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Ricinoleic Acid as a Marker for Ergot Impurities in Rye and Rye Products

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Ergot alkaloid and ricinoleic acid contents of 63 ergot sclerotia samples from rye throughout Germany of the harvest years 2006−2009 were determined. Alkaloid contents were analyzed by means of high-performance liquid chromatography with fluorescence detection (HPLC-FLD) and ricinoleic acid contents by means of gas chromatography with flame ionization detection (GC-FID). Ergot alkaloid amounts ranged from 0.01 to 0.2 g/100 g of sclerotia with an average amount of 0.08 g/100 g. Ergotamine and ergocristine were identified as lead alkaloids representing 57% (w/w) of the total alkaloid content. The average ricinoleic acid amount in the ergot sclerotia was 10.3 g/100 g. Because of the low variation of ricinoleic acid content in the ergot sclerotia, a new method for the determination of ricinoleic acid in rye products as a marker for ergot contaminations was developed. This method allows the determination of ergot impurities as low as 0.01% (w/w). Furthermore, 29 rye products (flours, bread mix, bread) were investigated for their ricinoleic acid and ergot alkaloid contents.

KEYWORDS: Ergot alkaloids; ricinoleic acid; rye; ergot sclerotia; ergot impurities; alternative determination method; HPLC-FLD; GC-FID

INTRODUCTION

The term ergot describes the dark-colored sclerotia, the overwintering form of the filamentous fungus *Claviceps* spp. After infection of a host plant by the fungus, the sclerotia replace the kernels on grain ears. The most common host is rye as a cross-pollinating species (*1*, *2*), but other grains can also be infected. The sclerotia contain several toxic alkaloids, the ergot alkaloids. The most prominent in occurrence and toxicity are the lysergic acid (8-(*R*)) ergot alkaloids ergometrine, ergosine, ergotamine, ergocornine, ergocryptine, and ergocristine and their related isolysergic acid forms, the 8-(*S*)-epimers or -inine forms (**Figure 1**). Fresh ergot sclerotia contain predominantly lysergic acid alkaloids; however, isomerization takes place during storage. This isomerization also occurs in alkaline or acidic milieu or during thermal treatment and is reversible (*3*). Hence, it is very important to determine the lysergic acid form as well as the isolysergic acid form. The amount of alkaloids in the sclerotia varies considerably. An average amount of 0.2% (0.01−0.5%) (w/w) is given by Lorenz (*4*).

At present, there is no legal regulation in the European Union (EU) for the alkaloid content in food and feed but for the amount of ergot impurities in feed materials containing unground cereals (Directive 2002/32/EC) (*5*). The maximum level is set to 0.1% (w/w) ergot sclerotia present in feed material. For food a reference point of 0.05% (w/w) as maximum amount of ergot impurities in rye exists according to good agricultural practice. This physical determination is often inaccurate because the size, weight, and composition of the sclerotia vary considerably. Moreover, this method is inapplicable for processed materials. Therefore, chemical analysis methods are required as well as more data about the ergot alkaloid patterns in Europe (*6*).

As there are only few data about the actual ergot alkaloid concentrations in ergot sclerotia available, it was an objective of this study to collect some data of the alkaloid concentrations in ergot sclerotia from recent harvests. Therefore, the ergot alkaloid contents in 63 ergot samples throughout Germany, and some samples from Poland and Lithuania, of the harvest years 2006−2009 were determined.

Besides the toxic ergot alkaloids, the sclerotia consist of a high amount of fat (ca. 30% (w/w)) with ricinoleic acid ((*R*)-12-hydroxy-(*Z*)-9-octadecenoic acid) as a characteristic fatty acid. The ricinoleic acid amount in the fat is reported as approximately 30% (w/w) (*7*−*10*). Ricinoleic acid is also the main fatty acid of castor oil. However, a contamination of grain with castor beans seems to be very unlikely. Therefore, ricinoleic acid may be useful as a marker for quantifying ergot in processed food and feed materials. Accordingly, the development of a method for the determination of ricinoleic acid in rye products (flour, bread) was a second objective. Ergot alkaloid contents were analyzed according to the method developed by Müller et al. (*11*) with slight improvements. Furthermore, the ricinoleic acid and ergot alkaloid contents in rye flour and bread samples from German rye mills and from German supermarkets were compared.

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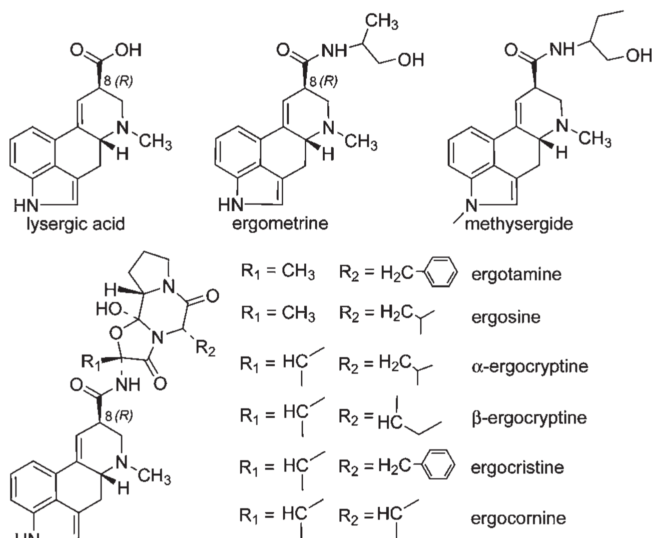

Figure 1. Chemical structures of lysergic acid ergot alkaloids and the synthetic derivative methysergide, used as internal standard.

