Comparison of Key Odorants Generated by Thermal Treatment of Commercial and Self-Prepared Yeast Extracts: Influence of the Amino Acid Composition on Odorant Formation

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Application of an aroma extract dilution analysis (AEDA) on a flavor concentrate isolated from a heat-processed (145 °C, 20 min) aqueous solution of a commercial yeast extract (CYE) revealed 2-furanmethanethiol, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 2-methoxyphenol, and 3- and 2-methylbutanoic acid as the key odorants among the 16 odor-active compounds of the intensely roasty, sweet smelling solution. Compared with CYE, in a thermally treated autolysate prepared under laboratory conditions from baker's yeast (SPYA) several odorants, e.g., methional, 2-acetyl-2-thiazoline, 3-hydroxy-4,5-dimethyl-2(5H)-furanone (Sotolon), phenylacetic acid, and 2,3-diethyl-5-methylpyrazine, showed higher flavor dilution (FD) factors, whereas the reverse was found for 2-furanmethanethiol (FMT). The amounts of its precursor amino acid cysteine in the CYE and the SPYA were well correlated with the different odor activities of the FMT in both solutions. Detailed model studies on the formation pathways of FMT indicated the binary mixtures 2-furaldehyde/cysteine as well as mercapto-2-propanone/hydroxyacetaldehyde as important intermediates in FMT formation. Heat treatment of a water-soluble, low molecular weight fraction isolated from baker's yeast cells predominantly generated the roast odorant 2-acetyl-1-pyrroline (ACPY). Under certain fermentation conditions, the amounts of its precursor ornithine in the yeast were increased, leading to higher odor activities of ACPY after thermal treatment of the extract.

INTRODUCTION

Yeast extracts (YE) are widely used to render especially meaty, broth-like flavors e.g., to soups, snacks, or cheese products (Nagodawithana, 1992). These food flavorings are produced from either brewer's spent yeast, baker's yeast, or torula yeast, respectively, by using autolytic, plasmolytic, or hydrolytic processes. The extracts are finally concentrated or dried, e.g., by spray-drying (Bronn, 1996).

Up to now, only a few studies have been performed on the volatile fraction of yeast extracts. Davidek et al. (1979) and Hajslova et al. (1980) identified a total of 29 acidic and 17 neutral/basic volatiles in a nonheated commercial yeast extract. Heating of the extract (100 °C; 10 min) led to a significant change in the overall odor. However, the compounds responsible for this flavor change were not reported. Ames and MacLeod (1985) identified more than hundred volatiles formed by heating (100 °C; 2 h) of a commercial yeast extract composition, manufactured from YE, salt, spices and undefined vegetable extracts. The authors suggested that among the volatiles especially sulfur compounds, such as the 2-methyl-3-(methylthio)furan, exhibiting a meaty odor note, may contribute to the overall odor of the processed material. More recently, in a very comprehensive work, Werkhoff et al. (1991) extended the knowledge especially on sulfur-containing volatiles generated by thermal treatment (100 °C, 6 h) of a commercial yeast extract. On the basis of their meat-like,

roasty odors, it was assumed that some of them are important contributors to the odor of the extract.

A systematic approach to evaluate which odorants significantly contribute to the flavors of either unheated or heated yeast extracts has not yet been performed. However, such data would be helpful to elucidate (i) flavor precursors in the yeast extracts and (ii) to gain insights into formation pathways leading to characteristic odorants.

Using aroma extract dilution analysis, a method which elucidates key odorants in complex volatile extracts (cf. reviews by Grosch, 1994, and Schieberle, 1995), the aim of the following study was, therefore, to identify the most important odorants in a nonheated and a heat-treated commercial yeast extract. The odorants were compared with those generated by thermal treatment of yeast extracts prepared under laboratory conditions. Furthermore, first experiments were undertaken to gain insights into the precursors and formation pathways leading to selected key odorants.

EXPERIMENTAL PROCEDURES

Chemicals. The following compounds were obtained commercially from the sources given in parentheses: compounds 1−7, 10, 12, 13, 15, 17−19, 21, 22, 22a, 23−26, 29, 30, 32, and 34−36 (Aldrich, Steinheim, Germany); compound 14 (Merck, Darmstadt, Germany).

The following odorants were synthesized as reported in the literature given in parentheses: no. 8 (Buttery and Ling, 1982), no. 9 (Asinger et al., 1964), no. 27 (Sen et al., 1991a), and no. 33 (Hofmann, 1995). The deuterium-labeled [²H]-2-furanmethanethiol used in the quantification experiments was synthesized as described by Sen and Grosch (1991b).

Commercial Yeast Extract. Baker's yeast and the commercial yeast extract (CYE) were supplied by German manufacturers.

