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Generation of Thiols by Biotransformation of Cysteine−Aldehyde Conjugates with Baker's Yeast

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Baker's yeast was shown to catalyze the transformation of cysteine−furfural conjugate into 2-furfurylthiol. The biotransformation's yield and kinetics were influenced by the reaction parameters such as pH, incubation mode (aerobic and anaerobic), and substrate concentration. 2-Furfurylthiol was obtained in an optimal 37% yield when cysteine−furfural conjugate at a 20 mM concentration was anaerobically incubated with whole cell baker's yeast at pH 8.0 and 30 °C. Similarly to 2-furfurylthiol, 5-methyl-2-furfurylthiol (11%), benzylthiol (8%), 2-thiophenemethanethiol (22%), 3-methyl-2-thiophenemethanethiol (3%), and 2-pyrrolemethanethiol (6%) were obtained from the corresponding cysteine−aldehyde conjugates by incubation with baker's yeast. This work indicates the versatile bioconversion capacity of baker's yeast for the generation of thiols from cysteine−aldehyde conjugates. Thanks to its food-grade character, baker's yeast provides a biochemical tool to produce thiols, which can be used as flavorings in foods and beverages.

KEYWORDS: Aroma; baker's yeast; cysteine conjugates; 2-furfurylthiol; benzylthiol; 5-methyl-2-furfurylthiol; 2-thiophenemethanethiol; 3-methyl-2-thiophenemethanethiol; 2-pyrrolemethanethiol

INTRODUCTION

High consumer demand for natural flavors has stimulated a number of research activities aimed at developing novel biocatalytically processed products with the use of microbial cells or isolated enzymes. Recently, a number of review articles have been published about the possibilities of using microorganisms to perform biochemical conversions (*1*−*7*). These studies showed that the microbial processes seem to be the most promising ways to produce natural flavor compounds or complex mixtures thereof (*8*−*10*). Many microorganisms are able to produce flavor compounds by fermentation starting from simple nutrients such as sugars, amino acids, and fatty acids. These readily available natural precursors can be converted to valuable flavors such as carboxylic acid esters or products of lipid oxidation (e.g., alcohols, aldehydes, and ketones), which contribute to the aroma of food (*11*−*13*). Little data is, however, available on the biogeneration of sulfur-containing compounds, e.g., thiols, heterocyclic thiols, and disulfides (*14*, *15*). The occurrence of such biotransformations requires more specific precursors and follows specific biochemical pathways (*11*).

Many thiols have been isolated from cooked foods, including meat and chocolate. Some of them are believed to be particularly important in roasted coffee aroma (*16*, *17*). For savory flavors, thiols are among the most important aroma compounds due to their odor characters and their low odor thresholds (*18*).

Furans such as 2-furfurylthiol and 5-methyl-2-furfurylthiol are well-known aroma compounds, which have been identified among the potent volatile flavors of roasted coffee (*19*). Hofmann et al. reported 5-methyl-2-furfurylthiol as the most potent odorant of thermally treated rhamnose/cysteine solution (*20*). This compound was also identified among volatiles isolated from yeast extracts (*21*).

Thiophenes have been identified in a wide range of food systems in which they significantly contribute to the sensory properties. They are responsible for the mild sulfurous odor of cooked meat (*22*) and chicken (*23*). The formation of thiophenes in various foods and in model systems has been described (*24*−*27*). The application of thiophene derivatives in foodstuffs to enhance their flavor has also been reported (*28*).

Pyrrole and derivatives are a group of nitrogen-containing heterocyclic compounds found among the Maillard reaction products in processed foods. The formation of pyrroles is thought to proceed via the Strecker degradation involving proline or hydroxyproline and dicarbonyl compounds (*29*). Thiol-containing pyrroles have been chemically synthesized (*30*). However, their application as a flavoring material has not received much attention (*31*, *32*).

