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Development of Stable Isotope Dilution Assays for the Simultaneous Quantitation of Biogenic Amines and Polyamines in Foods by LC-MS/MS

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Supporting Information

ABSTRACT: Microbial amino acid metabolism may lead to substantial amounts of biogenic amines in either spontaneously fermented or spoiled foods. For products manufactured with starter cultures, it has been suggested that certain strains may produce higher amounts of such amines than others; however, to support efforts of food manufacturers in mitigating amine formation, reliable methods for amine quantitation are needed. Using 10 isotopically labeled biogenic amines as the internal standards, stable isotope dilution assays were developed for the quantitation of 12 biogenic amines and of the 2 polyamines, spermine and spermidine, in one LC-MS/MS run. Application of the method to several foods revealed high concentrations of, for example, tyramine and putrescine in salami and fermented cabbage, whereas histamine was highest in Parmesan cheese and fermented cabbage. On the other hand, ethanolamine was highest in red wine and Parmesan cheese. The results suggest that different amino acid decarboxylases are active in the respective foods depending on the microorganisms present. The polyamine spermine was highest in salami and tuna.

KEYWORDS: *biogenic amines, [$^2H_2$]-tyramine, [$^2H_2$]-spermine, [$^2H_4$]-spermidine, benzoyl derivatives*

■ INTRODUCTION

Biogenic amines are known to occur in many foods,[1−5] and the highest concentrations are reported especially in products that undergo a fermentation process, such as cocoa and chocolate,[1] cheeses,[6−9] dry sausages,[10,11] beer,[12] or wine.[13−18] The amines are generated by certain microbial strains by an enzymatic decarboxylation of free amino acids, and selected amines are contrasted to their parent amino acids in Table 1. In addition,

Table 1. Examples of 12 Parent Amino Acids and Their Corresponding Biogenic Amines

amino acid	biogenic amine
tyrosine	tyramine
histidine	histamine
2-phenylalanine	2-phenylethylamine
tryptophan	tryptamine
lysine	cadaverine
ornithine	putrescine
valine	methylpropylamine
leucine	3-methylbutylamine
isoleucine	2-methylbutylamine
methionine	3-(methylthio)propylamine
serine	ethanolamine
aspartic acid	*β*-alanine

also the thermal degradation of amino acids in a Strecker-type reaction has recently been shown as an additional pathway in their formation.[18]

Although the toxicological relevance of biogenic amines is lower as compared to other food-related toxicants, such as mycotoxins or nitrosamines, their presence in foods still constitutes a public health concern due to well-known physiological effects.[19−21] However, although for most foods only recommendations for upper concentration limits do exist, currently commercial starter cultures are evaluated for their ability to form biogenic amines and, thus, fast and reliable methods to monitor their content in foods fermented with commercial starter cultures, such as dry sausages (salami) or dairy products, are needed.

Numerous methods for the quantitation of biogenic amines have already been published.[4] Because amines are very polar, commonly a derivatization of the amino group is performed to reduce their polarity and, also, to provide a chromophore for UV or fluorescence detection. The most common derivatization agents for LC analysis are benzoyl chloride[5,22−24] and dansyl chloride,[7,18,25,26] and the derivatives are usually monitored by either fluorescence or diode array detection.[4] However, in more recent studies liquid chromatography−mass spectrometry (LC-MS) was applied,[6,15,27] in particular, to increase the reliability of amine identification.

In most cases either only one internal standard of different chemical structure or an external calibration was used in the methods reported. However, the extraction of such polar compounds and the uncertain degree of derivatization may cause erroneous results. The use of isotopically labeled internal standards is known as the most reliable method in the quantitation of trace compounds in complex matrices.[28] To our knowledge, only in one recent study[29] have stable isotope dilution assays been developed for the quantitation of biogenic

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amines in seafood and salami samples, but using only three isotopically labeled internal standards.

The European Food Safety Association (EFSA) has previously proposed the concept of "Qualified Presumption of Safety" and has requested reliable data on the potential of microbial starter cultures used in food manufacturing to generate undesirable components.[30] Because strains of, for sample, staphylococci and lactobacilli are used in the production of fermented sausages and other foods, the study was aimed at developing stable isotope dilution assays for the quantitation of 12 biogenic amines and 2 polyamines and to apply the method to several food samples.

MATERIALS AND METHODS

Chemicals. [$^2H_4$]-2-Phenylethylamine and [$^2H_9$]-*n*-butylamine were from Dr. Ehrenstorfer (Augsburg, Germany). [$^2H_4$]-Tryptamine, [$^2H_4$]-histamine, and [$^2H_4$]-putrescine were from CDN Isotopes (Quebec, Canada). [$^{13}C_2$]-Ethanolamine and [$^{13}C_3$,^{15}N]-β-alanine were from Sigma-Aldrich (St. Louis, MO, USA).

2-Phenylethylamine, tryptamine, histamine, putrescine, ethanolamine, β-alanine, spermidine, spermine, cadaverine, 2-methylbutylamine, 3-methylbutylamine, 2-methylpropylamine, 3-(methylthio)-propylamine, benzyl chloroformate, benzoyl chloride, 4-aminobutanoic acid, and (*E*)-2-*N*,*N'*-bis(3-aminopropyl)-2-butene-1,4-diamine were from Sigma-Aldrich (Steinheim, Germany). 3-Aminopropionitrile was from Alpha Aesar (Karlsruhe, Germany). The purity of the amines was checked by NMR and by LC-MS after benzoylation (see below).

