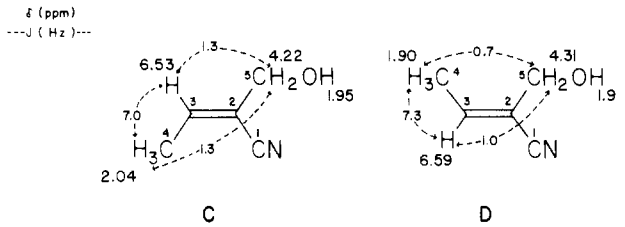

Figure 4. ^{1}H NMR data for (*Z*)- and (*E*)-2-(hydroxymethyl)-2-butenonitrile [(*Z*)-**2**, (*E*)-**2**].

Compounds C and D. The HRMS suggested the molecular formula of compound C to be C_5H_7NO. The IR spectrum of compound C indicated absorptions characteristic of alcohol (3390 cm^{-1}) and nitrile (2220 cm^{-1}). The ^{1}H NMR spectrum of compound C showed the presence of a hydroxy proton, a methyl group attached to an olefinic carbon, an olefinic proton, and a methylene proton adjacent to oxygen. The mass, IR, and ^{1}H NMR spectra of compound **D** were quite similar to those of compound C. It was concluded from these spectral data that compounds C and D were (*Z*) and (*E*) isomers of 2-(hydroxymethyl)-2-butenonitrile (**2**). In the ^{1}H NMR spectra, the chemical shift of the methylene proton adjacent to oxygen in compound D (4.31 ppm) was lower than that of compound C (4.22 ppm) due to the influence of the adjacent cis methyl group. The coupling constant values for $J_{4,5}$ were 1.3 and 0.7 Hz for compounds C and D, respectively. These ^{1}H NMR data indicate a cis configuration of C_4 and C_5 in compound D (Chamberlain, 1974). Accordingly, compound C was determined as (*Z*)-**2** and compound D as (*E*)-**2** as shown in Figure 4 (Hearne and La France, 1952).

Compound **1** (diastereomers) has a faint odor. Compound (*Z*)-**2** possesses a sweet and *p*-cresol derivative like odor, whereas compound (*E*)-**2** has a sweet, powdery, and mild odor.

Registry No. **1** (isomer 1), 83968-05-2; **1** (isomer 2), 83968-04-1; (*E*)-**2**, 107407-88-5; (*Z*)-**2**, 107407-87-4.

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Model Reactions on Roast Aroma Formation. 1. Reaction of Serine and Threonine with Sucrose under the Conditions of Coffee Roasting and Identification of New Coffee Aroma Compounds

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The composition of volatiles from roasting mixtures of sucrose, serine, and threonine were analyzed by capillary GC/MS. About 350 compounds (alkyl-, alkenyl-, and acyl-substituted furans, pyrroles, pyrazines, pyridines, oxazoles, and others, among them nine pyrrolylalkanols) were identified. With the exception of furans and oxazoles, most of them were also formed after heating serine and threonine in the absence of sucrose. This reaction demonstrates the importance of pyrolytic reactions occurring in addition to the Maillard reaction. After roasting of green coffee, about 160 compounds were identified, 53 of which, up to now, have not been described as constituents of coffee aroma.

Roast aromas are well-known to consist of a great many compounds. For instance, more than 700 compounds have been reported in roast coffee aroma (Clifford, 1985). In the aromas of roasted meat or peanuts, about 500 and 300 compounds, respectively, have been identified at this time. Therefore, it is clear that the investigation of roast aromas is very difficult and time consuming. In the same manner, the analysis of one of the various roast aroma essences produced by the flavour industry is a very hard task.

These essences are mostly produced by heating of specially treated proteins with sugars, lipids, selected amino acids, and fruity acids. Short overviews are given by Baltes (1979, 1980). A great many aroma compounds formed by these processes are of heterocyclic structure. They are formed by mechanisms that are postulated to involve the Maillard reaction (Baltes, 1980). But on the other hand, heating to about 250 °C also enhances pyrolytic degradation.