MATERIALS AND METHODS

Chemicals and Reagents. 15-Hydroxypentadecanoic acid was purchased from Alfa Aesar (Karlsruhe, Germany). Ricinoleic acid, ergometrine maleate (= ergonovine maleate), ergotamine-D-tartrate, α-ergocryptine, and ergocornine were purchased from Sigma-Aldrich (Steinheim, Germany). Ergosine, ergosinine, ergocristine, ergocristinine, ergometrinine, ergotaminine, ergocorninine, and α-ergocryptinine were obtained from Alfarma (Černošice, Czech Republic). Methysergide maleate was from Biotrend (Wangen, Switzerland). α-Amylase was purchased as powder (35 U/mg) from Fluka (Sigma-Aldrich) and dissolved in water or as solution (Termamyl 120 KNU/G) from Novo Nordisk (Bagsvaerd, Denmark). Ammonium carbamate, *N*,*O*-bis(trimethylsilyl)acetamide, trimethylchlorosilane (TMCS), and trimethylsilylimidazole (TMSI) were from Fluka (Sigma-Aldrich, Steinheim, Germany). Sodium hydroxide pellets, hydrochloric acid, and aqueous ammonia solution (25%) were from Grüssing (Filsum, Germany). 1,1,2-Trichloro-1,2,2-trifluoroethane (TCTFE) was purchased from LGC Promochem (Wesel, Germany). Orthophosphoric acid, ethyl acetate, 1-butanol, toluol, and *tert*-butyl methyl ether (tBME) were from Roth (Karlsruhe, Germany). Methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Purified water was generated by a Milli-Q Gradient A10 system (Millipore, Schwalbach, Germany). Solid phase extraction cartridges Bond Elut AL-B and Bond Elut NH2, sorbent weight 500 mg, were purchased from Varian (Darmstadt, Germany). Syringe filters Rotilabo with PVDF membrane with 0.45 μm pore size and 15 mm diameter were purchased from Roth.

Materials. Ergot sclerotia from rye and whole rye grain samples were kindly provided by German rye mills. In most cases, ergot sclerotia were of the discharge of the Sortex (grain cleaning by color). They were analyzed in the corresponding crop year (except for the ergot sample of 2005, which was analyzed in 2007). Fresh ergot (50 g at the minimum) was ground in a water-cooled mill and sieved through a sieve with 0.5 mm mesh size. Whole rye grains were ground under liquid nitrogen and then sieved through a sieve with 0.5 mm mesh size. Rye flour samples were obtained from German rye mills and from local supermarkets. Bread mix and bread samples were from local supermarkets. All data given are based on fresh weight.

Total Fat Content in Ergot Sclerotia. To 1 g of ground ergot sclerotia (particle size < 0.5 mm) were added 9 mL of water, 5 mL of hydrochloric acid (25%), and 5 mL of toluene, and the mixture was heated at 120 °C for 2 h. The amount of fatty oil in 1 mL of toluene was determined gravimetrically after removal of the solvent (*12*).

Ricinoleic Acid in Ergot Sclerotia. To 1 g of ground ergot sclerotia (particle size < 0.5 mm) were added 1 mL of water, 2 mL of sodium hydroxide (15 mol/L), and 2 mL of internal standard solution (25 mg/mL 15-hydroxypentadecanoic acid in 1-butanol), and the mixture was heated at 95 °C for 1 h. After acidification with 2 mL of phosphoric acid and centrifugation, 100 μL of the organic phase was dried under nitrogen at 70 °C and reconstituted in 1 mL of tBME and 300 μL of TMSI. After 10 min of silylation at 70 °C, the solution was cooled to room temperature and diluted 1:10 with tBME and TCTFE for measurement with GC-FID.

Ricinoleic Acid in Rye Flour/Rye Products. To 2 g of rye flour (other rye products were ground to particle size < 0.5 mm) were added 5 mL of water and 600 μL of heat stable α-amylase, and the mixture was heated at 95 °C for 30 min for hydrolysis of the starch. Afterward, 5 mL of sodium hydroxide (15 mol/L) and 5 mL of internal standard solution (15 μg/mL 15-hydroxypentadecanoic acid in 1-butanol) were added, and the mixture was heated at 95 °C for 1 h. After acidification with 5 mL of phosphoric acid and centrifugation, 1 mL of the organic phase was dried under nitrogen and reconstituted in 200 μL of methanol (pH 6, with acetic acid) for clean up by solid phase extraction (SPE): Amino phase cartridges (Bond Elut NH_2) were flushed with 2 mL of methanol, loaded with the fatty acid extract, and then washed with 5 mL of ethyl acetate/dichloromethane (1:1, v/v). The ricinoleic acid was extracted with 5 mL of tBME/acetic acid (98:2, v/v) (*13*). This solution was dried under nitrogen at 40 °C and reconstituted in 450 μL of tBME/TCTFE and 150 μL of *N*,*O*-bis(trimethylsilyl)acetamide (BSA) with 1% TMCS, which appeared to be a better silylating reagent for the rye extracts than TMSI.

