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Quantitation of *S*-Methylmethionine in Raw Vegetables and Green Malt by a Stable Isotope Dilution Assay Using LC-MS/MS: Comparison with Dimethyl Sulfide Formation after Heat Treatment

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The potent odorant dimethyl sulfide (**1**), showing a low odor threshold of 0.12 μg/L in water, is known to contribute to the aromas of various foods. Its cabbage-like odor plays an important role, particularly, in cooked vegetables, such as cabbage, celery, or asparagus. On the other hand, in fruit juices or beer, **1** may generate off-flavors. *S*-Methylmethionine (**2**) has previously been characterized as precursor of **1** during thermal processing, and several methods for its quantitation have been proposed. Using deuterium-labeled **2** as the internal standard, a stable isotope dilution assay (SIDA) using LC-MS/MS was developed for the fast quantitation of **2** in vegetables and malt. Application of the method to different foods revealed amounts between 2.8 mg (fresh tomatoes) and 176 mg (celery) of **2** per kilogram. To correlate the amount of **1** formed upon processing with the amounts of **2** present in the raw material, **1** was quantified before and after a thermal treatment of the same raw materials by a SIDA. Concentrations between 1.1 mg/kg (fresh tomatoes) and 26 mg/kg (celery) were determined in the processed samples. The quantitation of **2** during steeping, germination, and malting of barley, and a correlation of the data with the amounts of **1** formed after thermal treatment of the malt, resulted in yields between 24 and 27 mol % calculated on the basis of the amounts of **2**. The results suggested that the extent of the formation of **1** can be predicted, for example, in plant materials, from the amount of **2** present in the raw foods.

KEYWORDS: *S*-Methylmethionine; dimethyl sulfide; stable isotope dilution assay; tomato; asparagus; celery

INTRODUCTION

Dimethyl sulfide **1** (**Figure 1**), eliciting a cabbage-like odor at the low odor threshold of 0.12 μg/kg in water, has been suggested for decades to play an important role in the aroma of many cooked vegetables, such as tomato, cabbage, or asparagus (*1*−*3*). On the other hand, in fruit juice or beer, **1** may be the cause of an off-flavor (*4*−*7*). Challenger and Hayward (*7*) were the first to identify the amino acid *S*-methylmethionine **2** (**Figure 1**) in asparagus, and several studies have shown that **2** is easily degraded into **1** during food storage and/or a thermal treatment (*2*, *3*, *8*−*11*).

In many food manufacturing processes, heating is necessary to generate the typical aroma attributes of a product (e.g., asparagus), to prevent bacterial spoilage, or to concentrate liquids, for example, in orange or apple juice processing. Thus, it is of great interest to predict the potential of the raw material in the formation of **1** on the basis of the quantitation of **2** in the unprocessed raw materials.

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First estimations of **2** in foods were based on gravimetric approaches (*11*). Later, Kovatscheva and Popova (*3*) tried to photometrically determine the amino acid after separation by thin-layer chromatography. Further attempts to develop a direct assay for the quantitation of **2** by HPLC−fluorescence detection (*12*) as well as by NIR spectroscopy (*13*) resulted in unsatisfying data. Thus, in particular in the brewing industry, the quantitation of **2** is still performed by indirect methods, for example, by quantifying **1** formed upon heating of the raw material under alkaline conditions (*6*, *14*−*18*). However, following such procedures the very volatile **1** is often quantified by headspace gas chromatography without the use of an internal standard, which might lead to erroneous results.

Recently, Loscos et al. (*19*) used a stable isotope dilution analysis (SIDA) for the quantitation of dimethyl sulfide in grape must. To identify its precursor, **2**, in the unprocessed must, they used MALDI-TOF-MS, which was suggested as an appropriate tool to quantify **2** by means of deuterium-labeled L-methylmethionine. However, in this study, the labeled standard was not added to the grape before the isolation of the amino acid fraction, and, also, no validation of the method was performed.