Syntheses. *[$^2H_2$]-Spermine.* The target compound was synthesized by deuteration of the respective unsaturated butane-1,4-diamine derivative (Figure 1). (*E*)-2-*N*,*N'*-Bis(3-aminopropyl)-2-butene-1,4-diamine (10 mg, 0.050 mmol) was dissolved in [$^2H_4$]-methanol (10 mL) and deuterated with deuterium gas in an autoclave (2 bar) in the presence of 10% palladium on charcoal (10 mg) for 1 h. The filtered solution was evaporated to dryness, and the residue was taken up in water (2 mL) and acidified with 40 μL of H_3PO_4 for solid phase extraction on a Strata X-C column (Phenomenex, Aschaffenburg, Germany). After conditioning and equilibration, the sample was loaded, and impurities were eluted with 0.1% H_3PO_4 (2 mL) followed by removal of the neutral and acidic components with methanol. [$^2H_2$]-Spermine was finally isolated by elution with 5% NH_4OH in methanol (2 mL).

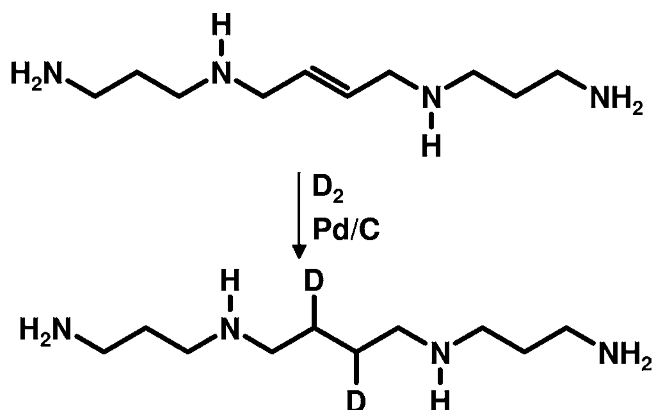

Figure 1. Synthetic route used in the preparation of [$^2H_2$]-spermine.

[$^2H_4$]-Spermidine. The target compound was synthesized in a four-step procedure (Figure 2) as modified from a published synthesis of the unlabeled compound.[31] 4-Aminobutanoic acid (1.3 g, 10 mmol) was dissolved in a mixture of water (4 mL), acetone (4 mL), and 10% NaOH (6 mL) and cooled to 0 °C. Then, a solution of benzyl chloroformate (2.60 g, 15.2 mmol) dissolved in acetone (4 mL) was added dropwise to the stirred mixture during 1 h. The pH was maintained at ~10 using aqueous sodium hydroxide (10%), and the mixture was stirred at room temperature for another 16 h. Then, water (30 mL) was added and the acetone was evaporated. The reaction mixture was acidified with HCl, and 3-aminopropionitrile (1.2 mg, 14 mmol) and *N*-[2-(dimethylamino)ethyl]-*N'*-ethylcarbodiimide (2.0 g, 12.8 mmol) were added to the suspension. After 20 h at room temperature, the target compound, benzyl {4-[(2-cyanoethyl)amino]-butanoyl}carbamate, was generated. To remove the carbobenzoxy group, the intermediate was hydrogenated in an autoclave (2 bar) for 1 h at room temperature, the filtered solution was evaporated, and 4-[(2-cyanoethyl) amino]butanoylamide was obtained as a colorless oil. The material was dissolved in diethyl ether (10 mL), then slowly added to a suspension of $LiAlD_4$ (50 mg, 1.2 mmol) in diethyl ether (10 mL), and finally stirred for 18 h at room temperature. The target compound was isolated by solid phase extraction using a Strata XC column (Phenomenex) as detailed above for spermine.