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Autolysis of Baker's Yeast (Bronn, 1996). Baker's yeast (300 g = 90 g dry weight), sodium chloride (5.0 g), and ethyl acetate (4.5 g) were mixed with tap water (1 L) and stirred for 24 h at 50 °C. The pH of the mixture decreased from 5.6 to 5.2. The suspension was then centrifuged for 15 min at 4 °C (40000*g*), and the supernatant was freeze-dried to obtain the yeast autolysate (SPYA).

Fermentation of Baker's Yeast in the Presence of NaCl and Nutrients. Commercial baker's yeast (100 g) was suspended in 1 L of tap water containing the following ingredients: glucose (100 g), sodium chloride (35 g), $ZnSO_4$ (28.17 g), $(NH_4)H_2PO_4$ (10 g), KH_2PO_4 (4 g), $CuSO_4$ (1.35 g), sodium citrate (1.14 g), $MgSO_4$ (0.8 g), $FeSO_4(NH_4)_2SO_4$ (15 mg), thiamine (44 mg), biotine (200 mg), pyridoxal (12 mg), *m*-inosit (100 mg), and calcium panthothenate (5 mg). The suspension (pH 5.0) was stirred for 12 h at 32 °C under oxygen. To isolate the yeast cells, the suspension was then centrifuged at 4 °C (40000*g*) for 15 min, the supernatant was decanted, and the cells were washed with tap water. After centrifugation, this procedure was repeated twice.

Isolation of the Low Molecular Weight Compounds from Yeast Cells. Either fresh baker's yeast or the fermented baker's yeast cells (portions of 60 g wet weight) were mixed with glass powder (0.25–0.56 mm diameter; Roth, Karlsruhe, Germany) and phosphate buffer (14 mL; pH 7.0; 0.1 M) and the viscous suspension treated for 10 min in a cell mill (type VI 3; Buehler, Tübingen, Germany) with permanent water cooling. The liquid suspension obtained was then taken up in another 86 mL of the phosphate buffer, the glass powder was removed by aspiration over a cellulose filter, and the cell walls were separated by centrifugation (4 °C, 15 min at 40000*g*). To isolate the low molecular weight compounds (LMW-YE), the supernatant was filtered over a membrane (MW cutoff, <1000; Amicon, Witten, Germany) and, finally, freeze-dried. The materials obtained from either the fermented baker's yeast or from fresh baker's yeast were analyzed for free amino acids (cf. Table 7) and thermally treated to generate the flavor compounds (cf. Table 8).

Thermal Treatment; Isolation of the Volatiles. The commercial yeast extract (CYE; 100 g), the freeze-dried yeast autolysate (SPYA; 120 g from 1200 g of fresh baker's yeast) or the low molecular weight fractions isolated from either fresh or fermented baker's yeast (LMW-YE; 5.1 g from 240 g of fresh baker's yeast) were taken up in tap water (1:5, weight/volume) and reacted in a laboratory autoclave (Type II; Roth, Karlsruhe, Germany) for 20 min at 145 °C. After being cooled with ice-water, the volatiles were isolated by extraction with diethyl ether, and, after drying over Na_2SO_4, the organic phase was concentrated to 100 mL by distilling off the solvent at 37 °C. The volatiles were then freed from the nonvolatile material by sublimation in vacuo as described recently (Sen et al., 1991a).

Separation into Acidic and Neutral/Basic Volatiles. The organic phase obtained (150 mL) was treated with an aqueous $NaHCO_3$ solution (pH 8.1; 0.5 M; total volume, 300 mL) followed by treatment with brine (total volume, 200 mL). The organic phase containing the neutral/basic volatiles was then dried over Na_2SO_4. To liberate the acidic compounds from their salts, the combined aqueous solutions were adjusted to pH 3.0 with hydrochloric acid (2 M) and the acidic volatiles extracted with diethyl ether (total volume, 300 mL). After being washed with brine (total volume, 200 mL), the organic phase was dried over Na_2SO_4.

Both extracts were concentrated to 100 μL by distilling off the solvent as described previously (Schieberle, 1991).

Aroma Extract Dilution Analysis (AEDA). The flavor dilution (FD) factors of the odor-active compounds were determined by AEDA (Ullrich and Grosch, 1987; Schieberle and Grosch, 1987) of the following dilution series: the original extract (100 μL) was stepwise diluted with diethyl ether (1:1). HRGC/olfactometry was then performed with aliquots (0.5 μL) using capillary FFAP (acidic fraction) and capillary DB-5 (neutral/basic fraction), respectively.

Determination of Free Amino Acids. Aliquots of either the commercial yeast extract or the freeze-dried material obtained from the self-prepared autolysate or extracts (SPYA

and LMW-YE), respectively, containing approximately 200 μmol of each free amino acid, were taken up in distilled water (100 mL) and ultrafiltrated over a membrane (MW cutoff, 1000). The ultrafiltrate was diluted (1:19 by volume) with acetate buffer (pH 2.2; 0.1 M) and 20 μL of this solution was analyzed by means of an amino acid analyzer (Type LC 3000; Biotronik, Maintal, Germany) using ninhydrin as the derivatizing reagent.