We have been engaged for some time in investigating model reactions of roast aroma formation by treating one or two selected amino acids with sugars or sugar degradation products under food-processing conditions to study the compounds formed as well as their precursors.

Recently, we gave a report on our experiments on the reaction of serine and threonine with sucrose under the conditions of coffee roasting, because we were interested in the contribution of these compounds to coffee aroma (Baltes and Bochmann, 1986). In this paper we give more details about the experiments and some special compounds identified.

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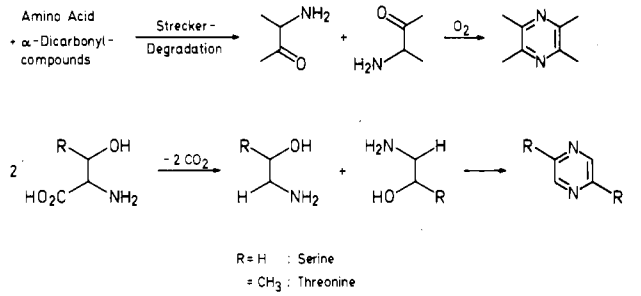

Figure 1. Formation of pyrazines via Strecker degradation and pyrolysis of hydroxyamino acids.

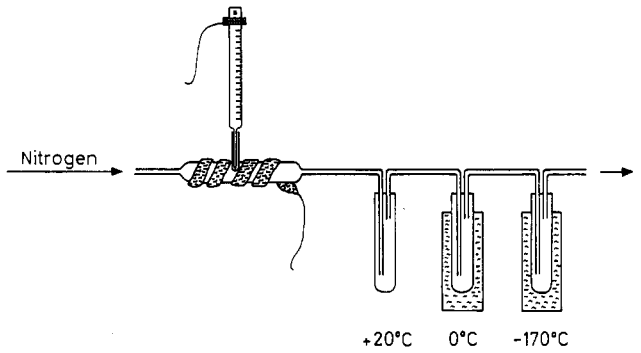

Figure 2. Roasting apparatus.

Raw coffee contains about 7% sucrose (Feldman et al., 1969) while the concentration of serine and threonine together amounts to about 1% (Thaler and Gaigl, 1963). After coffee roasting, these ingredients are nearly totally degraded. It was of further interest to study the contribution of pyrolytic mechanisms to the formation of aroma compounds. It has been reported that about 20 alkylpyrazines are formed after pyrolysis of hydroxyamino acids (Kato et al., 1970; Wang and Odell, 1973). Indeed, serine and threonine can undergo decarboxylation and react directly by dimerization to yield pyrazines, shown in Figure 1. Other amino acids normally can form pyrazines only via Strecker degradation.

Independently, serine and threonine were discussed to be the precursors of oxazoles in coffee aroma (Vitzthum and Werkhoff, 1974). Using information generated in these model system, we then investigated the aroma composition of coffee to ascertain which of these compounds are also present in coffee.

METHODS

Model Reactions. Mixtures: 1, 1.2 g of threonine + 3.4 g of sucrose; 2, 1.1 g of serine + 3.4 g of sucrose; 3, 0.6 g of threonine + 0.5 g of serine + 3.4 g of sucrose; 4, 1.2 g of threonine + 1.1 g of serine + 1.7 g of sucrose; 5, 1.2 g of threonine + 1.1 g of serine. Every mixture was thoroughly stirred in a mortar with 10% water and 10% sand and was layered in the reaction tube together with quartz wool. After the tube was covered with a heater band and a contact thermometer, it was connected with the cooling traps in the apparatus shown in Figure 2.

The roasting was carried out by heating the tube at a rate of 20 °C/min to a final temperature of 225 °C. The volatiles were carried over to the cooling traps by means of a nitrogen stream of 50 mL/min. The water-containing condensates were extracted with ether, and the ether fractions were combined, dried over Na_2SO_4 and molecular sieves, respectively, and concentrated by a nitrogen stream to a volume of 2–5 mL.