The majority of the thiols mentioned above was produced by organic synthesis or by process flavors (Maillard reaction). 2-Furfurylthiol is the only heterocyclic-containing aroma thiol, which has been generated via a biochemical process. This aroma compound was produced by the biotransformation of cysteine−furfural conjugate with various bacteria possessing β-(C−S)

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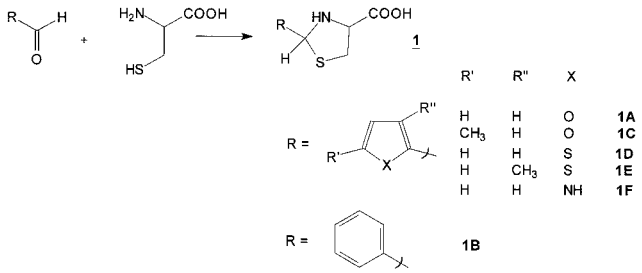

Figure 1. Chemical structure of cysteine–aldehyde conjugates used for incubation with baker's yeast.

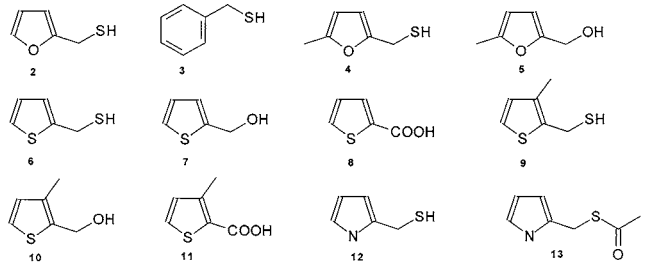

Figure 2. Chemical structures of biogenerated aroma compounds.

lyase enzymatic activity (*33*). However, these bacteria are not food-grade.

Not many food-grade microorganisms are known to possess β-(C−S) lyase activity (*14*, *32*). Only in *Saccharomyces cerevisiae* is β-cystathionase known to occur (*35*). It cleaves the C−S bond of the cysteine moiety in cystathionine. Therefore, the capability of baker's yeast was investigated for the generation of thiols from related cysteine−aldehyde conjugates. The aim was to find a biochemical tool to produce thiols with the use of a food-grade microorganism. Results of this study are reported here focusing on the production of thiols possessing furan, benzyl, thiophene, and pyrrole backbones.

MATERIALS AND METHODS

Materials. All chemicals were of analytical grade and commercially available. Furfural, 5-methyl-2-furfural, 2-thiophenecarboxaldehyde, 3-methyl-2-thiophenecarboxaldehyde, 2-pyrrolecarboxaldehyde, and benzaldehyde were purchased from Fluka (Buchs, Switzerland), and L-cysteine was purchased from Aldrich (Buchs, Switzerland). Fresh yeast cream was from Hefe Schweiz AG (Stettfurt, Switzerland). Organic solvents were purified by distillation using a Vigreux column (60 cm × 3 cm).

Preparation of Cysteine−Aldehyde Conjugates. Cysteine−aldehyde conjugates were prepared following a previously reported method (*36*) with some modifications. Aldehyde (11 mmol, neat or dissolved in ethanol) was added dropwise to a solution of cysteine (10 mmol) in distilled water (30 mL). After it was stirred for 1 h at room temperature, the resulting precipitate was filtered, washed with water and ethanol (or a mixture thereof), and then dried under vacuum. The products were analyzed by thin-layer chromatography (TLC) using *n*-butanol/acetic acid/water (2:1:1) as eluant. The following cysteine−aldehyde conjugates (**Figure 1**) were prepared, and pure products (as shown by TLC) were tentatively characterized by mass spectrometry.

*Cysteine−Furfural (**1A**).* Yield, 86%. MS-EI *m*/*z* (relative intensity): 199 (48), 153 (14), 127 (15), 107 (100), 94 (56), 80 (46), 43 (76).

*Cysteine−Benzaldehyde (**1B**).* Yield, 97%. MS-EI *m*/*z* (relative intensity): 209 (33), 176 (11), 164 (31), 137 (100), 130 (46), 117 (69), 104 (100), 77 (42).

*Cysteine−5-Methyl-2-furfural (**1C**).* Yield, 65%. MS-EI *m*/*z* (relative intensity): 213 (100), 181 (11), 167 (28), 141 (14), 125 (33), 121 (60), 109 (75), 94 (35).

*Cysteine−2-Thiophenecarboxyaldehyde (**1D**).* Yield, 88%. MS-EI *m*/*z* (relative intensity): 215 (26), 170 (20), 143 (90), 84 (20).

*Cysteine−3-Methyl-2-thiophenecarboxyaldehyde (**1E**).* Yield, 40%. MS-EI *m*/*z* (relative intensity): 229 (94), 183 (35), 157 (55), 97 (38).