Determination of Free Cysteine (Friedman et al., 1970; Köhler et al., 1991). Aliquots of the extracts containing approximately 20 μg of cysteine were dissolved in phosphate buffer (1 mL; 0.1 M; pH 7.5) and reacted with 4-vinylpyridine (4 mg of freshly distilled 4-vinylpyridine in 80 μL of isopropyl alcohol) for 2 h in the dark. The mixture was then freed from the solvent in a stream of nitrogen and taken up in acetate buffer (1 mL; pH 2.2; 0.1 M), and the pyridylethyl cysteine (PEC) formed was finally quantified by means of the amino acid analyzer. The response factor was determined by using pure PEC as the reference.

High-Resolution Gas Chromatography/Olfactometry (HRGC/O); Mass Spectrometry (MS). 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 HRGC/O, at the end of the capillary, the effluent was split 1:1 (by vol) into an FID and a sniffing port using deactivated but uncoated fused silica capillaries (50 cm × 0.32 mm). The FID and the sniffing port were held at 210 °C. Linear retention indices (RI) of the compounds were calculated from the retention times of *n*-alkanes. MS analysis was performed with an MS-8230 (Finnigan, Bremen, Germany) in tandem with the capillaries described above. 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 with isobutane as reactant gas.

Model Studies on the Formation of 2-Furanmethanethiol. The precursor mixtures detailed in Tables 4 to 6 were reacted for 20 min at 145 °C in phosphate buffer (100 mL; 0.5 M). The 2-furanmethanethiol formed was determined by a stable isotope dilution assay according to Schieberle (1996).

RESULTS AND DISCUSSION

Key Odorants in a Commercial Yeast Extract (CYE). In a first experiment, the volatile fraction of a commercial yeast extract exhibiting a weak yeasty, cheese-like odor note was isolated by extraction of an aqueous solution of the CYE with diethyl ether, followed by sublimation in vacuo. Application of the aroma extract dilution analysis revealed 16 odor-active regions, and the odorants responsible for the odors detected in the HRGC effluent were subsequently identified (cf. Table 1, footnote *a*). The results of the identification experiments, in combination with the flavor dilution (FD) factors (extract A; Table 1), revealed butanoic acid (no. 22; parmesan cheese-like), 3- and 2-methylbutanoic acid (no. 24; sweaty), and the caramel-like smelling 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (no. 32) as the most odor-active compounds. In addition, due to their comparatively high FD factors, acetic acid (no. 14), propanoic acid (no. 18), 2-methylpropanoic acid (no. 19), 2-phenylethanol (no. 30), and 2-phenylacetic acid (no. 35) also contributed to the overall yeasty, cheese-like odor of the unheated yeast extract.

After the extract was boiled for 1 h at 100 °C, in addition to the cheese-like, yeasty odor note, a more

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Table 1. Comparison of Key Odorants (FD ≥ 16) Present in a Commercial Baker's Yeast Extract (A) and Formed after Heat Treatment of the Extract in Water (B, 1 h, 100 °C; C, 20 min, 145 °C), Respectively

no.	odorant[a]	odor quality	RI on FFAP	RI on SE-54	FD factor[b] in A	B	C
9	3-mercapto-2-pentanone[c]	sulfury	1345	900	<2	<2	64
13	2-furanmethanethiol	roasty, coffe-like	1414	910	4	32	2048
14	acetic acid	sour, pungent	1436	645	16	64	64
16	methional	cooked potato	1449	906	2	16	64
18	propanoic acid	sour, cheese-like	1528	748	16	32	32
19	2-methylpropanoic acid	cheese-like	1548	789	16	32	32
22	butanoic acid	like parmesan-cheese	1612	821	32	32	64
22a	phenylacetaldehyde	honey-like	1640	1047	<2	8	64
24	3- and 2-methylbutanoic acid	sweaty	1651	873	32	32	256
29	2-methoxyphenol	burnt	1840	1090	2	32	256
30	2-phenylethanol	flowery	1898	1122	16	32	32
31	unknown	roasty	1932		<1	4	64
32	4-hydroxy-2,5-dimethyl-3(2H)-furanone	caramel-like	2030	1071	32	32	512
33	4-hydroxy-2,5-dimethyl-3(2H)-thiophenone	roasty, caramel	2044	1170	<1	4	64
34	3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon)	seasoning-like	2183	1082	8	16	64
35	phenylacetic acid	hot-chocolate, sweet	2468	1262	16	16	64

[a] The compound was identified by comparing it with the reference substance on the basis of the following criteria: retention index (RI) on the two stationary HRGC phases given in the table, mass spectra obtained by MS/EI and MS/CI, and odor quality and odor intensity perceived at the sniffing port. Odorants showing FD factors >8 in at least one of the experiments are given. [b] FD factor determined by aroma extract dilution analysis (cf. review by Schieberle, 1995). [c] The MS signals were too weak for an unequivocal interpretation. The compound was identified on the basis of the remaining criteria given in footnote *a*.

pronounced roasty odor became evident. The flavor difference between the unheated and the boiled yeast extract (cf. extracts A and B, Table 1) is reflected especially by a significant increase in the FD factors of the three odorants 2-furanmethanethiol (no. 13, Table 1), with a roasty, coffee-like note, methional (no. 16), with an odor reminiscent of cooked potato, and 2-methoxyphenol (no. 29; burnt).

