato crisps, linseed and rapeseed oil still contained the highest amounts of acrolein after frying. By applying both methods on different thermally treated fats and oils, nearly identical quantitative data were obtained.

KEYWORDS: acrolein, stable isotope dilution assay, fat and oil, foodborne toxicant

INTRODUCTION

During high-temperature cooking, the oxidative deterioration of fats and oils is known to generate several volatile compounds with significant reactivity, for example, unsaturated aldehydes. In addition, fatty acids and alcohols are well-known degradation products of processed oils.[1−3] Some of these volatile aldehydes may be harmful to humans, for example, acrolein, which has been associated with an adverse effect on human health and is recognized to play a role in diseases such as atherosclerosis, carcinogenesis, pathologies, and aging.[4−6] Furthermore, acrolein is known for its irritating power and its ability to depress the respiratory immune response.[7] Thus, acrolein has for many years been considered as a priority pollutant according to the U.S. Environmental Protection Agency (EPA).[8] The toxicity of the aldehyde is due to its ability to interfere with cell metabolisms and to modify proteins and nucleic acids by introducing inter- and intramolecular cross-links.[9−11]

In recent years, acrolein has been identified in several foodstuffs, biological samples, or tobacco smoke, respectively. Its formation has been found to be governed by several factors, such as temperature and time of heating, oxygen concentration, and type of oil.[12−14] Takeoka et al.[15] pointed out that oils with higher levels of polyunsaturated fatty acids generally produced more polar compounds when heated to 190 and 240 °C, respectively, for 8 h/day.

In the literature, a thermal degradation of glycerol formed by hydrolysis of triacylglycerides at higher temperatures (230 °C) is assumed as an important step in the generation of acrolein.[16−18] However, in contrast, Fujisaki et al.[12] showed that an increase of acrolein concentration after heating of an oil correlates with the increase of the oxygen concentration in the air, and they suggested an oxidative yet unknown reaction pathway in acrolein generation. On the basis of their observations of acrolein formation under nitrogen,[19−21] other groups proposed that acrolein formation is due to a free radical mechanism. However, the formation pathway of acrolein from unsaturated triacylglycerides is still unclear.

Exact quantitative data are the prerequisite in studies on formation mechanisms of food constituents. However, the analysis of acrolein is a challenge due to its high volatility and reactivity as well as the lack of a chromophore. A proper tool to enhance the recovery rate and, thus, the sensitivity of compounds with low boiling points is a derivatization step. Reagents such as 2,4-dinitrophenylhydrazine followed by HPLC analysis [13,22,23] as well as *o*-2,3,4,5,6-(pentafluorobenzyl)hydroxylamine hydrochloride [24] or pentafluorophenylhydrazine (PFPH)[25,26] prior to gas chromatography are commonly used in the analysis of volatile aldehydes. However, these analytical methods require extensive workup procedures, because the derivatives need to be isolated prior to chromatography. Another method for the isolation of substances with a very low boiling point is the static or dynamic headspace analysis. However, although no sample cleanup is necessary prior to GC analysis, commonly one drawback of this method is coelution with other volatiles.

Stable isotope dilution assays have been established as appropriate methods to quantitate volatile and/or unstable analytes, for example, in aroma analysis.[27,28] However, to the best of our knowledge, no method for the quantitation of acrolein using a stable isotopically labeled internal standard has been reported yet. Because it is a common trend in high-temperature cooking to use vegetable oils with a high degree of unsaturation, the initial objectives of the present work were (i) to develop a stable isotope

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dilution analysis for the quantitation of acrolein, (ii) to compare the data obtained by two different techniques, namely, headspace analysis and liquid−liquid extraction combined with a high-vacuum distillation after the derivatization step, and (iii) to apply these methods to thermally treated oils, as well as oils used to fry potato crisps.

MATERIALS AND METHODS

Food Samples. Potatoes and vegetable fats and oils differing in their fatty acid composition were purchased at local supermarkets: coconut oil, extra virgin olive oil, rapeseed oil, safflower oil, and linseed oil. Furthermore, a hydrogenated fat, particularly recommended for roasting, baking, and frying, was investigated.

Chemicals. [$^{13}C_3$]-Acrolein (99%) was obtained from Isotec (Ohio, IL) and acrolein from Fluka (Steinheim, Germany). Pentafluorophenylhydrazine (97%; PFPH) was from Sigma-Aldrich (Steinheim, Germany). All other reagents were of analytical grade.