Validation Parameters of the GC-FID Method for the Determination of Ricinoleic Acid in Rye Products. Calibrant solutions of 10 different concentration levels of ricinoleic acid and 15-hydroxypentadecanoic acid in butanol were prepared. Concentration levels ranged from 0.4 to 80 μg/mL. These solutions were also used as spiking solutions for the rye flour, corresponding to 1−200 mg/kg. Standard calibration solutions were measured three times; spiked rye flour samples were extracted in repeat determination and measured three times each. The resulting peak areas were plotted against the concentrations. The resulting calibration curves were calculated by linear regression. The recovery was determined by the function of recovery from the standard calibration curve and the matrix calibration curve (*14*). The limit of detection (LOD) and the limit of quantification (LOQ) were estimated according to the calibration method of the German Standard DIN 32645 from the matrix calibration (*15*; see also ref *16*, eq 29). An α error of 0.05 and a confidence range of ±33.3% (k = 3) were applied.

GC-FID. A HP 6890 series gas chromatograph with flame ionization detector (Hewlett-Packard, Agilent, Böblingen, Germany) was used for the determination of ricinoleic acid. The chromatographic separation was performed on a 30 m × 0.25 mm i.d. fused silica, 0.1 μm Rtx-35 column (Restek, Bad Soden, Germany), using 0.6 mL/min hydrogen as carrier gas. The injector temperature was set to 280 °C, and injection volume was 1 μL with split injection (1:10 for ergot samples, 1:5 for rye samples). The column temperature was held for 2 min at 200 °C in the beginning and than raised at 10 °C/min to 240 °C for 2 min and at 60 °C/min to 300 °C for 5 min. The detector temperature was set to 300 °C. Data acquisition was performed with Chemstation software (Agilent). Identification was done via retention time and reference substances. Quantitation was done via peak area and internal standard calibration with 15-hydroxypentadecanoic acid as internal standard by the response factor.

Ergot Alkaloids in Ergot Sclerotia. One gram of ground ergot sclerotia (particle size < 0.5 mm) was extracted with 50 mL of ethyl acetate/methanol/ammonium hydroxide (25%) (75:5:7, v/v/v) by shaking for 1 h according to the method by Müller (*11*). As internal standard methysergide (**Figure 1**) (1 μg/mL) was added to the extraction solvent according to the method described in the "Schweizerisches Lebensmittelbuch" (*17*). For the measurement 100 μL was dried under nitrogen at maximum 40 °C and reconstituted in 1.4 mL of HPLC eluent.

Ergot Alkaloids in Rye Flour/Rye Products. Ten grams of rye flour (other rye products ground to particle size < 0.5 mm) were extracted with 50 mL of ethyl acetate/methanol/ammonium hydroxide (25%) (75:5:7, v/v/v) by shaking for 1 h according to the method by Müller (*11*). As internal standard methysergide (10 ng/mL) was added to the extraction solvent. The extract was cleaned up by passing an aliquot of 5 mL through an alumina oxide column (Bond Elut AL-B). For the measurement 1 mL was dried under nitrogen at maximum 40 °C and reconstituted in 0.75 mL of HPLC eluent.

HPLC-FLD. The sample extracts were reconstituted in acetonitrile/ammonium carbamate buffer (50:50, v/v) and the ergot alkaloids separated

Table 1. Values of Ricinoleic Acid and Total Alkaloid Contents by Year

	ricinoleic acid (mg/100 g)					ergot alkaloids (μg/100 g)					
	total	2005	2006	2007	2008	total	2005	2006	2007	2008	2009
n	55	1	3	37	14	63	1	3	37	15	7
mean value	10.3	11.3	8.7	10.0	11.5	75.7	38.7	44.6	68.1	121.9	35.9
SD	1.2		0.5	0.8	1.2	51.1		30.9	36.6	64.5	7.4
min	8.0		8.2	8.0	9.5	11.6		21.5	11.6	29.5	19.8
max	12.9		9.4	12.3	12.9	236.2		88.3	136.1	236.2	42.3
median	10.2		8.6	9.9	11.7	48.7		24.0	48.7	137.0	39.7
90th percentile	12.3		9.2	10.7	12.8	157.3		75.4	128.3	185.7	41.4
CV in %	11.4		5.7	8.3	10.1	67.5		69.3	53.7	52.9	20.5

on a 250 × 4.6 mm i.d., 5 μm, Omnispher C18 column (Varian, Darmstadt, Germany). The HPLC system consisted of a binary pump (Merck-Hitachi L-7100, Tokyo, Japan), an autosampler (Merck-Hitachi AS-2000A), and a fluorescence detector (Merck-Hitachi FLD F-1050). The injection volume was 10 μL. The binary mobile phase consisted of acetonitrile (mobile phase A) and ammonium carbamate buffer, 0.2 g/L (mobile phase B). The initial gradient conditions were 35% mobile phase A, which was increased at a linear rate to 60% over the next 18 min, held for 1 min, then increased further to 70% over 3 min, held for 4 min, and finally equilibrated to the starting conditions for 8 min with a total run time of 35 min. The flow rate was 1 mL/min. The ergot alkaloids were detected with a fluorescence detector at an excitation wavelength of 330 nm and an emission wavelength of 415 nm. Data acquisition was performed with Merck-Hitachi D-7000 HSM HPLC System Manager software. Identification was done via retention time and reference substances. Quantitation was done via peak area and internal standard calibration with methysergide as internal standard by response factors. α- and β-ergocryptinine were integrated as one peak and calculated as α-ergocryptinine.