Gas Chromatography/Mass Spectrometry. Columns: (1) 50-m fused silica column of 0.3-mm inner diameter, coated with Carbowax 20 M; (2) 90-m fused silica column of 0.3-mm inner diameter, dynamically coated with a 10% Carbowax TPA solution. Other conditions: gas chromatograph Carlo Erba 2150 with fid and P-N detector, Model 793; GC/MS system, Finnigan MAT 4500 with INCOS data system; Transline 230 °C, ion source 120 °C; cyclic scan, 0.6 s; mass range, (EI) m/e 35–220, (CI, methane as reaction gas) m/e 75–330; retention index values, calculated according to Van den Dool and Kratz (1963). A standard of n-alkanes C_6–C_{26} was added to each reaction mixture.

H–D Isotope Exchange. Ether extract (1 mL) was shaken with 3 mL of D_2O for 3 h, separated, and analyzed by GC/MS.

Quantitative Determination. The amounts of reaction products were calculated by an integration program of the INCOS data system based on ion intensities after correction of the base line.

Roasting of Coffee. Raw coffee (Brazil Arabica; 50 g) was ground and used to fill the reaction tube as described. Roasting was performed in the same manner as the model reaction mixture. After extraction of the condensates, the reaction products were preseparated on a column fitted with Al_2O_3 (activity grade 3) and by subsequent elution by pentane, pentane/ether (7:3), pentane/ether (6:4), ether, and ethyl acetate. Separation and identification by GC/MS were as described previously. Pyrrolylalkanols were synthesized by procedure of Murahashi et al. (1974).

Trapping. For trapping special fractions, a glass column [7 m × 2 mm i.d., 5% Carbowax TPA on Chromosorb G AW DMCS (80–100 mesh)] was used. The fractions were collected in capillaries while being cooled with liquid air. They were tested sensorically and analyzed by GC/MS.

Table I. Composition of the Model Mixtures and Proportion of Total Volatile Compounds Identified

mixture	compd ident, %	molar ratio Ser	Thr	sucrose
1	91	0	1	1
2	90	1	0	1
3	86	0.5	0.5	1
4	70	1	1	0.5
5	59	1	1	0

RESULTS

Composition of the Volatiles. The composition of the reaction mixtures were determined by heating in a special roast apparatus (Figure 2) designed in a manner providing equimolar amounts of amino acids and sugars. In one experiment a fourfold excess of the amino acids was used. In order to test the possibility of a pyrolytic reaction mechanism, serine and threonine were also heated without sucrose. The thermolytic degradation of sucrose was already tested earlier (Baltes and Schmahl, 1978). Table I gives an overview over the molar reaction mixtures.

From the isolated volatiles, about 350 compounds were separated and their structures elucidated. These compounds represent about 59–91% of the quantities of all volatiles isolated after roasting the mixtures.

The absolute quantities obtained by these reactions were so small that GC/MS was the only suitable method for structure elucidation. For some additional aid we preseparated the carbonyl compounds by treating the condensates with $NaHSO_3$. Also very useful was the simultaneous use of a nitrogen detector in addition to the fid to characterize N-containing compounds in the gas chromatogram. Also helpful was the calculation of modified Kovats indices (Van den Dool and Kratz, 1963) after addition of an alkane standard C_6–C_{26}. The GC/MS measurements were carried

