JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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

RACHID BEL RHLID,* YVETTE FLEURY, IMRE BLANK, LAURENT B. FAY, DIETER H. WELTI, FRANCIA ARCE VERA, AND MARCEL A. JUILLERAT

Nestec Ltd., Nestlé Research Center, Vers-chez-les-Blanc, P.O. Box 44, 1000-Lausanne 26, Switzerland

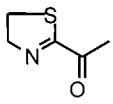
Roasted notes contribute to the flavor of thermally processed foods such as meat and bread. 2-Acetyl-2-thiazoline is one of the key volatile compounds responsible for the roasted and popcorn-like aroma character. We report here on the biogeneration of flavoring preparations with intense roasted notes, 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

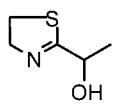
Several types of compounds are known to elicit roasted notes, mainly nitrogen-containing heterocyclic components formed in the course of the Maillard reaction, such as pyrazines (*1*), pyrrolines (*2*), pyridines (*3*), thiazolines (*4*) and thiazines (*5*). These heterocyclic compounds are important 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.



2-Acetyl-2-thiazoline

1



2-(1-Hydroxyethyl)-4,5-dihydrothiazole

2

The aim of the study reported here was to generate roasted notes based on aerobic incubation of cysteamine, ethyl-L-lactate, and D-glucose with baker's yeast. Sensory-directed chemical analysis was applied to characterize the key flavor compounds and the reaction intermediates. In addition, we report on the biogeneration of 2-(1-hydroxyethyl)-4,5-dihydrothiazole by microbial reduction of 2-acetyl-2-thiazoline, the stability of 2-(1-hydroxyethyl)-4,5-dihydrothiazole, and its potential as aroma precursor to increase the roasted note of pizza dough after baking.

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 Hefe 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

* To whom correspondence should be addressed (Phone (+41) 21 785 8634, Fax (+41) 21 785 8549, e-mail: rachid.bel-rhlid@rdls.nestle.com).

10.1021/jf011170d CCC: $22.00
Published on Web 03/05/2002

(500 mL) equipped with an electrode and a magnetic stirrer (500 rpm). 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 D-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 15% 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 3×10^{-3} mbar (*16*). The contents of the traps were combined, dried, and concentrated to about 1 mL for chromatographic analyses.

Sensory Evaluation of Samples A, B, and C. The aroma of each sample was evaluated by sniffing the headspace of the freshly prepared samples (A, B, C). Ten assessors were asked to describe the aroma quality and intensity. For descriptive analysis, a limited number of aroma descriptors were provided to the panel in order to reduce the number of attributes and simplify the aroma characterization. The intensity range of the aroma was scored from 1 (weak) to 3 (intense).

Microbiological reduction of 2-Acetyl-2-thiazoline into 2-(1-Hydroxyethyl)-4,5-dihydrothiazole. Commercial yeast cream (400 g) was centrifuged, and the supernatant (290 g) was discarded. The biomass was then resuspended in distilled water (290 g). This yeast suspension (400 g) was then used in the same manner as described above. The pH was adjusted to the desired value (6.5, 7.5, or 8.5) with 2 M NaOH solution before addition of 800 μL of 2-acetyl-2-thiazoline (7.4 mmol). During the biotransformation, the pH was kept constant using a pH-stat system, and the temperature was controlled at 30 °C.

Analyses by Gas Chromatography. GC−Olfactometry (GC−O) analyses were performed using a Carlo Erba gas chromatograph (Mega 2, GC 8000, Fisons Instruments, via Brechbühler, Schlieren, Switzerland) equipped with an automatic cold on-column injector, FID, and sniffing port (*17*). Fused silica capillary columns (DB-1701 and DB-FFAP) were used, both 30 m × 0.32 mm i.d. and film thickness 0.25 μm (J&W Scientific, Folsom, CA). The temperature program for the DB-1701 was 35 °C (2 min), 40 °C/min to 50 °C (1 min), 6 °C/min to 240 °C (10 min), and for the FFAP was 50 °C (2 min), 6 °C/min to 180 °C, and 10 °C/min to 240 °C (10 min). Linear retention indices (RI) were calculated according to van den Dool and Kratz (*18*).

