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Chemoenzymatic Synthesis of Aroma Active 5,6-Dihydro- and Tetrahydropyrazines from Aliphatic Acyloins Produced by Baker's Yeast

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Twenty-five acyloins were generated by biotransformation of aliphatic aldehydes and 2-ketocarboxylic acids using whole cells of baker's yeast as catalyst. Six of these acyloins were synthesized and tentatively characterized for the first time. Subsequent chemical reaction with 1,2-propanediamine under mild conditions resulted in the formation of thirteen 5,6-dihydropyrazines and six tetrahydropyrazines. Their odor qualities were evaluated, and their odor thresholds were estimated. Among these pyrazine derivatives, 2-ethyl-3,5-dimethyl-5,6-dihydropyrazine (roasted, nutty, 0.002 ng/L air), 2,3-diethyl-5-methyl-5,6-dihydropyrazine (roasted, 0.004 ng/L air), and 2-ethyl-3,5-dimethyltetrahydropyrazine (bread crustlike, 1.9 ng/L air) were the most intensive-smelling aroma active compounds.

KEYWORDS: Pyrazine derivatives; acyloins; baker's yeast; carboligation; flavor

INTRODUCTION

Alkylpyrazines are aroma active molecules that impart nutty, roasted, or earthy tonalities (*1*−*3*). Their identification, characterization, synthesis, and application in food products have been widely studied *(4*−*6)*. Alkylpyrazines have been identified in plants (*7*), insects (*8*), and fermented and processed foods (*6*). In fermented foodstuffs, such as soy sauce, sake, and vinegar, pyrazines arise from microbial processes (*9*). In processed foods, the generation of alkylpyrazines is well-known to be associated with the Maillard reaction (*10*) or pyrolysis reactions (e.g., roasted coffee) (*11*). The closely related 5,6-dihydropyrazines, however, have received little attention. They have been produced from diketones and diamines at ambient temperatures (*12*) and described as precursors of the corresponding pyrazines (*13*, *14*). A systematic study of the aroma qualities and aroma thresholds of these compounds has not been undertaken. Tetrahydropyrazines have never been reported as aroma active compounds so far.

In the present work, thirteen 5,6-dihydropyrazines and six tetrahydropyrazines were generated by a two step chemoenzymatic approach. In the first step, a pool of acyloins was obtained by biotransformation of aliphatic aldehydes and 2-ketocarboxylic acids using whole cells of baker's yeast as the catalyst. The second step consisted of a chemical reaction of these acyloins with 1,2-propanediamine under mild conditions. Some of the generated pyrazine derivatives showed pronounced roasted or earthy aromas with low odor thresholds.

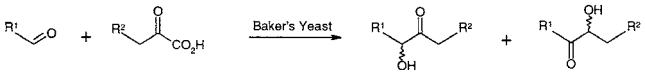

Figure 1. Biogeneration of acyloins using whole cells of baker's yeast.

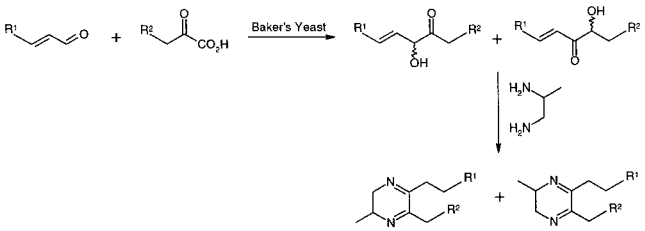

Figure 2. Chemoenzymatic synthesis of 5,6-dihydropyrazines.

MATERIALS AND METHODS

Materials. Dried baker's yeast was purchased from Hefe Schweiz (Stettfurt, Switzerland). All chemicals were from Sigma Aldrich Chemical Co. (Buchs, Switzerland) and of analytical grade quality. Organic solvents were distilled using a Vigreux column (1 m × 1 cm) before their use.

