Quantitative Studies on the Formation of Key Odorants in Thermally Treated Yeast Extracts Using Stable Isotope Dilution Assays

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Eighteen compounds recently identified as key odorants in yeast extracts were quantified by stable isotope dilution assays, and their odor activity values (OAV; ratio of concentration to odor threshold) were calculated. In a thermally treated commercial yeast extract (CYE) as well as in a self-prepared bakers' yeast extract (SPYE), both exhibiting roasty, meat-like odors, 2-methyl-3-furanthiol, 2-furfurylthiol, 3-methylbutanal, and methional showed the highest OAVs. Phenylacetaldehyde was high in the CYE but not in the SPYE, whereas the reverse was true for 2,3-diethyl-5-methylpyrazine. Thermal processing of a yeast extract containing the water-soluble, low molecular weight compounds (LMW-YE) isolated from mechanically disrupted fresh bakers' yeast cells resulted in a bread-crust-like odor. In this extract high OAVs for 3-methylbutanal (malty) and 2-acetyl-1-pyrroline (popcorn-like) were measured, suggesting a significant aroma contribution of both odorants. Both were much increased when the bakers' yeast had been pretreated with sodium chloride and nutrients (12 h at 32 °C). The results were well correlated with a significant increase in the respective precursor amino acids leucine (3-methylbutanal) and ornithine and proline (2-acetyl-1-pyrroline). Results of further model studies suggested Strecker degradation as one of the key reaction pathways in the formation of key odorants during thermal treatment of yeast extracts.

Keywords: *Strecker degradation; amino acids; stable isotope dilution assay; phenylacetaldehyde; 3-methylbutanal; methional; 2-acetyl-1-pyrroline; 2-methyl-3-furanthiol; 2-furfurylthiol*

INTRODUCTION

Yeast extracts and yeast autolysates are rich in free amino acids (Höhn and Solms, 1975; Schieberle, 1990a) and find widespread use as precursor blocs in the production of the processed flavors. Mostly savory, meat-like odors are reported to be generated upon thermal treatment of such materials, and, therefore, the identification of sulfur-containing aroma compounds bearing these odor qualities has gained much interest (Werkhoff et al., 1991). Intensely smelling sulfur-containing food odorants, such as 5-methylfurfurylthiol, 2-methyl-3-furanthiol, or 3-mercapto-2-butanone, have been identified in thermally treated yeast extracts (Werkhoff et al., 1991) and have as well been established as key odorants in Maillard model systems containing cysteine (Hofmann and Schieberle, 1995, 1997a). It is, therefore, quite obvious that cysteine is one of the most important precursor amino acids in yeast extracts.

The intensely popcorn-like-smelling 2-acetyl-1-pyrroline (2-AP) was identified among the key odorants of a thermally treated bakers' yeast extract (Schieberle, 1990b). Model experiments confirmed (Schieberle, 1990a) the rare amino acid ornithine, occurring in relatively high yields in bakers' yeast, as another very effective precursor of odorants in yeast extracts. Sulfur-containing compounds known to be generated from cysteine were, however, not detected among the aroma compounds identified (Schieberle, 1990b).

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The key odorants responsible for the overall flavors of processed yeast extracts are often unclear. Furthermore, although a great number of model Maillard studies have extended our knowledge on the general role of several amino acids as flavor precursors, up to now, systematic quantitative correlations between the amounts of specific precursor amino acids, such as ornithine or cysteine present in yeast extracts, and the amounts of odorants generated upon their thermal treatment have not been performed. Such data are, however, the prerequisite either to enhance the concentration of certain aroma precursors (e.g., by the yeast itself) or to assemble a tailor-made precursor bloc, which would then liberate the wanted aroma upon thermal treatment.

Aroma extract dilution analysis (AEDA) ranks volatiles present in an aroma extract on the basis of their odor activities in air (Schieberle, 1995). Using AEDA, we recently identified the key odorants generated upon thermal treatment of several commercial and self-prepared yeast extracts (Münch et al., 1997). The differences in the aromas generated showed correlations with the flavor dilution (FD) factors of odorants eliciting such odors. For example, the roasty, meat-like note of a commercial extract was shown to be correlated with a high FD factor of 2-furfurylthiol (FFT). In contrast, in a self-prepared yeast extract (SPYE) having a bread-crust-like odor, 2-acetyl-1-pyrroline (popcorn-like) showed a high FD factor, whereas FFT was not detected among the odor-active compounds.

