Generation of Roasted Notes Based on 2-Acetyl-2-thiazoline and Its Precursor, 2-(1-Hydroxyethyl)-4,5-dihydrothiazole, by Combined Bio and Thermal Approaches

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Roasted notes contribute to the flavor of thermally processed foods such as meat and bread. 2-Acetyl-2-thiazoline is one of the key volatile compounds responsible for the roasted and popcorn-like aroma character. We report here on the biogeneration of flavoring preparations with intense roasted notes, which are characterized by a high content of 2-acetyl-2-thiazoline. These flavoring preparations were obtained by fermentation of cysteamine, ethyl-L-lactate, and D-glucose with baker’s yeast. The precursor of 2-acetyl-2-thiazoline, 2-(1-hydroxyethyl)-4,5-dihydrothiazole, was prepared under mild conditions by microbial reduction of the carbonyl group of 2-acetyl-2-thiazoline using baker’s yeast as biocatalyst. The addition of 2-(1-hydroxyethyl)-4,5-dihydrothiazole as aroma precursor to pizza dough resulted in an increase of the roasted note.

KEYWORDS: Roasted notes; bioflavor; baker’s yeast; baked flavor; 2-acetyl-2-thiazoline; 2-(1-hydroxyethyl)-4,5-dihydrothiazole; GC–Olfactometry; GC–MS

INTRODUCTION

Several types of compounds are known to elicit roasted notes, mainly nitrogen-containing heterocyclic components formed in the course of the Maillard reaction, such as pyrazines (1), pyrrolines (2), pyridines (3), thiazolines (4) and thiazines (5). These heterocyclic compounds are important constituents of many foods, such as bread (6), cooked and roasted meat, chocolate, coffee, and beer (7). Among these aroma volatiles, thiazolines and thiazoline derivatives play a key role in roasted flavors, particularly in meat products (8), and they have received increasing research attention (9, 10).

One of the most important thiazolines, which exhibits an intense roasted aroma character, is 2-acetyl-2-thiazoline, 1. It was reported for the first time as a volatile constituent of beef broth (11) and later identified as a sensory relevant constituent of roasted beef (12). Several methods to generate 2-acetyl-2-thiazoline by organic synthesis (13) and the Maillard reaction (14) have been published. Münch et al. (15) obtained 2-acetyl-2-thiazoline by thermal treatment of commercial and self-prepared yeast extracts. Moreover, Hofmann and Schieberle (14) proposed a reaction pathway involving cysteamine and methylglyoxal as substrates to produce 2-(1-hydroxyethyl)-4,5-dihydrothiazole, 2, which was then transformed to 2-acetyl-2-thiazoline by heat treatment in a model reaction. However, the impact of 2-(1-hydroxyethyl)-4,5-dihydrothiazole as a potential precursor in a food product has never been demonstrated.

MATERIALS AND METHODS

Materials. All chemicals were of analytical grade and were purchased from Fluka (Buchs, Switzerland; cysteamine, ethyl-L-lactate) or from Merck (Darmstadt, Germany; D-glucose, sodium chloride, and sodium sulfate). The baker’s yeast cream was purchased from Hef Schweiz AG (Stettfurt, Switzerland). Diethyl ether was purified by distillation using a Vigreux column (1 m × 1 cm).

Fermentation: General Procedure. Commercial yeast cream (1 L) was centrifuged for 15 min, and the supernatant was discarded. The biomass was resuspended in a sodium bicarbonate buffer (1 L, 0.2 M, pH 9.8). This yeast cream solution (150 mL) was then placed in a flask...
The flask was kept at 35 °C using an oil bath, and the pH was adjusted to 9.8 with sodium hydroxide (2 M). The pH was automatically maintained throughout the reaction using a pH-stat device (Impulsomat 614, Metrohm, Herisau, Switzerland). Cysteamine (385 mg, 5 mmol) and ethyl-L-lactate (590 mg, 5 mmol) were then added. Aliquots of α-glucose (10 g and 5 g, respectively) were added after 4 h and 24 h of incubation. After 48 h of reaction, the mixture was centrifuged, and the supernatant was further treated.