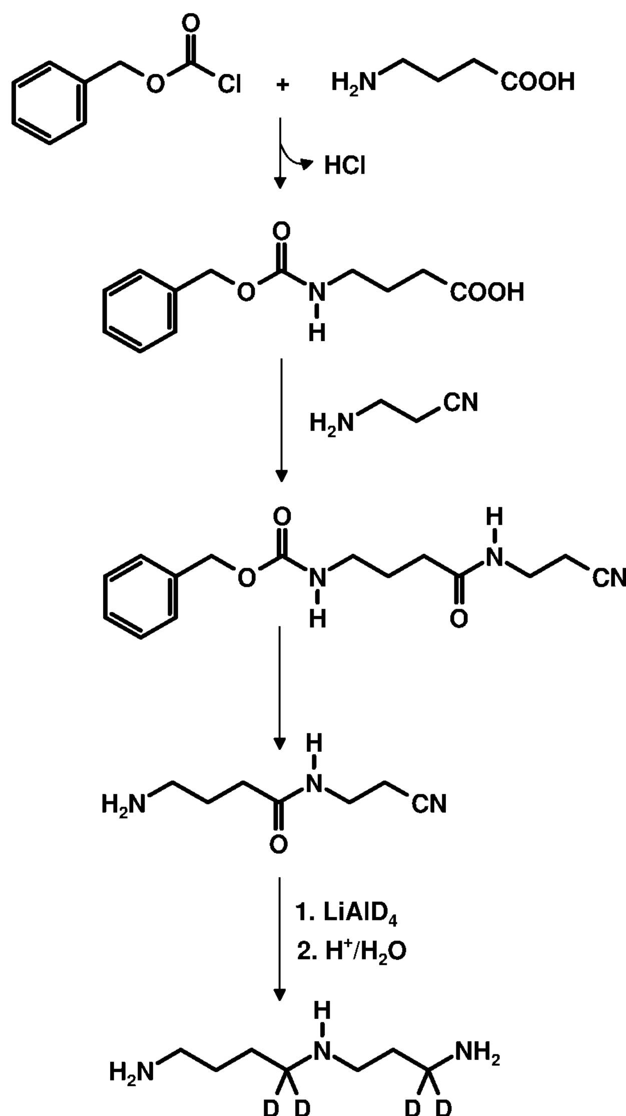

Figure 2. Synthetic route used in the preparation of [$^2H_4$]-spermidine.

[$^2H_2$]-Tyramine. The target compound was synthesized by reducing 4-hydroxybenzyl cyanide with $LiAlD_4$ (Figure 3). To a solution of 4-hydroxybenzylcyanide (1.0 g, 7.5 mmol), dissolved in tetrahydrofuran, was added a suspension of $LiAlD_4$ (0.21 g, 5 mmol) in tetrahydrofuran, and the mixture was stirred for 18 h. After the addition of ethyl acetate, followed by water, the aqueous phase was extracted with diethyl ether, and the combined organic phases were evaporated to dryness. The residue was dissolved in acetonitrile, and

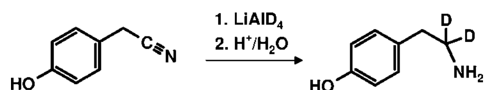

Figure 3. Synthetic route used in the preparation [$^2H_2$]-tyramine.

the target compound was isolated by preparative HPLC on a Phe-Hex stationary phase (25 cm × 0.8 cm).

Food Samples. Italian salami, Dutch Leerdammer cheese, Italian Merlot red wine, canned tuna, canned fermented cabbage (sauerkraut), chocolate (75% cocoa), yogurt (plain, 3.5% fat), and Parmesan cheese were purchased at local stores in Munich, Germany.

Method Development. 2-Phenylethylamine, tryptamine, histamine, putrescine, ethanolamine, β-alanine, spermidine, spermine, cadaverine, and the labeled standards [$^2H_4$]-2-phenylethylamine, [$^2H_9$]-*n*-butylamine, [$^2H_4$]-tryptamine, [$^2H_4$]-histamine, [$^2H_4$]-putrescine, [$^{13}C_2$]-ethanolamine, [$^{13}C_3$,$^{15}N_1$]-β-alanine, [$^2H_2$]-spermine, [$^2H_2$]-spermidine, and [$^2H_2$]-tyramine (100 nmol each) were each dissolved in aqueous sodium bicarbonate (30 mL), and the solution was adjusted to pH 10 by adding sodium hydroxide (2 mol/L). Solutions were stored at 4 °C for a maximum of 2 weeks.

Preparation of Benzamide Derivatives. The benzamidines were synthesized by reacting each amine singly with benzoyl chloride to obtain the derivatives as exemplified for 2-phenylethylamine and

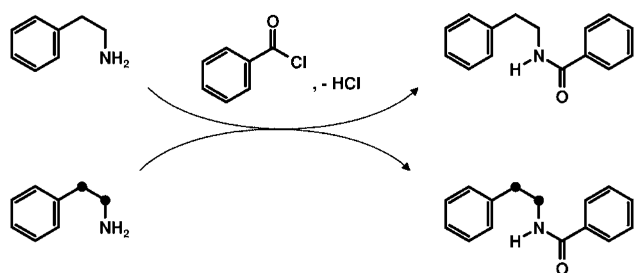

Figure 4. Derivatization of 2-phenylethylamine and [$^2H_4$]-2-phenylethylamine by benzoylchloride, forming the respective benzamides.

[$^2H_4$]-2-phenylethylamine in Figure 4. Each amine (~100 μg) was reacted with benzoyl chloride (20 mg) dissolved in acetonitrile (20 mL) for 2 h at room temperature with stirring. The benzamidines formed were extracted with dichloromethane and, after concentration, the solutions were subjected to LC-MS/MS analysis.

Liquid Chromatography–Tandem Mass Spectrometry. Mass spectra were recorded using the 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 autosampler and a 5 μm Aqua C18 125 Å HPLC column (1.5 cm × 0.2 cm; Phenomenex). The column was kept at 30 °C and connected to a 4 × 2 mm polar RP precolumn (Phenomenex). The sample (10 μL) was separated at a flow rate of 0.2 mL/min. The solvent system was composed of water (A) and acetonitrile (with 0.1% formic acid, v/v) (B). A linear gradient was applied by increasing the concentration of B from 0 to 50% within 35 min and then increased to 100% within 13 min. The mass spectrometer was operated in the positive electrospray ionization mode (ESI$^+$) with a spray needle voltage of 3.5 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 (both nitrogen) was adjusted to 40 and 10 arbitrary units, respectively. The collision cell was operated at a collision gas (argon) pressure of 0.13 Pa.