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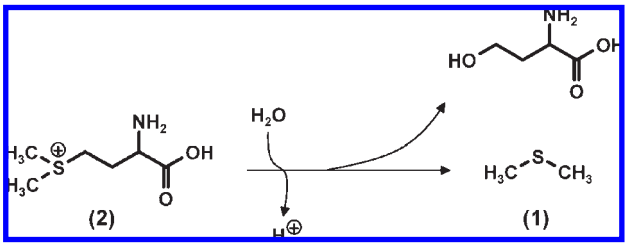

Figure 1. Reaction scheme indicating the formation of dimethyl sulfide (**1**) from a thermal degradation of *S*-methylmethionine (**2**).

Thus, the purpose of the present study was, first, to develop a SIDA in combination with LC-MS/MS to quantify **2** in food samples and, second, to correlate the results with the amounts of dimethyl sulfide **1** formed upon thermal treatment of the same samples. Besides the determination of the aroma contribution of **1** to, for example, vegetables and juices, such data might also be useful to predict the amounts of **1** that might potentially be formed during storage or thermal processing of foods on the basis of the concentrations of **2** present in the raw materials.

MATERIALS AND METHODS

Chemicals. [$^2H_6$]-Dimethyl sulfide ([$^2H_6$]-**1**), dimethyl sulfide, and [$^2H_3$]-methyl iodide were from Aldrich (Steinheim, Germany). *S*-Methylmethionine was obtained from Fluka (Steinheim, Germany). Methanol, ethyl acetate, and acetic acid were from VWR International (Darmstadt, Germany).

[$^2H_3$]-*S*-Methylmethionine ([$^2H_3$]-**2**) was synthesized by modifying a recently published procedure (*20*).

Food Samples. Leek, cabbage, turnip cabbage, beetroot, celery, asparagus, tomatoes, orange juice, and strawberry juice were purchased from local supermarkets. Hand-squeezed orange juice was produced in the laboratory.

Malting Procedure. Barley var. Barke (crop 2004, unstained, Herzogenaurach, Germany) was malted in a laboratory scale as follows: For the first steeping, barley kernels (150 g) were soaked in demineralized water (450 mL) and stored for 5 h at 18 °C at 70% relative humidity. After removal of the water, the material was stored for another 19 h at 13 °C at 100% relative humidity. For the second steeping, the procedure was applied again.

For germination, the kernels were stored for 4 days at 15 °C and 100% relative humidity. Malting was then performed as a three-step procedure, treating the germinated kernels first for 14 h at 50 °C, then for 2 h at 65 °C, and, finally, for 9 h at 85 °C.

Model Studies on the Formation of Dimethyl Sulfide (1) from *S*-Methylmethionine (2). To elucidate the influence of the pH of the foods under investigation on the conversion of **2** into **1** during a thermal treatment, aqueous solutions of **2** (10 μg in 20 mL of buffer) were heated in closed glass vessels at 100 °C for 10, 20, or 30 min, respectively. Either a sodium acetate buffer (5 mmol/L) at pH 4.4, similar to the pH of tomatoes, or a citric acid buffer (5 mmol/L) at pH 6.8, similar to the pH of asparagus and other vegetables, was used. To check the influence of the matrix, in a second series of experiments, **2** (40 μg/20 g of sample), dissolved in tap water (200 μL), was added to either orange or tomato juice, respectively. Both juices were adjusted to pH 4.4 and heated at 100 °C for 30 min. The amount of **1** was determined before and after the thermal treatment of the samples as described below.

Quantitation of *S*-Methylmethionine (2) by a SIDA. *Sample Preparation*. Vegetables (10−40 g) were homogenized in water (≈10 mL), and the pH was adjusted to 4.4 using acetic acid (2 mol/L). Malt (5−40 g) was finely ground, suspended in water, and further analyzed without adjustment of the pH. The internal standard [$^2H_3$]-*S*-methylmethionine ([$^2H_3$]-**2**), dissolved in water (2 mL containing 100−1000 μg, depending on the amounts of **2** determined in preliminary experiments) was added, and after the addition of methanol (90 mL), the sample was stirred for 30 min. After centrifugation (4500 rpm, 30 min, 4 °C) (Multifuge $3_{L\text{-}R}$, Heraeus, Germany), the clear extract was collected, and the residue was extracted again with methanol/water (70:30, v/v; 40 mL). Finally, the solvent was evaporated from the combined extracts at 40 °C by means of a rotary evaporator. The aqueous solution obtained was washed with ethyl acetate (50 mL), and, finally, filtered through an Amicon-Diaflo-YM-1-membrane (Millipore, Schwalbach, Germany) (cutoff M_r > 1000). The solution obtained was diluted 1:500 with acetonitrile/water (50:50, v/v) for LC-MS/MS measurements.