Preparation of the Samples A, B, and C. A 30-ml portion of the liquid phase (supernatant), obtained as described above, was saturated with sodium chloride and extracted with diethyl ether to give sample A. Another 30-ml aliquot of the liquid phase was acidified to pH 6.5 with 5% hydrochloric acid and refluxed for 30 min in a 50-mL flask equipped with a reflux condenser and magnetic stirrer to obtain sample B. A third part of the supernatant (30 mL) was refluxed in the same manner as above, but at pH 10, to obtain sample C. After cooling to room temperature, the aqueous solutions (samples B and C) were evaluated sensorially, then saturated with sodium chloride and extracted with diethyl ether overnight using a rotary perforator (liquid—liquid extraction). The organic phases were dried over anhydrous sodium sulfate and purified by high vacuum distillation at 30 °C. The organic phases were redissolved in 99.95% deuterated DMSO-d6, with TMS as an internal shift reference. The inner coil of the 5-mm broadband multineulcear probehead was tuned for 13C detection without decoupler heating, and the temperature in the probehead was 23.3 °C. The intervals between the hard pulses with a pulse angle of ca. 67° were chosen relatively long (11.5 and 14.6 s, respectively) to obtain a good representation of quadrupolar carbons and near-quantitative 1H NMR integral values. Additional one- and two-dimensional spectra were acquired for complete structure elucidation (DEPT 135, undecoupled 13C NMR, 1H COSY, direct and long range 13C–1H HETCOR, optimized for 145 and 8 Hz coupling constants, respectively).

1H NMR (360 MHz, DMSO-d6), 7.88 (br t, 1H, slowly D2O-exchangeable, −NH), 5.51 (br s, 1H, instantly D2O-exchangeable, −OH), 3.95 (s, 3H, J = 6.4 Hz, CH−OH), 3.22 (d, 2H, J = 6.7 Hz, −CH2−), 2.53 (t, 2H, J = 7.1 Hz, −CH2−), 2.35 (br s, ca. 1H, instantly D2O-exchangeable, −SH), 1.20 (d, 3H, J = 6.8 Hz, −CH3). The curly (‘•’) denote an approximate description of the coupling pattern. 13C NMR (90.56 MHz, DMSO-d6) δ 174.43 (s, C=C=O), 67.15 (d, −CH−OH), 41.45 (t, −CH−CH2), 2.31 (t, −CH2−SH), 20.97 (q, −CH3). The multiplicities given refer to the one-bond CH couplings. The 1H COSY and 13C–1H HETCOR spectra confirmed the molecular structure. The molecule was found to be unstable in DMSO-d6 solution. A dimer was formed over time, and the initially fast exchange rates of the mobile protons slowed (first the −SH, then the −OH protons), leading to changes of the signal multiplicities in the proton spectrum.

Biological Function of 2-Acetyl-2-thiazoline into 2-(1-Hydroxyethyl)-4,5-dihydrothiazole. As described in the Sensory Evaluation, the application of 2-(1-hydroxyethyl)-4,5-dihydrothiazole was performed using pizza dough as a food model. A water solution of 2-(1-hydroxyethyl)-4,5-dihydrothiazole (1.6 mg/mL) was mixed with other ingredients of the dough and partially replacing the water involved in the recipe keeping the final moisture constant. The 2-(1-hydroxyethyl)-4,5-dihydrothiazole concentration was 5 mg per 50 g of raw dough. For the frozen pizza, samples were prebaked for 8 min at 220 °C, wrapped in plastic bags without modified atmosphere, and kept frozen for 2 weeks. For the refrigerated pizza, samples were wrapped in plastic bags with modified atmosphere (50% N2, 50% O2) and kept at 8 °C for 1 week. Frozen and refrigerated pizza samples were baked for 8 and 15 min, respectively, at 200 °C in a rotary convection oven.

Sensory evaluation by triangle test procedures was performed on the baked pizza dough. Samples spiked with 2-(1-hydroxyethyl)-4,5-dihydrothiazole were compared to the corresponding reference. Tasting sessions were performed under red light to avoid visual identification of the different product. Panelists (30) were asked to identify which pizza sample out of the three was different. The samples were presented in the following two schemes: one reference, two 2-(1-hydroxyethyl)-4,5-dihydrothiazole spiked samples or two references and one 2-(1-hydroxyethyl)-4,5-dihydrothiazole spiked sample.