GC−mass spectrometry (GC−MS) analyses were carried out using a MAT-8430 mass spectrometer (Finnigan, Bremen, Germany) using the same GC conditions as described above. The MS−EI spectra were generated at 70 eV, and MS−CI spectra were generated at 150 eV, with ammonia as the reagent gas.

High-Performance Liquid Chromatography Analysis of 2-(1-Hydroxyethyl)-4,5-dihydrothiazole and 2-Acetyl-2-thiazoline. HPLC analyses were performed using a Hewlett-Packard chromatograph (series 1100, Agilent, Geneva, Switzerland) equipped with a diode array detector. Separation was performed on a ChromCart Cartridge column (250 × 4 mm i.d.) packed with reversed-phase Nucleosil 100, RP-18, 5 μm, Macherey-Nagel 721662-40 (Oensingen, Switzerland). Elution was carried out at 1 mL/min with a linear gradient from 10 to 50% acetonitrile in 0.1% trifluoroacetic acid (TFA) over 10 min. The temperature of the column was maintained at 40 °C, and detection was achieved at three different wavelengths: 306 nm for 2-acetyl-2-thiazoline, 232 nm for 2-(1-hydroxyethyl)-4,5-dihydrothiazole, and 205 nm for other reaction products.

Liquid Chromatography/Mass Spectrometry (LC/MS). An analytical HPLC column (Nucleosil 100-5, RP-18, Macherey-Nagel) was coupled to an LCQ−MS detector (Finnigan, Bremen, Germany) using positive electrospray ionization (ESI). HPLC conditions were the same as described above, and the UV signal was recorded only at 205 nm. Optimized tuning was performed by direct introduction of 2-acetyl-2-thiazoline solution into the mass spectrometer before LC−MS analysis.

NMR Analyses of Compound 25. ^{1}H NMR and composite pulse decoupled ^{13}C NMR spectra were acquired under standard conditions on a Bruker DPX-360 NMR spectrometer. The sample was dissolved in 99.95% deuterated DMSO-d_6, with TMS as an internal shift reference. The inner coil of the 5-mm broadband multinuclear probehead was tuned for ^{13}C detection without decoupler heating, and the temperature in the probehead was 22.3 °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 quaternary carbons and near-quantitative ^{1}H NMR integral values. Additional one- and two-dimensional spectra were acquired for complete structure elucidation (DEPT 135, nondecoupled ^{13}C NMR, ^{1}H COSY, direct and long range ^{13}C−^{1}H HETCOR, optimized for 145 and 8 Hz coupling constants, respectively).

^{1}H NMR (360 MHz, DMSO-d_6) δ 7.88 (br t, 1H, slowly D_2O-exchangeable, −NH), 5.51 (br s, 1H, instantly D_2O-exchangeable, −OH), 3.95 (sl br q, 1H, $J \geq 6.4$ Hz, CH−OH), 3.22 ("q", 2H, $J_{avg} = 6.7$ Hz, HN−CH_2−), 2.53 (t, 2H, $J = 7.1$ Hz, −CH_2−SH), 2.35 (br s, ca. 1H, instantly D_2O− exchangeable, −SH), 1.20 (d, 3H, $J = 6.8$ Hz, −CH_3). The quotes (" ") denote an approximate description of the coupling pattern. ^{13}C NMR (90.56 MHz, DMSO-d_6) δ 174.43 (s, C=O), 67.15 (d, −CH−OH), 41.45 (t, −HN−CH_2), 23.31 (t, CH_2−SH), 20.97 (q, CH_3). The multiplicities given refer to the one-bond CH couplings. The ^{1}H COSY and ^{13}C−^{1}H HETCOR spectra confirmed the molecular structure. The molecule was found to be unstable in DMSO-d_6 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.