Gas Chromatography (GC) Analyses. GC-olfactometry (GC-O) analyses were performed on a Agilent 6890 Series equipped with a splitless injector and a sniffing port. The separation of volatiles was performed using helium as the carrier gas (1.5 mL min^{-1}) and nitrogen (45 kPa) as the makeup gas for the flame ionization detector (FID, 250 °C). The gas flow was divided at a point 200 mm before reaching the detector. A part of the gas flow was deviated to the FID, while the other part was led to a sniffing port. The sniffing port was heated to 200 °C in order to prevent condensation of the molecules and ended into a glass funnel. For the separation of the compounds, a DB-1 or a DB-Wax capillary column (both 30 m × 0.25 mm, film thickness 0.25 μm, J & W Scientific) was used. The temperature program for the DB-1

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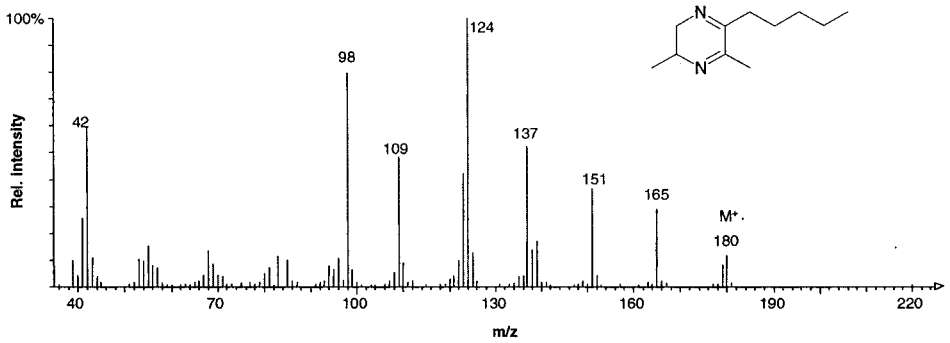

Figure 3. GC-MS/EI spectrum of 2-pentyl-3,5-dimethyl-5,6-dihydropyrazine.

Table 1. Pool of Acyloins Produced by Baker's Yeast

substrate	acyloin	yield (%)	RI[a]
acrolein[b]	4-hydroxy-1-penten-3-one	0.5	1372
	3-hydroxy-pent-1-en-4-one	2.5	1381
acrolein[c]	4-hydroxy-1-hexen-3-one	0.2	1430
	3-hydroxy-1-hexen-4-one	0.3	1453
but-(*E*)-2-enal[b]	3-hydroxy-(*E*)-4-hexen-2-one	4.9	1481
pent-(*E*)-2-enal[b]	3-hydroxy-(*E*)-4-hepten-2-one	21	1586
	2-hydroxy-(*E*)-4-hepten-3-one	14	1619
hex-(*E*)-2-enal[b]	3-hydroxy-(*E*)-4-octen-2-one	28	1683
	2-hydroxy-(*E*)-4-octen-3-one	31	1698
hept-(*E*)-2-enal[b]	3-hydroxy-(*E*)-4-nonen-2-one	7.2	1776
	2-hydroxy-(*E*)-4-nonen-3-one	4.5	1796
oct-(*E*)-2-enal[b]	3-hydroxy-(*E*)-4-decen-2-one	6.1	1946
	2-hydroxy-(*E*)-4-decen-3-one	3.7	1959
propanal[b]	2-pentanon-3-ol	0.5	1344
	3-pentanon-2-ol	3.9	1361
butanal[b]	2-hexanon-3-ol	3.8	1430
	3-hexanon-2-ol	1.6	1423
pentanal[b]	2-heptanon-3-ol	9.1	1560
	3-heptanon-2-ol	3.8	1551
hexanal[b]	2-octanon-3-ol	55	1655
	3-octanon-2-ol	19	1647
heptanal[b]	2-nonanon-3-ol	21	1768
	3-nonanon-2-ol	9.2	1757
octanal[b]	2-decanon-3-ol	4.5	1889
	3-decanon-2-ol	1.7	1874

[a] Linear RI on a DB-Wax column. [b] Pyruvate was used as the cosubstrate. [c] 2-Ketobutyric acid was used as the cosubstrate.

column was 5 min isothermal at 40 °C, then raised to 260 °C at 4 °C min^{-1}, and kept at 260 °C for 5 min, while that for the DB-Wax column was 5 min isothermal at 40 °C, then raised to 220 °C at 5 °C min^{-1}, and kept at 220 °C for 5 min. Linear retention indices (RI) were calculated according to van den Dool and Kratz (*15*). GC-MS analyses were performed on a Finnigan MAT-8430 mass spectrometer combined with an HP 5890 gas chromatograph using the same DB-1 and BD-Wax columns and the same chromatographic conditions as described above. The mass spectrometer was operated in both electron impact mode at 70 eV and positive chemical ionization 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 v 8.7 (MSP Friedli, Koeniz, Switzerland).