RESULTS AND DISCUSSION

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Table 1. Odor-Active Compounds Identified in Sample A

<table>
<thead>
<tr>
<th>no</th>
<th>compound</th>
<th>FFAP</th>
<th>DB-1701</th>
<th>aroma quality (GC–O)</th>
<th>aroma intensity (GC–O)</th>
</tr>
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<tbody>
<tr>
<td>3</td>
<td>isobutanol</td>
<td>1085</td>
<td>725</td>
<td>malty</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>3-methyl-1-butanol*</td>
<td>1203</td>
<td>848</td>
<td>metallic, musty, malty</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>2-methyl-3-furanthiol*</td>
<td>1305</td>
<td>932</td>
<td>meaty, roasty</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>3-mercapto-2-pentanone†</td>
<td>1354</td>
<td>1021</td>
<td>catty, sulfury</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>3-isopropyl-2-methoxypryrazine*</td>
<td>1398</td>
<td>1149</td>
<td>roasty</td>
<td>1–2</td>
</tr>
<tr>
<td>8</td>
<td>2-methyl-2-thiazoline*</td>
<td>1418</td>
<td>1025</td>
<td>putrid, amine-like</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>2-ethyl-3,5-dimethylpyrazine*</td>
<td>1453</td>
<td>1149</td>
<td>roasty, earthy</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>butanolic acid*</td>
<td>1625</td>
<td>970</td>
<td>sweaty, yeasty</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>isovaleric acid*</td>
<td>1665</td>
<td>1024</td>
<td>sweaty, rancid, yeasty</td>
<td>2–3</td>
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<tr>
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<td>N-acetyl cysteamine**</td>
<td>1748</td>
<td>1245</td>
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<td>2–3</td>
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<tr>
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</tr>
<tr>
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<td>1278</td>
<td>roasty</td>
<td>1–2</td>
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<tr>
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<td>1905</td>
<td>n.d.</td>
<td>spicy, almond-like</td>
<td>1–2</td>
</tr>
<tr>
<td>16</td>
<td>N-acetyl cysteamine**</td>
<td>2040</td>
<td>1450</td>
<td>roasty</td>
<td>2</td>
</tr>
</tbody>
</table>

A Baker’s yeast was incubated with ethyl lactate and cysteamine for 24 h at pH 9.8. *Identification was based on retention index (RI), mass spectrometry (MS), and/or reference compounds (Ref) († RI, MS, Ref; † RI, Ref; † RI, MS; ‡ MS, Ref). † The following capillary columns were used: FFAP and OV-1701. ‡ The aroma intensity was estimated from 1 (weak), to 2 (medium), and 3 (high).

Methylthiazolidine 8, isovaleric acid 11, and 2-acetyl-2-thiazoline 1 were the most intensely smelling compounds identified in this sample.

Several other odorants were also identified in sample A, i.e., 2-methyl-3-furanthiol 5, 3-mercapto-2-pentanone 6, and 2-ethyl-3,5-dimethylpyrazine 9. These odorants have been cited as characteristic constituents of boiled and roasted meat (11, 12). As shown in this study, these aroma impact compounds can also be generated by fermentation using suitable precursors and without applying any heat treatment. Several, mainly cyclic, sulfur-containing compounds were also identified, such as thiazolines and thiazolidines. Three thiazolines were identified as 2-acetyl-2-thiazoline 1, 2-(1-hydroxyethyl)-4,5-dihydrothiazole 2, and 2-methyl-2-thiazoline 8. 2-Acetyl-2-thiazoline was the most intensely smelling odorant and is described as having a pleasant roasted, popcorn-like odor. This compound was identified by GC–O and GC–MS, and the sensory and chromatographic properties of this compound were identical to those of the reference compound.

In sample A, we identified 2-(1-hydroxyethyl)-4,5-dihydrothiazole by matching retention indices and mass spectra with those published in the literature (14). This is the first time that these two compounds (1 and 2) have been generated via fermentation and without applying any heat treatment.