*Cysteine−2-Pyrrolecarboxyaldehyde (**1F**).* Yield, 75%. MS-EI *m*/*z* (relative intensity): 198 (16), 154 (10), 132 (12), 120 (40), 111 (98).

General Bioconversion Procedure. Five liters of commercial fresh yeast cream (20% dry weight) was centrifuged, and the supernatant was discarded. The yeast cells were resuspended in 1 L of 0.1 M phosphate buffer. The yeast suspension (300 mL) was then placed in a 500 mL flask equipped with an electrode and a magnetic stirrer (500 rpm). The flask was immersed in an oil bath heated at 30 °C. When the anaerobic assays were performed, the yeast suspension was flushed for 15 min with a nitrogen flow and the whole apparatus was kept under nitrogen during the incubation period. The suspension was adjusted to the desired pH, which was automatically maintained throughout the reaction with 2 M sodium hydroxide solution using a Metrohm pH-stat device model 691 (Metrohm, Herisau, Switzerland). The cysteine−aldehyde conjugate (6 mmol, 20 mM) was then added all at once.

Five milliliters of the reaction mixture was sampled at different incubation times. The sample was adjusted to pH 4.0 with 2 M hydrochloric acid. Five hundred microliters of a solution of benzylthiol in diethyl ether (2000 ppm) was then added as an internal standard. In the case of the biogeneration of benzylthiol, a solution of 2-furfurylthiol in diethyl ether (2000 ppm) was used as the internal standard. After sodium chloride (3 g) was added, the sample was extracted with diethyl ether (3 × 15 mL). The diethyl ether extracts were separated from the mixture by centrifugation (15 min, 5000 rpm), combined, dried over sodium sulfate, and concentrated to a volume of 2 mL using a Vigreux column (30 cm × 1 cm). The concentrated solution was then analyzed by various gas chromatographic techniques. The yields of all thiols are given in mol % relative to original corresponding cysteine conjugates (20 mM). The concentrations of targeted thiols were calculated on the basis of their peak area (gas chromatography-flame ionization detection (GC-FID) and GC-FPD) relative to that of the internal standard assuming that their response factors were equal to that of the internal standard.

GC Analyses (GC, GC-O, and GC-MS). GC and 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 a cold on-column injector, FID and FPD detectors, and a sniffing port (*37*). Fused silica capillary columns (DB-1701, DB-Wax, and DB-FFAP) were used, all of 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 column was 35 °C (2 min), 40 °C/min to 50 °C (1 min), 6 °C/min to 240 °C (10 min) and for the DB-Wax and DB-FFAP columns, 35 °C (2 min), 6 °C/min to 180 °C, 10 °C/min to 240 °C (10 min). The injected volume for GC and GC−O was 0.2 μL. Linear retention indices (RI) were calculated by linear interpolation according to van den Dool and Kratz (*38*).

GC-MS analyses were performed on a MAT-8430 mass spectrometer (Finnigan, Bremen, Germany) combined with an HP 5890 gas chromatograph using the above GC conditions. The mass spectrometer was operated in both electron mode at 70 eV and positive chemical ionization mode at 150 eV with ammonia as the reagent. The interface temperature was 220 °C, and the source temperature was set at 180 °C. All data were processed with MassLib (MSP Friedli, Koeniz, Switzerland). The known compounds were identified by comparison of their RI and MS with literature data. All new compounds were tentatively identified on the basis of their MS data analysis.

RESULTS AND DISCUSSION

Biogeneration of 2-Furfurylthiol. To evaluate the capacity of baker's yeast to generate thiols, the cysteine−furfural conjugate **1A** was used as a model substrate targeting the biogeneration of 2-furfurylthiol **2** (**Figure 2**) because of its occurrence in food and reaction flavors and its typical sulfury, roasted, and coffeelike odors. 2-Furfurylthiol **2** was generated from cysteine conjugate **1A** in a bioconversion yield of about