The samples obtained from the model studies described above were prepared using the same procedure.

Liquid Chromatography−Tandem Mass Spectrometry (LC-MS/MS). LC-MS/MS was performed by means of a Finnigan Surveyor Plus HPLC-System (Thermo Electron Corp., Waltham, MA) equipped with an autosampler. Chromatography was carried out on a Discovery HS F5 column (15 cm × 2.1 mm, 3 μm) (Supelco, Bellefonte, PA) using a flow rate of 0.2 mL/min. For separation, the mobile phase (50% water and 50% acetonitrile containing 0.1% formic acid; v/v), was linearly increased to 100% acetonitrile within 15 min. After injection of an aliquot of the samples (10 μL), the effluent was monitored using a triple-quadrupole mass spectrometer TSQ Quantum Discovery (Thermo Electron Corp.) running in the ESI^+ mode. Spray voltage was set to 4000 V, sheath gas pressure was 30 arb ("arbitrary unit" according to equipment producer), auxiliary gas pressure was 10 arb, and capillary temperature was 280 °C.

The limit of detection (LoD) was determined by analyzing food samples and by correlation of the signal intensity and background noise on the basis of a factor of 3:1.

Validation of the LC-MS/MS Method for *S*-Methylmethionine (2). The precision of the LC-MS/MS method was determined by six times injecting an extract from asparagus stacks. The relative standard deviation (RSD) was found to be 5.5%. The recovery was validated by spiking tomatoes with known concentrations of **2**. The results showed that 102.5% of the theoretical amounts were recovered. Finally, a calibration curve was monitored by injecting standard solutions containing **2** and [$^2H_3$]-**2** in different ratios (from 1:10 to 10:1) covering concentrations of **2** between 0.1 and 1.9 μg/mL.

Quantitation of Dimethyl Sulfide (1) by a SIDA in Combination with SPME. *Sample Preparation*. Vegetables (2−10 g) were homogenized in water (5 mL) and adjusted to pH 4.4 using acetic acid (2 mol/L). Fruit juices (10 g) and malt (1−4 g) were suspended in water (5 mL) and analyzed without adjustment of the pH. The samples were heated at 100 °C for 30 min in septum-sealed glass vessels and then cooled by placing the vessels in an ice bath. After addition of the internal standard [$^2H_6$]-dimethyl sulfide ([$^2H_6$]-**1**) through the septum (0.3−30 μg dissolved in 1 mL of water), depending on the amounts of **1** determined in preliminary experiments, the samples were stirred for 30 min for equilibration, and after dilution with water by 1:100, an aliquot (0.5 mL) was transferred into a vial filled with aqueous saturated sodium chloride solution (9.5 mL). The vial was immediately sealed with a septum and a steel lid for SPME measurements (Grace Alltech, Deerfield, IL) and was maintained at 22 °C for 30 min. The raw samples were treated the same way.