The predominant volatile compound generated in sample A was identified as N-acetyl cysteamine 16. This compound, which smells burnt, yeasty, and musty, is probably the precursor of 2-methyl-2-thiazoline 8 which was generated upon storage, most likely via intramolecular cyclization followed by elimination of water (Figure 1). However, the contribution of 2-methyl-2-thiazoline to the overall aroma appeared to be rather low.

A reference sample was obtained using the same incubation conditions as for sample A, but without addition of cysteamine and ethyl-L-lactate. After extraction and concentration, the odor of the sample was described as only yeasty and musty. GC analyses did not show any of the sulfur-containing compounds found in sample A, thus indicating that the sulfur compounds were generated from cysteamine.

The thermal treatment of the supernatant (obtained after fermentation) at pHs 6.5 and 10 resulted in samples B and C, respectively. The aroma qualities of these samples were described as roasted, popcorn, and bread crust-like, and both were found to have high odor intensities. However, sample B was more intense than C, thus suggesting the importance of the pH to flavor formation during heat treatment. The aqueous solutions obtained after heating were extracted with diethyl ether. The extracts were then concentrated and analyzed by GC–O on two capillary columns of different polarities (FFAP and DB-1701).

As shown in Table 2, 2-acetyl-2-thiazoline was the dominant aroma compound in both samples B and C. This result is in good agreement with the sensory evaluation of the two samples, which were clearly described as roasted and popcorn-like. The amount of 2-acetyl-2-thiazoline in sample B was three times as much as that in sample C estimated on the basis of peak areas. However, sample C contained more 2-methylthiazolidine 8, 2-ethyl-3,5-dimethylpyrazine 9, and trimethylpyrazine 19 than sample B.

Microbiological Reduction of 2-Acetyl-2-thiazoline into 2-(1-Hydroxyethyl)-4,5-dihydrothiazole. 2-(1-Hydroxyethyl)-4,5-dihydrothiazole 2 has been proposed as a potential precursor of 2-acetyl-2-thiazoline in a model reaction (14). However, the impact of 2-(1-hydroxyethyl)-4,5-dihydrothiazole as a precursor to improve the roasted notes of baked goods has never been demonstrated in food models. In this study, 2-(1-hydroxyethyl)-4,5-dihydrothiazole was prepared by microbial reduction of the carbonyl group of 2-acetyl-2-thiazoline using baker’s yeast as a biocatalyst. This approach was identified as the most appropriate way to produce 2-(1-hydroxyethyl)-4,5-dihydrothiazole in a single step, under mild conditions and using a food-grade substrate and biocatalyst (baker’s yeast). The microbial reduction was performed at 30 °C and different pH values. Best results were obtained at pH 6.5. Indeed, after 6 h reaction time at pH 6.5, most of the 2-acetyl-2-thiazoline was transformed, and
positive chemical ionization. The compound confirmed by LC-MS analysis and by GC-MS working in positive chemical ionization. The compound at this pH. Indeed, at pH 4.5, two other compounds were obtained at pH 4.5 could be explained by the instability of this compound at this pH. Moreover, the yield of generated 2-(1-hydroxyethyl)-4,5-dihydrothiazole was very low at pH 4.5 as compared to that at the other pH conditions studied. However, no significant difference was observed in the pH range from 6.5 to 8.5. The highest yield was obtained at pH 6.5 (60%). The low yield of 2-(1-hydroxyethyl)-4,5-dihydrothiazole obtained at pH 4.5 could be explained by the instability of this compound at this pH. Indeed, at pH 4.5, two other compounds were identified in the reaction mixture by HPLC and GC, and were tentatively characterized on the basis of their mass spectrometry data (Figure 3) as N-lactoyl cysteamine 25 and S-acetyl-N-lactoyl cysteamine 26. The molecular ions were confirmed by LC-MS analysis and by GC-MS working in positive chemical ionization. The compound 25 was purified by HPLC and its structure was confirmed by NMR data analysis; however, the characterization of compound 26 by NMR was not possible because of the presence of several co-eluting byproducts.

Compound 25 was obtained in high amounts when the biotransformation of 2-acetyl-2-thiazoline was performed under acidic conditions. A hypothetical pathway leading to the formation of compounds 25 and 26 is proposed in Figure 4. Acid-catalyzed addition of water to 2-(1-hydroxyethyl)-4,5-dihydrothiazole leads to the corresponding dihydroxy intermediate with subsequent ring opening, which gives rise to N-lactoyl cysteamine 25. Further acetylation leads to compound 26.