Biogeneration of Aliphatic Acyloins Using Whole Cells of Baker's Yeast. One gram of glucose (5.6 mmol) and 140 mg of sodium pyruvate or 155 mg of sodium 2-ketobutyric acid (1.25 mmol) were added to 50 mL of 0.1 M citrate buffer (pH 6.0), containing 10 g of dried baker's yeast, 2 mM thiamine pyrophosphate, and 20 mM $MgSO_4$. One out of several alkanals or (*E*)-2-alkenals (1.25 mmol) was added per assay, and the mixture was incubated at 23 °C. After 1 h, the reaction mixture was centrifuged and the cells were discarded. An aqueous solution, containing 145 μg of 4-hydroxy-4-methyl-pentan-2-one (1.25 μmol), was added as an internal standard to the supernatant, and the mixture was then extracted continuously with 150 mL pentane/CH_2Cl_2 (2:1) overnight. The organic phase was dried over Na_2SO_4 and concentrated

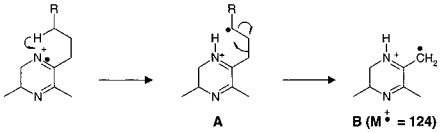

Figure 4. Suggested mechanism leading to mass fragment 124 in GC-MS/EI spectra of 5,6-dihydropyrazines.

using a Vigreux column at 40 °C. The extracts were analyzed by GC-FID and GC-MS using a DB-Wax capillary column.

Chemoenzymatic Synthesis of 5,6-Dihydropyrazines from (*E*)-2-Alkenals. One gram of glucose (5.6 mmol) and 140 mg of sodium pyruvate or 155 mg of sodium 2-ketobutyric acid (1.25 mmol) were added to 50 mL of 0.1 M citrate buffer (pH 6.0), containing 10 g of dried baker's yeast, 2 mM thiamine pyrophosphate, and 20 mM $MgSO_4$. One out of several (*E*)-2-alkenals (1.25 mmol) was added per assay, and the mixture was incubated at 23 °C. After 1 h, 925 mg of 1,2-propanediamine (12.5 mmol) was added. After it was further incubated for 90 min, the reaction mixture was centrifuged, and an aqueous solution containing 10 mg of trimethylpyrazine (82 μmol) was added to the supernatant as an internal standard. The mixture was extracted twice with diethyl ether. The combined etheral solutions were dried over Na_2SO_4 and concentrated using a Vigreux column at 40 °C. The extracts were analyzed by GC-O and GC-MS, using a DB-1 capillary column.

Chemoenzymatic Synthesis of Tetrahydropyrazines from Alkanals. One gram of glucose (5.6 mmol) and 140 mg of sodium pyruvate (1.25 mmol) were added to 50 mL of 0.1 M citrate buffer (pH 6.0), containing 10 g of dried baker's yeast, 2 mM thiamine pyrophosphate, and 20 mM $MgSO_4$. One out of several alkanals (1.25 mmol) was added per assay, and the mixture was incubated at 23 °C. After 1 h, the reaction mixture was centrifuged, and the supernatant was extracted continuously with 150 mL of pentane/CH_2Cl_2 (2:1) overnight. The organic phase was dried over Na_2SO_4, concentrated using a Vigreux column at 40 °C, and adjusted to a volume of 5 mL. A 925 mg amount of 1,2-propanediamine (12.5 mmol) was slowly added, and the mixture was further incubated for 90 min. An etheral solution containing 10 mg of trimethylpyrazine (82 μmol) was added as an internal standard, and the etheral phase was washed two times with water. The extracts were analyzed by GC-O and GC-MS, using a DB-1 capillary column.

Chemical Synthesis of 5,6-Dihydropyrazines. The synthesis of 5,6-dihydropyrazines was performed according to the protocol reported by Flament and Stoll (*12*). To 81.4 mg of 1,2-propanediamine (1.1 mmol) in 5 mL of ether, 1 mmol of one out of several diketones (2,3-butanedione, 2,3-pentanedione, 2,3-hexanedione, or 3,4-hexanedione) was slowly added. After 1 h of reaction at room temperature, the organic phases were washed twice with one volume of water, dried over Na_2SO_4, concentrated, and analyzed by GC-FID and GC-MS, using a DB-1 capillary column.