To study the influence of higher temperatures, in a further experiment an aqueous solution of the same amount of the CYE was thermally treated by pressure cooking in an autoclave at 145 °C. This treatment resulted in an intensely roasty, sulfury smelling solution.

With the exception of the newly detected 3-mercapto-2-pentanone (no. 9, Table 1), the odor-active compounds identified in this experiment (extract C) agreed with those generated at lower temperatures (extract B). However, the FD factors, especially of the three odorants 2-furanmethanethiol (no. 13), 2-methoxyphenol (no. 29), and 4-hydroxy-2,5-dimethyl-3(2H)-furanone (no. 32), were very significantly increased. In addition, the FD factors of 2- and 3-methylbutanoic acid (no. 24), 2-phenylacetic acid (no. 35), 4-hydroxy-2,5-dimethyl-3(2H)-thiophenone (no. 33), and methional (no. 16) were also somewhat higher in the pressure-cooked extract.

The results revealed that especially the roasty smelling 2-furanmethanethiol, 4-hydroxy-2,5-dimethyl-3(2H)-furanone with a caramel-like odor, and 2-methoxyphenol (burnt) were mainly responsible for the overall roasty, sweet note of the pressure-cooked extract. Furthermore, the data clearly indicate that effective precursors for these flavor compounds are undoubtedly present in the yeast extract.

Key Odorants in a Self-Prepared Yeast Autolysate (SPYA). It might be assumed that the drying processes used in the production of commercial yeast extracts (cf. Bronn, 1996) may significantly influence the flavor, because on the one hand volatiles might be evaporated while on the other hand odorants will already be generated by the thermal treatment.

To gain first insights into the influence of heat processing on the generation of odorants from precursors, a yeast autolysate was prepared from baker's yeast

under defined conditions in a laboratory scale and the water-solubles were then freeze-dried and finally heat-treated for 20 min at 145 °C in aqueous solution. Application of the AEDA on the intensely roasty, yeast-like smelling flavor extract revealed 33 odor-active compounds in the FD factor range 16−2048 (Table 2), 30 of which could be identified. The results of the identification experiments (Table 2) showed that 2- and 3-methylbutanoic acid (no. 24; sweaty) and 2-phenylethanol (no. 30) with a flowery odor note appeared with the highest FD factors, followed by butanoic acid (no. 22), 4-hydroxy-2,5-dimethyl-3(2H)-furanone (no. 32), and methional (no. 16). Additional odorants showing higher FD factors were 2,3-diethyl-5-methylpyrazine (no. 17), 2-methylpropanoic acid (no. 19), 2-acetyl-2-thiazoline (no. 26), 2-methoxyphenol (no. 29), 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon; no. 34), and 2-phenylacetic acid (no. 35).

A comparison with the data obtained by heating the commercial yeast extract under identical conditions (cf. C in Table 1 with Table 2) revealed a different composition of the key odorants, e.g., the roasty and/or potato-like smelling odorants 2-acetyl-2-thiazoline (no. 26) and 2,3-diethyl-5-methylpyrazine (no. 17) were generated in significant amounts in the self-prepared yeast autolysate, but were not detected among the key odorants in the heat-processed commercial extract (Table 1). On the other hand, the very potent roast odorant 2-furanmethanethiol (no. 13) appearing with the highest FD factor among the odorants of the commercial yeast extract (C in Table 1) was present only with a comparatively low odor activity in the self-prepared, thermally treated yeast autolysate (Table 2).

These results suggest different precursor concentrations for the generation of 2-furanmethanethiol in both yeast-derived materials and prompted us to study its formation in more detail.

Amounts of Free Cysteine in the Yeast Extracts. It is generally accepted in the literature that the amino acid cysteine is involved in the formation of the 2-furanmethanethiol during Maillard-type reactions (cf. e.g., Hofmann and Schieberle, 1995). To establish the role of cysteine in the formation of the thiol, first, the amounts of cysteine in the commercial yeast extract

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Table 2. Key Odorants (FD ≥ 16) Generated by Thermal Treatment of a Self-Prepared Yeast Autolysate (SPYA)