Validation Parameters of the HPLC-FLD Method for Ergot Alkaloids in Rye Products. Calibrant solutions of five different concentration levels of ergometrine, ergometrinine, ergosine, ergosinine, ergotamine, ergotaminine, ergocornine, ergocorninine, ergocryptine, ergocryptinine, ergocristine, ergocristinine, and the internal standard methysergide were prepared. Concentration levels ranged from 2.5 to 50 ng/mL. Blank rye flour samples were spiked with these solutions, corresponding to 12.5−250 μg/kg. Standard calibration solutions were measured twice; spiked rye flour samples were worked up and measured once. The resulting peak areas were plotted against the concentrations. Calibration curves were calculated by linear regression. The recovery was determined by the function of recovery from the standard calibration curve and the matrix calibration curve (*14*). The blank rye flour sample was worked up twice and measured twice each. The LOD and LOQ were estimated according to the blank method of the German Standard DIN 32645 (*15*) (see eqs **1** and **2**). An α error of 0.05 and a confidence range of ±33.3% (k = 3) were applied. A detailed validation study of the method is given in the method description by Müller (*11*).

$$x_{LOD} = \frac{s_{bl}}{b} \times t_{f,\alpha} \times \sqrt{1+\frac{1}{n}} \quad (1)$$

$$x_{LOQ} = k \times \frac{s_{bl}}{b} \times t_{f,\alpha} \times \sqrt{1+\frac{1}{n}} \quad (2)$$

f = degrees of freedom ($f = n - 1$), $1/k$ = confidence range, t = critical value of the Student distribution, α = level of significance, s_{bl} = standard deviation of the blank values, and b = slope of the calibration curve's regression.

RESULTS AND DISCUSSION

Ricinoleic Acid and Alkaloid Contents in Ergot Sclerotia. The analyzed ergot sclerotia contained a total amount of fat varying from 26.3 to 44.7 g/100 g with an average amount of 34.6 g/100 g (n = 51, SD = 3.2 g/100 g, CV = 9.2%). The average ricinoleic acid content was 10.3 g/100 g with only little variation from 8.0 to 12.9 g/100 g (n = 55, SD = 1.2 g/100 g, CV = 11.4%). This gives an average amount of ricinoleic acid of 29.8 g/100 g of fat (24.0−34.8 g/100 g, n = 51, SD = 2.1 g/100 g, CV = 7.2%), which corresponds to the ricinoleic acid amounts in ergot oil previously described in the literature (*7−10*). The low amount of variation of the ricinoleic acid content in the ergot sclerotia indicates that the ricinoleic acid concentration correlates with the amount of ergot sclerotia and is therefore a good marker for ergot impurities in grain products. The mean values, standard deviation (SD), minimum and maximum values, 90th percentile and the coefficient of variation (CV) of the ricinoleic acid contents in the sclerotia differentiated by year are given in **Table 1**.

In contrast, the alkaloid contents varied considerably from 11.5 to 236.2 mg/100 g (0.01−0.24% (w/w)) with a mean value of 75.7 mg/100 g (n = 63, SD = 51.1 mg/100 g, CV = 67.5%) (**Figure 2**). These amounts of alkaloids are much lower compared to literature data, where an average amount of 0.2% (w/w) with a variation from 0.01 to 0.5% (w/w) is given (*4*). In **Table 1** the mean values, the standard deviation (SD), the minimum (min) and maximum (max) levels, the median, the 90th percentile, and the coefficients of variation (CV) of the total alkaloid contents of the harvest years 2005−2009 are compared. The sclerotia of the harvest year 2008 with a minor ergot infestation of rye (ergot impurities on rye: 2007, 0.04%; 2008, 0.02% (*18*)) had a higher amount of ergot alkaloids than the sclerotia of the harvest year 2007.

All sclerotia samples contained the ergot alkaloids ergometrine, ergosine, ergotamine, ergocornine, α-ergocryptine, and ergocristine and the related -inine forms. These are the six ergot alkaloids the EFSA (*6*) considered as the most important ones. In addition to these six ergot alkaloids, only one ergot alkaloid, β-ergocryptine, was contained in appreciable amounts. Between the alkaloid patterns no significant differences were detectable. There was a tendency that sclerotia samples from southern Germany (Bavaria, Baden-Wuerttemberg, also Hesse) had higher total alkaloid contents than samples from northern Germany (Bremen, Hamburg, Lower Saxony, Mecklenburg-Western Pomerania) and central Germany (North Rhine Westfalia, Thuringia, Saxony, Saxony-Anhalt) (**Figure 2**). However, not all samples could be allocated to specified regions as the mills usually mix grain from different regions so that a regional analysis was not possible. The amounts of the lysergic acid alkaloids were higher than the amounts of the isolysergic acid alkaloids. Ergotamine and ergocristine were the main alkaloids in all 63 analyzed ergot sclerotia samples. In a few samples ergosine could also count as a major alkaloid. The amounts of ergotamine, ergocristine, and their isomers ergotaminine and ergocristinine represent 57% of the total alkaloid content with little variation (SD = 8.9%, n = 63). The correlation of the total ergot alkaloid content and the amount of ergotamine/-inine plus ergocristine/-inine is shown in **Figure 3**. The coefficient of determination (R^2) was 0.901. The one outlier with the highest total ergot alkaloid content had an exceptionally high amount of ergosine. In the 1980s Scott and