Fatty Acid Composition Analysis. The fatty acid composition was determined by GC-FID after derivatization with methanol/sodium methanolate to obtain the corresponding fatty acid methyl esters closely following the IUPAC standard method.[29]

Aliquots of the oil (100 mg) were accurately weighed into screw-capped centrifuge tubes, and tetrahydrofuran (1 mL) was added. After the oil had been dissolved by shaking, sodium methanolate (2 mL; 0.5 mol/L) was added and the solution was heated for 1 h at 50 °C. Then, water (5 mL) and hexane (5 mL) were added, the suspension was mixed using a vortexer for 1 min, and the organic layer was separated and, finally, dried over anhydrous sodium sulfate. Aliquots (2 μL) of the solutions were analyzed by means of a Thermo Quest gas chromatograph (Thermo Finnigan, Egelsbach, Germany), equipped with a DB-5 column (WCOT fused silica, 30 m × 0.25 mm i.d., 0.25 μm film thickness) (J&W Scientific, Agilent Technologies, Santa Clara, CA) using the cold on-column technique at 60 °C. After 2 min, the temperature was raised at 10 °C/min to 240 °C and, finally, held for 10 min isothermally. The flow of the carrier gas helium was 2.5 mL/min. A flame ionization detector was used at 250 °C. Identification was carried out using a reference mixture of fatty acid methyl esters.

Sample Preparation. To simulate the real conditions of headspace analysis in fats and oils, either acrolein or [$^{13}C_3$]-acrolein (100 ng each; dissolved in methanol) was added to unheated olive oil (1 g in 20 mL vials), which did not contain acrolein above the detection limit. After 20 min of continuous stirring at room temperature, the samples were subjected to mass spectrometric analysis as described below.

Oil Heating Process. Oil samples (30 g) were weighed into glass tubes (200 × 35 mm i.d.) and were heated in a metal block. Heating was controlled by a thermocouple placed inside the oil. Samples were singly heated at 100, 140, 180 (regular deep-frying temperature), 220, and 260 °C, respectively, for 24 h each. In a further series of experiments, the samples were heated at 180 °C for 2, 6, 16, 24, 48, and 96 h, respectively. Immediately after the oils had cooled to room temperature, [$^{13}C_3$]-acrolein (120−900 μg, dissolved in methanol; depending on the amounts determined in preliminary experiments) was added, and the glass tubes were closed and kept at −20 °C prior to analysis.

Deep-Frying of Potatoes. Thin slices (peeled, 1.5 mm of cross section) were fried for 2.5 min at 180 °C in an electrical deep-fryer DF320 (Kenwood, Heusenstamm, Germany) containing 2.3 L of oil, which was preheated for 10 min prior to frying. The potato-to-oil mass ratio was about 1:7. After three, six, and nine frying cycles, respectively, aliquots (10 mL) of the oil were withdrawn and, after cooling to room temperature, amended with [$^{13}C_3$]-acrolein (5−35 μg).

Quantitation by Two-Dimensional HRGC-MS-Stable Isotope Dilution Assay Using Headspace Isolation (Method I). Aliquots (500 μL) of the headspace volume were injected into the hot PPKD injector (Thermo Finnigan, Hamburg, Germany) of a 2000 series Trace GC (Thermo Finnigan, Hamburg, Germany) using a Combi Pal autosampler (CTC Analytics, Zwingen, Switzerland), held at 20 °C, equipped with a 1 mL gastight syringe (SGE Analytic Science, Darmstadt, Germany). After each injection, a possible carry-over into the syringe was eliminated by an automatic syringe flush (helium). The headspace autosampler conditions were set as follows: incubation temperature, 20 °C; syringe temperature, 35 °C; syringe injection volume, 250 μL; syringe injection speed, 0.1 mL/s. The effluent was quantitatively transferred into a cold trap (initial temperature, −120 °C for 0.1 min; heating rate, 12 °C/s; final temperature, 240 °C for 3 min) (SGE Analytic Science) using a moving column stream switching system (Thermo Finnigan, Hamburg, Germany). After the cooling was turned off, the trapped analyte was transferred onto a WCOT fused silica HP-5 MS column of 30 m × 0.25 mm i.d.; 0.5 μm film thickness (J&W Scientific). The oven was heated from −5 °C at 4 °C/min to 50 °C and then to 220 °C at 10 °C/min. The effluent was monitored by an ion trap mass spectrometer Saturn 2000 (Varian, Darmstadt, Germany) running in the chemical ionization mode (70 eV ionization energy) with methanol as the reagent gas. Acrolein and [$^{13}C_3$]-acrolein were first located by means of their molecular masses obtained in the full scan mode (m/z 57 for the analyte and m/z 60 for the labeled standard, respectively) and by their retention indices. Acrolein concentrations were calculated from the area counts obtained from the mass chromatograms using the equation

$$m(\text{acrolein}) = \text{Rf} \times \frac{m([^{13}C_3]\text{-acrolein}) \times A(\text{acrolein})}{A([^{13}C_3]\text{-acrolein})}$$

where A(acrolein) is the area of unlabeled acrolein, A([$^{13}C_3$]-acrolein) is the area of [$^{13}C_3$]-acrolein, and m([$^{13}C_3$]-acrolein) is the amount of added [$^{13}C_3$]-acrolein. The response factor Rf is determined by analysis of defined mixtures of acrolein and [$^{13}C_3$]-acrolein as described below.