Determination of the Yields and Estimation of the Odor Threshold Values. The concentrations and yields of dihydropyrazines and tetrahydropyrazines were determined by GC using trimethylpyrazine as an internal standard. Approximate odor threshold values of tetrahydropyrazines and dihydropyrazines were estimated by GC-O using the

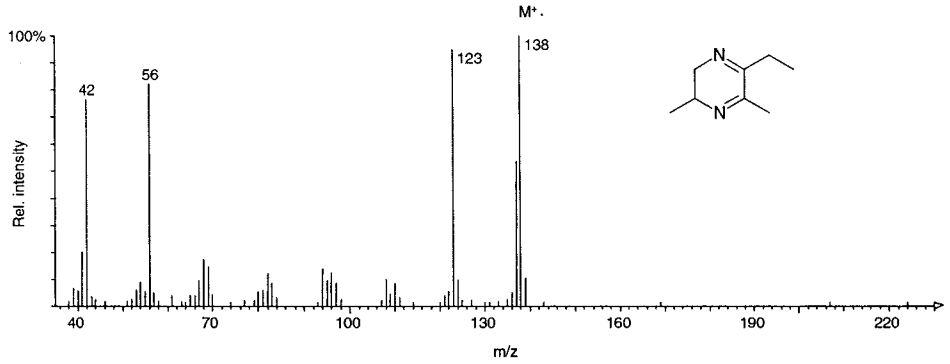

Figure 5. GC-MS/EI spectrum of 2-ethyl-3,5-dimethyl-5,6-dihydropyrazine.

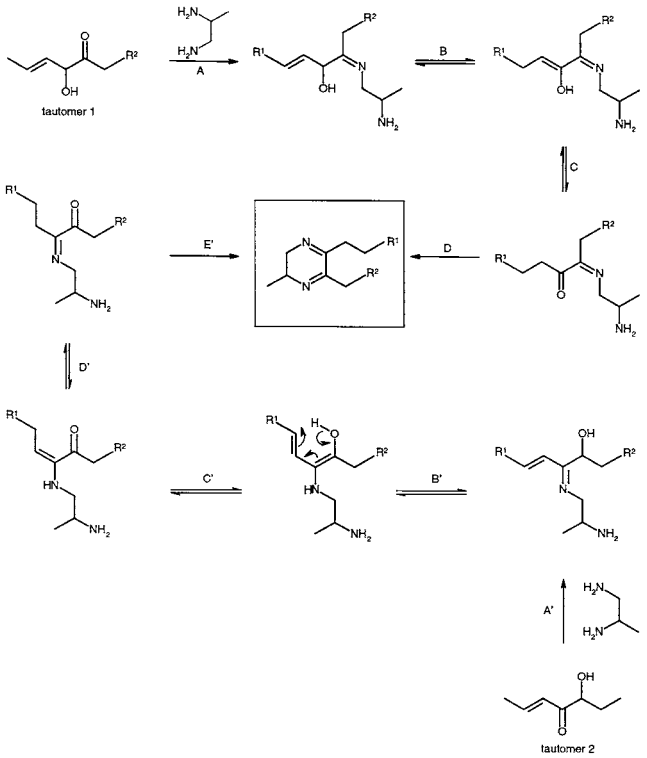

Figure 6. Suggested mechanisms for the formation of 5,6-dihydropyrazines from α,β-unsaturated acyloins and 1,2-propanediamine.

well-known method described in the literature (*16*, *17*). Three test persons participated in the sniffing. The values listed in **Tables 2** and **3** specify the odor threshold ranges at which at least two of the three assessors agreed.

RESULTS AND DISCUSSION

Biogeneration of Acyloins. The scope and limitations of baker's yeast-mediated carboligation reaction were investigated. Several reactions using aliphatic aldehydes and 2-ketocarboxylic acids (pyruvate or 2-ketobutyric acid) as substrates were carried out (**Figure 1**). After they were incubated, for all aldehydes except but-(*E*)-2-enal, a pair of acyloins was identified by GC-MS (**Table 1**). Where available, the molecular structures of the acyloins were determined by comparison of their GC-MS data with those described in the literature (*18*, *19*). The molecular structure of 3-hydroxy-1-penten-4-one was confirmed by comparing its analytical data (GC-MS, ^{1}H NMR, ^{13}C NMR) with those of a synthesized reference compound (*20*). The structures of 4-hydroxy-1-hexen-3-one, 3-hydroxy-(*E*)-4-nonen-2-one, 2-hydroxy-(*E*)-4-nonen-3-one, 3-hydroxy-(*E*)-4-decen-2-one, and 2-hydroxy-(*E*)-4-decen-3-one were tentatively suggested on the basis of their GC-MS data. As shown in **Table 1**, under the used experimental conditions, the yields of generated acyloins