Calibration Curves. Ten mixtures containing all 14 analytes and the 10 internal standards in different concentrations of each pair varying in ratios between 50:1 (100 μg labeled vs 2 μg unlabeled) and 1:50 (2 μg unlabeled vs 100 μg labeled) were benzoylated as described above and were then analyzed by LC-MS/MS. Calibration curves were constructed as exemplified for 2-phenylethylamine in the Supporting Information. The curves showed a very good linearity over the entire concentration range and allowed the calculation of MS response factors (Table 2). Methylpropylamine, 2- and 3-methylbutylamine, and 3-(methylthio)propylamine were quantitated using [$^2H_9$]-*n*-butylamine as the internal standard.[18] For the quantitation of cadaverine, the labeled putrescine was used.

Analysis of Food Samples. Foods were frozen in liquid nitrogen and then ground in a laboratory mill type A10 (Jahnke & Kunkel, IKA-Labortechnik, Staufen, Germany). Defined amounts of the 10 labeled internal standards (Figure 5), dissolved in aqueous trichloroacetic acid (10%), were added to the powder (2–10 g), which was suspended in 20 mL of aqueous trichloroacetic acid (10%). For equilibration, the sample was stirred for 30 min, then homogenized for 3 min using an Ultraturrax (Jahnke & Kunkel, IKA-Labortechnik), and ultrasonified for another 10 min. The suspension obtained was centrifuged (18000 rpm) for 20 min at 4 °C and, finally, filtered. The pH of the filtrate was adjusted to 10 with aqueous sodium hydroxide (2 mol/L) and, after the addition of benzoyl chloride (20 mg), dissolved in acetonitrile (20 mL); this mixture was finally stirred for 2 h at room temperature. The benzamides were then extracted twice with dichloromethane (total volume = 20 mL), and the organic phases were combined, dried over Na_2SO_4, and evaporated to dryness at 30 °C. The residue was dissolved in a mixture of acetonitrile and 0.1% aqueous formic acid (20:80, v/v) (2 mL), and after filtration over a syringe filter (0.45 μm; Spartan 13/0.45 RC; Schleicher & Schuell, Dassel, Germany), the solution was diluted with water and analyzed by LC-MS/MS as described above.

RESULTS AND DISCUSSION

Because the labeled internal standards for the quantitation of tyramine, spermine, and spermidine were commercially not available, these were synthesized as shown in Figures 1–3; details of the synthetic procedure can be found under Materials and Methods. The seven further internal standards (Figure 5) were commercially available.

Method Development. Each of the 14 amines and the 10 labeled internal standards was derivatized with benzoyl chloride as exemplified for 2-phenylethylamine in Figure 4, and their mass spectra were recorded in the full scan mode. For all benzoylated amines, the $[M + H]^+$ peak was obtained as base peak (Table 2). The respective parent ion was then subjected to MS/MS. The most intense fragments of the parent ions (collision energy = 35 V) were investigated and, by performing a series of runs with different collision energies and flow rates of the sheath and auxiliary gas, the yields were optimized (Table 2).

Application of stable isotope dilution assays requires, in particular, data on the influence of the equilibration time after standard addition and also on the time span used for derivatization. For this purpose, the same amounts of internal standards were administered to three different samples of a model salami assigned as A–C (Supporting Information). Sample A was equilibrated for 30 min, and time for derivatization was 2 h; sample B was equilibrated for 60 min, and derivatization time was 2 h; sample C was equilibrated for 30 min, and derivatization time was 10 min.

To check the reproducibility of the method, three different samples of the same batch underwent the workup procedure for each of the three different experiments A–C. To determine the precision of the method, three replicates of each experiment were analyzed (Supporting Information).

The standard deviation (RSD; %) for the seven amines selected was calculated between 1.0 (2-phenylethylamine) and 5.1 (cadaverine) over a broad range of concentration (sample B; Supporting Information). The results also confirmed that an

Table 2. Mass Spectrometric Parameters Used in the Stable Isotope Dilution Assays

amine and labeled isotopologue	collision energy (V)	first transition m/z (parent ion; $[M^\circ + H]^+$) to m/z (product ion)	collision energy (V)	second transition m/z (parent ion; $[M^\circ + H]^+$) to m/z (product ion)	response factor
$[^{13}C_3,^{15}N]$-*β*-alanine	14	198–136	18	198–105	
β-alanine	14	194–134	18	194–105	0.82
$[^{13}C_2]$-ethanolamine	18	168–105	37	168–77	
ethanolamine	18	166–105	37	166–77	1.00
$[^2H_4]$-tryptamine	18	269–148	26	269–105	
tryptamine	18	265–144	26	265–105	0.95
$[^2H_4]$-histamine	18	220–95	26	220–105	
histamine	18	216–95	26	216–105	0.88
$[^2H_4]$-phenylethylamine	16	230–105	34	230–109	
phenylethylamine	20	226–105	38	226–103	0.88
$[^2H_2]$-tyramine	16	244–105	21	244–123	
tyramine	16	242–105	21	242–121	0.80
$[^2H_4]$-putrescine	16	301–180	28	301–105	
putrescine	16	297–176	28	297–105	0.85
cadaverine[a]	16	311–190	32	311–105	0.91
$[^2H_9]$-*n*-butylamine	18	187–123	19	187–105	
methylpropylamine[b]	18	178–122	19	178–122	0.77
2-methylbutylamine[b]	18	192–122	19	192–105	0.83
3-methylbutylamine[b]	18	192–122	19	192–105	0.82
3-(methylthio)propylamine[b]	10	210–162	19	210–105	0.67
$[^2H_2]$-spermine	28	621–499	40	621–162	
spermine	28	619–497	40	619–162	0.81
$[^2H_4]$-spermidine	20	462–340	32	462–162	
spermidine	20	458–336	32	458–162	0.69