Bakery Application and 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% N_2, 50% O_2) 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.

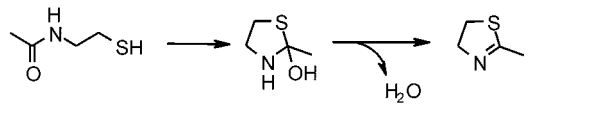
RESULTS AND DISCUSSION

Biogeneration of Flavoring Samples. After fermentation of cysteamine, ethyl-L-lactate, and D-glucose with baker's yeast, the reaction mixture was centrifuged, and the aqueous phase was extracted with diethyl ether. Ten assessors described the aroma quality of the freshly concentrated extract (sample A) as roasted, dried sausage and sausage skin-like of high odor intensity. As shown in **Table 1**, sixteen odor-active volatiles were detected by GC−O using two capillary columns of different polarities. Thirteen odorants were identified by matching retention indices, odor qualities, and mass spectra with those of reference compounds, if available, or with literature data. 2-

Table 1. Odor-Active Compounds Identified in Sample A[a]

no	compound[b]	linear retention index[c] FFAP	linear retention index[c] DB-1701	aroma quality (GC–O)[d]	aroma intensity (GC–O)[d]
3	isobutanol*	1085	725	malty	2
4	3-methyl-1-butanol*	1203	848	metallic, musty, malty	2
5	2-methyl-3-furanthiol†	1305	932	meaty, roasty	2
6	3-mercapto-2-pentanone†	1354	1021	catty, sulfury	2
7	3-isopropyl-2-methoxypyrazine†	1398	1149	roasty	1–2
8	**2-methyl-2-thiazolidine**‡	1418	1025	**putrid, amine-like**	**3**
9	2-ethyl-3,5-dimethylpyrazine*	1453	1149	roasty, earthy	2
10	butanoic acid*	1625	970	sweaty, yeasty	2
11	isovaleric acid*	1665	1024	sweaty, rancid, yeasty	2–3
1	**2-acetyl-2-thiazoline***	1748	1245	**roasty, popcorn**	**2–3**
2	2-(1-hydroxyethyl)–4,5-dihydrothiazole*	1909	1270	roasty, amine-like	1–2
12	unknown	1774	n.d.	roasty	1–2
13	unknown	1790	1276	roasty	1–2
14	2-phenylethanol*	1905	n.d.	spicy, almond-like	1–2
15	unknown	2040	1450	roasty	2
16	*N*-acetyl cysteamine≠	2200	n.d.	burnt, yeasty, musty	1–2

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



N-Acetylcysteamine 2-Methyl-2-thiazoline

16 **8**

Figure 1. Hypothetical formation of 2-methylthiazoline from *N*-acetyl cysteamine.

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-dimethyl-pyrazine **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

Table 2. Odor-Active Compounds Identifed in Samples B and C[a]

		linear retention index[c]			aroma intensity[e] (GC–O)[d]	
no	compound [b]	FFAP	DB-1701	aroma quality (GC–O)[d]	sample B	sample C
17	diacetyl†	990	680	buttery, sweet	2	-
3	isobutanol*	1084	725	malty	1	1
4	3-methyl-1-butanol*	1201	845	metallic, musty, malty	2	1–2
18	2-acetyl-1-pyrroline†	1328	1013	roasty	1–2	1
19	trimethylpyrazine†	1397	1078	roasty, earthy	-	2
8	2-methyl-2-thiazolidine‡	1415	1025	putrid, amine-like	1	2
20	unknown	1437	n.d.	roasty, earthy	-	1–2
21	unknown	1448	n.d.	savory	1–2	-
9	2-ethyl-3,5-dimethylpyrazine*	1451	1149	roasty, earthy	1–2	2
22	unknown	1473	n.d.	pyridine-like	-	1
23	(*E*)-2-nonenal†	1528	1272	fatty	-	1–2
11	isovaleric acid*	1664	1024	sweaty, rancid	1	1
1	**2-acetyl-2-thiazoline***	1748	1255	**roasty, popcorn**	**3**	**3**
14	2-phenylethanol*	1905	1275	spicy, almond-like	1	2
24	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone†	2025	1250	caramel-like	-	1–2