FD factors are only a rough estimate of the relative concentrations of odorants in a given extract. The

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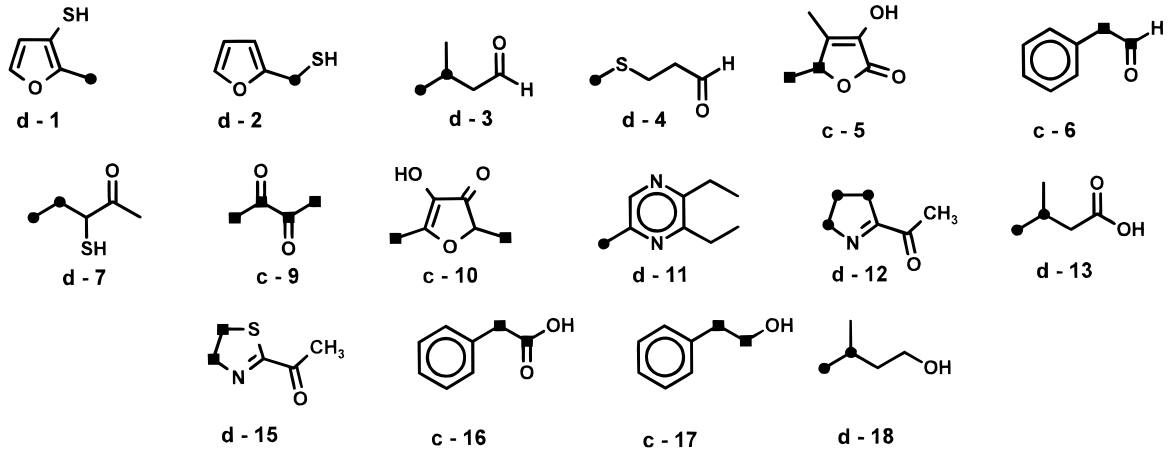

Figure 1. Structures of the labeled internal standards used in the isotope dilution assays: (●) deuterium label; (■) carbon-13 label.

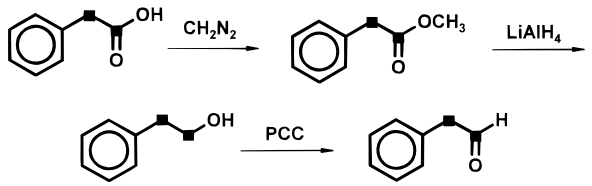

Figure 2. Synthesis of carbon-13-labeled (■) 2-phenylethanol and 2-phenylacetaldehyde (Schnermann and Schieberle, unpublished results).

purpose of the following study was, therefore, to quantify the 18 most odor-active compounds previously identified in four yeast extracts having different odors (Münch et al., 1997) and to calculate their odor activity values (OAVs). Furthermore, first model studies were undertaken to reveal factors influencing the formation of selected odorants from their precursor amino acids in yeast extracts.

EXPERIMENTAL PROCEDURES

Commercial Yeast Extract (CYE). Bakers' yeast (Fa. Wieninger, Passau, Germany) and the CYE were supplied by German manufacturers.

Chemicals. The following compounds were obtained commercially from the sources given in parentheses: compounds 1−3, 5, 6, 8−11, and 13−18 (Aldrich, Steinheim, Germany); compound 4 (Sigma, Munich, Germany). Compound 7 was synthesized as described by Asinger et al. (1964) and compound 12 according to the method of Buttery and Ling (1982). The labeled internal standards (cf. Figure 1) were synthesized as reported recently: d-1, d-2, d-4, and d-7 (Sen and Grosch, 1991); d-3 (Schieberle and Grosch, 1992); c-5 (Blank et al., 1992); c-6 and c-17 (Schnermann and Schieberle, unpublished results; cf. Figure 2); c-9 (Hofmann and Schieberle, 1997b); c-10 (Sen et al., 1991); d-11 and d-15 (Cerny and Grosch, 1993); d-12 (Schieberle and Grosch, 1987); d-13 and d-18 (Guth and Grosch, 1994); c-16 was supplied by Aldrich.

Autolysis of Bakers' Yeast (SPYE). According to a technical procedure (Bronn, 1996), bakers' yeast (90 g dry weight = 300 g) was suspended in tap water (1 L) containing sodium chloride (5 g) and ethyl acetate (4.5 g) and then stirred for 24 h at 50 °C. The pH of the suspension decreased from 5.6 to 5.2. The mixture was centrifuged at 4 °C for 15 min (40000*g*); the residue was washed twice with water and repeatedly centrifuged. The combined supernatants were freeze-dried.

Fermentation of Bakers' Yeast (Malaney and Tanner, 1988). Fresh bakers' yeast (30 g of dry weight = 100 g wet weight) was suspended in tap water (1 L) containing glucose (100 g), sodium chloride (35 g), zinc sulfate (28.2 g), $(NH_4)H_2PO_4$ (10 g), KH_2PO_4 (4 g), $CuSO_4$ (1.35 g), sodium citrate (1.14 g), $MgSO_4$ (0.4 g), pyridoxal (12 mg), *meso*-inosit (100 mg), and calcium panthothenate (5 mg). The suspension (pH 5.0) was stirred for 12 h at 32 °C under a stream of pure oxygen. The mixture was then centrifuged for 15 min at 4 °C (40000*g*), the supernatant was discarded, and the residue washed twice with tap water. The low molecular weight fraction was finally isolated as described above. A total of four fermentations was performed omitting either glucose, sodium chloride, or both (control).