Quantitation by Two-Dimensional HRGC-MS-Stable Isotope Dilution Assay after Derivatization with PFPH (Method II). *Preparation of Reference Substances.* Unheated olive oil (10 g) was spiked with acrolein and [$^{13}C_3$]-acrolein (5 μg each; dissolved in methanol). After the addition of PFPH (2 mg; dissolved in 1 mL of 0.1 mol/L HCl), the solution was stirred for 20 min at room temperature using a magnetic stirrer.

Then, the water-in-oil emulsion was extracted with dichloromethane (50 mL) by continuous liquid−liquid extraction. The PFPH derivatives of acrolein and [$^{13}C_3$]-acrolein were separated from the bulk of the fat by solvent-assisted flavor evaporation (SAFE)[30] at 48 °C under vacuum (5×10^{-3} Pa). The distillate was dried over anhydrous sodium sulfate and concentrated to a final volume of ∼1 mL using a Vigreux column (100 cm × 3 cm) at 48 °C. Aliquots (2 μL) of the solution were analyzed by GC-MS as described below.

Analysis of Oil Samples. These were prepared as described above for method I. To aliquots (approximately 3 g) was added PFPH (2 mg; dissolved in 0.1 mol/L HCl), and the mixture was stirred for 20 min at room temperature. Further workup was done as described above for the reference substances.

Instrumental Conditions. Using a Combi Pal autosampler (CTC Analytics), an aliquot (2 μL) of the sample was injected into the hot PPKD injector of a 2000 series Trace GC and transferred onto an FFAP column (30 m × 0.25 mm i.d., 0.5 μm) (J&W Scientific). The temperature was programmed from 50 to 220 °C at 6 °C/min. The effluent was monitored using an ion trap mass spectrometer Saturn 2000 running in the chemical ionization mode (70 eV ionization energy) with methanol as the reagent gas.

The derivatives of acrolein and [$^{13}C_3$]-acrolein were characterized by means of their molecular masses obtained in the full scan mode (m/z 237 for the analyte and m/z 240 for the labeled standard, respectively) and by their retention indices.

Calibration Curves. Defined mixtures of labeled and unlabeled acrolein in different mass ratios were prepared for both methods as described above. The ratios of the area counts of the analyte (m/z 57, method I; or m/z 237, method II) and the labeled standard (m/z 60, method I; or m/z 240, method II) were plotted against the ratio of the concentrations. In total, seven mixtures of known amounts of acrolein and [$^{13}C_3$]-acrolein (molar ratios 10:1 to 1:10) were measured, and the response factor was calculated according to ref 27. Calibration was performed each day prior to sample measurement.

Limit of Detection (LOD) and Limit of Quantitation (LOQ). The calculation of the LOD and LOQ was carried out on the basis of a correlation between the intensity of the respective ions and the background noise with a minimum ratio of 3:1. Unheated oil samples were spiked with known amounts of acrolein in decreasing concentrations (100, 50, 25, 20, 15, 10, and 5 μg/kg) and analyzed in triplicates using both methods. A control run without the addition of acrolein and [$^{13}C_3$]-acrolein was performed to ensure the absence of the respective mass signals in the oil samples.

Influence of Storage Time on Acrolein Stability. To prove the stability of acrolein during storage, rapeseed oil was heated at 180 °C for 72 h in sealed glass tubes. After cooling, the samples were stored for 48 h, 1 week, and 3 months, respectively, either at room temperature or at 4 °C. Prior to analysis, aliquots (10 g) of the oil were spiked with [$^{13}C_3$]-acrolein (400 μg) and analyzed as described above.

RESULTS AND DISCUSSION

Development of the Analytical Procedure. *Method I.* In a first experiment, acrolein and [$^{13}C_3$]-acrolein (2 μg; dissolved in methanol) were singly placed into two 20 mL headspace vials, and after 20 min of equilibration, the solutions were analyzed by headspace GC-MS. The mass chromatogram of acrolein obtained by chemical ionization showed a peak at m/z 57. The labeled isotopologue revealed a clear peak at the same retention index with the mass fragment m/z 60. The isotopic purity in [$^{13}C_3$]-acrolein was >98%. Next, either acrolein or [$^{13}C_3$]-acrolein (100 ng each; dissolved in methanol) was singly added to unheated olive oil (1 g in 20 mL vials). The analyte and the labeled analogue were characterized by selected ion monitoring for acrolein (m/z 57) and [$^{13}C_3$]-acrolein (m/z 60) and by their retention index. The results indicated that the fatty matrix neither contained coeluting compounds showing either m/z 57 or 60, respectively, nor contained acrolein above the detection limit.