Shelf Life Stability of 2-(1-Hydroxyethyl)-4,5-dihydrothiazole in Water as a Function of pH. The thermal transformation of 2-(1-hydroxyethyl)-4,5-dihydrothiazole and the stability of 2-acetyl-2-thiazoline under heat treatment have already been studied (14). However, the stability of 2-(1-hydroxyethyl)-4,5-dihydrothiazole has not been reported in the literature. In this study, several aqueous solutions of 2-(1-hydroxyethyl)-4,5-dihydrothiazole were stored at room temperature and at different pHs (4.5, 6.5, 7.5, and 8.5) for a 40-day period. As shown in Figure 5, a significant degradation (75 %) of 2-(1-hydroxyethyl)-4,5-dihydrothiazole was observed after only 1 day at pH 4.5. However, a gradual decrease of the 2-(1-hydroxyethyl)-4,5-dihydrothiazole concentration was shown at pH values of 6.5, 7.5, and 8.5, and the half-life of 2-(1-hydroxyethyl)-4,5-dihydrothiazole was obtained in 60% yield.

Influence of the pH on the Microbiological Reduction of 2-Acetyl-2-thiazoline. Figure 2 shows the kinetic curves representing the biotransformation of 2-acetyl-2-thiazoline and the biogeneration of 2-(1-hydroxyethyl)-4,5-dihydrothiazole at pHs 4.5, 6.5, 7.5, and 8.5. The pH of the baker’s yeast suspension was adjusted before the addition of 2-acetyl-2-thiazoline and was automatically maintained throughout the reaction. As shown in Figure 2, the biotransformation rates of 2-acetyl-2-thiazoline were quite similar at pHs 6.5, 7.5, and 8.5, whereas the reaction rate was slightly slower at pH 4.5. The low yield of 2-(1-hydroxyethyl)-4,5-dihydrothiazole was obtained at pH 4.5 could be explained by the instability of this compound at this pH. Indeed, at pH 4.5, two other compounds were identified in the reaction mixture by HPLC and GC, and were tentatively characterized on the basis of their mass spectrometry data (Figure 3) as N-lactoyl cysteamine 25 and S-acetyl-N-lactoyl cysteamine 26. The molecular ions were confirmed by LC-MS analysis and by GC-MS working in positive chemical ionization. The compound 25 was purified by HPLC and its structure was confirmed by NMR data analysis; however, the characterization of compound 26 by NMR was not possible because of the presence of several co-eluting byproducts.

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Dihydrothiazole at these pH conditions and room temperature was estimated to be about 20 days.

Applications of 2-(1-Hydroxyethyl)-4,5-dihydrothiazole in Pizza Dough and Sensory Evaluation.

2-(1-Hydroxyethyl)-4,5-dihydrothiazole has been proposed as a potential precursor of 2-acetyl-2-thiazoline in a model reaction (14). In this study, the impact of this aroma precursor to improve the roasted notes of baked goods was evaluated using two types of pizzas: frozen and chilled. The aqueous solution of 2-(1-hydroxyethyl)-4,5-dihydrothiazole (1.6 mg/mL) was mixed with the classical ingredients of the pizza recipe to reach 5 mg per 50 g of raw dough. Thirty assessors were asked to describe the aroma quality of the freshly prepared samples by smelling the headspace above the sample. Taste and texture were not considered in this study. The addition of 2-(1-hydroxyethyl)-4,5-dihydrothiazole resulted in an increase in the roasted, toasted and popcorn-like notes as compared to the reference with 99.9% confidence level in triangle test.

In conclusion, the fermentation of cysteamine, ethyl-L-lactate, and D-glucose with baker’s yeast resulted in a flavoring preparation which was described as dried sausage-like. When this reaction mixture was heated under acidic or alkaline conditions, the resulting samples exhibited attractive and intense roasted, popcorn and bread crust-like notes. High amounts of 2-acetyl-2-thiazoline were detected in these samples by different chromatographic techniques. Moreover, 2-(1-hydroxyethyl)-4,5-dihydrothiazole seems to be a promising precursor to increase roasted note of baked goods.

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