Isolation of a Yeast Fraction Containing the Water-Soluble, Low Molecular Weight (MW <1000) Aroma Precursors. Fresh bakers' yeast (18 g dry weight) and glass powder (0.25−0.56 mm i.d.; Roth, Karlsruhe, Germany) were mixed with phosphate buffer (14 mL; pH 7.0; 0.1 mol/L) and treated for 75 s in a cell mill (MSK; Braun Biotech, Melsungen, Germany) under cooling with liquid carbon dioxide. The suspension was diluted with the same phosphate buffer (86 mL), the glass powder and the unsoluble materials were filtered off by using a paper filter, and the mixture was finally centrifuged. To isolate the low molecular weight fraction, the supernatant was filtered over a membrane (MW cutoff < 1000; Amicon, Witten, Germany) and the filtrate was freeze-dried.

Determination of Free Amino Acids. The free amino acids present in the yeast extracts were determined as described recently (Münch et al., 1997).

Thermal Treatment; Quantification of Odorants. The freeze-dried material (5−50 g) was suspended in water (1 + 3 by weight) and thermally treated for 20 min at 145 °C in a laboratory autoclave (type II; Roth, Karlsruhe, Germany). After cooling, known amounts of the labeled internal standards (cf. Figure 1; 5-10 μg each, dissolved in ethanol) were added to aliquots of the reacted mixture. The volume of the aliquots used depended on the concentrations of the respective odorant under investigation and was determined in preliminary experiments. The mixtures were extracted with diethyl ether and the volatiles and the internal standards isolated by sublimation in vacuo (Münch et al., 1997). After separation by HRGC, the concentrations of the odorants were determined by mass chromatography (Sen et al., 1991) using the selected ions and response factors summarized in Table 1. For statistical analysis, a model mixture containing known amounts of the complete set of the odorants and the labeled internal standards was worked up three times. The values differed by not more than ±5%.

Quantification of 2-Furfurylthiol and 2-Methyl-3-furanthiol. After cooling, the thermally treated extract (5−20 g) was spiked with d-1 and d-2 (cf. Figure 1; 10 μg each). Due to the easy oxidation of the labeled 2-methyl-3-furanthiol into its disulfide, an aliquot of the stock solution was treated with $LiAlH_4$ prior to use. The concentration of the $[^2H]_3$MFT was then determined by HRGC using unlabeled 2-furfurylthiol as the internal standard.

Table 1. Selected Ions, Response Factors, and HRGC Columns Used in the Stable Isotope Dilution Assays/Mass Chromatography

odorant	*m/z*	labeled internal standard (cf. Figure 1)	*m/z*	response factor	stationary HRGC phase
2-acetyl-1-pyrroline	112	[$^2H_{2-6}$]-2-acetyl-1-pyrroline (**d-12**)	114−118[a]	0.98	SE-54
2-acetyl-2-thiazoline	130	[$^2H_4$]-2-acetyl-2-thiazoline (**d-15**)	134	1.00	SE-54
2,3-butanedione	101	[$^{13}C_4$]-2,3-butanedione (**c-9**)	105	1.00	DB-Wax
2,3-diethyl-5-methylpyrazine	151	[$^2H_3$]-2,3-diethyl-5-methylpyrazine (**d-11**)	154	0.96	SE-54
2-furfurylthiol	81	[$^2H_2$]-2-furfurylthiol (**d-2**)	83	0.89	SE-54
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	129	[$^{13}C_2$]-3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**c-5**)	131	1.00	FFAP
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	129	[$^{13}C_2$]-4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (**c-10**)	131	1.00	FFAP
methional	105	[$^2H_3$]methional (**d-4**)	108	0.98	SE-54
3-mercapto-2-pentanone	119	[$^2H_2$]-3-mercapto-2-pentanone (**d-7**)	121	0.88	SE-54
2-methylbutanal	69	[$^2H_3$]-3-methylbutanal (**d-3**)	71−72	0.54	DB-Wax
3-methylbutanal	69	[$^2H_3$]-3-methylbutanal (**d-3**)	71−72	0.94	DB-Wax
2-/3-methylbutanoic acid	85	[$^2H_{2-3}$]-3-methylbutanoic acid (**d-13**)	87−88[a]	0.85	FFAP
3-methylbutanol	71	[$^2H_2$]-3-methylbutanol (**d-18**)	73	0.95	FFAP
phenylacetaldehyde	121	[$^{13}C_2$]phenylacetaldehyde (**c-6**)	123	1.00	SE-54
phenylacetic acid	137	[$^{13}C_2$]phenylacetic acid (**c-16**)	139	1.00	FFAP
phenylethanol	105	[$^{13}C_2$]phenylethanol (**c-17**)	107	1.00	FFAP
[β-(4-pyridyl)ethyl]-(2-furfuryl) thioether[b]	220	[$^2H_2$]-[β-(4-pyridyl)ethyl]-(2-furfuryl) thioether (**d-2**)	222	1.00	SE-54
[β-(4-pyridyl)ethyl]-(2-methyl-3-furyl) thioether[b]	220	[$^2H_2$]-[β-(4-pyridyl)ethyl]-(2-methyl-3-furyl) thioether (**d-1**)	223	1.00	SE-54