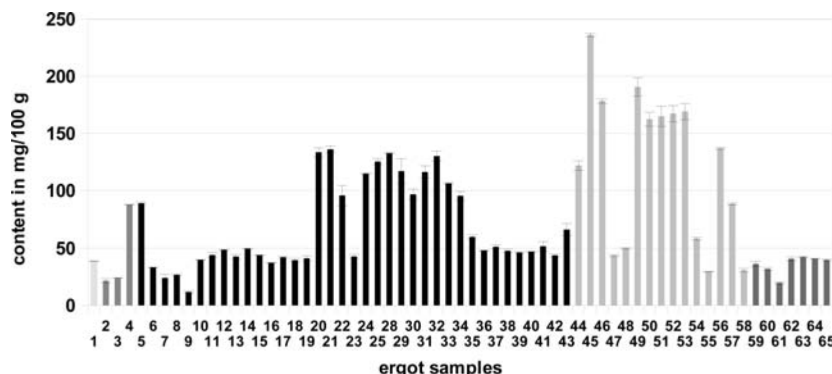

Figure 2. Total ergot alkaloid contents in ergot sclerotia samples (given as the sum of the amounts of ergometrine, ergosine, ergotamine, ergocornine, α-ergocryptine, ergocristine, and the related -inine forms, in mg/100 g): 1, 2005; 2−4, 2006; 5−43, 2007; 44−58, 2008. Sample number region: 1−2, Lower Saxony; 3, Mecklenburg-Western Pomerania; 4, mixture; 5, North Rhine-Westphalia; 6−9, Lower Saxony; 10, 14, 15, 18, 20, 23, Poland; 11, 17, 19, 21, Lithuania; 12, 13, 16, 22, Germany; 24, 25, Bavaria; 28, Hesse; 29−40, Lower Saxony; 41, Thuringia; 42−46, Bavaria; 47, Germany; 48, Saxony; 49−53, 56, Hesse; 54, 57, North Rhine-Westphalia; 55, Mecklenburg-Western Pomerania; 58, Saxony-Anhalt.

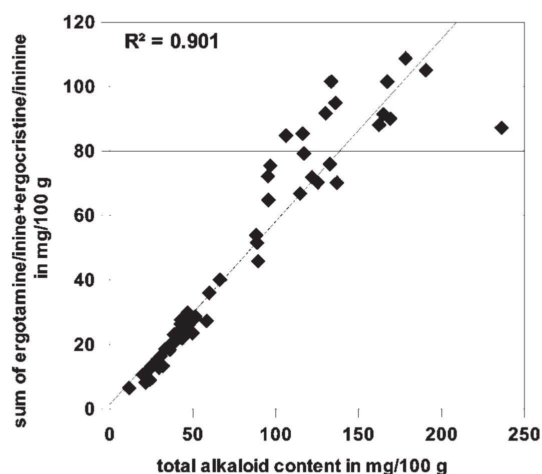

Figure 3. Correlation between the amounts of ergotamine/-inine and ergocristine/-inine and the total alkaloid content.

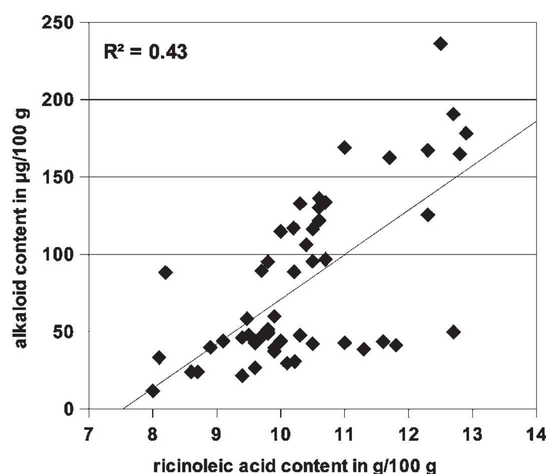

Figure 4. Total ergot alkaloid content in correlation to the ricinoleic acid content in ergot sclerotia.

Lawrence (*19*) and Young (*20*) also reported ergotamine and ergocristine as major alkaloids in Canadian ergot, Baumann et al. (*21*) in Swiss ergot, and Porter et al. (*22*) in American ergot; Klug (*23*) mentioned ergotamine as a typical alkaloid for central European ergot. Appelt and Ellner (*24*) analyzed 19 ergot samples from rye of the harvest years 2007 (n = 13) and 2008 (n = 6). They observed likewise ergotamine and ergocristine as main alkaloids in rye ergot and higher total alkaloid contents in samples from southern Germany.

Summing up, it is not possible to establish a correlation between the ricinoleic acid content and the total ergot alkaloid content. This relationship is shown in **Figure 4**. The bad coefficient of determination (R^2) of 0.43 reflects the diversity of the ricinoleic acid and the ergot alkaloid concentrations.