Table 1. Volatile Components Identified in the Diethyl Ether Extract from the Biotransformation of the Cysteine–Furfural Conjugate under Aerobic Conditions[a]

compound[b]	linear retention index FFAP	linear retention index DB-1701	aroma quality[c] (GC-O)
S-methylthioacetate	1046	758	cheese, eggy
S-ethylthioacetate	1080		sulfury, garlic, onion
2-methyl-1-propanol	1084	840	
thioacetic acid	1124	711	sulfury, onion, garlic
diethyl disulfide		981	fruity, pungent, sulfury
3-methyl-1-butanol	1204	844	
2-methyl-1-butanol	-	951	
acetic acid	1425	785	
2-furfurylthiol	1435	998	burnt, roasted, onion
2-furfural	1457	908	almond, caramel-like
2-furfurylmethyl sulfide	1485	1089	garlic, eggy
2-mercapto-1-ethanol	1498		
butanoic acid	1618	980	
2-furfuryl alcohol	1665	1012	cooked sugar, ethereal
3-methylbutanoic acid	1669	1037	
S-furfuryl thioacetate	1769	1275	burnt, roasted, savory
2-furanoic acid	2433[d]		
bis(2-furfuryl) disulfide	2600	1862	fried onion, burnt coffee

[a] The reaction was carried out aerobically at pH 6.9 and 30 °C. The substrate concentration was 20 mM. [b] Identification was based on comparison of RI and MS with literature data using the MassLib database. [c] GC-O has been performed only for the main aroma compounds. [d] DB-wax capillary column was used to determine the RI.

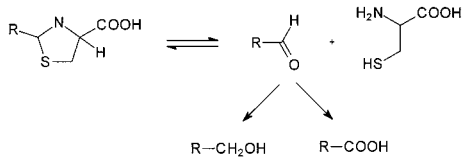

Figure 3. Hypothetical pathways leading to alcohols and acids from cysteine–aldehyde conjugates.

25% after 24 h of incubation with baker's yeast (conditions: 20 mM substrate, pH 6.9, aerobic mode). Besides 2-furfurylthiol, several other sulfur-containing compounds were detected in the reaction mixture (**Table 1**). However, their concentrations were quite low (<1%) except for thioacetic acid (10%) and *bis*(2-furfuryl) disulfide, the dimer of 2-furfurylthiol (10%). Besides the common metabolites from baker's yeast itself, furfural and in particular furfuryl alcohol were identified in the reaction mixture by GC-MS. Furfural, possibly originating from hydrolysis of cysteine−furfural conjugate **1A**, was enzymatically reduced into 2-furfuryl alcohol (**Figure 3**).

To better understand the biotransformation of cysteine−furfural conjugate **1A** into 2-furfurylthiol **2**, the influence of the incubation conditions (e.g., incubation mode (aerobic, anaerobic), pH, and substrate concentration) on the biotransformation kinetic and yield were investigated.

Influence of Aerobic and Anaerobic Incubation Mode. Biotransformation of the cysteine−furfural conjugate **1A** (20 mM) was performed with baker's yeast at pH 6.9 under aerobic and anaerobic conditions. As shown in **Tables 1** and **2**, many compounds, which are common metabolites from baker's yeast, were mostly generated under aerobic rather than anaerobic conditions. Acetic acid and furfuryl alcohol were the major compounds identified in both assays. As shown in **Figure 4**, 2-furfurylthiol **2** was generated with an optimal yield of 28% after 48 h of reaction time and under nitrogen, while the yield was only about 25% under aerobic conditions and after 24 h of incubation. Moreover, the degradation of 2-furfurylthiol was much more pronounced under aerobic than anaerobic mode. This difference can be explained by the chemical oxidative dimerization of 2-furfurylthiol **2** into *bis*(2-furfuryl) disulfide (10%, data not shown), which is favored under oxidizing conditions.

Table 2. Volatile Components Identified in the Diethyl Ether Extract from the Biotransformation of the Cysteine–Furfural Conjugate under Anaerobic Conditions[a]

compound[b]	linear retention index FFAP	linear retention index DB-1701	aroma quality[c] (GC-O)
2-methyl-1-propanol	1084	840	
3-methyl-1-butanol	1204	844	
acetic acid	1425	785	
2-furfurylthiol	1435	998	burnt, roasted, onion
2-furfural	1457	908	
2-furfurylmethyl sulfide	1485	1089	garlic, eggy
1-mercapto-2-propanol			
2-mercapto-1-ethanol	1498		
2-furfuryl alcohol	1665	1012	
3-methylbutanoic acid	1669	1037	
S-furfuryl thioacetate	1769	1275	burnt, roasted, savory
2-furanoic acid	2433[d]		
bis(2-furfuryl) disulfide	2600	1862	fried onion, burnt coffee

[a] The reaction was carried out anaerobically at pH 6.9 and 30 °C. The substrate concentration was 20 mM. [b] Identification was based on comparison of RI and MS with literature data using the MassLib database. [c] GC-O was performed only for the main aroma compounds. [d] DB-wax capillary column was used to determine the RI.