Two-Dimensional Gas Chromatography−Mass Spectrometry (GC/GC-MS). The samples were placed at 20 °C into the tray of a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland) equipped with an 85 μm SPME fiber (CAR/PDMS, Supelco, Sigma-Aldrich Chemie). The volatiles were thermally desorbed onto a GC column DB-FFAP (fused silica, 30 m × 0.32 mm i.d., 0.25 μm film thickness) (J&W Scientific, Waldbronn, Germany) by placing the fiber in the hot injector (250 °C) (PPKD injector, Thermo Finnigan, Egelsbach, Germany) of a gas chromatograph (Trace GC 2000 series, Thermo Finnigan). The temperature of the first oven was set to 37 °C and held for 4 min, and then the temperature was raised to 230 °C at 40 °C/min. The analyte and the internal standard were transferred into a cold trap (−80 °C using a moving column stream switching system (*21*). Then, cooling was turned off and the trapped material was transferred onto a DB-1701 capillary column (fused silica, 30 m × 0.32 mm i.d., 0.25 μm film thickness) (J&W Scientific) placed in a second GC (CP 3800, Varian, Darmstadt, Germany). The temperature of the second oven was set at 37 °C and held for 5 min, and then the temperature was raised to 200 °C at 40 °C/min. The effluent was recorded by means of an ion trap mass spectrometer (Saturn 2000, Varian) running in the chemical ionization mode (MS-CI) with

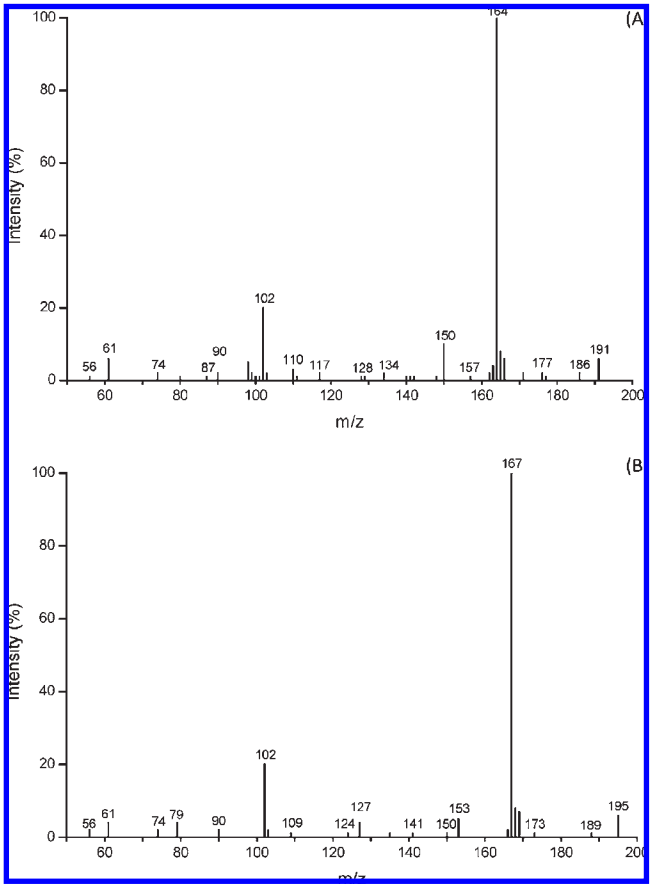

Figure 2. Mass spectra of *S*-methylmethionine (**A**) and the labeled internal standard [$^2H_3$]-*S*-methylmethionine (**B**).

Table 1. Influence of the pH and Heating Time on the Degradation of *S*-Methylmethionine (**2**) in Model Systems

			% degradation after[a]			
expt	model system	pH	0 min	10 min	30 min	60 min
1	citric acid buffer[b]	6.8	0	9	20	33
2	sodium acetate buffer[b]	4.4	0	4	8	19
3	tomato juice[c]	4.4	na[d]	na	18	na
4	orange juice[c]	4.4	na	na	28	na

[a] Data are mean values of triplicates. The results differed by not more than ±2%. [b] *S*-Methylmethionine (10 μg) dissolved in 20 mL of buffer was heated at 100 °C in a closed vessel. [c] The food sample (20 g) was spiked with **2** (40 μg) and heated in a closed vessel. [d] Not analyzed.

methanol as the reactant gas. Concentrations were calculated from the area counts of **1** and [$^2H_6$]-**1** obtained from the mass chromatograms as recently reported for linalool (*21*).

The LoD was estimated by correlating the signal intensity and the background noise on the basis of a factor of 3:1.