[a] The sum of the isotopomers was used. [b] Labeled derivatives obtained after treatment of 2-furfurylthiol and 2-methyl-3-furanthiol with 4-vinylpyridine.

After the pH was adjusted to 7.5 using sodium hydroxide (2 mol/L), freshly distilled 4-vinylpyridine (2 g) was added. The solution was stirred for 2 h in the dark and, after the addition of cysteine (3 g), stirred for 1 h to bind the excess of 4-vinylpyridine. The solution was extracted with diethyl ether (total volume = 150 mL), and the combined organic phases were treated with aqueous sodium hydroxide (1 mol/L) to remove acidic compounds. The [β-(4-pyridyl)ethyl] thioethers formed from the labeled and unlabeled thiols were finally isolated from the organic phase by extraction with hydrochloric acid (1 mol/L, total volume = 150 mL). The combined aqueous extracts were adjusted to pH 12.0 using sodium hydroxide (1 mol/L) and the target compounds isolated by extraction with diethyl ether (total volume = 150 mL). After drying over Na_2SO_4, the extracts were concentrated to 100 μL for mass chromatography (cf. Table 1).

High-Resolution Gas Chromatography (HRGC)/Mass Chromatography (MC). HRGC was performed with a type 5160 gas chromatograph (Fisons Instruments, Mainz, Germany) by using the following capillaries: FFAP [30 m × 0.32 mm fused silica capillary, FFAP (free fatty acid phase), 0.25 μm; J&W Scientific, Fisons Instruments, Mainz, Germany] and SE-54 (30 m × 0.32 mm fused silica capillary DB-5, 0.25 μm; J&W Scientific). The samples were applied by the on-column injection technique at 40 °C. After 2 min, the temperature of the oven was raised at 40 °C/min to 50 °C (SE-54) or 60 °C (FFAP), respectively, held for 2 min isothermally, then raised at 6 °C/min to 240 °C, and held for 10 min. The flow of the carrier gas helium was 2.5 mL/min. For mass chromatography, the columns were coupled to an ITD 800 (Finnigan, Bremen, Germany) running in the chemical ionization mode with methanol as the reactant gas. The selected ions of the labeled standard and the aroma compound (cf. Table 1) were monitored and their intensities calculated by means of a computer program. Mass spectra in the electron impact mode (MS-EI) were generated at 70 eV and in the chemical ionization mode (MS-CI) at 115 eV.

RESULTS AND DISCUSSION

Application of the AEDA on aroma distillates obtained by thermal treatment of four different yeast extracts had recently revealed high FD factors for 17 compounds (compounds 2−18) listed in Table 2 (Münch et al., 1997). However, their FD factors differed significantly depending on the extract used.

Table 2. Odor Thresholds of Compounds Recently Identified as Key Odorants in Yeast Extracts (Münch et al., 1997)

no.	odorant	odor quality	odor threshold[a] (μg/L of water)
1	2-methyl-3-furanthiol	meat-like	0.007
2	2-furfurylthiol	roasty, coffee-like	0.01
3	3-methylbutanal	malty	0.4
4	methional	cooked potato	1.8
5	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	seasoning-like	0.3
6	phenylacetaldehyde	honey-like	4
7	3-mercapto-2-pentanone	sulfury	0.7
8	2-methylbutanal	malty, green	4
9	2,3-butanedione	buttery	15
10	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	caramel-like	25
11	2,3-diethyl-5-methylpyrazine	potato-like	0.09
12	2-acetyl-1-pyrroline	popcorn-like	0.1
13	3-methylbutanoic acid	rancid, sweaty	750
14	2-methylbutanoic acid	rancid, sweaty	540
15	2-acetyl-2-thiazoline	roasty, popcorn	1
16	phenylacetic acid[b]	honey, chocolate-like	1000
17	2-phenylethanol	flowery	1000
18	3-methylbutanol	malty	1000

[a] Odor thresholds were taken from Rychlik et al. (1998). [b] The odor threshold was determined by means of the triangle test (Schieberle and Hofmann, 1997b).

FD factors rank aroma compounds on the basis of their odor threshold in air. To gain insights into the flavor contribution of an aroma compound in an aqueous medium, the OAVs (ratio of concentration to odor threshold; Schieberle, 1995) were determined.