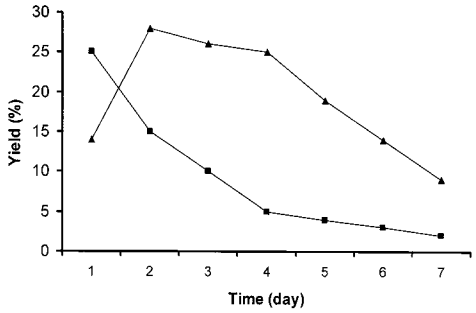

Figure 4. Influence of incubation mode on the biogeneration of 2-furfurylthiol: aerobic (■) and anaerobic (▲).

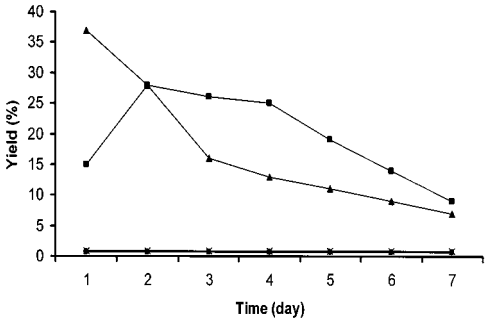

Figure 5. Influence of the pH on the biogeneration of 2-furfurylthiol at pH 6.0 (×), pH 6.9 (■), pH 8.0 (▲), and pH 9.0 (●).

Influence of pH. To determine the optimal pH for the biotransformation of cysteine−furfural conjugate **1A** (20 mM) with baker's yeast, anaerobic incubation trials were performed at different pH values (e.g., 6.0, 6.9, 8.0, and 9.0). As shown in **Figure 5**, the yield of 2-furfurylthiol **2** was less than 1% at pH 6.0 and pH 9.0, while at pH 6.9 and pH 8.0 it was 15 and 37%, respectively, after 24 h of incubation. However, the amounts of **2**, which were optimally obtained after 24 h of incubation at pH 8.0, decreased more dramatically with longer incubation

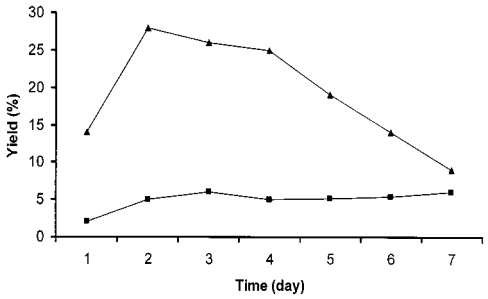

Figure 6. Effect of substrate concentration on the biogeneration of 2-furfurylthiol at 40 (■) and 20 (▲) mM.

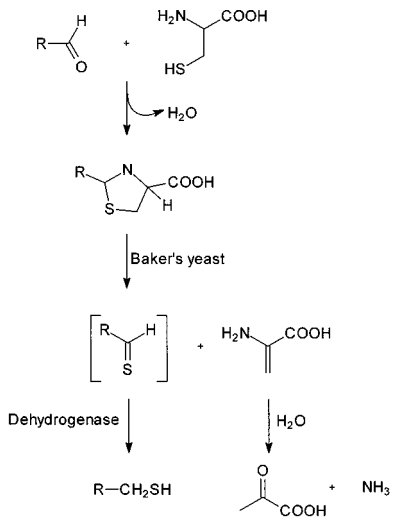

Figure 7. Hypothetical pathways leading to thiols from cysteine–aldehyde conjugates using baker's yeast as the biocatalyst.

times as compared to pH 6.9 (**Figure 5**). This decrease could be partially attributed to the increase of dimerization of **2** into *bis*(2-furfuryl) disulfide, which was favored at pH 8.0 as compared to pH 6.9 (data not shown).