			RI on		
no.	odorant[a]	odor quality	FFAP	SE-54	FD factor[b]
1	2-methylpropanal	malty	821	552	32
2	ethyl acetate	fruity	900	655	32
3	3-methylbutanal	malty	930	652	64
4	ethyl-2-methylpropanoate	fruity	974	758	32
5	2,3-butanedione	buttery	978	592	32
6	2,3-pentanedione	buttery	1052	696	64
8	2-acetyl-1-pyrroline	roasty, popcorn-like	1323	926	16
9	3-mercapto-2-pentanone	sulfury	1345	900	16
11	unknown	sweet, earthy	1367		32
12	2,3,5-trimethylpyrazine	potato-like	1386	1000	64
13	2-furanmethanethiol	roasty, coffee-like	1414	910	64
14[c]	acetic acid	sour, pungent	1436	645	64
15	2-ethyl-3,5-dimethylpyrazine	potato-like	1440	1079	256
16	methional	cooked potato	1449	906	1024
17	2,3-diethyl-5-methylpyrazine	roasted potato	1481	1158	512
18[c]	propanoic acid	sour, cheese-like	1528	748	16
19[c]	2-methylpropanoic acid	cheese-like	1548	789	512
20	unknown	roasty, meat-like	1587		16
21	2-acetylpyrazine + unknown	roasty	1608	1022	512
22[c]	butanoic acid	like parmesan-cheese	1612	821	1024
23	2-acetylthiazol	roasty	1620	1020	16
24[c]	2- and 3-methylbutanoic acid	sweaty, cheese-like	1651	873	2048
25[c]	pentanoic acid	sweaty	1723		16
26	2-acetyl-2-thiazoline	roasty	1738	1106	512
27	(*E*)-β-damascenone	cooked apple	1806	1368	128
29	2-methoxyphenol	burnt	1840	1090	512
30	2-phenylethanol	flowery	1898	1122	2048
31	unknown	roasty	1932		32
32[c]	4-hydroxy-2,5-dimethyl-3-(2*H*)-furanone	caramel-like	2030	1071	1024
33[c]	4-hydroxy-2,5-dimethyl-3(2*H*)-thiophenone	roasty, caramel	2044	1170	32
34[c]	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon)	seasoning-like	2183	1082	512
35[c]	2-phenylacetic acid	hot chocolate, sweet	2468	1262	512
36	4-hydroxy-3-methoxy-benzaldehyde (vanillin)	vanilla-like	2538	1406	32

[a] Cf. footnote *a* in Table 1. [b] Cf. footnote *b* in Table 1. [c] The FD factor was determined in the fraction of the acidic volatiles.

Table 3. Concentrations of Cysteine in the Commercial Yeast Extract (CYE) and the Self-Prepared Yeast Autolysate (SPYA)

	cysteine	
sample	mg/g[a]	mg reacted[b]
CYE	2.13	209
SPYA	0.21	76

[a] Cysteine was determined after derivatization with 4-vinylpyridine. Concentrations are based on the dry weight of baker's yeast or the commercial yeast extract, respectively. [b] The amounts were calculated from the total amount of the yeast material reacted in the respective experiments (cf. Experimental Procedures).

(CYE) and the self-prepared yeast autolysate (SPYA) were compared.

The data displayed in Table 3 revealed 10 times higher amounts of cysteine in the CYE. A calculation of the amounts of cysteine reacted in the processed solutions (C in Tables 1 and 2) on the basis of these data indicated that about three times more cysteine had been processed using the CYE. Assuming cysteine to be the precursor, it was possible to explain the higher FD factor of 2-furanmethanethiol in this extract compared with the self-prepared autolysate (cf. C in Table 1 with Table 2).

Model Studies on the Formation of 2-Furanmethanethiol. To study the formation of the 2-furanmethanethiol (FMT) in more detail, three different carbohydrates were reacted with cysteine at three different pH values. The results showed (Table 4) that the FMT is most significantly formed from cysteine in the presence of ribose, especially at pH 3.0. At pH 5.0, which is the pH used in the processing experiments of the yeast extracts, also glucose generates the FMT from cysteine, whereas rhamnose was not an effective pre-

Table 4. Formation of 2-Furanmethanethiol (FMT) in Different Model Systems Containing Cysteine (3.3 mmol) and a Carbohydrate

	FMT (μg) at pH		
carbohydrate (10 mmol)	3.0	5.0	7.0
ribose	22.9	12.1	1.2
glucose	0.7	2.8	0.6
rhamnose	0.2	0.8	0.1

Table 5. Amounts of 2-Furanmethanethiol (FMT) Generated from 2-Furaldehyde in the Presence of Cysteine or Hydrogen Sulfide, Respectively

	2-furaldehyde (1 mmol)	FMT generated	
expt	reacted in the presence of	μg	mol %
1	cysteine (1 mmol)	25.3	0.02
2	hydrogen sulfide (0.25 mmol)	130.1	0.12
3	hydrogen sulfide (1 mmol)	550.8	0.48

cursor. It is interesting to note that the maximum concentration of FMT from the pentose is generated at pH 3.0, while the hexoses showed a maximum at pH 5.0 (Table 4).