Validation of the SPME-GC-MS Method for Dimethyl Sulfide (1). The precision of the GC-MS method was determined by injecting six times an extract from asparagus stacks. The RSD was found to be 2.72%. The recovery was validated by spiking the tomato juice with known concentrations of **1**. Results showed that 95.2% of the theoretical amounts of **1** was recovered. The calibration curve was monitored by injecting the standard solutions containing **1** and [$^2H_6$]-**1** in different ratios covering concentrations between 0.03 and 0.15 μg/mL.

RESULTS AND DISCUSSION

Method Development for the Quantitation of *S*-Methylmethionine by a SIDA. First, deuterium-labeled *S*-methylmethionine ([$^2H_3$]-**2**), which was commercially not available, was synthesized from L-methionine and [$^2H_3$]-methyl iodide by modifying a recently published procedure (*20*). The mass spectrum obtained is contrasted to the spectrum of unlabeled **2** in **Figure 2**. The incorporation of the labeling was confirmed by the base peak at *m*/*z* 167 for [$^2H_3$]-**2** (**Figure 2B**) in comparison to the base peak of unlabeled **2** at *m*/*z* 164 (**Figure 2A**).

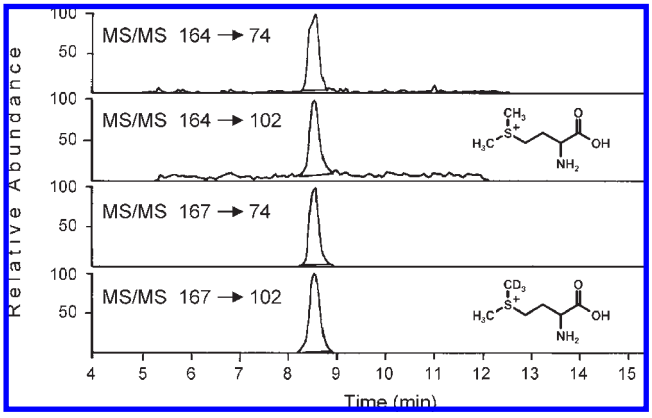

Figure 3. LC-MS/MS chromatogram of an orange juice sample containing ≈1.2 mg of *S*-methylmethionine (**2**)/kg.

Table 2. Concentrations of *S*-Methylmethionine (**2**) and Dimethyl Sulfide (**1**) in Raw Vegetables: Comparison with the Amounts of **1** Formed after Cooking[a]

		concn of **1**[b] (mg/kg)	
sample	concn of **2**[b] (mg/kg)	before	after cooking
tomato	2.8	<0.002	1.1 (105)[c]
cabbage	81.0	<0.002	10.0 (33)[c]
turnip cabbage	124.0	<0.002	15.0 (33)[c]
beetroot	89.0	0.05	11.0 (33)[c]
celery	176.0	<0.002	25.0 (40)[c]
leek	94.0	<0.002	9.5 (27)[c]

[a] After addition of water, the material was refluxed for 30 min. [b] Mean values of triplicates. [c] Yield (in mol %) as compared to the amount of **2**.

For the determination of a response factor, first a 1:1 mixture of [$^2H_3$]-**2** and **2** was analyzed by LC-MS/MS. From the spectra, the most abundant ion transitions were selected as *m*/*z* 164 to *m*/*z* 74 and *m*/*z* 164 to *m*/*z* 102 for **2** as well as *m*/*z* 167 to *m*/*z* 74 and *m*/*z* 167 to *m*/*z* 102 for [$^2H_3$]-**2**. To obtain the maximum intensities of the parent and product ions, the collision energy was systematically optimized during MS/MS measurements. Finally, 12 mixtures containing different concentrations of both the analyte and the internal standard were analyzed. On the basis of the calibration curve obtained (data not shown), a response factor of 0.75 was calculated.