Table 2. 5,6-Dihydropyrazines Tentatively Identified on the Basis of Their MS Data Analysis

5,6-dihydropyrazines[a]	odor quality	odor threshold (ng/L air)	RI[b]	mass spectra *m/z* (%)	yield (%)[c]
2-ethyl-3,5-dimethyl-	roasted, nutty	0.002	1038	138 (100), 137 (50), 123 (75), 68 (19), 56 (83), 42 (70)	24
3-ethyl-2,5-dimethyl-	roasted, popcornlike	0.227	1031	138 (100), 137 (45), 123 (67), 82 (20), 56 (79), 42 (74)	24
2,3-diethyl-5-methyl-	roasted	0.004	1090	152 (43), 151 (20), 137 (68), 56 (100), 42 (20)	44
2-propyl-3,5-dimethyl-	roasted	2.5	1110	152 (75), 137 (67), 124 (89), 123 (30), 109 (36), 70 (100), 42 (94)	21
3-propyl-2,5-dimethyl-	*d*	>2000	1100	152 (81), 137 (51), 124 (67), 123 (85), 109 (10), 70 (100), 42 (63)	19
2-butyl-3,5-dimethyl-	roasted	16.8	1215	166 (48), 151 (44), 137 (57), 124 (100), 123 (69), 84 (82), 42 (47)	23
3-butyl-2,5-dimethyl-	*d*	>2000	1198	166 (50), 151 (40), 137 (48), 124 (100), 123 (80), 84 (50), 42 (68)	23
2-pentyl-3,5-dimethyl-	roasted	3.2	1311	180 (10), 165 (28), 151 (40), 137 (52), 124 (100), 123 (42), 109 (49), 98 (80), 42 (60)	24
3-pentyl-2,5-dimethyl-	*d*	>2000	1298	180 (12), 165 (17), 151 (37), 137 (38), 124 (100), 123 (67), 109 (22), 98 (48), 42 (58)	22
2-hexyl-3,5-dimethyl-	*d*	>2000	1393	194 (10), 179 (21), 151 (28), 137 (40), 124 (100), 123 (41), 112 (39), 42 (37)	24
3-hexyl-2,5-dimethyl-	*d*	>2000	1380	194 (12), 179 (18), 151 (22), 137 (28), 124 (100), 123 (66), 112 (40), 42 (38)	24
2-heptyl-3,5-dimethyl-	*d*	>2000	1511	208 (12), 193 (22), 151 (30), 137 (41), 126 (28), 124 (100), 123 (41), 109 (38), 42 (40)	27
3-heptyl-2,5-dimethyl-	*d*	>2000	1498	208 (11), 193 (18), 151 (20), 137 (42), 126 (31), 124 (100), 123 (59), 109 (22), 42 (37)	26

[a] All unknown molecules are tentatively identified on the basis of their MS data analysis. [b] Linear RI on a DB-1 column. [c] Reaction yields were determined using trimethylpyrazine as the internal standard. [d] Odorless under the experimental conditions applied.

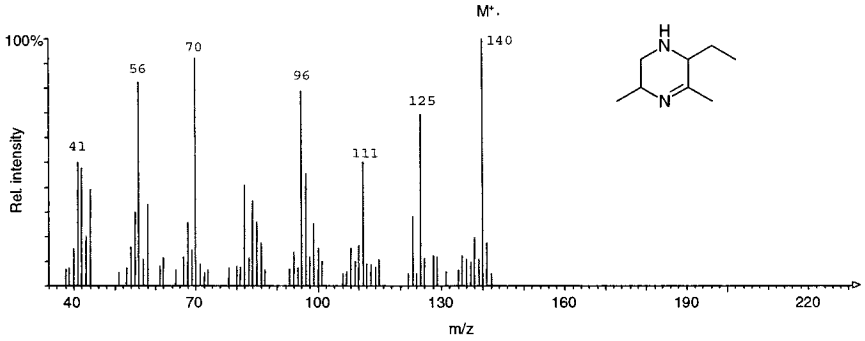

Figure 7. GC-MS/EI spectrum of 2-ethyl-3,5-dimethyltetrahydropyrazine.