[a]Cadaverine was quantified using $[^2H_4]$-putrescine as the internal standard. [b]Methylpropylamine, 2- and 3-methylbutylamine, and 3-(methylthio)propylamine were quantitated using $[^2H_9]$-*n*-butylamine as the internal standard.[18]

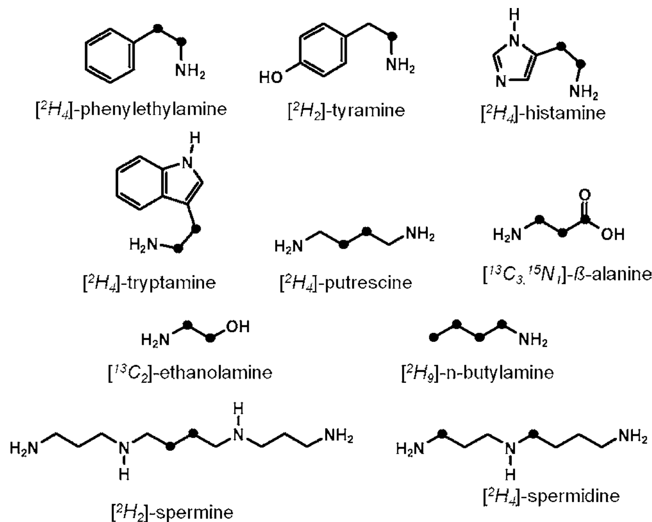

Figure 5. Structures of the isotopically labeled standards used in the stable isotope dilution assays.

equilibration time of 30 min (sample B) was sufficient, because for all amines tested the results were in very good agreement with data obtained for sample A, which was equilibrated for 60 min. Furthermore, the data indicated that with equilibration times of 30 and 10 min for derivatization (sample C), the same precise results were obtained as compared to a derivatization of time 2 h (sample B).

To verify the precision by another approach, control samples were spiked with tyramine, putrescine, and phenylethylamine in three different concentrations. The mean recoveries of the three amines were 98.2, 103.7, and 96.7%, respectively (data not shown).

To ensure that the accuracy of the analysis was maintained, a control sample was spiked with tyramine, histamine, and 2-phenylethylamine and was analyzed with each set of foods.

The limit of quantitation was calculated on the basis of the correlation between intensity of the respective ions and the background noise. For this purpose an amine-free matrix was simulated, containing 35% sunflower oil in phosphate buffer (0.026 mol/L KH_2PO_4, 0.041 mol/L Na_2HPO_4). Mixtures of all analytes and internal standards in various concentrations were added and monitored by LC-MS/MS after isolation as described above for food analysis. The limit of quantitation (LoQ) for all amines was determined to be 0.05 μg/kg.

Analysis of Food Samples. In a first experiment, the 10 internal standards were added to a salami sample (Figure 6). A typical UV chromatogram and the results of monitoring of selected masses by LC-MS are shown exemplified for tyramine and histamine as well as for the two labeled internal standards. The data showed that MS/MS detection enabled unambiguous peak identification and quantitation. Tyramine, histamine, and the labeled standards could unequivocally be determined by MS/MS, whereas in the LC-UV chromatogram, for example, tyramine was not detectable at all. The mass range was almost free of background interferences, emphasizing the selectivity of the LC-MS/MS in comparison to an LC-UV method.

Then, the method was applied to salami, chocolate, fermented cabbage, and red wine (Table 3). In salami, the highest concentrations among the 14 amines analyzed were found for tyramine followed by putrescine, spermine, ethanolamine, and *β*-alanine. In the canned fermented cabbage, also high concentrations of putrescine, tyramine, histamine, and *β*-