Method II. The crucial factor with headspace analysis of low molecular weight compounds such as acrolein is, however, the low specifity of the mass traces. This prompted us to develop an additional method to confirm the reliability of the results obtained by the headspace method similar to the development of a derivatization method using 2-mercaptobenzoic acid for the quantitation of acrylamide, also having an m/z signal of 72 (MS-CI) with a low specifity.[31]

In preliminary trials, acrolein was derivatized with different nucleophiles (e.g., 2,4-dinitrophenylhydrazine or 2-mercaptobenzoic acid), and the respective adducts were analyzed. As a result, PFPH was found to convert acrolein best within 30 min and with high yields into a stable hydrazone, which could be detected by GC-MS. Cecinato et al.[32,33] also reported the use of PFPH for the successful analysis of volatile aldehydes in environmental air.

For method development, first the derivatives were synthesized by reacting acrolein as well as [$^{13}C_3$]-acrolein singly with PFPH to obtain stable derivatives (Figure 1). The samples were separated by GC and monitored by MS-CI. As PFPH itself has a lower retention time compared to its acrolein derivative, the MS detector was switched off during its elution to avoid contamination of the ion trap. The mass spectra of the PFPH derivatives of acrolein and [$^{13}C_3$]-acrolein are given in Figure 2. Various ions were obtained from the 1:1 mixture, which are postulated to be $[C_5F_3]^+$, $[C_5F_5]^+$, and $[C_6F_5NH]^+$, respectively. The fragment m/z 182 in the spectrum is attributable to the loss of $-NH_2$ from the hydrazine moiety, whereas the fragments m/z 155 and 117 may be formed by rearrangement of the aromatic ring. Intense $[M + 1]^+$ ions of the unlabeled (m/z 237) and the labeled acrolein derivative (m/z 240), however, confirmed the successful derivatization of acrolein and its isotopologue. In contrast to the assumption that two peaks might be generated, that is, the syn and the anti forms of the enamine, double peaks were not detected in the chromatogram, probably because the two isomers did not separate on the stationary phase used.

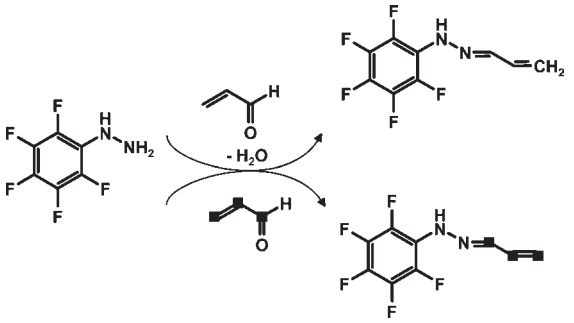

Figure 1. Derivatization scheme applied in the conversion of acrolein (analyte) and [$^{13}C_3$]-acrolein (internal standard) into pentafluorophenylhydrazine derivatives prior to the stable isotope dilution assay (method II).

Next, unheated oil samples (10 g) were spiked with acrolein and [$^{13}C_3$]-acrolein (each 5 μg, dissolved in methanol), and the solutions were derivatized with PFPH. The best results to remove interfering compounds from the PFPH derivatives were obtained by means of a high-vacuum distillation technique, SAFE.[30] Thus, mass spectra could be obtained for the extract of fats and oils identical to those shown in Figure 2 obtained from the model solution. Thus, the workup procedure was proven to be suitable for the quantitation of acrolein. Finally, similar to the development of method I, unheated olive oil was analyzed without adding either acrolein or [$^{13}C_3$]-acrolein, respectively. Again, no signals for m/z 237 and 240 could be detected in the respective unspiked samples, revealing again that acrolein is, if at all, only present below its detection limit and that [$^{13}C_3$]-acrolein is an appropriate internal standard.

Calibration Curves, LOD, and LOQ. A calibration curve was generated for each method by analyzing seven model mixtures in unheated olive oil, containing defined amounts of labeled and unlabeled acrolein in different molar ratios (10:1 to 1:10). The mixtures were worked up for each method and were analyzed by GC-MS. The $[M + 1]$ mass ratios of the area counts of the analyte (m/z 57 for method I and m/z 237 for method II, respectively) and the labeled standard (m/z 60 for method I and m/z 240 for method II, respectively) were plotted against the concentration ratios. From these data, two response curves were obtained showing a good linearity (see the Supporting Information).