[a] Baker's yeast was incubated with ethyl lactate and cysteamine for 24 h at pH 9.8. [b] Identification was based on retention index (RI), mass spectrometry (MS), and reference compounds (Ref). (*RI, MS, Ref; †RI, Ref; ‡RI, MS; ≠MS, Ref). [c] The following capillary columns were used: FFAP and OV-1701. [d] The aroma intensity was estimated from 1 (weak), to 2 (medium), and 3 (high). [e] Sample B was boiled under acidic conditions (pH 6), and sample C was boiled under alkaline conditions (pH 10).

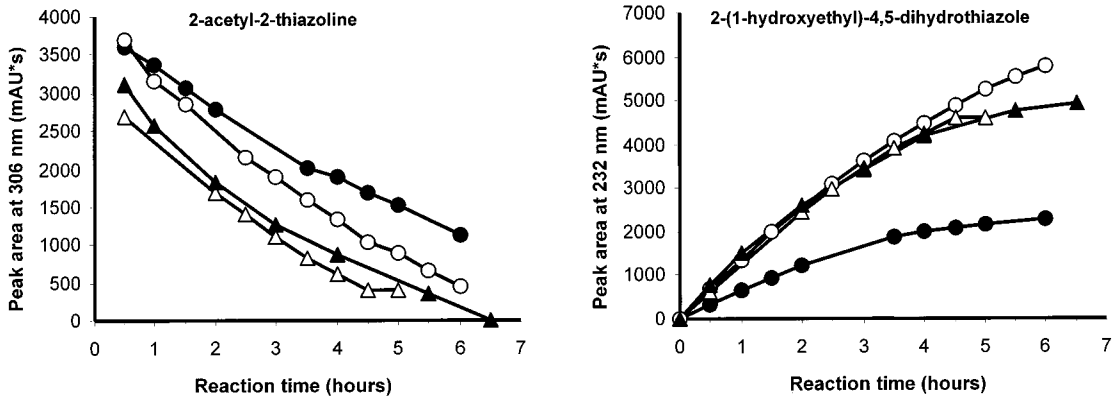


Figure 2. Biotransformation of 2-acetyl-2-thiazoline into 2-(1-hydroxyethyl)-4,5-dihydrothiazole as function of pH (●, pH 4.5; ○, pH 6.5; △, pH 7.5; and ▲, pH 8.5).

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. 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-