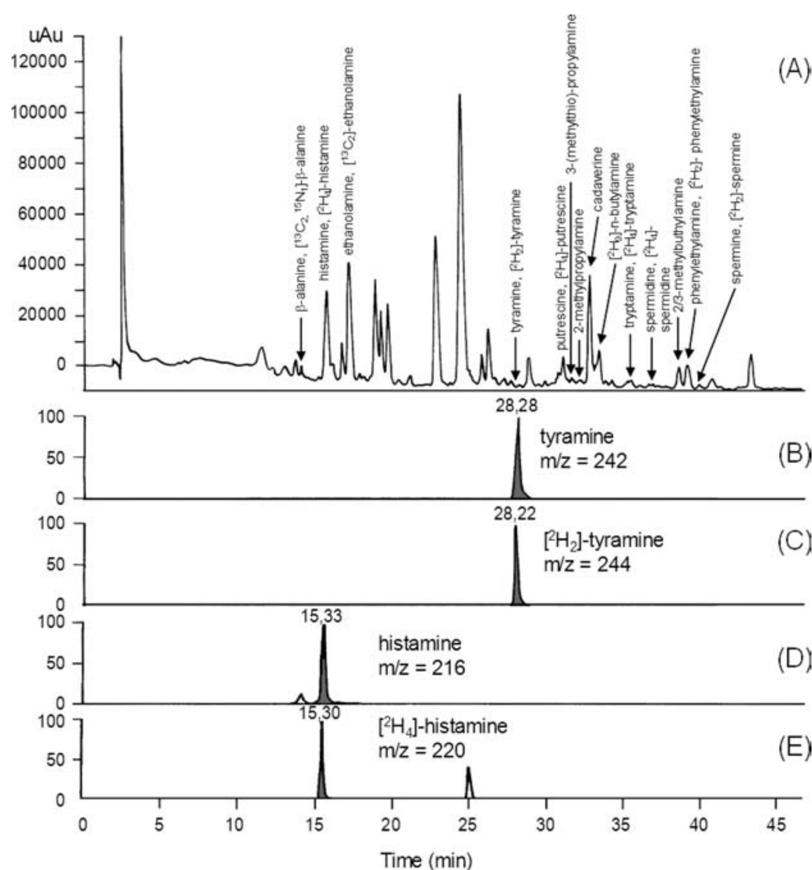

Figure 6. (A) HPLC-UV chromatogram of a derivatized extract from salami in comparison to single mass traces of analytes and the corresponding internal standards (B−E).

Table 3. Concentrations (Milligrams per Kilogram)[a] and Relative Standard Deviations (in Parentheses) for 12 Biogenic Amines and 2 Polyamines in Salami, Fermented Cabbage, Chocolate, and Red Wine

	salami	fermented cabbage	chocolate	red wine
tyramine	77.14 (1.8)	60.66 (2.3)	3.11 (3.0)	1.93 (2.9)
tryptamine	1.63 (2.8)	0.18 (2.9)	1.43 (2.7)	0.03 (1.6)
histamine	8.54 (1.3)	37.01 (1.9)	0.26 (0.6)	3.67 (1.7)
2-phenylethylamine	3.2 (1.6)	0.73 (1.0)	2.67 (2.1)	0.61 (2.0)
cadaverine	6.54 (0.6)	21.5 (4.5)	0.75 (4.9)	0.4 (1.5)
putrescine	61.57 (2.7)	108.9 (2.9)	0.8 (2.8)	8.5 (0.6)
ethanolamine	13.4 (0.2)	13.01 (0.4)	7.22 (2.6)	19.8 (0.7)
β-alanine	11.43 (2.9)	22.68 (2.6)	0.33 (2.6)	0.03 (1.6)
methylpropylamine	0.39 (1.9)	0.05 (2.5)	0.84 (3.7)	0.02 (1.9)
3-methylbutylamine	0.02 (2.8)	0.02 (1.1)	2.9 (0.2)	0.42 (0.7)
2-methylbutylamine	0.01 (1.9)	0.07 (1.9)	0.81 (2.6)	0.01 (2.5)
3-(methylthio)propylamine	0.07 (2.1)	0.23 (2.2)	nd[b]	nd
spermine	14.09 (1.7)	1.2 (0.9)	1.95 (2.6)	0.07 (2.6)
spermidine	3.11 (3.0)	10.98 (2.7)	7.40 (3.4)	2.08 (3.6)

[a]Mean values of triplicates. [b]nd, not determined.

alanine were monitored. Histamine and cadaverine were measured in substantially higher amounts than in the salami. Tyramine is known to cause the so-called "cheese" reaction, a hypertensive crisis induced when monoamine oxidase inhibitor drugs are medicated.[32]

In the literature, tyramine concentrations of 10−1500 mg/kg have been reported in fermented sausages.[11,32] Thus, the value in the sausage analyzed is at the lower end of the reported range. However, the extremely differing literature results indicate the strong influence of the starter cultures and processing conditions on the formation of biogenic amines.

In chocolate, spermidine, ethanolamine, tyramine, and 2-phenylethylamine were the most abundant biogenic amines (Table 3). However, although cocoa, the main ingredient of chocolate, undergoes a quite long spontaneous fermentation, the concentrations of tyramine and of most of the other biogenic amines were lower as compared to, for example, salami or the fermented cabbage, respectively. In fermented, roasted cocoa beans, a concentration of ∼10 mg of 2-phenylethylamine

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Table 4. Concentrations (Milligrams per Kilogram)[a] and Relative Standard Deviations (in Parentheses) of 12 Biogenic Amines and 2 Polyamines in 2 Cheeses, a Yogurt, and Tuna

	Leerdamer	Parmesan	yogurt	tuna
tyramine	nd[b]	3.75 (2.2)	nd	0.06 (2.5)
tryptamine	0.04 (2.4)	0.07 (2.5)	nd	0.02 (1.4)
histamine	0.02 (0.5)	40.64 (2.6)	nd	0.33 (1.8)
2-phenylethylamine	0.005 (1.7)	0.2 (1.8)	0.001 (2.7)	0.03 (1.2)
cadaverine	0.01 (3.1)	1.98 (1.2)	0.38 (3.3)	0.07 (1.1)
putrescine	0.07 (2.8)	0.83 (1.1)	0.03 (2.8)	0.35 (2.1)
ethanolamine	5.67 (2.2)	19.4 (0.7)	4.86 (1.3)	6.54 (1.7)
β-alanine	nd	nd	nd	0.13 (0.2)
2-methylpropylamine	0.67 (1.7)	0.11 (2.6)	0.07 (1.8)	0.14 (2.5)
3-methylbutylamine	0.01 (2.8)	0.15 (1.8)	0.003 (1.7)	0.05 (1.3)
2-methylbutylamine	nd	0.01 (2.2)	0.004 (1.6)	>0.01
3-(methylthio)propylamine	nd	nd	0.0004 (2.8)	>0.01
spermine	0.65 (2.8)	0.82 (3.4)	0.17 (1.3)	11.25 (1.8)
spermidine	0.95 (3.1)	0.83 (0.4)	0.47 (1.9)	7.6 (2.6)