For both methods, LODs were calculated on the basis of a signal-to-noise ratio of 3:1. For this purpose, unheated olive oil

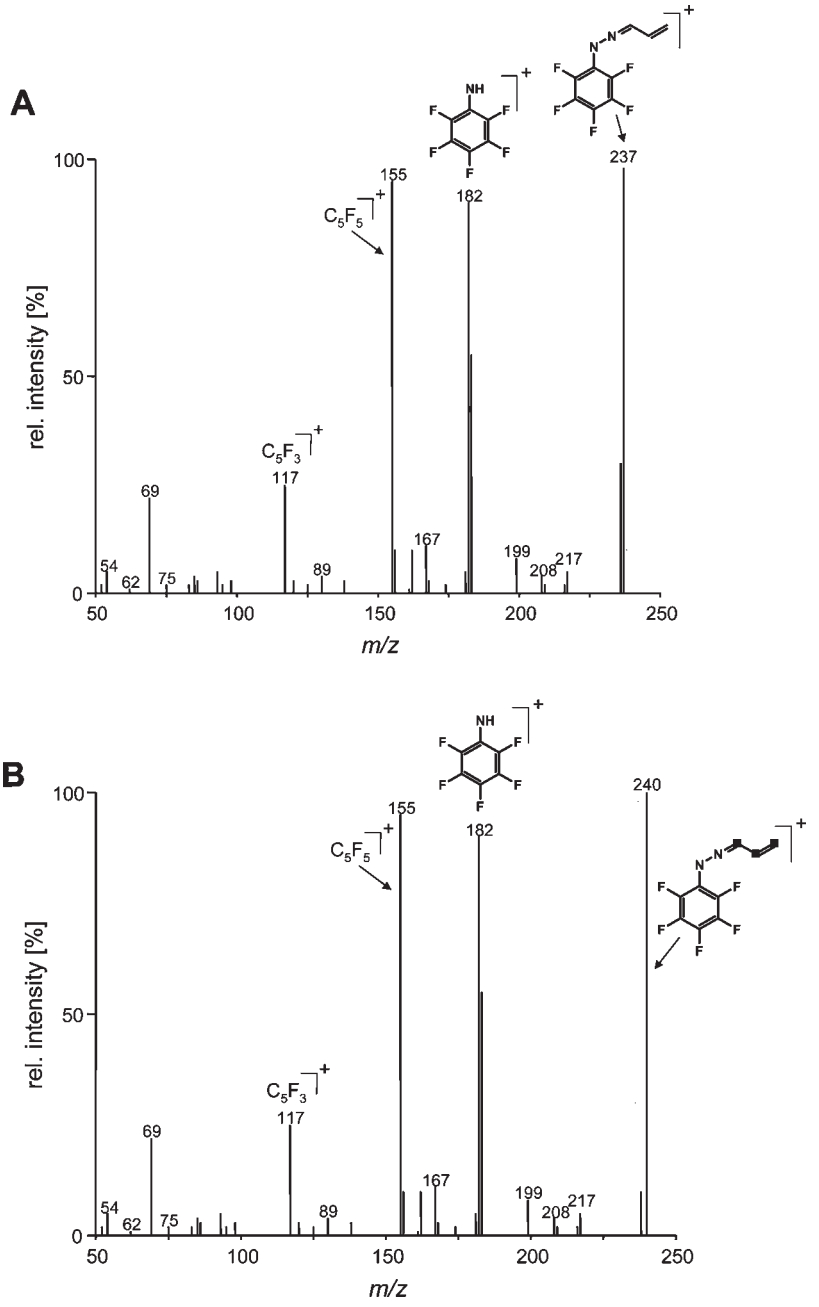

Figure 2. Mass spectra (MS-CI) of the PFPH derivatives of acrolein (A) and [$^{13}C_3$]-acrolein (B).

samples were spiked with different amounts of acrolein (5−100 μg/kg) and, after workup, measured by GC-MS. The results allowed the calculation of LODs of 15 μg/kg (method I) and 20 μg/kg (method II), respectively, and LOQs of 45 μg/kg (method I) and 60 μg/kg (method II), respectively. Interestingly, both methods revealed nearly the same LOD. The comparable sensitivity of both methods might be explained by the following reasons: First, the high-vacuum distillation, which is an appropriate tool for the separation of interfering compounds, bears the possibility that part of the derivatized acrolein as well as [$^{13}C_3$]-acrolein is lost. This, however, affects only the sensitivity, not the quantitative data, because acrolein as well as [$^{13}C_3$]-acrolein would be lost in the same ratio. Second, the high volatility and reactivity of acrolein may result in losses during the derivatization procedure. Nevertheless, the goal of this method development, using selective quantifier fragments of m/z 237 (analyte) and m/z 240 (internal standard) to check the correctness of the results obtained with the headspace GC-MS method (method I) using less selective quantifier signals of m/z 57 (analyte) and m/z 60 (internal standard), was achieved.