Quantitation of Dimethyl Sulfide (1) by a SIDA Using Two-Dimensional HRGC and SPME Isolation. Due to losses during the workup procedure, the exact quantitation of odor-active volatiles with low boiling points is a continuous challenge. However, combining SPME with a SIDA has recently been proven as a reliable tool for the quantitation of such volatile aroma compounds. In particular, the time-consuming optimization of temperature, salt addition, and extraction time can be significantly shortened by the use of isotopically labeled standards (*21*).

For method development, an MS response factor was determined by analyzing five mixtures of known amounts of labeled [$^2H_6$]-**1** and unlabeled **1** in different mass ratios (from 1:3 to 3:1). Plotting the ratio of the area counts of [$^2H_6$]-**1** (*m*/*z* 69) and **1** (*m*/*z* 63) against the ratio of the concentrations allowed the calculation of a response factor of 0.99 (data not shown).

Table 3. Concentrations of *S*-Methylmethionine (**2**) in Green and White Asparagus from Different Geographical Origins: Amounts of Dimethyl Sulfide (**1**) Formed after Cooking[a]

		concn of **2**[b] (mg/kg) in		concn of **1**[b] (mg/kg) in processed	
expt	sample	stalks	spears	stalks	spears
1	white asparagus (Peru)	86.0	161.0	5.7 (18)[c]	14 (23)[c]
2	white asparagus (Germany)	68.0	252.0	4.1 (16)[c]	21 (22)[c]
3	white asparagus (Greece)	101.0	113.0	6.2 (16)[c]	9.4 (22)[c]
4	green asparagus (Peru)	109.0	234.0	7.5 (18)[c]	18 (21)[c]
5	green asparagus (Germany)	53.0	94.0	4.9 (25)[c]	9.3 (26)[c]
6	green asparagus (Mexico)	64.0	134.0	6.0 (25)[c]	13 (26)[c]

[a] After addition of water, the vegetable was refluxed for 30 min. [b] Data are mean values of triplicates. [c] Yield of **1** (in mol %) as compared to the amount of **2**.

Table 4. Concentration of *S*-Methylmethionine (**2**) and Dimethyl Sulfide (**1**) in Orange Juice and Strawberry Juice

			concn of **1**[a] (μg/kg)	
expt	sample	concn of **2**[a] (mg/kg)	before	after cooking
1	commercial orange juice	0.9	3	40
2	commercial strawberry juice	1.8	50	200
3	hand-squeezed orange juice	1.2	<2	200

[a] Data are mean values of triplicates.

Model Studies on the Thermal Stability of 2. To determine the stability of *S*-methylmethionine during thermal processing, the amino acid was dissolved in two buffers simulating either the pH of the juices or the vegetables under investigation, and the time course of the degradation after a thermal treatment was followed (**Table 1**). Whereas the amino acid was continuously degraded with time under all conditions, the pH showed a clear effect, because 20% of **2** was degraded at pH 6.8, whereas only 8% was decomposed at pH 4.4 after 30 min at 100 °C. To elucidate the influence of the matrix, **2** was administered to a freshly prepared tomato or orange juice, respectively, and the degradation was measured after 30 min at 100 °C. The amount of **1** already present in the two juices after heating was subtracted. The results showed a substantial influence of the matrix, because the degradation of **2** in the juices (expts 3 and 4, **Table 1**) was higher as compared to an aqueous buffer of the same pH (expt 2, **Table 1**).

Quantitation of *S*-Methylmethionine (2) and Dimethyl Sulfide (1) in Foods. In the next series of experiments, the SIDAs were used for the quantitation of **2** and **1** in raw and processed foods, respectively. In **Figure 3**, an LC-MS/MS chromatogram obtained for **2** in an extract from orange juice, showing the lowest concentrations among the samples analyzed, is exemplarily shown. From this sample, the LoD for **2** was calculated to be 50 μg/kg.

Application of the method to different kinds of vegetables revealed concentrations of **2** in the milligrams per kilogram range (**Table 2**). Among the samples analyzed, cabbage, turnip cabbage, beetroot, celery, and leek showed high concentrations with celery containing the highest amounts, whereas fresh tomatoes showed the lowest concentration of 2.8 mg/kg.