Ricinoleic Acid and Ergot Alkaloid Contents in Rye Samples. *Ricinoleic Acid in Rye Samples.* The fatty acid spectrum of ergot oil differs from that of rye by the absence of linolenic acid and the presence of ricinoleic acid. With this assumption, a method for the determination of ricinoleic acid in rye samples up to an amount of ergot impurities of 0.01% (w/w) was developed. Cleanup of the fatty acid extract was done by SPE using aminopropyl columns as it is well-known that these columns are suitable for the separation of lipid classes (*13*, *25*−*27*). The LOD was 1.93 mg/kg for ricinoleic acid and 2.13 mg/kg for the internal standard 15-hydroxypentadecanoic acid, and the LOQ was 7.00 mg/kg for ricinoleic acid and 7.68 mg/kg for 15-hydroxypentadecanoic acid. The CV of the procedure (V_{x0}) was 3.21% for ricinoleic acid, and the recovery was 90% with a coefficient of determination (R^2) for the regression of the recovery function of 0.9995. The response factor for ricinoleic acid relating to the internal standard 15-hydroxypentadecanoic acid was 1.04 at a concentration level of 20 μg/mL (50 mg/kg). **Figure 5** shows a typical GC-FID chromatogram of a rye flour sample with a ricinoleic acid content of 49 mg/kg.

Additionally, rye flour samples were spiked with ergot powder with known ricinoleic acid content in concentrations of 0.01 and 0.05% (w/w), so that the expected ricinoleic acid contents in the mixtures could be calculated. The ricinoleic acid contents were determined in repeat determination. The recovery for the flour with 0.01% ergot was 97% (SD = 1.5%) and for the flour with 0.05% ergot, 92% (SD = 0.1%).

With the described method ergot impurities can be determined reliably by using simple standard procedures within short chromatographic run times. Just two common standard substances are required, which are quite stable during sample preparation and storage. Storage experiments over 3 months under different conditions and bread-baking experiments showed no degradation of the ricinoleic acid (data not shown). The good correlation between the ricinoleic acid and ergot contents allows ergot impurities as low as 0.01% (w/w) to be determined.

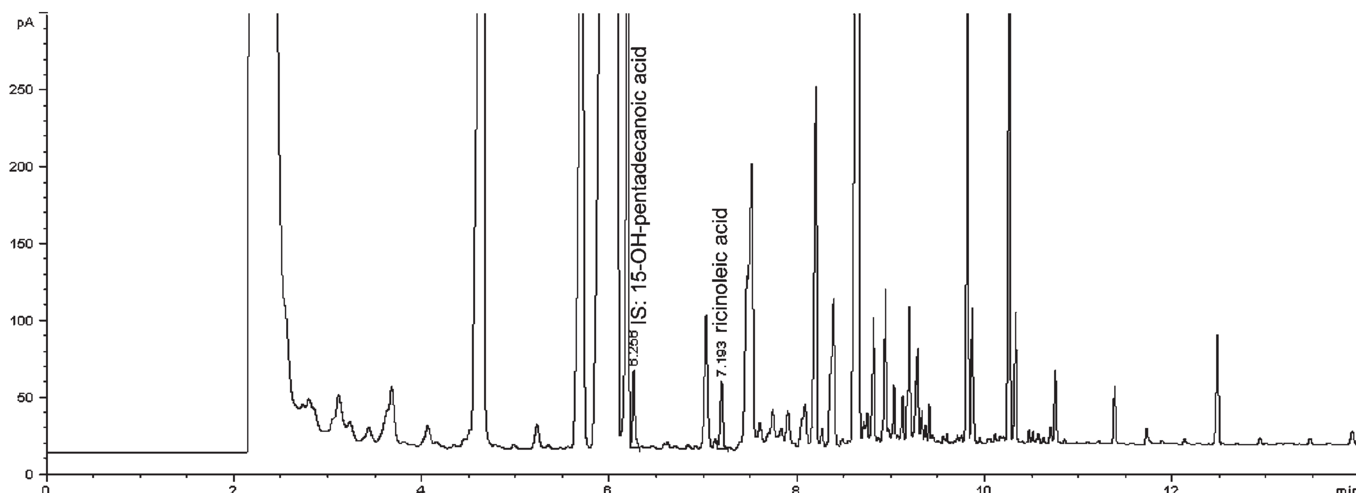

Figure 5. GC-FID chromatogram of a rye flour sample with a ricinoleic acid amount of 49 mg/kg.

Table 2. Response Factors for the Ergot Alkaloids Relating to the Internal Standard Methysergide, in Order of Elution

ergot alkaloid	response factor	ergot alkaloid	response factor
ergometrine	1.03	ergocristine	2.88
ergometrinine	1.36	ergosinine	1.72
ergosine	2.82	ergotaminine	1.97
ergotaminine	3.06	ergocorninine	2.04
ergocornine	2.51	α-ergocryptinine	1.74
α-ergocryptine	2.65	ergocristinine	2.19

Table 3. Limits of Detection (LOD) and Quantification (LOQ), Calculated by Blank Method (DIN 32645), for the Ergot Alkaloids, in Order of Elution

ergot alkaloid	LOD (μg/kg)	LOQ (μg/kg)
ergometrine	0.65	2.60
ergometrinine	0.70	2.85
ergosine	1.15	4.55
ergotamine	0.35	1.40
ergocornine	1.65	6.65
α-ergocryptine	0.85	3.45
ergocristine	1.00	4.10
ergosinine	0.25	1.10
ergotaminine	1.65	6.65
ergocorninine	0.45	1.85
α-ergocryptinine	0.85	3.55
ergocristinine	0.75	3.10
methysergide (IS)	0.55	2.17

Ergot Alkaloids in Rye Samples. Rye samples were analyzed according to the method developed by Müller et al. (*11*) with slight modifications such as halved sample weight and volume of extraction solvent and the use of the internal standard methysergide for quantification. The response factors for each quantified ergot alkaloid are shown in **Table 2**.