Storage Stability. To investigate the influence of ambient conditions on the stability of acrolein during storage of the heated oils, heated rapeseed oil (180 °C for 72 h) was analyzed after different periods of storage time (48 h, 1 week, 1 month, respectively) and at two different temperatures (room temperature and 4 °C, respectively). Furthermore, the influence of light was investigated using amber or clear glass vessels. For each type of oil, an unheated sample was used as the reference. The results

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showed that neither temperature nor storage time influenced the acrolein concentration in the oils. Also, no difference between the oils stored in amber or clear glass was found, indicating that, once formed, acrolein will not react with other fat ingredients during storage at low temperatures (data not shown).

Generation of Acrolein during Thermal Processing of the Oils. To estimate the influence of the fatty acid composition on the amounts of arolein formed after heating, six different oil samples varying in their fatty acid compositions were heated at 180 °C for 24 h, and analyses were carried out using both methods. First, the fatty acid composition of the fat and oil samples was determined (Table 1). In agreement with the literature, coconut oil was dominated by lauric and myristic acid, whereas olive oil mainly consisted of oleic acid. Also in rapeseed oil, oleic acid was the most prominent fatty acid, whereas safflower oil showed the highest amount of linoleic acid and linseed oil was highest in linolenic acid. On the other hand, the hydrogenated fat contained mainly lauric acid, but was also high in oleic and linoleic acid. To check the influence of heat processing on the fatty acid composition, the six fats were heated for various times (up to 96 h) at 180 °C and were analyzed again. Only a marginal decrease, particularly in the unsaturated fatty acids, was observed (data not shown).

Typical chromatograms of heated oil samples obtained by method I (Figure 3A) as well as by method II (Figure 3B) are exemplarily shown for a thermally processed olive oil sample, indicating clear peaks for acrolein and the internal standard.

The quantitative results obtained for the six different oils by both methods are summarized in Table 2. A first observation was that both methods resulted in very similar data, proving that the direct headspace measurement can be used without interference of coeluting compounds (m/z 57 and 60 in MS-CI). At first glance it seems that heating of oils with higher levels of unsaturated fatty acids, in particular linolenic acid (rapeseed and linseed oil; Table 1), generated higher amounts of acrolein.

Table 1. Fatty Acid Composition of the Unheated Fat and Oil Samples

	concn[a] (mg/100 mg fat)					
sample	C12:0[b]	C14:0[c]	C16:0[d]	C18:1[e]	C18:2[f]	C18:3[g]
coconut oil	50	18	9	7	<1	<1
olive oil	<1	<1	11	79	7	<1
rapeseed oil	<1	<1	4.6	63	19.4	8.6
safflower oil	<1	<1	6.3	12.8	73	<1
linseed oil	<1	<1	5.4	20.4	14.9	55.3
frying fat	48	<1	12	25	14	<1

[a] Data are mean values of triplicates. [b] C12:0, lauric acid. [c] C14:0, myristic acid. [d] C16:0, palmitic acid. [e] C18:1, oleic acid. [f] C18:2, linoleic acid. [g] C18:3, linolenic acid.

Table 2. Comparison of the Concentrations of Acrolein in Six Oil Samples after Heating at 180 °C for 24 h

	concn[a] (mg/kg)	
sample	method I	method II
coconut oil	8.1 (7.7/8.5)	7.4 (7.1/7.7)
olive oil	29.3 (27.5/31.1)	27.4 (26.5/28.3)
rapeseed oil	156.4 (159.7/153.1)	152.2 (155.2/149.2)
safflower oil	46.3 (39.2/53.4)	39.3 (42.4/36.2)
linseed oil	207.4 (198.9/215.9)	198.1 (204.7/191.5)
frying fat	55.8 (53.2/58.4)	56.5 (54.1/58.9)

[a] Mean values (concentration range) based on two independent heat processes.