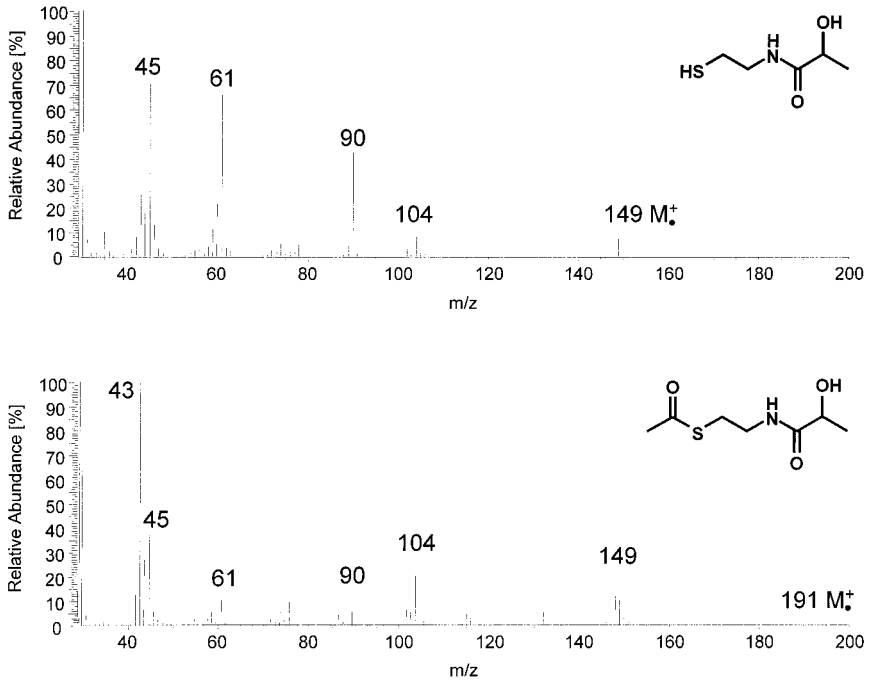


Figure 3. Mass spectra of compounds *S*-lactoyl cysteamine **25** and *N*-acetyl-*S*-lactoyl cysteamine **26**.

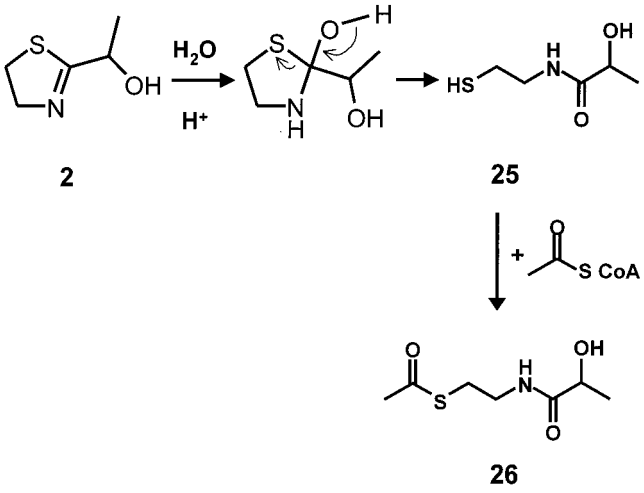


Figure 4. Hypothetical pathway leading to *S*-lactoyl cysteamine **25** and *N*-acetyl-*S*-lactoyl cysteamine **26**.

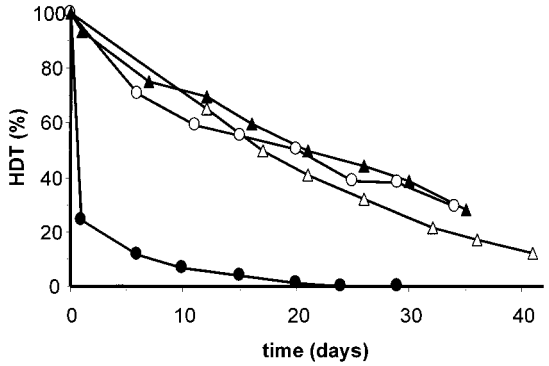


Figure 5. Stability of a 2-(1-hydroxyethyl)-4,5-dihydrothiazole solution at different pHs (●, pH 4.5; ○, pH 6.5; △, pH 7.5; and ▲, pH 8.5).

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.

ACKNOWLEDGMENT

We are grateful to S. Devaud, M. Bilat, F. Beaud, and S. Metairon for their help and expert technical assistance.

LITERATURE CITED

(1) Yoo, S. S.; Ho, C.-T.; Raghavan, S. K. Pyrazine generation from the Maillard reaction of mixed amino acids in model systems. *Perfum. Flavor.* **1997**, *22*, 49−52.

(2) Hofmann, T.; Schieberle, P. Flavour contribution and formation of the intense roast-smelling odorants 2-propionyl-1-pyrroline and 2-propionyltetrahydropyridine in Maillard-type reactions. *J. Agric. Food Chem.* **1998**, *46*, 2721−2726.