Table 3. Tetrahydropyrazines Tentatively Identified on the Basis of Their MS Data Analysis

tetrahydropyrazines[a]	odor quality	odor threshold (ng/L air)	RI[b]	mass spectra *m/z* (%)	yield[c]
trimethyl-	roasted	135	1070	126 (100), 111 (27), 96 (33), 82 (62), 70 (34), 56 (25), 42 (93)	24
2-ethyl-3,5-dimethyl-	bread crustlike	1.9	1255	140 (100), 125 (70), 111 (53), 96 (79), 82 (43), 70 (93), 56 (82), 42 (48)	21
3-ethyl-2,5-dimethyl-	cooked ricelike	70	1174	140 (88), 125 (100), 111 (52), 96 (70), 82 (40), 70 (96), 56 (97), 42 (76)	11
2,3-diethyl-5-methyl-	roasted	4.3	1159	154 (100), 139 (54), 126 (23), 111 (33), 96 (31), 84 (43), 70 (63), 56 (70), 42 (19)	10
2-propyl-3,5-dimethyl-	*d*	>2000	1268	154 (90), 139 (34), 125 (100), 110 (38), 96 (42), 84 (40), 70 (72), 42 (43)	11
3-propyl-2,5-dimethyl-	*d*	>2000	1245	154 (79), 139 (12), 125 (70), 110 (38), 96 (45), 84 (90), 70 (100), 42 (69)	11

[a] All unknown molecules are tentatively identified on the basis of their MS data analysis. [b] Linear RI on a DB-1 column. [c] Reaction yields were determined using trimethylpyrazine as the internal standard. [d] Odorless under the experimental conditions applied.

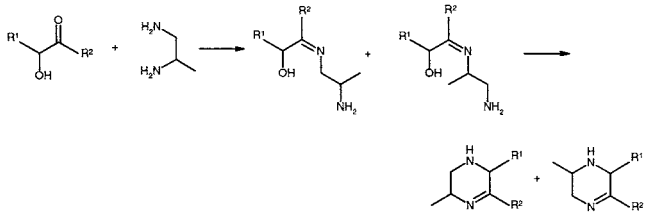

Figure 8. Tetrahydropyrazines synthesized from acyloins.

varied drastically (from 0.2 to 55%) depending on the molecular structure of the aldehyde substrates. The pair of C8 acyloins, formed from hexanal and hex-(*E*)-2-enal, was produced with the highest total yields of 74 and 59%, respectively. C7 and C9 acyloins were generated in moderate total yields of 11.7−35%. However, shorter acyloins than C7 or longer than C9 were generated with yields of less than 10%. A similar influence of the substrate structure on the yield was observed earlier when PDC from *Zygosaccharomyces bisporus* was used in an analogous reaction (*19*).

Chemoenzymatic Synthesis of 5,6-Dihydropyrazines. (*E*)-2-Alkenals and 2-ketocarboxylic acids (pyruvate or 2-ketobutyric acid) were incubated with whole cells of baker's yeast. 1,2-Propanediamine was then added to the reaction media, and the mixtures were left under stirring at room temperature. After extraction with diethyl ether and GC-MS analysis of the organic phases, two isomeric forms of 5,6-dihydropyrazines were identified for each studied combination of aldehyde and 2-ketocarboxylic acids (**Figure 2**). The molecular structures of 2-ethyl-3,5-, 3-ethyl-2,5-, 2-propyl-3,5-, and 3-propyl-2,5-dimethyl-5,6-dihydropyrazines as well as that of 2,3-diethyl-5-methyl-5,6-dihydropyrazine were determined by comparison of their RI and GC-MS spectra with those of reference substances, which were synthesized according to Flament and Stoll (*12*). The structures of the butyl-, pentyl-, hexyl-, and heptyldimethyl-5,6-dihydropyrazines were tentatively suggested on the basis of their GC-MS data. The MS/EI spectra of these pyrazine derivatives as well as those of the propyl derivatives contain a prominent mass fragment of 124 (**Figure 3**). The fragment can be explained by transfer of the γ-hydrogen from the alkyl chain to the pyrazine nitrogen, which leads to the formation of reactive intermediate A, which easily fragments to the allyl radical-stabilized intermediate B (**Figure 4**). In agreement with this mechanism, 2-ethyl-3,5- and 3-ethyl-2,5-dimethyl-5,6-dihydropyrazine as well as 2,3-diethyl-5-methyl-5,6-dihydropyrazine do not have a mass fragment 124, due to the missing γ-hydrogen of the alkyl chain (**Figure 5**). A reaction mechanism was proposed in order to explain the formation of the 5,6-dihydropyrazines (**Figure 6**). It is thought that for acyloin tautomer **1** Schiff base formation (A) is followed by a double bond migration (B), which results in the formation of a energetically more favorable conjugated π system. The resulting molecule has an enol function, which is in tautomeric equilibrium (C) with a keto function. The keto group reacts with the other free amino group of 1,2-propanediamine (D) to give 5,6-dihydropyrazines. The other dihydropyrazine regioisomer could be formed when the other amino group of 1,2-propanediamine attacks first. However, for acyloin tautomer **2**, Schiff base formation (A′) is thought to be followed by imine/enamine tautomerization (B′), a rearrangement (C′), a second tautomerization (D′), and dihydropyrazine formation (E′). Furthermore, it cannot be excluded that tautomerization between the acyloins occurs. The possibility that baker's yeast might be involved in the proposed imine/enamine tautomerization was excluded by the reaction of acyloins and 1,2-propanediamine in the absence of yeast cells.