[a]Mean values of triplicates. [b]nd, not determined.

was previously reported.[18] Because the chocolate analyzed in this study contained 75% cocoa, the concentration of the amine is in the expected order of magnitude. A series of older studies[1] reports on a correlation between the consumption of chocolate or cheese, respectively, and the occurrence of migraine, and 2-phenylethylamine is believed to be a main source causing the headache. However, if this were true, also salami containing the amine in the same order of magnitude should be a cause of migraine attacks.

As found for chocolate, ethanolamine, the degradation product of the amino acid serine (Table 1), was the most abundant biogenic amine in red wine followed by putrescine, histamine, and tyramine. However, the overall concentrations of most of the other amines in red wine were much lower as compared to, for example, the fermented cabbage. The prevalence of ethanolamine in different wines was also recently reported, and our data are very close to the concentrations reported in the literature.[16]

Next, the 14 biogenic amines were quantitated in two cheeses and a yoghurt sample. The Dutch Leerdamer cheese contained very low concentrations of all amines analyzed, except ethanolamine. On the other hand, in Parmesan cheese, in particular histamine, followed by ethanolamine and tyramine, was the most abundant amine (Table 4). The histamine concentrations were much higher than, for example, in salami (Table 3). Finally, in the yogurt sample, only ethanolamine was present in the milligrams per kilogram range.

For histamine, the European Union has recommended upper limits of 100 mg/kg in raw fish. The focus of legislation is on fish because it contains relatively high concentrations of free histidine, which can be converted into histamine during storage by associated microorganisms. However, in a sample of canned tuna only very low amounts of histamine were measured (Table 4), whereas spermine, spermidine, and ethanolamine were the most abundant biogenic amines in the fish sample. These results were in quite good agreement with Oguri et al.,[33] who also found only 1.3 mg/kg in tuna.

The results showed that it is possible to quantitate 14 of the most important biogenic amines in foods in one run. It is, however, interesting to note that in foods, certain amino acids, such as leucine, isoleucine, valine, and methionine, are obviously not converted into their biogenic amines, whereas in particular tyrosine, histamine, ornithine, serine, and aspartic acid seem to be better substrates for the microbial metabolism. To clarify the influence of selected starter cultures and processing parameters on the formation of biogenic amines, further studies are underway.

ASSOCIATED CONTENT

Supporting Information

Figure S1 and Table S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) Askar, A.; Treptow, H. *Biogenic Amines in Foods* (in German); Eugen Ulmer: Stuttgart, Germany, 1986.

(2) Silla Santos, M. H. Biogenic amines: their importance in foods. *Int. J. Food Microbiol.* **1996**, *29*, 213−231.

(3) Lange, J.; Thomas, K.; Wittmann, C. Comparison of a capillary electrophoresis method with high-performance liquid chromatography for the determination of biogenic amines in various food samples. *J. Chromatogr., B* **2002**, *779*, 229−239.

(4) Oenal, A. A review: Current analytical methods for the determination of biogenic amines in foods. *Food Chem.* **2007**, *103*, 1475−1486.

(5) Karovicova, J.; Kohajdova, Z. Biogenic amines in food. *Chem. Papers* **2003**, *59*, 70−79.

(6) Calbiani, F.; Careri, M.; Elviri, L.; Mangia, A.; Pistarà, L.; Zagnoni, I. Rapid assay for analyzing biogenic amines in cheese: matrix solid-phase dispersion followed by liquid chromatography-electrospray-tandem mass spectrometry. *J. Agric. Food Chem.* **2005**, *53*, 3779−3783.

(7) Innocente, N.; Biasutti, M.; Padovese, M.; Moret, S. Determination of biogenic amines in cheese using HPLC technique and direct derivatization of acid extract. *Food Chem.* **2007**, *101*, 1285−1289.

(8) Gianotti, V.; Chiuminatto, U; Mazzucco, E.; Gosetti, F.; Bottaro, M.; Frascarolo, P.; Gennaro, M. C. A new hydrophilic interaction liquid chromatography tandem mass spectrometry method for the simultaneous determination of seven biogenic amines in cheese. *J. Chromatogr., A* **2008**, *1185*, 296−300.