2-Furaldehyde is one of the major products generated by reacting pentoses in the presence of amino acids (Ledl and Schleicher, 1990). Furthermore, hydrogen sulfide is known to be formed as a Strecker degradation product of cysteine. To elucidate the role of both intermediates in the formation of FMT, 2-furaldehyde was reacted in the presence of either cysteine or hydrogen sulfide, respectively, and the amounts of FMT generated were quantified. The results showed (Table 5) that the aldehyde is an important intermediate in the formation of FMT, because, compared with ribose (cf. Table 4 at pH 5.0) 20-fold more FMT (on a molar basis) was formed in the presence of cysteine. Substitution of cysteine by

Scheme 1. Hypothetical Pathway Leading to 2-Furanmethanethiol via 2-Furaldehyde and Hydrogen Sulfide as the Intermediates

Table 6. Amounts of 2-Furanmethanethiol (FMT) Generated from Mercapto-2-propanone and Hydroxyacetaldehyde (1 mmol Each) at Different pH Values[a]

		FMT	
expt	pH	μg	mol %
1	3.0	26.1	0.02
2	5.0	40.5	0.04
3	7.0	58.2	0.05
4	5.0	9.2	0.007

[a] The mercapto-2-propanone was substituted by 2-oxopropanal (1 mmol) and cysteine (4 mmol).
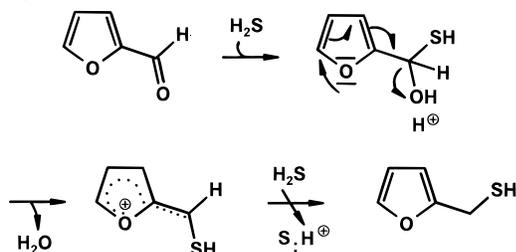
hydrogen sulfide then led to a very significant increase in the formation of FMT, with increasing amounts of hydrogen sulfide leading to increasing concentrations of FMT (cf. experiments 2 and 3; Table 5). Assuming 2-furaldehyde to be the precursor formed by degradation of ribose in the processed yeast extracts, the data corroborate the above findings that high cysteine concentrations lead to higher FD factors or amounts, respectively, of the FMT (cf. no. 13; extract C in Tables 1 and 2).

Scheme 1 shows a hypothetical pathway showing the formation of FMT from 2-furaldehyde and H$_2$S. The three-step process assumes the formation of a thio-ketal, which subsequently loses water to yield an oxycation. Reduction of this intermediate, e.g., by hydrogen sulfide itself, then gives rise to FMT.

The formation of 2-furaldehyde cannot be explained from a hexose. However, as shown in Table 4, the FMT is also generated in a processed glucose/cysteine solution.

Scheme 2 shows an alternative pathway leading to FMT from the carbohydrate degradation products 2-oxopropanal and hydroxyacetaldehyde in the presence of hydrogen sulfide. At the first step the formation of mercapto-2-propanone from a reaction of 2-oxopropanal with hydrogen sulfide is suggested. An aldol-type addition of hydroxyacetaldehyde to the mercapto-2-propanone then yields an intermediate, which by elimination of two molecules of water results in FMT formation.

To prove this hypothesis, in a first series of experiments (nos. 1−3; Table 6), mercapto-2-propanone was reacted with hydroxyacetaldehyde and the FMT formed was quantified by a stable isotope dilution assay. The results showed that significant amounts of the FMT are generated from both precursors, with the highest yields
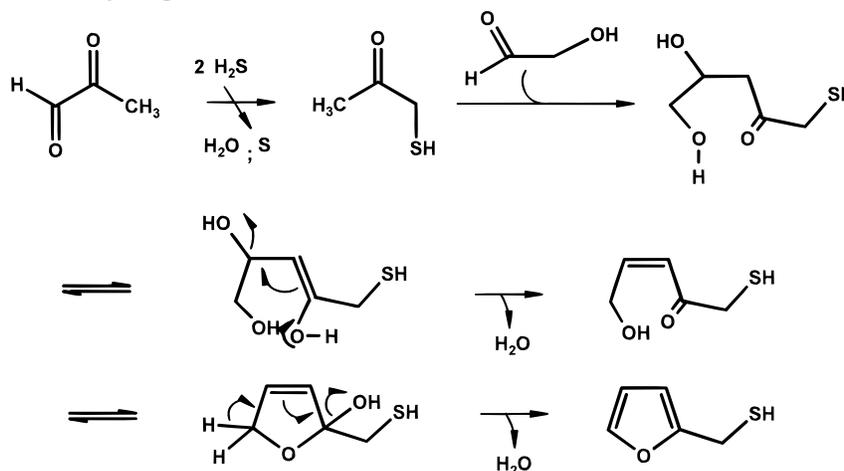
being produced at pH 7.0. Reacting the ternary mixture 2-oxopropanal, cysteine, and hydroxyacetaldehyde also generated distinct but lower amounts of the flavor compound (experiment 4, Table 6). Although these reactants were less effective than the 2-furaldehyde/hydrogen sulfide mixture (cf. Tables 5 and 6), the results indicate that different pathways may lead to FMT formation, e.g., in thermally treated yeast extracts. In combination with the significant differences in the odor activities of FMT found in the CYE and the SPYE (cf. Tables 1 and 2), these results suggest that, in addition to the different amounts of cysteine (Table 3), the amounts or the type of carbohydrate might also be different in both yeast extracts. Furthermore, the results clearly indicate that the pretreatment of the yeast extracts might significantly influence the precursor concentration and, consequently, the flavor formed after thermal treatment.