The odor qualities of the 5,6-dihydropyrazines were characterized by GC-O, and the odor thresholds were estimated using the method reported in the literature (*16*, *17*). Some of the 5,6-dihydropyrazines had pleasant roasted odor properties (**Table 2**). The lowest odor thresholds (approximate range) were determined for 2-ethyl-3,5-dimethyl-5,6-dihydropyrazine and 2,3-diethyl-5-methyl-5,6-dihydropyrazine. These values were as

low as those of the well-known pyrazines: 2,3-diethyl-5-methylpyrazine and 2-ethyl-3,5-dimethylpyrazine (*3*). Both dihydropyrazines showed roasted odor characteristics and odor thresholds in the range of 0.002 ng/L air. Seven 5,6-dihydropyrazines with longer alkyl substituents showed no characteristic odor at concentrations up to 2000 ng/L (**Table 2**).

Chemoenzymatic Synthesis of Tetrahydropyrazines. Saturated acyloins were generated with baker's yeast except 4-hydroxy-3-hexanone, which was produced according to Bel Rhlid et al. (*21*). The chemical reaction of these acyloins with 1,2-propanediamine was carried out at room temperature in diethyl ether. After incubation, tetrahydropyrazines were identified in the reaction mixture. These compounds were tentatively characterized on the basis of their GC-MS data (**Figure 7**). From the theoretical point of view, the condensation of 1,2-propanediamine with each acyloin could result in two tetrahydropyrazine isomers (**Figure 8**). However, in our experiments, for each acyloin, only one peak corresponding to the tetrahydropyrazines was identified in the reaction mixture by GC-MS analysis. This result could be explained by the fact that either the tetrahydropyrazine isomers could not be separated by GC under the conditions applied or, in contrast to the theoretical prediction, only one isomer was formed. As shown in **Table 3**, some of these tetrahydropyrazines imparted roasted, cooked ricelike, and bread crustlike aromatic characteristics. However, their estimated odor thresholds were significantly higher than those of the 5,6-dihydropyrazine counterparts.

CONCLUSIONS

The results obtained in this study show that baker's yeast is a versatile biocatalyst, which is capable of accepting several aliphatic aldehydes and at least two 2-ketocarboxylic acids as substrates for acyloins generation. The acyloins formation was followed by chemical reaction with 1,2-propanediamine under mild conditions and led to the production of 5,6-dihydropyrazines and tetrahydropyrazines with yields for some of the isomer pairs of up to 50% (**Table 2**). While two of the six produced tetrahydropyrazines were odorless under the experimental conditions applied, four of them exhibited roasted, bread crustlike, or cooked ricelike aroma tonalities. Some of the synthesized 5,6-dihydropyrazines can be placed among the pyrazine derivatives with the lowest threshold values described in the literature (*3*).

LITERATURE CITED

(1) Gallois, A. Pyrazines in Foods. State of the Art. *Sci. Aliments* **1984**, *4*, 145−166.

(2) Seitz, E. W. Fermentation production of pyrazines and terpenoids for flavors and fragrances. In *Bioprocess Production of Flavor, Fragrance, and Color Ingredients*; Gabelmann, A., Ed.; Wiley: New York, 1994.