(3) Ames, J M.; Guy, R. C. E.; Kipping, G. J. Effect of pH, temperature, and moisture on the formation of volatile compounds in glycine/glucose model systems. *J. Agric. Food Chem*. **2001**, *49*, 4315−4323.
(4) Whitfield, F. B.; Mottram, D. S. Heterocyclic volatiles formed by heating cysteine or hydrogen sulfide with 4-hydroxy-5-methyl-3(2H)-furanone at pH 6.5. *J. Agric. Food Chem*. **2001**, *42*, 816−822.
(5) Huang, T.-C.; Su, Y.-M.; Ho, C.-T. Mechanistic studies on the formation of thiazolidines and structurally related thiazines in a cysteine/2,3-butandione model system. *J. Agric. Food Chem*. **1998**, *46*, 664−667.
(6) Grosch, W.; Schieberle, P. Bread. In *Volatile Compounds in Foods and Beverages*; Maarse, H., Ed.; Marcel Dekker: New York, 1991; pp 41−73.
(7) Hartmann, G. J.; Jin, Q. Z.; Lee, K. N.; Ho, C. T.; Chang, S. Nitrogen-containing heterocyclic compounds identified in the volatile flavor constituents of roasted beef. *J. Agric. Food Chem.* **1983**, *31*, 1030−1033.
(8) Sagaguchi, M.; Shibamoto, T. Formation of heterocyclic compounds from the reaction of cysteamine and D-glucose, acetaldehyde, or glyoxal. *J. Agric. Food Chem*. **1978**, *26*, 1179−1183.
(9) Kerler, J.; van der Ven, J. G. M.; Weenen, H. α-Acetyl-*N*-heterocycles in the Maillard reaction. *Food Rev. Int*. **1997**, *13*, 553−575.
(10) Hofmann, T.; Schieberle, P. Identification of key aroma compounds generated from cysteine and carbohydrates under roasting conditions. *Z. Lebensm.-Unters.-Forsch*. **1998**, *207*, 229−236.
(11) Tonsbeek, C. H. T.; Copier, H.; Plancken, A. J. Components contributing to beef flavour. Isolation of 2-acetyl-2-thiazoline from beef broth. *J. Agric. Food Chem*. **1971**, *19*, 1014−1016.
(12) Cerny, C.; Grosch, W. Evaluation of potent odorants of roasted beef by aroma extract dilution analysis. *Z. Lebensm.-Unters.-Forsch*. **1992**, *194*, 322−325.
(13) Flament, I. U.S. Patent 3,881,025, 1975.
(14) Hofmann, T.; Schieberle, P. Studies on the formation and stability of the roast-flavor compound 2-acetyl-2-thiazoline. *J. Agric. Food Chem*. **1995**, *43*, 2946−2950.
(15) Münch, P.; Hofmann, T.; Schieberle, P. Comparison of key odorants generated by thermal treatment of commercial and self-prepared yeast extracts: Influence of the amino acid composition on odorant formation. *J. Agric. Food Chem*. **1997**, *45*, 1338−1344.
(16) Sen, A.; Laskawy, G.; Schieberle, P.; Grosch, W. Quantitative determination of β-damascenone in foods using a stable isotope dilution assay. *J. Agric. Food Chem.* **1991**, *39*, 757−759.
(17) Blank, I.; Lin, J.; Arce Vera, F.; Welti, D. H.; Fay, L. B. Identification of potent odorants formed by autoxidation of arachidonic acid − Structure elucidation and synthesis of (*E,Z,Z*)-2,4,7-tridecatrienal. *J. Agric. Food Chem.* **2001**, *49*, 2959−2965.
(18) van den Dool, H.; Kratz, P. A generalization of the retention index system including linear temperature programmed gas−liquid partition chromatography. *J. Chromatogr*. **1963**, *11*, 463−471.

Received for review August 29, 2001. Revised manuscript received January 10, 2002. Accepted January 14, 2002.

JF011170D