The LOD and LOQ were estimated according to the blank method of the German Standard DIN 32645 and are listed in **Table 3**. An α error of 0.05 and a confidence range of ±33.3% (k = 3) were applied. Recoveries were between 95 and 100% for each single alkaloid and 120% for the internal standard methysergide.

Figure 6 shows a HPLC-FLD chromatogram of a contaminated rye flour sample in which all ergot alkaloids were detectable. The total ergot alkaloid content of this sample was 400 μg/kg. In **Figure 7** the ricinoleic acid and total ergot alkaloid contents of 29 analyzed rye samples are shown. Total ergot alkaloid contents were summed from the amounts of the individual ergot alkaloid contents (the concentrations of each alkaloid are given in the Supporting Information). Most of the samples contained low concentrations of ergot alkaloids and ricinoleic acid. Except for two samples, the ergot contamination was <0.05% (w/w) in all rye products, whereby an amount of 0.05% ergot impurities in grain corresponds to 50 mg/kg ricinoleic acid and 1 mg/kg total ergot alkaloids (based on an average alkaloid content of 0.2%), respectively.

In the rye flour samples ergotamine and ergocristine were the major ergot alkaloids as well. However, the sum of these two ergot alkaloids and their related -inine forms was not 57% of the total alkaloid content as it was in the ergot sclerotia samples. Instead, the sum of ergotamine, ergocristine, ergotaminine, and ergocristinine varied from 29 to 78% of the total alkaloid content, depending on the degree of contamination and the sample type. In rye flour samples where all ergot alkaloids were detectable the amount of the sum of ergotamine, ergocristine, ergotaminine, and ergocristinine of 50% (variation from 41 to 57%) fit best to the amount in the ergot sclerotia. One reason for the discrepancy between the amount in the sclerotia and the flour samples might be that ergot alkaloids accumulate variably in the different milling fractions, which affects different flour types. Wolff et al. (*28*) observed the distribution of ergometrine in different milling fractions. They recognized the highest ergometrine amount in the semolina flour. This implies that it would be hardly possible to choose lead alkaloids for the quantification of the total alkaloid content in rye samples.

Comparison of the ricinoleic acid contents and the alkaloid contents in the rye samples of the German market indicates that there is no direct coherence. The presence of ricinoleic acid is an indicator for ergot impurities and correlates with the amount of ergot sclerotia, but it does not allow exact conclusions about the alkaloid content. However, based on the 90th percentile of the alkaloid content of sclerotia (0.16 g/100 g), it would be possible to set maximum levels for ergot, which could easily be controlled by determining the ricinoleic acid content.

With the described method for the determination of ricinoleic acid in rye samples, a chemical alternative analysis method for the determination of ergot impurities was developed. Due to the well-corresponding ricinoleic acid amount with ergot impurities, it is a reliable method for processed food and feed materials. The method requires less chromatographic effort, short run times, and no difficulties with the standard substances as they are common fatty acids. Concerning the ergot alkaloids, the data