Determination of OAVs. In a first experiment, a CYE was thermally treated in aqueous solution and 18 of the odorants identified previously (Münch et al., 1997) were quantified (Table 3). A calculation of the OAVs revealed FFT and 2-methyl-3-furanthiol (MFT) as the key odorants in the extract. The MFT had not been detected during AEDA of the same extract (Münch et al., 1997), which was undoubtedly caused by the instability of this odorant during the workup procedure. A stable isotope dilution assay using derivatization of the thiol group with 4-vinylpyridine, which was recently

Table 3. Concentrations and Odor Activity Values (OAVs) of 18 Key Odorants in a Thermally Treated Commercial Yeast Extract (CYE)

no.	odorant	concn[a] (μg/kg)	OAV[b]
1	2-methyl-3-furanthiol	530	75710
2	2-furfurylthiol	580	58000
3	3-methylbutanal	1456	3640
4	methional	1095	608
5	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	117	390
6	phenylacetaldehyde	1250	313
7	3-mercapto-2-pentanone	170	243
8	2-methylbutanal	965	241
9	2,3-butanedione	1932	129
10	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	2471	99
11	2,3-diethyl-5-methylpyrazine	2.7	30
12	2-acetyl-1-pyrroline	2.2	22
13	3-methylbutanoic acid	3714	5
14	2-methylbutanoic acid	1486	3
15	2-acetyl-2-thiazoline	2.8	3
16	phenylacetic acid	2660	3
17	2-phenylethanol	476	0.5
18	3-methylbutanol	55	0.1

[a] Based on the dry weight of the yeast extract. [b] OAV were calculated by dividing the concentrations by the odor thresholds given in Table 1.

Table 4. Concentrations and Odor Activity Values (OAVs) of 18 Key Odorants in a Thermally Treated Self-Prepared Extract of Bakers' Yeast (SPYE)

no.	odorant	concn[a] (μg/kg)	OAV[b]
1	2-methyl-3-furanthiol	22	3140
2	2-furfurylthiol	29	2900
3	3-methylbutanal	617	1543
4	methional	287	159
5	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	64	213
6	phenylacetaldehyde	1	0.3
7	3-mercapto-2-pentanone	nd[c]	nd
8	2-methylbutanal	370	93
9	2,3-butanedione	190	13
10	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	1352	54
11	2,3-diethyl-5-methylpyrazine	53	589
12	2-acetyl-1-pyrroline	6	60
13	3-methylbutanoic acid	3043	4
14	2-methylbutanoic acid	1217	2
15	2-acetyl-2-thiazoline	8	8
16	phenylacetic acid	932	1
17	2-phenylethanol	43500	43
18	3-methylbutanol	5535	6

[a] Based on the dry weight of the fresh bakers' yeast. [b] OAV were calculated by dividing the concentrations by the odor thresholds given in Table 1. [c] nd, not determined.

Table 5. Concentrations and Odor Activity Values (OAVs) of 15 Selected Key Odorants Formed by Thermal Treatment of Water-Soluble Low Molecular Weight Fractions (MW < 1000) Isolated from Fresh Bakers' Yeast (I) or Bakers' Yeast Stored for 12 h in the Presence of Nutrients and Sodium Chloride (II)

odorant	concn[a] (μg/kg) in		OAV[b] in	
	I	II[c]	I	II[c]
2-furfurylthiol	<0.1	<0.1	<10	<10
3-methylbutanal	100	801	250	2000
methional	92	225	51	125
phenylacetaldehyde	81	351	20	88
2-methylbutanal	84	527	21	132
2,3-butanedione	467	1314	31	87
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	25	316	1	13
2,3-diethyl-5-methylpyrazine	1	2	11	24
2-acetyl-1-pyrroline	5	33	50	330
3-methylbutanoic acid	5915	5714	8	8
2-methylbutanoic acid	2366	2286	4	4
2-acetyl-2-thiazoline	13	2	13	2
phenylacetic acid	180	165	0.2	0.2
2-phenylethanol	2470	3737	3	4
3-methylbutanol	4556	1921	5	2

[a,b] Cf. Table 3. [c] A suspension of bakers' yeast was stored for 12 h at 32 °C in an aqueous medium containing yeast nutrients and sodium chloride (Münch et al., 1997).

shown to be an effective method for quantifying this labile odorant (Schieberle and Hofmann, 1997), confirmed a significant contribution of MFT to the overall odor (Table 3).

Further important odorants in the CYE were 3-methylbutanal, methional, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon), and phenylacetaldehyde. On the basis of very low OAVs, 3-methylbutanoic acid, phenylacetic acid, and 2-phenylethanol, which had been identified with higher FD factors in the extract (Münch et al., 1997), did not contribute much to the overall odor. These data were well in line with the overall roasty, meat-like odor impression of the processed CYE extract.