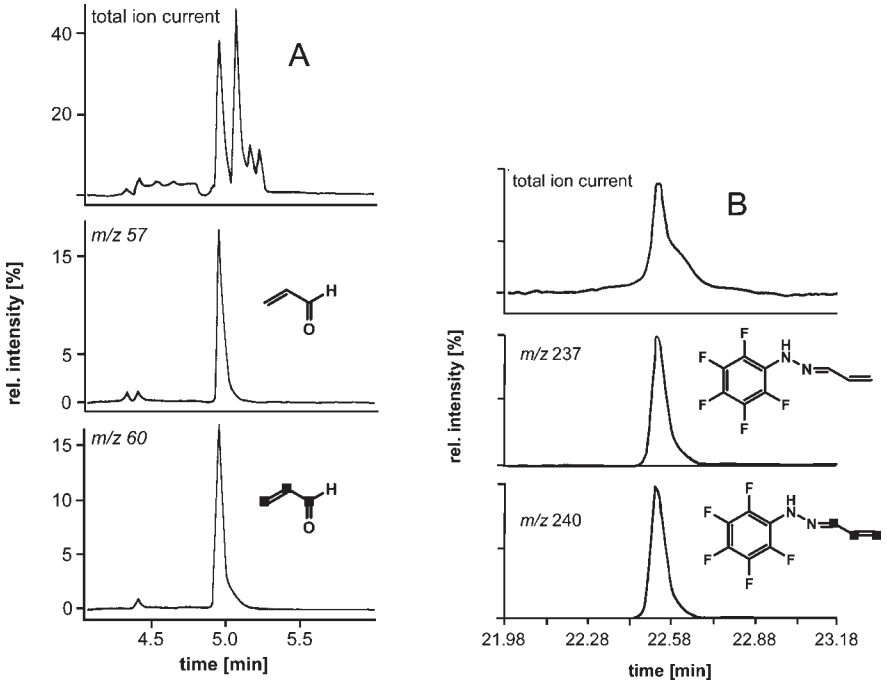

Figure 3. Mass spectra (MS-CI) obtained in the analysis of acrolein (about 15 mg/kg) in an olive oil sample using method I (without derivatization; A) and method II (with derivatization using PFPH; B).

Table 3. Influence of Temperature on Amounts of Acrolein Formed from the Six Oils after Heating for 24 h

	concn[a] (mg/kg)											
	coconut oil		olive oil		rapeseed oil		safflower oil		linseed oil		hydrogenated fat	
temp (°C)	method I	method II	method I	method II	method I	method II	method I	method II	method I	method II	method I	method II
100	12.4	13.1	3.3	2.7	76.4	85.4	33.4	29.1	174.4	168.8	64.2	63.9
140	6.7	7.1	15.3	12.2	162.1	165.2	36.5	37.1	242.3	232.2	69.3	65.6
180	8.1	7.4	29.3	27.4	156.4	152.2	46.3	39.3	207.4	198.1	55.8	56.5
220	6.4	7.2	6.4	5.1	38.6	40.1	21.1	17.9	47.5	47.2	54.1	58.1
260	47.8	46.4	33.0	30.9	6.0	6.4	22.9	23.0	36.9	31.4	88.1	85.9

[a] Data are mean values of triplicates. Standard deviations amounted to not more than 16% for different samples.

Table 4. Influence of Heating Time on Amounts of Acrolein Formed from the Six Oils at 180 °C

	concn[a] (mg/kg)											
	coconut oil		olive oil		rapeseed oil		safflower oil		linseed oil		hydrogenated fat	
heating time (h)	method I	method II	method I	method II	method I	method II	method I	method II	method I	method II	method I	method II
2	1.3	1.1	10.2	11.8	103.1	106.1	16.1	25.1	94.1	85.7	62.2	58.9
6	5.4	5.1	8.5	8.1	59.3	67.6	8.4	16.3	101.2	94.2	74.5	71.1
16	7.2	6.6	15.3	16.5	75.8	81.3	15.6	19.5	99.6	89.3	61.7	59.6
24	8.1	7.4	29.3	27.4	156.5	146.2	46.8	39.3	207.8	190.1	55.8	56.5
48	4.3	5.3	13.2	12.3	95.6	87.3	15.6	19.0	75.5	70.3	43.5	39.4
96	6.9	6.1	3.1	3.2	44.1	39.0	3.4	4.1	65.6	61.3	65.5	71.2

[a] Data are mean values of triplicates. Standard deviations amounted to not more than 16% for different samples.

However, safflower oil, containing by far more linoleic acid as compared to,for example, rapeseed oil, showed a remarkably lower acrolein content. Although its main fatty acid is linoleic acid, safflower oil commonly contains a high level of antioxidants, for example, tocopherols,[34] which might explain its higher stability with respect to acrolein formation. Because saturated or monounsaturated fatty acids are more stable to thermal oxidation, this may explain the significantly lower acrolein generation from coconut and olive oil.