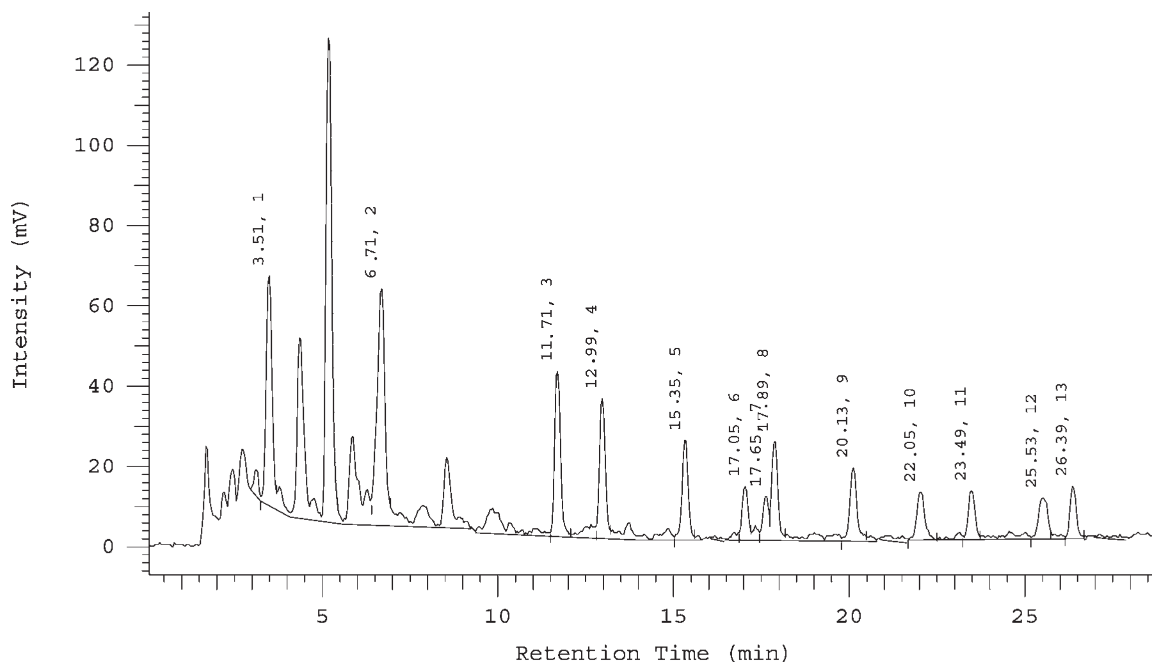

Figure 6. HPLC-FLD chromatogram of a rye flour sample with a total alkaloid content of 400 μg/kg: 1, ergometrine; 2, methysergide (IS); 3, ergosine; 4, ergotamine; 5, ergocornine; 6, α-ergocryptine; 7, β-ergocryptine; 8, ergocristine; 9, ergosinine; 10, ergotaminine; 11, ergocorninine; 12, α- + β-ergocryptinine; 13, ergocristinine.

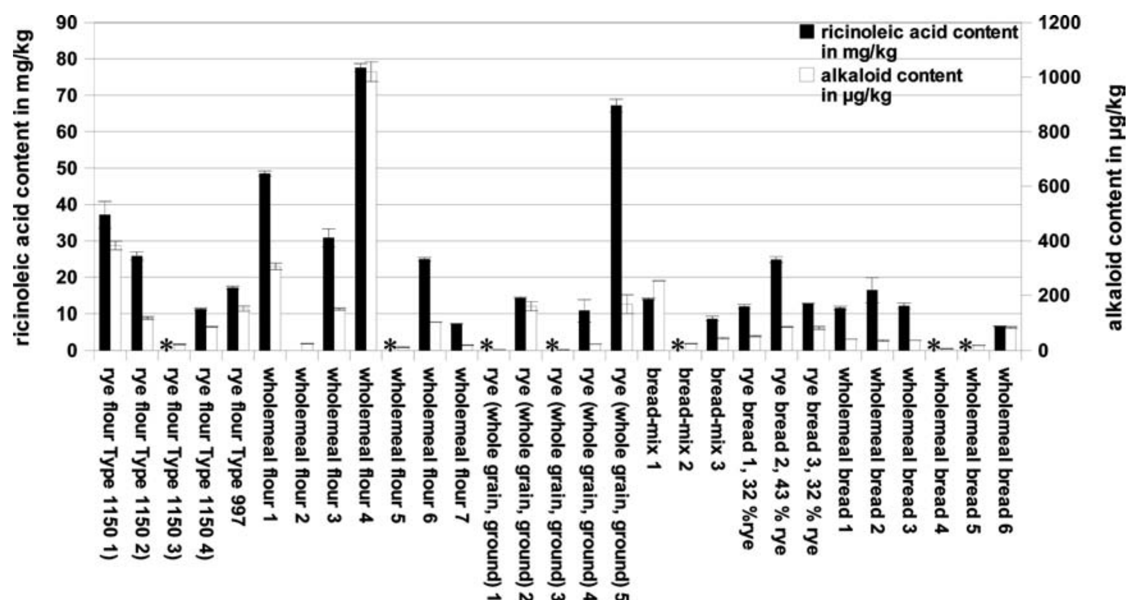

Figure 7. Ergot alkaloid and ricinoleic acid contents in rye samples. $*$, <LOQ.

of ergot sclerotia samples from 2006 to 2009 show that the average ergot alkaloid content of 78.7 mg/100 g (0.08% (w/w)) is much lower than the published average ergot alkaloid content of 200 mg/100 g (0.2% (w/w)) (*4*). Nevertheless, for risk calculations an ergot alkaloid content of 200 mg/100 g would be a good basis as the highest determined amount was 236.2 mg/100 g and the 90th percentile was 157.3 mg/100 g. Ergotamine and ergocristine were the main alkaloids. However, the constant values of ergometrine and ergocristine in the ergot sclerotia are not exactly transferable to rye samples. Therefore, they could not be used as lead alkaloids for rye products. The ricinoleic acid content is a good marker for ergot impurities and correlates with the amount of ergot sclerotia in rye; however, it does not allow a direct conclusion about the toxicity as the ergot alkaloid content varies considerably.

ABBREVIATIONS USED

BSA, *N*,*O*-bis(trimethylsilyl)acetamide; FID, flame ionization detector/detection; FLD, fluorescence detector/detection; IS, internal standard; LOD, limit of detection; LOQ, limit of quantification; R^2, coefficient of determination (Bravais and Pearson); SPE, solid phase extraction; tBME, *tert*-butyl methyl ether; TCTFE, 1,1,2-trichloro-1,2,2-trifluoroethane; TMCS, trimethylchlorosilane; TMSI, trimethylsilylimidazole.

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Supporting Information Available: Figure 8 and Tables 4 and 5, the total ergot alkaloid contents are calculated from the single

ergot alkaloid amounts; α- and β-ergocryptinine were integrated as one peak and calculated as α-ergocryptinine. This material is available free of charge via the Internet at http://pubs.acs.org.

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