In a thermally treated SPYE, 33 odorants had been detected, among which 2-phenylethanol, 3-methylbutanoic acid, butanoic acid, 2,3-diethyl-5-methylpyrazine, and methional showed high FD factors (Münch et al., 1997). On the basis of the quantitative results and a calculation of the OAVs (Table 4), 2-methyl-3-furanthiol (1), 2-furfurylthiol (2), 3-methylbutanal (3), 2,3-diethyl-5-methylpyrazine (11), sotolon (5), and methional (4) were elucidated as the key odorants in the SPYE. Compared to the CYE (cf. Table 3) the following significant differences were observed among the 10 key odorants (OAVs > 100): MFT, FFT, and 2,3-butanedione were 20- or 10-fold lower in the SPYE, and phenylacetaldehyde was not among to the key odorants. On the other hand, 3-methylbutanal, 2-methylbutanal, methional, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, and sotolon were only slightly reduced. In contrast, 2,3-diethyl-5-methylpyrazine was much increased in the SPYE to become fourth in rank. The significant reduction especially of phenylacetaldehyde might be explained by an enzymic reduction into 2-phenylethanol during the extract preparation. This is corroborated by the fact that the alcohol was much increased in the SPYE compared with the CYE. Similar data were obtained for the pair 3-methylbutanal/3-methylbutanol (cf. Tables 3 and 4).

Influence of Yeast Pretreatment. In the previous study (Münch et al., 1997) we showed that a significantly different set of odorants was formed when a fraction containing the water-soluble, low molecular weight compounds isolated from bakers' yeast (LMW-YE) was heated. Furthermore, it was shown that the FD factors of several of the odorants increased much when the bakers' yeast cells had been fermented for 12 h at 32 °C in the presence of nutrients and sodium chloride prior to the isolation of the LMW-YE.

In the next experiment the odorants formed by heating of two different LMW-YEs were quantified. LMW-YEs were isolated from either fresh bakers' yeast (LMW-YE-I) or fresh bakers' yeast fermented in the presence of nutrients and sodium chloride (LMW-YE-II). After freeze-drying, the extracts were thermally treated and 15 of the 18 odorants under investigation were quantified in both materials. The results indicated that, on the basis of high OAVs (Table 5), 3-methylbutanal followed by 2-acetyl-1-pyrroline, methional, 2-methylbutanal, phenylacetaldehyde, and 2,3-butanedione are the key odorants generated from the LMW-YE-I (Table

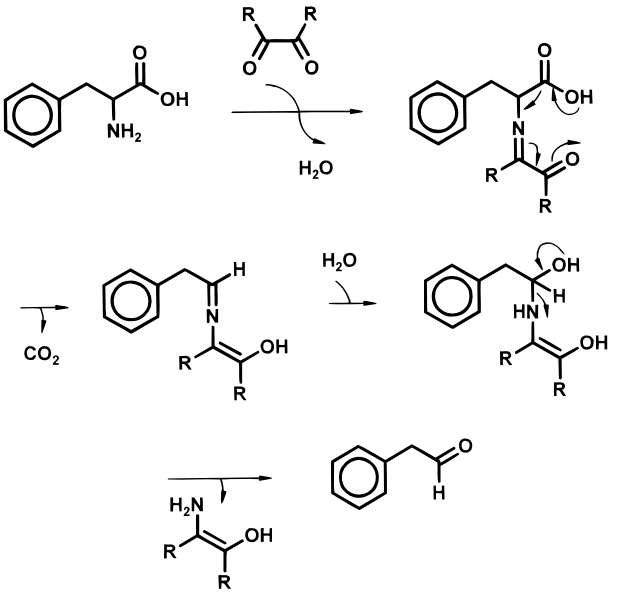

Figure 3. Strecker degradation of phenylalanine yielding phenylacetaldehyde.

5). In the LMW-YE from yeast, which had been fermented in the presence of sodium chloride (LMW-YE-II; Table 5), much higher concentrations of the six odorants were generated. On the other hand, for example, 3-methylbutanoic acid, phenylacetic acid, 2-phenylethanol, or 3-methylbutanol showed only slight differences in their concentrations in I and II.

These results are in good agreement with the more intense bread-crust-like odor of II compared to I, because 2-acetyl-1-pyrroline (popcorn-like), 3-methylbutanal (malty), and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (caramel-like), which have previously been characterized as key odorants in wheat bread crust (Schieberle and Grosch, 1992), were increased by factors of 6−12, respectively.

Influence of the Amino Acid Concentration on Odorant Formation. An oxidative decarboxylation of amino acids catalyzed by α-dicarbonyls, the so-called Strecker degradation, is known to yield several potent aroma compounds. As exemplified for phenylalanine in Figure 3, phenylacetaldehyde is formed from this precursor amino acid.