(9) Mayer, H. K.; Fiechter, G.; Fischer, E. A new ultra-pressure liquid chromatography method for the determination of biogenic amines in cheese. *J. Chromatogr., A* **2010**, *1217*, 3251−3257.
(10) Vanderekerckhove, P. Amines in dry fermented sausages. *J. Food Sci.* **1977**, *42*, 283−285.
(11) Latorre-Moratalla, M. L.; Veciana-Nogués, T.; Bover-Cid, S.; Garriga, M.; Aymerich, T.; Zanardi, E.; Ianieri, A.; Fraquenza, M. J.; Patarata, L.; Drosinos, E. H.; Lauková, A.; Talon, R.; Vidal-Carou, M. C. Biogenic amines in traditional fermented sausages produced in selected European countries. *Food Chem.* **2008**, *107*, 912−921.
(12) Fernandes, J. O.; Judas, I. C.; Oliveira, M. B.; Ferreira, I. M. P. L. V. O.; Ferreira, M. A. A GC-MS method for quantitation of histamine and other biogenic amines in beer. *Chromatogr. Suppl.* **2001**, *53*, 327−331.
(13) Romero, R.; Gázquez, D.; Gabur, M. G.; Sánchez-Viñas, M. Optimization of chromatographic parameters for the determination of biogenic amines in wines by reversed-phase high-performance liquid chromatography. *J. Chromatogr., A* **2000**, *87*, 75−83.
(14) Landete, J. M.; Ferrer, S.; Polo, L.; Pardo, J. Biogenic amines in wine from three Spanish regions. *J. Agric. Food Chem.* **2005**, *53*, 1119−1124.
(15) Millan, S.; Sampedro, M. C.; Unceta, N.; Goicolea, M. A.; Barrio, R. J. Simple and rapid determination of biogenic amines in wine by liquid chromatography-electrospray ionization ion trap mass spectrometry. *Anal. Chim. Acta* **2007**, *584*, 145−152.
(16) Del Prete, V.; Constantini, A.; Cecchini, F.; Morassut, M.; Garcia-Moruno, E. Occurrence of biogenic amines in wine: the role of grapes. *Food Chem.* **2009**, *112*, 474−481.
(17) Kelly, M. T.; Blaise, A.; Larroque, M. Rapid automated high performance liquid chromatography method for simultaneous determination of amino acids and biogenic amines in wine, fruit, honey. *J. Chromatogr., A* **2010**, *1217*, 7385−7392.
(18) Granvogl, M.; Bugan, S.; Schieberle, P. Formation of amines and aldehydes from parent amino acids during thermal processing of cocoa and model systems: new insights into pathways of the Strecker reaction. *J. Agric. Food Chem.* **2006**, *54*, 1730−1739.
(19) Steve, L.; Jayne, E.; Nordlee, A. Histamine poisoning (scrombroid fish poisoning): an allergy-like intoxication. *Clin. Toxicol.* **1989**, *27*, 225−240.
(20) Nout, M. J .R. Fermented foods and food safety. *Food Res. Int.* **1994**, *27*, 291−298.
(21) Shalaby, A. Significance of biogenic amines to food safety and human health. *Food Res. Int.* **1996**, *29*, 675−690.
(22) Hwang, D. F.; Chang, S. H.; Shiua, C. Y.; Chai, T. High performance liquid chromatographic determination of biogenic amines in fish implicated in food poisoning. *J. Chromatogr., B* **1997**, *693*, 23−30.
(23) Oedestan, O.; Ueren, A. A method for benzoyl chloride derivatization of biogenic amines for high performance liquid chromatography. *Talanta* **2009**, *78*, 1321−1326.
(24) Redmond, J. W.; Tseng, A. High pressure liquid chromatographic determination of putrescine, cadaverine, spermidine and spermine. *J. Chromatogr.* **1979**, *170*, 479−481.
(25) Dadáková, E.; Křížek, M.; Pelikánová, T. Determination of biogenic amines in food using ultra-performance liquid chromatography (UPLC). *Food Chem.* **2009**, *116*, 365−370.
(26) Mazzucco, E.; Gosetti, F.; Bobba, M.; Marengo, E.; Robotti, E.; Gennaro, M. C. High-performance liquid chromatography-ultraviolet detection method for the simultaneous determination of typical biogenic amines and precursor amino acids. *J. Agric. Food Chem.* **2010**, *58*, 127−134.
(27) Loukou, Z. M.; Zotou, A. Determination of biogenic amines as dansyl derivates in alcoholic beverages by high-performance liquid chromatography with flourimetric detection and characterization of the dansylated amijnes by liquid chromatography-atmospheric pressure chemical ionization. *J. Chromatogr., A* **2003**, *993*, 103−113.
(28) Rychlik, M.; Asam, S. Stable isotope dilution assays in mycotoxin analysis. *Anal. Biochem. Chem.* **2008**, *390*, 617−628.
(29) Bomke, S.; Seiwert, B.; Dudek, L.; Effkemann, S.; Karst, U. Determination of biogenic amines in food samples using derivatization followed by liquid chromatography/mass spectrometry. *Anal. Bioanal. Chem.* **2009**, *393*, 247−256.
(30) Scientific opinion on risk based control of biogenic amine formation in fermented foods. *EFSA J.* **2011**, *9*, 1−92.
(31) Leete, E. Spermidine: an indirect precursor of the pyrrolidine rings of nicotine and nornicotine in *Nicotiana glutinosa*. *Phytochemistry* **1986**, *24*, 957−960.
(32) McCabe-Sellers, B. J.; Staggs, C. G.; Bogle, M. L. Tyramine in foods and monoamine oxidase inhibitor drugs. *J. Food Compos. Anal.* **2006**, *19*, 58−65.
(33) Oguri, S.; Okuya, Y.; Yanase, Y.; Suzuki, S. Post-column derivatization capillary electrochromatography for detection of biogenic amines in tuna-meat. *J. Chromatogr., A* **2008**, *1202*, 96−101.