Using the same samples, then the amount of **1** was analyzed before and after the cooking process. Before heating, **1** was found only in raw beetroot, but in low concentrations of 50 μg/kg. In all other samples, the concentrations were below the detection limit of 2 μg/kg. However, after cooking, a very high concentration of 25 mg/kg were found in celery, which was the highest amount determined among all samples.

A correlation of the yields of **1** formed after heating with the amounts of **2** present in the raw materials revealed that between 27 mol % (leek) and 40 mol % (celery) of **2** was decomposed into **1** during cooking (**Table 2**). The very high "yields" of **1** in processed tomatoes (105%) might, however, indicate that **2** is not the only precursor of **1**. Because the amount of **2**, which might be bound in proteins, cannot be determined with this method, tomatoes might contain a higher level of protein-bound **2**, which in turn might generate higher amounts of **1**.

Table 5. Influence of the Malting Process on the Concentrations of *S*-Methylmethionine (**2**) and Dimethyl Sulfide (**1**) in Barley, Germinated Barley, and Malt: Comparison with the Amounts of **1** Formed after Cooking (30 min; 100 °C)

			concn of **1**[a] (mg/kg)	
expt	barley sample	concn of **2**[a] (mg/kg)	before	after cooking
1	unprocessed	0.9	0.008	0.09
2	after 1st steeping	4.8	0.004	0.1
3	after 2nd steeping	3.4	0.02	0.3 (24)[b]
4	after 1st day of germination	7.8	0.01	0.7 (24)[b]
5	after 2nd day of germination	10.0	0.01	1.0 (27)[b]
6	after 3rd day of germination	18.0	0.02	1.7 (25)[b]
7	after 4th day of germination	24.0	0.06	2.2 (25)[b]
8	after malting	2.4	8.2	8.6

[a] Data are mean value of triplicates. [b] Yield of **1** (in mol %) calculated from the amount of **2**.

According to the literature (*3*), asparagus has been suggested to contain high amounts of **2**. Thus, in a second series of experiments, the amino acid was quantified in green and white asparagus from different geographical origins. The amounts present in stalks and spears were separately measured. The results revealed concentrations of **2** between 53 and 109 mg/kg in the stalks of the asparagus samples, whereas concentrations between 94 and 252 mg/kg were found in spears (**Table 3**). Interestingly, the differences observed between the six samples were more pronounced between the origin of the samples as compared to green and white varieties. However, in all samples analyzed, the amounts of **2** were higher in the spears as compared to the stalks. For example, in the spears of German white asparagus higher concentrations by a factor of 3.5 were present as compared to the stalks. However, this observation was less pronounced in the Greek white asparagus.

To study whether the amounts of **2** were correlated with the concentrations of **1** formed after heating, the same samples were cooked in closed vessels and, after 30 min at 100 °C, the amounts of dimethyl sulfide **1** formed were quantified. As found for the other vegetables, the results revealed that the amounts of **2** present in the raw material and the concentrations of **1** formed after a thermal treatment were correlated to some extent. By assuming that the amino acid is the only precursor of **1**, yields between 16 and 26 mol %, respectively, could be calculated. However, interestingly, not only the yields but also the conversion rate of **2** into **1** was slightly higher from the spears, probably indicating an influence of the texture on the efficacy of the degradation of **2** into **1** (**Table 3**); for example, spears are softer than stalks.

In commercial orange and strawberry juices, 0.9 or 1.8 mg/kg, respectively, of **2** was determined (**Table 4**). Both juices also

Table 6. Comparison of the Measured Amounts of **1** in Barley Samples after Thermal Treatment (30 min, 100°C) and Predicted Amounts of **1** on the Basis of a Standardizied Conversion Rate[a]

		concn of **1**[b] (μmol/kg)	
expt	barley sample	measured	predicted
1	unprocessed	1.4	1.3
2	after 1st steeping	2.2	6.7
3	after 2nd steeping	5.1	4.6
4	after 1st day of germination	11	11
5	after 2nd day of germination	17	14
6	after 3rd day of germination	27	25
7	after 4th day of germination	35	34
8	after malting	6.1	3.5

[a] An average conversion rate of 25% was used. [b] Concentrations are based on fresh weight.