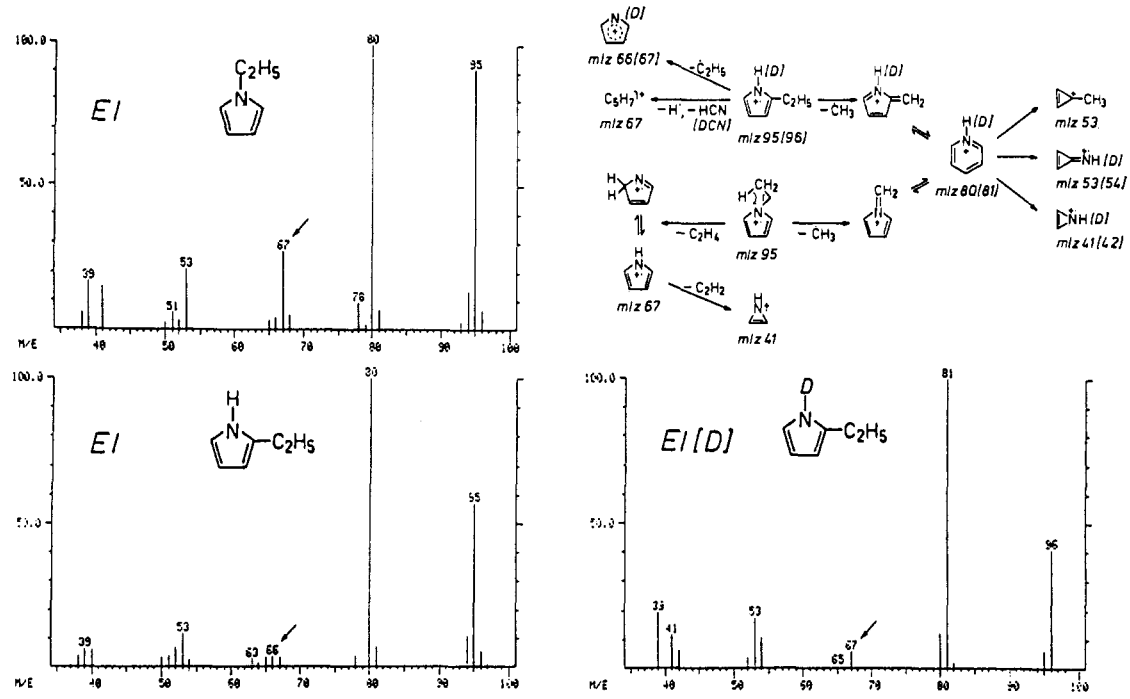

Figure 3. EI mass spectra and fragmentation pattern of 1- and 2-ethylpyrrole and 2-ethylpyrrole-*1-d*.

Table II. Quantities of Component Groups after Roasting Mixtures of Sucrose, Serine, and Threonine[a]

	1		2		3		4		5	
	a	b	a	b	a	b	a	b	a	b
alkylfurans	1.6	12	4.1	12	4.3	11	<1	8	0	0
alkenylfurans	<1	8	1.1	8	1.0	7	1.0	7	0	0
furanylalkanones	2.9	14	4.7	14	3.5	14	1.1	5	0	0
furan aldehydes	33	6	40	6	32	6	<1	2	0	0
furanylalkenones and -alkenals	1.0	7	<1	4	<1	7	<1	3	0	0
furfuryl alcohols	14	3	14	3	14	3	3.3	3	0	0
(hydroxymethyl)furfural	3.2	1	8.5	1	5.6	1	0	0	0	0
furfuryl alkanoates and alkyl furoates	<1	6	<1	6	<1	6	<1	2	0	0
N-furfurylamines	<1	1	0	0	<1	1	<1	2	0	0
furanylfurans and furfurylfurans	<1	5	<1	8	<1	5	0	0	0	0
furfurylpyrroles and furanylpyrroles	<1	7	<1	8	<1	7	<1	4	0	0
furanylpyrazines	<1	3	<1	3	1.0	3	<1	3	0	0
furfurylpyrazines	1.0	10	1.5	12	1.1	11	1.4	11	0	0
furanones	<1	10	1.6	13	<1	12	0	0	0	0
alkylpyrroles	<1	6	<1	2	<1	5	4.4	31	8.4	44
pyrrolylalkanones and pyrrole aldehydes	1.3	11	1.4	10	1.6	11	<1	6	0	0
N-pyrrolylalkanols	<1	1	<1	1	<1	2	8.1	9	16	9
dihydropyrrolizines	<1	1	<1	1	<1	2	<1	2	0	0
alkylpyrazines	22	31	5.4	26	11	30	38	47	32	51
alkenylpyrazines	<1	10	<1	11	<1	12	1.5	12	<1	7
acetylpyrazines	<1	3	<1	3	<1	3	<1	3	0	0
dihydrocyclopentapyrazines	3.2	9	<1	8	1.5	9	3.2	11	<1	8
tetrahydroquinoxalines	<1	5	<1	1	<1	2	<1	5	<1	2
quinoxalines	<1	9	<1	6	1.0	8	<1	9	<1	5
pyrrolopyrazines	<1	12	<1	2	<1	10	3.0	15	<1	11
alkylpyridines	<1	16	<1	6	<1	10	<1	18	1.5	23
acetylpyridines	<1	8	<1	5	<1	8	<1	4	0	0
oxazoles	<1	7	<1	7	<1	6	<1	4	0	0
phenols	<1	8	<1	8	<1	8	0	0	0	0
carbocyclic compounds	1.0	10	1.7	12	2.3	12	0	0	0	0
aliphatic compounds (alkanoates, amides)	<1	4	0	0	<1	3	1.0	5	1.2	6