Influence of Substrate Concentration. The effect of substrate concentration on the biogeneration of 2-furfuylthiol **2** by baker's yeast was studied under anaerobic mode at pH 6.9 and 30 °C. Two substrate concentrations, 20 and 40 mM, were used. As shown in **Figure 6**, compound **2** was generated in a yield of 28% after 48 h of reaction when the substrate concentration was 20 mM. This yield dropped to 6% when the concentration was doubled (i.e., 40 mM). Moreover, the yield of 6% was reached after 3 days of incubation time showing slower kinetics in the biogeneration of **2** at higher substrate concentrations. These results indicate that the baker's yeast enzymatic activities involved in the transformation of cysteine−furfural conjugate into 2-furfurylthiol are strongly influenced by substrate concentration.

Results from incubation trials with cysteine−furfural conjugate **1A** clearly indicate the high capacity of baker's yeast in the biogeneration of 2-furfurylthiol **2**. The biochemical pathways could involve β-(C−S) lyase and dehydrogenase of baker's yeast (**Figure 7**). The optimal incubation conditions were pH 8.0, substrate concentration 20 mM, and anaerobic incubation mode.

Biogeneration of Other Thiols. Positive results obtained with 2-furfurylthiol prompted us to investigate the capacity of baker's yeast to generate other thiols of various chemical structures. Emphasis was given to heterocyclic-containing aroma thiols occurring in food and food systems and useful as flavorings.

Biogeneration of Benzylthiol. As shown by GC-FPD analysis, benzylthiol **3** was the major sulfur compound of the reaction mixture from the cysteine−benzaldehyde conjugate **1B** incubated with baker's yeast. This aroma volatile was generated in a yield of 8% after 72 h of incubation. Among other sulfur-containing volatiles (**Table 3**), benzylmethyl sulfide and *S*-benzyl thioacetate, which are derivatives of benzylthiol, were also identified in the reaction mixture. As shown in **Table 3**, the volatile components containing no sulfur, which were identified in the diethyl ether extract of the reaction mixture, can be divided into two groups with respect to their origin. Compounds such as propanoic acid and phenyl ethanol are common metabolites from baker's yeast, while other compounds were generated from the cysteine conjugate **1B**. Benzyl alcohol for instance was probably generated by enzymatic reduction of benzaldehyde, which was itself released by hydrolysis of the conjugate **1B** (**Figure 3**). Other benzaldehyde derivatives were also detected in the reaction mixture, i.e., benzyl acetate and benzoic acid.

Table 3. Volatile Components Identified in the Diethyl Ether Extract from the Biotransformation of the Cysteine–Benzaldehyde Conjugate[a]

compound[b]	linear retention index FFAP	linear retention index DB-1701	aroma quality[c] (GC-O)
S-methylthioacetate	1046	758	cheese, eggy
thioacetic acid	1124	711	sulfury, onion, garlic
acetic acid	1425	785	acid, pungent
1-mercapto-2-propanol	1492		
2-mercapto-1-ethanol	1498		
benzaldehyde		1240	almond, sweet aromatic
2-methylthio-1-ethanol			green, sulfury
propanoic acid	1525	899	fruity, sour
benzylthiol	1626	1181	cabbage, roasted
benzylmethyl sulfide	1664		
2-methylthio acetic acid			
benzyl acetate		1256	green, fresh, fruity
benzyl alcohol	1875	1205	balsamic, fruity
phenylethanol	1909		fruity, sweet, floral
S-benzylthioacetate	1986	1450	
3-methylthiopropanoic acid	2298		
4-methylthiobutanoic acid	2384		
benzoic acid	2428	1345	1345

[a] The reaction was carried out anaerobically at pH 6.9 and 30 °C. The substrate concentration was 20 mM. [b] Identification was based on comparison of RI and MS with literature data using the MassLib database. [c] GC-O was performed only for the main aroma compounds.

Biogeneration of 5-Methyl-2-furfurylthiol. Biotransformation of cysteine−5-methyl-2-furfural conjugate **1C** with whole cell baker's yeast (pH 8.0, 30 °C and at 20 mM substrate) generated 5-methyl-2-furfurylthiol **4** in a yield of 11%. Thiol **4** was the most intense odorant among the important odor active volatiles identified in the reaction mixture by different chromatographic techniques (GC, GC-MS, GC-O) as listed in **Table 4**. Thiol **4** elicits an attractive coffeelike and roasted note. The odor quality as well as the chromatographic and spectroscopic properties (RI, MS) of this thiol were identical to those reported in the literature (*20*, *21*). The odor threshold value of this aroma compound **4** is very low (0.006 ng/L of air), which is comparable to that of 2-furfurylthiol **2** and 2-methyl-3-furanthiol known as very intense odorants eliciting coffeelike and cooked meat flavor notes (*17*, *18*).