On the basis of this pathway, an increase in the respective amino acid in the extract should, consequently, result in an increase in the respective Strecker aldehyde. Five of the odorants that were increased in the LMW-YE from NaCl-treated yeast (LMW-YE II) are known to be generated by Strecker-type reactions from the six distinct amino acids given in parentheses: 3-methylbutanal (from leucine); 2-acetyl-1-pyrroline (from ornithine and proline; Schieberle, 1990a); phenylacetaldehyde (from phenylalanine); 2-methylbutanal (from isoleucine); and methional (from methionine). Assuming the Strecker degradation as the key formation pathway in the formation of these odorants (cf. Table 5), there should be an increase in the respective precursor amino acid induced by the composition of the fermentation medium.

In Table 6, the concentrations of the six free amino acids determined in the respective LMW-YE fractions isolated after fermentation under different conditions are summarized. Fermentation of the yeast cells in the presence of all nutrients except glucose and NaCl (A in Table 6) resulted in relatively low amounts of free amino acids in the LMW-YE and, also, the addition of glucose was not very effective in enhancing the free amino acids (B, Table 6). However, when NaCl was present during fermentation, the amino acid concentrations were enhanced by factors of 4 (methionine) to 25 (proline), respectively. A very interesting effect was observed when both glucose and NaCl were present (D, Table 6): under these conditions, especially ornithine was further increased, whereas, for example, methionine and isoleucine were decreased (cf. C and D, Table 6).

Table 6. Influence of the Composition of the Fermentation Medium on the Concentrations of Selected Free Amino Acids in Bakers' Yeast Cells

	concn[a] (mg/g) in expt			
amino acid	A[b] (control)	B (A + glucose)	C (A + NaCl)	D (A + glucose + NaCl)
proline	0.04	0.05	1.00	1.52
ornithine	0.21	0.21	0.80	2.98
leucine	0.25	0.29	1.31	1.24
isoleucine	0.20	0.19	1.24	0.90
methionine	0.07	0.07	0.27	0.16
phenylalanine	0.19	0.19	1.02	0.82

[a] Based on dry weight. Data are mean values of triplicates. [b] The set of nutrients given under Experimental Procedures was used.

A correlation between the amounts of flavor compounds formed upon thermal treatment (Table 5) with the amino acid concentrations present (Table 6) revealed that an increase in the respective precursor amino acid results in an increase in the corresponding odorant as expected. For example, ornithine is increased by a factor of 14 in II (cf. A and D in Table 6), leading to an increase in the 2-AP by a factor of 6 (cf. I and II, Table 5, which are identical with A and D in Table 6). Also, leucine is increased by a factor of 5 (cf. A and D, Table 6), leading to an increase in 3-methylbutanal by a factor of 8 (cf. I and II in Table 5). These results indicate that obviously the degradation pathway of the respective precursor amino acids is not significantly influenced by other amino acids present in the extract.

Influence of Carbohydrates or Their Degradation Product 2-Oxopropanal on Odorant Formation. A comparison between the concentrations of free amino acids in the four extracts with the amounts of odorants generated thereof (on a molar basis) revealed that, in general, in the LMW-YEs the amino acids seem to be most effectively converted into the odorants (Table 7). This might probably be caused by the presence of dihydroxyacetone phosphate in the LMW-YEs (Schieberle, 1990b), which is not likely to occur in the CYE. The triose phosphate may easily liberate 2-oxopropanal, which would in turn catalyze the Strecker degradation (Figure 3). To study the influence of carbohydrates on the aroma formation, in a model experiment, first the CYE was reacted in the presence of either glucose or 2-oxopropanal, respectively, and the formation of seven selected odorants was measured. As summarized in Table 8, addition of glucose (A, Table 8) led to a slight increase in the amounts of the three Strecker aldehydes 3-methylbutanal, methional, and phenylacetaldehyde as well as that of 2-acetyl-1-pyrroline. In contrast, the two thiols FFT and MFT were significantly decreased. Addition of the α-dicarbonyl compound 2-oxopropanal (B, Table 8) significantly increased the amounts of the three Strecker aldehydes, with methional being increased 28-fold. However, both thiols were significantly decreased as found in the experiment with glucose.

Table 7. Concentrations of Precursor Amino Acids in the Four Different Yeast Extracts—Correlation with the Amounts[a] of Odorants[b] (in Parentheses) Formed after Thermal Treatment

amino acid	amount (mg/kg of dry wt) in extract							
	CYE		SPYE		LMW-YE-I		LMW-YE-II	
ornithine	2654	} (2×10^{-3})	2719	} (6×10^{-3})	210	}(5×10^{-3})	2980	} (33×10^{-3})
proline	3679		4004		40		1520	
leucine	14003	(1.46)	10280	(0.62)	250	(0.1)	1240	(0.80)
isoleucine	8748	(0.97)	6466	(0.37)	200	(0.08)	900	(0.53)
methionine	4280	(1.1)	2534	(0.29)	70	(0.09)	160	(0.23)
phenylalanine	9364	(1.25)	5447	(1×10^{-3})	190	(0.08)	820	(0.35)

[a] Data were taken from Tables 4 and 5. [b] For explanation see text.