Factors Influencing the Amino Acid Concentration in Yeast. During manufacturing of yeast extracts, sodium chloride usually is used to facilitate autolysis of the cells. To elucidate the influence of sodium chloride on the composition of the amino acids, baker's yeast cells were grown for 12 h in the presence of sodium chloride and, also, yeast nutrients. The water-soluble, low molecular weight fraction (LMW-YE) was isolated, the amino acids were quantified, and their concentrations were compared with those in the fresh baker's yeast. The results revealed (Table 7) that with the exception of alanine, all amino acids analyzed were increased after incubation (cf. I and II; Table 7). However, their relative concentrations were significantly changed. While alanine was by far the predominant amino acid in the extract prepared from the fresh yeast cells (II in Table 7), especially methionine, ornithine and lysine increased very significantly during

Scheme 2. Reaction Pathway Explaining the Formation of 2-Furanmethanethiol from 2-Oxopropanal, Hydroxyacetaldehyde, and Hydrogen Sulfide

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Table 7. Concentrations of Free Amino Acids in Aqueous Extracts Containing the Water Soluble, Low Molecular Weight Compounds (LMW-YE) of either Fermented Baker's Yeast (I) or Fresh Baker's Yeast (II)

amino acid	concn (mg/g dry weight)[a]		amounts (mg) reacted in each extract[b]	
	I	II	I	II
threonine	0.71	0.26	50	18
serine	1.58	0.71	111	50
proline	1.25	0.54	88	38
glycine	0.55	0.37	39	26
alanine	3.43	6.51	240	456
valine	1.98	0.62	139	43
methionine	2.94	0.76	206	53
isoleucine	1.01	0.26	71	18
leucine	1.07	0.17	75	12
tyrosine	0.62	0.13	43	9
phenylalanine	1.56	0.22	109	15
histidine	1.53	1.12	107	78
ornithine	2.73	0.74	191	52
lysine	2.01	0.17	141	12
arginine	1.94	0.30	136	21
cysteine	0.03	0.07	2.1	5

[a] The concentration was determined in the water-soluble low molecular weight fraction (LMW-YE) obtained by cell disruption, centrifugation and ultrafiltration (cf. Experimental Procedures). [b] For reaction conditions see Table 8.

fermentation to reach the highest concentrations next to alanine (I in Table 7). The highest increase was found for lysine. The latter result was well in line with data reported by Malaney and Tanner (1988).

To reveal whether the changes in the amino acid composition had an effect on the odorants generated during processing, the LMW fractions obtained from the fermented baker's yeast and from the fresh baker's yeast were thermally treated. The solution obtained by heating the LMW-YE of the fermented baker's yeast exhibited an attractive malty, bread crust-like, sweet odor which was significantly different from the odors obtained by reacting the yeast autolysate (SPYE) or the commercial yeast extract (CYE), respectively.

Application of the AEDA on a solvent extract focused the identification experiments on the 15 odorants summarized in Table 8 (extract I). The highest FD factors were determined for the roasty, popcorn-like smelling 2-acetyl-1-pyrroline (no. 8, Table 8). Additional compounds showing high odor activities were 2,3-butanedione (no. 5, buttery), 2-phenylethanol (no. 30, flowery), and 2- and 3-methylbutanoic acid (no. 24, sweaty) followed by methional (no. 16) and 2-phenylacetalde-

hyde (no. 22a, honey-like). A comparison of the FD factors with those determined in a processed extract of the fresh baker's yeast (extract II; Table 8) indicated that, with the exception of the methylbutanoic acids and 2-phenylethanol, the FD factors of all other odorants were increased at least 8 times in the yeast cells which had been fermented prior to heating.

In a previous study we (Schieberle, 1990) could show that the amino acids ornithine and proline are key precursors of 2-acetyl-1-pyrroline. A comparison of the amounts of ornithine and proline in both extracts (cf. Table 7) revealed an increase of both amino acids by factors of 3.7 and 2.3, respectively, being well in line with the increase in the FD factor of 2-acetyl-1-pyrroline in the incubated extract (no. 8, Table 8).

2-Acetyl-1-pyrroline was not detectable among the odorants of the processed commercial yeast extract (Table 1). The data suggest that both precursors, ornithine and proline, were not present in sufficient concentrations. However, quantification of both amino acids in the commercial extract revealed that higher amounts of both amino acids had been reacted than in the experiments using the low molecular weight fraction of baker's yeast (cf. CYE in Tables 9 and 7).