The major component identified in the reaction mixture was, however, 5-methyl-2-furfuryl alcohol **5**. It was obtained in a yield of 80% relative to the substrate cysteine conjugate **1C**. This alcohol likely originated from the enzymatic reduction of the corresponding aldehyde, itself released by hydrolysis of the cysteine conjugate **1C** (**Figure 3**). These results indicate a much

Table 4. Most Odor Active Volatiles Identified in the Diethyl Ether Extract from the Biotransformation of the Cysteine–5-Methyl-2-furfural Conjugate[a]

compound[b]	linear retention index FFAP	linear retention index DB-1701	aroma quality (GC-O)	MS-EI data *m/z* (relative intensity)
5-methyl-2-furfurylthiol	1497	1086	coffee, roasted	128 (18), 113 (2), 95 (100), 80 (5)
5-methyl-2-furfuryl alcohol	1723	1103		112 (100), 97 (35), 95 (85), 43 (57)
S-(5-methyl-2-furfuryl)thioacetate[c]	1840	1366	burnt, caramel	170 (10), 127 (2), 95 (100), 43 (12)
bis(5-methyl-2-furfuryl) disulfide		2026	sulfury, burnt	254 (5), 189 (3), 127 (10), 95 (100)

[a] The reaction was carried out anaerobically at pH 8.0 and 30 °C. The substrate concentration was 20 mM. [b] Identification was based on comparison of RI and MS with literature data using the MassLib database. [c] Tentatively identified on the basis of MS data analysis.

Table 5. Most Odor Active Volatiles Identified in the Diethyl Ether Extract from the Biotransformation of the Cysteine–2-Thiophenecarboxaldehyde Conjugate[a]

compound[b]	linear retention index FFAP	linear retention index DB-1701	aroma quality (GC-O)	MS-EI data *m/z* (relative intensity)
2-thiophenemethanethiol	1702	1197	meaty, roasted, grilled	130 (35), 97 (100)
2-thiophenemethanol	1950	1216	savory, sulfury	114 (100), 97 (45), 85 (50)
2-thiophenecarboxylic acid	2519	1484	sulfury, meaty, animal	128 (100), 111 (42), 83 (20), 45 (20)
bis(2-thiophenemethyl) disulfide	2641	2232	roasted, burnt	258 (38), 194 (100), 129 (8), 97 (60)

[a] The reaction was carried out anaerobically at pH 8.0 and 30 °C. The substrate concentration was 20 mM. [b] Identification was based on comparison of RI and MS with literature data using the MassLib database.

Table 6. Most Odor Active Volatiles Identified in the Diethyl Ether Extract from the Biotransformation of the Cysteine–3-Methyl-2-thiophenecarboxaldehyde Conjugate[a]

compound[b]	linear retention index FFAP	linear retention index DB-1701	aroma quality (GC-O)	MS-EI data *m/z* (relative intensity)
3-methyl-2-thiophenemethanethiol	1820	1294	coffee, roasted	144 (23), 129(2), 111 (100), 67 (6)
3-methyl-2-thiophenemethanol	2034	1316	fruity, pungent	128 (100), 113 (75), 111 (82), 99 (30)
3-methyl-2-thiophenecarboxylic acid	2501	1590	animal, spicy	142 (100), 125 (55), 97 (62), 45 (12)

[a] The reaction was carried out anaerobically at pH 8.0 and 30 °C. The substrate concentration was 20 mM. [b] Compounds tentatively identified on the basis of their MS data analysis.

Table 7. Most Odor Active Volatiles Identified in the Ether Extract from the Biotransformation of the Cysteine–2-Pyrrolecarboxaldehyde Conjugate[a]

compound [b]	linear retention index FFAP	linear retention index DB-1701	aroma quality (GC-O)	MS-EI data *m/z* (relative intensity)
2-pyrrolemethanethiol	2094	1309	earthy, sulfury	113 (50), 80 (100), 86 (12), 53 (38)
2-pyrrolemethyl thioacetate	2293	1492	roasted, savory	155 (85), 112 (10), 80 (100), 68 (35), 43 (40)

[a] The reaction was carried out anaerobically at pH 8.0 and 30 °C. The substrate concentration was 20 mM. [b] Compounds tentatively identified on the basis of their MS data analysis.

faster hydrolysis/reduction of the substrate as compared to its biotransformation into 5-methyl-2-furfurylthiol **4**.