Table 8. Influence of the Addition of Glucose (A) or 2-Oxopropanal (B) to the Commercial Yeast Extract (CYE) on the Amounts and Odor Activity Values (OAVs) of Selected Odorants Formed after Thermal Treatment

odorant	control[a]		A		B	
	concn (μg/kg)	OAV	concn (μg/kg)	OAV	concn (μg/kg)	OAV
3-methylbutanal	1456	3640	3418	8545	9391	23477
methional	1095	608	1707	948	29068	16149
phenylacetaldehyde	1250	313	3056	764	18510	4627
2-acetyl-1-pyrroline	2.2	22	12.3	123	nd[b]	
2-methyl-3-furanthiol	530	75714	122	17428	158	22571
2-furfurylthiol	580	58000	163	16300	122	12200

[a] No carbohydrate added (cf. Table 3). [b] nd, not determined.

Table 9. Concentrations of 3-Methylbutanal, Methional, and Phenylacetaldehyde Formed from Their Precursor Amino Acids in Model Mixtures[a]

precursor	Strecker aldehyde formed	concn (μg)	mol %
L-leucine	3-methylbutanal	343	0.4
L-leucine[b]	3-methylbutanal	3693	4.3
L-phenylalanine	phenylacetaldehyde	325	0.3
L-phenylalanine[b]	phenylacetaldehyde	1217	1.0
L-methionine	methional	299	0.3
L-methionine[b]	methional	989	0.7

[a] The amino acid (1 mmol) and glucose (3 mmol) were dissolved in phosphate buffer (30 mL; 0.1 mol/L; pH 7.0) and reacted for 20 min at 145 °C in an autoclave. [b] Glucose was substituted by 2-oxopropanal.

These data reveal that not only the amount of an amino acid present determines the amount of odorant generated after thermal treatment but also, much more, the type of the reactive carbohydrates present.

To study in more detail the influence of carbohydrates on the effectiveness of amino acids in generating the respective aldehyde by a Strecker reaction, the three amino acids leucine, phenylalanine, and methionine were singly reacted with a molar excess of glucose or 2-oxopropanal. The formation of the corresponding Strecker aldehyde in each of the six model systems was studied quantitatively. The data (Table 9) showed that leucine, when reacted with glucose, yielded 0.4 mol % of 3-methylbutanal, whereas the reaction with 2-oxopropanal increased the molar yield 11-fold (4.3 mol %). Phenylalanine or methionine yielded 0.3 mol % of the corresponding Strecker aldehydes phenylacetaldehyde and methional, respectively, when reacted with glucose. As found for leucine, substitution of glucose by 2-oxopropanal significantly increased the yield of the respective Strecker aldehydes. However, compared with leucine the molar yields were lower.

To gain an insight into the time course of the amino acid degradation, in a further experiment, phenylalanine was reacted with 2-oxopropanal and the formation of phenylacetaldehyde (PAA) as well as the degradation of phenylalanine (PA) was followed. The data (Table 10) revealed that within the first 90 min, an increasing reaction time also increased the formation of PAA. Within the first 20 min, a very fast decomposition of PA was observed, yielding 3.3 mol % of PAA as calculated from the amount of PA degraded. A further increase in the reaction time up to 90 min decreased the degradation rate of PA; however, the molar yield was significantly increased (7.6 %). This result indicates that obviously intermediates of the reaction pathway given in Figure 3 are formed, which are only temporarily stable. A further increase in the reaction time from 90 to 150 min did not much increase the amounts of PAA, although a further degradation of PA was observed. Obviously, at this stage in parallel with the formation of PAA also a degradation of the aldehyde occurs. In summary, this result indicates that the formation of Strecker aldehydes during thermal treatment of amino acids runs through a maximum under the conditions applied. In the model systems studied here, 7.6 mol % was the maximum yield of the Strecker aldehyde phenylacetaldehyde from phenylalanine.

Table 10. Influence of the Reaction Time on the Formation of Phenylacetaldehyde (PAA) from L-Phenylalanine (PA) and 2-Oxopropanal[a]

reaction time (min)	amount (PAA)		degradation of PA (%)	PAA[b] (mol %)
	μg	μmol		
20	1217	10.1	31	3.3
30	2216	18.5	40	4.6
60	2875	24.0	47	5.1
90	4992	41.6	55	7.6
120	5215	43.5	62	7.0
150	5241	43.7	70	6.2

[a] L-Phenylalanine (1 mmol) and 2-oxopropanal (3 mmol) were dissolved in phosphate buffer (30 mL; pH 7.0; 0.1 mol/L) and reacted for 20 min at 145 °C in an autoclave. [b] Calculated on the basis of the amount of amino acid degraded.

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