To study the influence of the temperature on acrolein formation, the six oil samples were examined after heating at five different temperatures. With the exception of coconut oil and the frying fat, increasing the temperature from 100 to 180 °C led to a clear increase in acrolein formation. However, from linseed and rapeseed oil, the maximum amount of acrolein was already formed at 140 °C (Table 3). Olive oil and safflower oil showed a maximum of acrolein formation at 180 °C, but the amounts were much lower as compared to the two first mentioned oils. For all oils, except for coconut oil and frying fat, the amounts of acrolein were clearly lower when heated at 220 °C. Probably, at this quite high temperature, acrolein already undergoes reactions with other degradation products. However, increasing the temperature to 260 °C increases the acrolein formation from only coconut oil and frying fat. These results confirmed that obviously the content of linolenic acid is crucial for the formation of acrolein during heating of oils, and the data support results of previous studies on acrolein release into the air during frying of foods in different oils.[21,34−36]

In a further series of experiments, the influence of the heating time on acrolein formation was studied. For this purpose, the oil samples were heated at 180 °C for various heating times from 2 to 96 h. Linseed and rapeseed oil showed quite high amounts of acrolein already after 2 h at 180 °C. Also, the frying fat was high in acrolein after 2 h (Table 4). With increasing heating time, for olive, rapeseed, and safflower as well as linseed oil, it was obvious that acrolein formation increased, especially between 16 and 24 h, whereas further heating (up to 96 h) led to a decrease in acrolein. By contrast, coconut oil as well as the frying fat revealed nearly constant acrolein concentrations over the time range applied.

The observed decrease in acrolein generation at temperatures between 140 and 220 °C as well as at heating times >24 h are probably due to the fact that at high temperatures and with an increase of time, either the reactant from which acrolein was formed has been depleted or another formation pathway becomes more dominant when more drastic conditions are applied.

To study the influence of the presence of food material in the oil during heating on the generation of acrolein, thin slices of potatoes were deep-fried for 2.5 min in each oil, preheated at 180 °C. After three, six, and nine frying cycles, aliquots of the frying oil were analyzed. As shown in Table 5, acrolein was detected in all samples and similar results were obtained for both methods. Furthermore, the data showed that frying in oils containing mainly saturated or monounsaturated fatty acids resulted in acrolein amounts of only 0.39 mg/kg (coconut oil) or 0.46 mg/kg (olive oil), respectively, after nine frying cycles (Table 5). However, the same rapeseed or linseed oil showed concentrations of 2.42 and 2.72 mg/kg, respectively, when heated without potato chips (Table 5). Nevertheless, partial inhibition of acrolein formation in the presence of chips was observed, because in control samples, which were heated under the same conditions but without any potato slices, much higher

Table 5. Amounts of Acrolein in the Six Oils after Frying of Potato Crisps

	concn[a] (mg/kg)											
	coconut oil		olive oil		rapeseed oil		safflower oil		linseed oil		hydrogenated fat	
frying cycles (2.5 min at 180 °C)	method I	method II	method I	method II	method I	method II	method I	method II	method I	method II	method I	method II
3	0.18	0.16	0.55	0.54	4.31	4.51	2.59	2.52	5.44	5.12	1.02	0.99
6	0.20	0.18	0.59	0.58	3.87	3.92	2.53	2.45	4.59	4.75	0.84	0.89
9	0.39	0.41	0.46	0.42	2.42	2.37	1.61	1.36	2.72	2.79	0.65	0.61
control sample[b]	0.90	0.93	2.41	2.35	5.03	4.99	3.93	3.51	7.72	7.52	2.94	2.84

[a] Mean values of triplicates. Standard deviations amounted to not more than 16% for different samples. [b] Oil samples, heated under similar frying conditions, but without food.

concentrations of acrolein were formed, for example, for olive oil, 2.41 mg/kg in comparison to about 0.46 mg/kg. Furthermore, with increasing frying time, the acrolein concentration of the oil gradually decreased, for example, in safflower oil, 2.59 mg/kg (three frying cycles) to 1.61 mg/kg (nine frying cycles) (Table 5). These results might be explained by the presence of air and water during the frying process. Thus, a number of degradation products can be formed by oxidative and hydrolytic reactions, which can react either with acrolein itself or with precursors favoring other reaction pathways. Nevertheless, also under real frying conditions, linseed and rapeseed oils generated the highest amounts of acrolein.

The results showed that both newly developed methods can be successfully applied to quantify the acrolein concentration in frying oils. Furthermore, the data suggest that linolenic acid may be an important precursor in acrolein formation during frying. Thus, frying oils containing this fatty acid should more carefully be recommended for frying purposes. Further studies on the formation pathway of acrolein are underway.

■ ASSOCIATED CONTENT

Supporting Information. Figure S1 (calibration curve of seven mixtures of acrolein and [$^{13}C_3$]-acrolein in a defined molar ratio using method I or II. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

Safety. Both acrolein and [$^{13}C_3$]-acrolein are hazardous and must be handled in a fume hood.

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