Biogeneration of 2-Thiophenemethanethiol. Bioconversion of cysteine−2-thiophene carboxaldehyde conjugate **1D** was performed at pH 8.0 and 30 °C. 2-Thiophenemethanethiol **6** was obtained in a yield of 22% after 40 h of incubation. It was characterized based on chromatographic properties and mass spectra in comparison with literature data (*39*, *40*). Thiol **6** was the most intense odorant with roasted, grilled, and meatlike odors among the volatiles identified in the diethyl ether extract as listed in **Table 5**. Other sulfur-containing volatiles were identified in the reaction mixture such as 2-thiophenemethanol **7**, 2-thiophenecarboxylic acid **8**, and the dimer of the thiol **6**. The alcohol **7** has been identified in many foods such as cooked beef (*41*), cooked asparagus (*42*), and corn (*43*). As found by GC-O (**Table 5**), the alcohol **7** possesses a savory and sulfury odor character, while sulfury, meaty, and animal odors were attributed to the carboxylic acid **8**. In a similar way as with other cysteine−aldehyde substrates, the hydrolysis of cysteine−2-thiophenecarboxaldehyde **1D** followed by enzymatic reduction and oxidation of the released aldehyde (**Figure 2**) might account for the generation of compounds **7** and **8**. 2-Thiophenemethanethiol **6** could be useful as a flavoring since it was described to impart a coffeelike odor when evaluated in a base of either syrup or soluble coffee (*28*).

Biogeneration of 3-Methyl-2-thiophenemethanethiol. The biotransformation of cysteine−3-methyl-2-thiophenecarboxaldehyde conjugate **1E** (20 mM) with baker's yeast at pH 8.0 produced 3-methyl-2-thiophenemethanethiol **9** in 3% yield after 40 h of incubation. Its odor character was found to be roasted and coffeelike by GC-O analysis (**Table 6**). The other compounds identified in the reaction mixture were 3-methyl-2-thiophenemethanol **10** and 3-methyl-2-thiophenecarboxylic acid **11**. Alcohol **10** was by far the most abundant volatile component

and was obtained in a yield of 45%. The odor quality of this volatile was pungent and fruity (GC-O data in **Table 6**). The generation of compounds **10** and **11** again suggests hydrolysis of the cysteine−aldehyde conjugate **1E** and the subsequent enzymatic reduction/oxidation of the released aldehyde.

Biogeneration of 2-Pyrrolemethanethiol. Biotransformation of cysteine−2-pyrrole carboxyaldehyde conjugate **1F** resulted in two main odor active volatiles: 2-pyrrole methanethiol **12** and 2-pyrrolemethyl thioacetate **13** as tentatively identified by GC-MS. Thiol **12** was obtained in a yield of 6% after 24 h of incubation. The aroma character of thiol **12** was earthy, fermented, and sulfury, while roasted and savory characters were attributed to thioacetate **13**, as analyzed by GC-O (**Table 7**). Thiol **12** and its acetate **13** newly generated with the use of baker's yeast could, therefore, be of use as flavorings in foods.

CONCLUSION

The results obtained in this work indicate that baker's yeast possesses the enzymatic capacity to generate thiols from cysteine−aldehyde conjugates. Thiols of various chemical structures were obtained, indicating that the baker's yeast enzymes involved in the biochemical transformation, in particular the β-(C−S) lyase, are not substrate specific. Beside thiols, the generation of alcohols and carboxylic acids of similar structure as the starting aldehydes suggested the involvement of baker's yeast dehydrogenases and oxidases to transform the aldehyde, which was likely released from cysteine−aldehyde conjugates by chemical hydrolysis (**Figure 3**). The biogeneration yield of thiols was high, in particular for 2-furfurylthiol **2** (37%). Further yield improvement could be expected by continuous removal of thiols upon generation, as demonstrated in previous work on microbial production of 2-furfurylthiol (*33*). The capacity of baker's yeast, as demonstrated by this work, provides a biochemical tool to produce thiols. These aroma compounds thus obtained with a food-grade microorganism (baker's yeast) could be of great potential use as flavorings in foods and beverages. These findings were included in a patent application (*44*).

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