To generate 2-acetyl-1-pyrroline from ornithine or proline, respectively, 2-oxopropanal needs to be present (Schieberle, 1990). Therefore, on the basis of the data presented here, it seems likely that precursors generating 2-oxopropanal during thermal treatment were not present in the commercial yeast extract or the self-prepared yeast autolysate, respectively. However, this has to be clarified by additional experiments.

On the contrary, 2-furanmethanethiol, which was detected as the key odorant in the processed commercial yeast extract, was sensorially not detectable in the processed LMW-YE of either fresh or fermented baker's yeast (cf. C in Tables 1 and 8). In the latter materials, by factors of 95 or 42, respectively, lower amounts of the precursor amino acid cysteine were reacted (cf. Tables 3 and 7) which might be an explanation for the absence of 2-furanmethanethiol. However, it is yet unclear why such large differences especially in the amounts of cysteine occur.

2-Phenylacetaldehyde (no. 22a, Table 8) and methional (no. 16) are well-known Strecker degradation products of the amino acids phenylalanine and methionine, respectively. The increase of both odorants in the fermented LMW-YE is, therefore, well in line with the increase in both amino acids by factors of 7.3 and

Table 8. Intense Odorants (FD ≥ 16) Generated by Thermal Treatment of Extracts Containing the Water Soluble, Low Molecular Weight Compounds (LMW-YE) of either Fermented Baker's Yeast (I) or Fresh Baker's Yeast (II)

no.	odorant[a]	odor quality	RI on		FD factor[b] in	
			FFAP	SE-54	I	II
1	2-methylpropanal	malty	821	552	16	2
3	3-methylbutanal	malty	930	652	16	4
5	2,3-butanedione	buttery	978	592	128	8
7	3-methylbutanol	malty, yeasty	1211	732	8	8
8	2-acetyl-1-pyrroline	roasty, popcorn	1323	926	256	16
10	2-methoxy-3-isopropylpyrazine	earthy, beany	1428	1097	16	16
14[c]	acetic acid	sour, pungent	1436	645	16	8
15	2-ethyl-3,5-dimethylpyrazine	potato-like	1440	1079	16	8
16	methional	cooked potato	1449	906	64	8
19[c]	2-methylpropanoic acid	cheese-like	1548	789	16	16
22[c]	butanoic acid	like parmesan-cheese	1612	821	32	16
22[a]	phenylacetaldehyde	honey-like	1640	1047	64	8
24[c]	2- and 3-methylbutanoic acid	sweaty	1651	873	128	128
30	2-phenylethanol	flowery	1898	1122	128	128
32[c]	4-hydroxy-2,5-dimethyl-3(2H)-furanone	caramel-like	2030	1071	32	4

[a] Cf. footnote a in Table 1. [b] Cf. footnote b in Table 1. [c] The FD factor was determined in the fraction of the acidic volatiles.

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Table 9. Concentrations of Free Amino Acids[a] in the Commercial Yeast Extract (CYE) and the Self-Prepared Yeast Autolysate (SPYA)

	mg/g		mg reacted by heating[b]	
amino acid	CYE	SPYA	CYE	SPYA
threonine	7.29	2.51	715	904
serine	14.29	5.84	1401	2102
proline	3.75	1.55	368	558
glycine	4.74	1.91	465	688
alanine	18.78	6.96	1841	2505
valine	9.56	3.23	937	1163
methionine	4.37	0.73	428	263
isoleucine	8.93	2.51	875	904
leucine	14.28	3.43	1400	1235
tyrosine	7.88	2.05	773	738
phenylalanine	9.55	2.24	936	806
histidine	4.74	1.91	465	688
ornithine	2.70	1.58	265	567
lysine	7.85	4.49	770	1616
arginine	7.82	3.10	767	1116

[a] Concentrations were calculated on the basis of the dry weight of the commercial yeast extract or the baker's yeast, respectively. [b] For reaction conditions see Experimental Procedures.

3.9, respectively, compared with the LMW-YE of the fresh baker's yeast (cf. Table 7, I and II).

CONCLUSIONS

The results have shown that, depending on the conditions used in the preparation of yeast extracts, significant differences in the key flavor compounds obtained after thermal treatment may occur. In particular, the amounts of certain precursor amino acids have been shown to be well correlated with the odor activities of some key odorants, e.g., the odor activity of the roasty, coffee-like smelling 2-furanmethanethiol in the processed extracts was well correlated with the amounts of free cysteine in the respective yeast material. However, the amounts of the precursor amino acid ornithine were not correlated with the formation of 2-acetyl-1-pyrroline.

To use the flavor potential of yeast extracts more effectively, exact quantitative studies on the amount of the key odorants formed are, therefore, a necessary further step to find correlations between the odor quality of the processed extracts and flavor precursors in the extracts.

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