[a] Key: a, percent of total ion current of the whole fraction; b, number of components in this group.

out with the short cycle time of 0.5–0.6 s/decade in the EI as well as in the CI mode. The fragmentation patterns of the mass spectra were studied in detail. The formulas of all identified compounds not mentioned here will be published elsewhere together with their modified Kovats index values, their mass spectra, and their quantities (Baltes and Bochmann, 1987). Structures were confirmed by spectra from the literature and by comparison with reference standards. The exchange of active hydrogen atoms against deuterium by shaking the unseparated condensates with D_2O was also helpful. This method was indispensable for structural differentiation of alkylated pyrroles, as the mass spectra of the 1- and 2-substituted compounds often do not differ. This is illustrated in Figure 3. Table II gives a summary of the compounds formed from all experiments as well as their numbers and quantities calculated by a special integration program of the data system. These calculations use the proportional ion

Table III. Mass Spectral Data and Modified Kovats Indices of Pyrrolylalkanols

compound	retention index	MS	references
3-(1-pyrrolyl)propan-2-ol	1828	EI: 126 (5), 125 (56), 110 (1), 93 (1), 82 (6), 81 (44), 80 (100), 78 (7), 68 (23), 67 (8), 54 (12), 53 (21), 45 (16), 41 (8), 39 (9) EI (D): 126 (35), 125 (15), 83 (6), 82 (32), 81 (51), 80 (100), 78 (8), 69 (20), 68 (15), 55 (14), 54 (13), 53 (36), 46 (18), 41 (13), 39 (18)	Grünewald (1983) and standard
3-(2-resp-3-methyl-1-pyrrolyl)propanol	1883	EI: 140 (4), 139 (39), 95 (19), 94 (100), 93 (12), 82 (22), 80 (14), 78 (6), 67 (7), 65 (5), 53 (8), 45 (5), 41 (9), 39 (7) EI (D): 141 (4), 140 (33), 139 (8), 97 (4), 96 (11), 95 (37), 94 (100), 93 (10), 83 (23), 82 (11), 81 (14), 80 (9), 78 (9), 68 (5), 67 (10), 65 (7), 53 (13), 46 (7), 42 (7), 41 (21), 39 (16)	
3-(3-resp-2-methyl-1-pyrrolyl)propan-1-ol	1917	EI: 139 (43), 95 (14), 94 (100), 82 (31), 80 (12), 78 (7), 67 (5), 65 (5), 53 (12), 45 (4), 41 (8), 39 (11)	
2-(1-pyrrolyl)ethan-1-ol	1934	EI: 111 (70), 93 (2), 80 (100), 78 (10), 68 (20), 67 (8), 53 (35), 41 (8), 39 (10) EI (D): 112 (45), 111 (18), 94 (2), 80 (100), 78 (10), 69 (20), 68 (18), 53 (35), 41 (18), 39 (18)	Grünewald (1983) and standard
3-(dimethyl-1-pyrrolyl)propan-1-ol	1962	EI: 153 (33), 109 (16), 108 (100), 96 (20), 94 (15), 92 (5), 67 (6), 65 (5), 53 (4), 45 (2), 41 (10), 39 (7)	
3-(dimethyl-1-pyrrolyl)propan-1-ol	1967	EI: 153 (41), 109 (14), 108 (100), 96 (20), 94 (15), 92 (7), 81 (4), 67 (7), 65 (5), 53 (7), 45 (2), 41 (11), 39 (4)	
2-(2-resp-3-methyl-1-pyrrolyl)ethan-1-ol	1991	EI: 125 (60), 106 (2), 94 (100), 82 (20), 80 (18), 78 (16), 67 (15), 53 (13), 41 (13), 39 (8) EI (D): 126 (40), 125 (18), 94 (100), 83 (15), 67 (15), 53 (18), 41 (22), 39 (18)	
2-(dimethyl-1-pyrrolyl)ethan-1-ol	2077	EI: 139 (54), 109 (11), 108 (100), 96 (9), 94 (19), 92 (8), 67 (9), 65 (6), 53 (4), 45 (3), 41 (12), 39 (12)	