(3) Wagner, R.; Czerny, M.; Bielohradsky, J.; Grosch. W. Structure-odour-activity relationships of alkylpyrazines. *Z. Lebensm.-Unters. Forsch.* **1999**, *208*, 308−316.

(4) Maga, J. A.; Sizer, C. E. Pyrazines in foods. *CRC Crit. Rev. Food Technol.* **1973**, *4*, 39−115.

(5) Vernin, G. *Chemistry of Heterocyclic Compounds in Flavors and Aromas*; Wiley: New York, 1981.

(6) Rizzi, G. P. The biogenesis of food-related pyrazines. *Food Rev. Int.* **1988**, *4*, 375−400.

(7) Maga, J. A. Pyrazine update. *Food Rev. Int.* **1992**, *8*, 479−558.

(8) Brophy, J. J.; Cavill, G. W. K.; Plant, W. D. Volatile constituents of an Australian ponerine ant *Rhytidoponera metallica*. *Insect Biochem.* **1981**, *11*, 307.

(9) Kosuge, T.; Zenda, H.; Tsuji, K.; Yamamoto, T.; Narita, H. Studies on Flavor Components of Foodstuffs. Part I: Distribution of Tetramethylpyrazine in Fermented Foodstuffs. *Agric. Biol. Chem.* **1971**, *35*, 693−696.

(10) Hwang, H.-I.; Hartman, T. G.; Rosen, R. T.; Lech, J.; Ho, C.-T. Formation of pyrazines from the Maillard reaction of glucose and lysine-α-amine-^{15}N. *J. Agric. Food Chem.* **1994**, *42*, 1000−1004.

(11) Vitzthum, O. G.; Werkhoff, P. Newly discovered nitrogen-containing heterocycles in coffee aroma. *Z. Lebensm.-Unters. Forsch.* **1974**, *156*, 300−307.

(12) Flament, I.; Stoll, M. Synthesis of 3-hydroxy-2-methylpyrazines by condensation of 1,2-propanediamine and saturated 2,3-diketones. *Helv. Chim. Acta* **1967**, *50*, 1754−1758.

(13) Akiyama, T.; Enomoto, Y.; Shibamoto, T. A new method of pyrazine synthesis for flavor use. *J. Agric. Food Chem.* **1978**, *26*, 1176−1179.

(14) Masuda, H.; Tanaka, M.; Akiyama, T.; Shibamoto, T. Preparation of 5-substituted 2,3-dimethylpyrazines from the reaction of 2,3-dimethyl-5,6-dihydropyrazines and aldehydes and ketones. *J. Agric. Food Chem.* **1980**, *28*, 244−246.

(15) Van den Dool, H.; Kratz, P. Generalization of the retention index system including linear temperature programmed gas−liquid partition chromatography. *J. Chromatogr.* **1963**, *11*, 463−471.

(16) Boelens, M. H.; van Gemert, L. J. Structure−activity relationship of natural volatile nitrogen compounds. In *Developments in Food Flavours*; Birch, G. G, Lidley, M. G., Eds.; Elsevier Applied Science: Amsterdam, 1986; pp 23−49.

(17) Ullrich, F.; Grosch, W. Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. *Z. Lebensm.-Unters. Forsch.* **1987**, *184*, 277−282.

(18) Neuser, F.; Zorn, H.; Berger, R. G. Generation of odorous acyloins by yeast pyruvate decarboxylase and their occurrence in sherry and soy sauce. *J. Agric. Food Chem.* **2000**, *48*, 6191−6195.

(19) Neuser, F. Enzymatic formation of aroma-active α-hydrosy ketones with yeast *Zygosaccharomyces bisporus*. Ph.D. Thesis, University of Hannover, 1999.

(20) Kurniadi, T. H.; Bel Rhlid, R.; Juillerat, M. A.; Schüler, M.; Berger, R. G. Enantiogenic synthesis of (*R*)-(−)-3-hydroxy-1-penten-4-one. *Tetrahedron: Asymmetry* **2003**, *14*, 363−366.

(21) Bel Rhlid, R.; Renard, M. F.; Veschambre, H. Microbial reduction of 3,4-diketones and α-ketothioacetals. Application to a chemoenzymatic synthesis of the *exo*- and *endo*- brevicomin enantiomers. *Bull. Soc. Chim. Fr.* **1996**, *133*, 1011−1021.

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