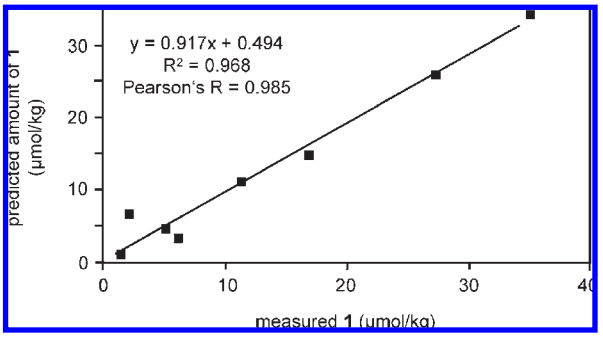

Figure 4. Correlation of the concentration of **1** measured after heat treatment with amounts predicted from concentrations of **2** in unheated barley samples.

contained **1**, but in somewhat lower amounts. According to Guadagni and Miers (*22*, *23*), **1** should generate an off-flavor in tomato juice when present in concentrations above 2 mg/kg. However, because the odor threshold of **1** in water was reported to be 0.12 μg/L (*24*), in the commercial strawberry or orange juice, **1** should contribute to the overall aroma, because the odorant showed an odor activity value (OAV; ratio of concentration to odor threshold) of 350 or 15, respectively. These data corroborated the significant influence of **1** on the aromas of thermally processed foods, even at very low concentrations, because, for example, in a hand-squeezed orange juice, **1** was increased to 200 μg/kg after the application of a heat process. Thus, in general, pasteurization processes may lead to the generation of **1**, which may then contribute to a "cooked" aroma note in fruit juices.

Generation of *S*-Methylmethionine (2) during Barley Fermentation and Malting. In the manufacturing process of beer, it is considered to be very important to degrade *S*-methylmethionine already during malt production to avoid off-flavors in the final beer. Because in the brewing industry the amounts of **2** are commonly estimated on the basis of the amounts of **1** formed after heating under alkaline conditions, the SIDA for **2** was applied on barley/malt samples produced by a laboratory procedure.

According to the literature (*25*), **2** is formed by L-methionine *S*-methyltransferase during germination. However, the results displayed in **Table 5** show that the generation of **2** is already initiated during steeping. After 4 days of germination, the amounts increased to 24 mg/kg, and finally, in the pale malt (expt 8), the amounts of **2** were reduced by almost 90% as compared to the green malt (expt 7) germinated for 4 days, which was undoubtedly caused by the heating process.

A correlation of the amounts of **1** formed after heat treatment with the amounts of **2** present in the raw material (expts 3−7; **Table 5**) revealed a quite good correlation, suggesting that between 24 and 27 mol % of **2** is degraded into **1** under the conditions applied.

These quantitative data were finally used in a statistical calculation. An ANOVA was carried out using Sigma Stat 3.5 software to compare the mean concentrations of the measured amounts of **1** in malt with the amounts predicted from the concentrations of **2** (**Table 6**). **Figure 4** illustrates these results by comparing the amounts of **1** measured to the amounts predicted on the basis of the amounts of **2** present using an average yield of 25 mol % (**Table 5**). The Pearson correlation coefficient was 0.985 for barley ($p < 0.001$, 95%). Thus, the results of the statistical test proved that the quantitation of **2** can be used to predict the formation of **1** in a quite reliable way.

In summary, the SIDA developed for the quantitation of **2** by LC-MS/MS in malt and vegetables was proven to be a method with high selectivity and high sensitivity, showing a low LoD (30−85 μg per kg). In addition, the recovery rate of 102.5% determined in tomato juice as well as an average standard deviation of ≤6% confirmed good reproducibility. The quantitative results suggest that the presence of *S*-methylmethionine in any raw material will undoubtedly lead to a significant generation of the potent aroma compound dimethyl sulfide after a thermal treatment. Thus, this sulfur compound should be an important aroma contributor to numerous processed vegetables.

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