current of every compound; therefore, the values are only approximations. Most of the compounds were already mentioned in the literature in connection with roast aromas. The alkyl groups possess chain lengths of up to C_5.

As expected, furans were formed when the reaction mixture contained at least an equimolar amount of sucrose. Most prominent were furan aldehydes, furan alcohols, alkylfurans, and furan alkanones. These are also the main compounds of sugar pyrolysis. Moreover, oxazoles, furanones, and acetyl-, formyl-, furanyl-, and furfuryl-substituted derivatives of N-heterocyclic compounds (pyrazines, pyrroles, pyridines) were formed. Threonine seems to be more reactive than serine. It formed the fourfold amount of alkylpyrazine when both amino acids were separately treated with sucrose. Also dihydrocyclopentapyrazines were formed in higher amounts from the reaction mixture containing threonine plus sucrose. Possibly the higher reactivity is due to the additional methyl group of this amino acid (in comparison to serine).

Corresponding to its composition, the volatiles of reaction mixtures 1–3 predominantly smelled of roast aromas. On the other hand, burnt aromas resulted from roasting reaction mixtures 4 and 5 where the amino acids were present in excess or where sucrose was absent. Pyrrolylalkanols seem to be predominatly responsible for this effect. The relatively high yields of pyrrolylalkanols were astonishing to us. Compounds of this type have been described by Ruiter (1973) referring to a reaction of glyoxal with aminoethanol and by Grünewald (1983) as roast products after reaction of glucose with hydroxyproline. Table III shows the composition, mass spectra, relative Kovats indices, and relative amounts of pyrrolylalkanol compounds found after roasting of our reaction mixtures. While only 1-(1-pyrrolyl)-2-ethanol is obviously formed from serine, the corresponding isopropyl alcohol isomer could be found exclusively after reaction of threonine (Figure 4).

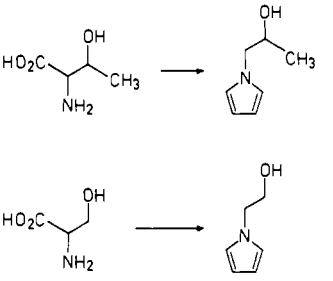

Figure 4. Precursors of pyrrolylethanol and pyrrolylpropan-2-ol.

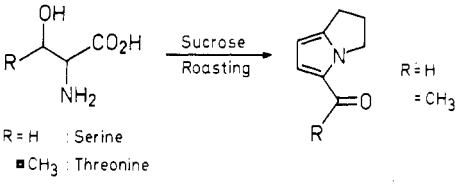

Figure 5. Formyl- and acetylpyrrolizine and its probable precursors.

The precursor also can be recognized in the structures of two 2,3-dihydro-1*H*-pyrrolizines, which are formed predominantly by roasting equimolar amounts of serine and threonine, respectively, with sucrose. Thus, threonine reacted to form a compound containing an acetyl group, while after reaction of serine a formyl moiety in the 5-position was identified. Up to now, proline was suggested to be the only precursor of this compound class (Figure 5).

By pyrolysis of serine and threonine under conditions of coffee roasting, a great many compounds are formed. From roasting serine and threonine without sucrose, we identified more than 160 compounds corresponding to 78% to the amount of all volatiles. The portion of alkylpyrazine amounted to 38% and 32%, respectively, being higher than in the presence of sucrose. These results mean that many

Table IV. Probable New Aroma Compounds of Roast Coffee

compound	retention index	MS	references
2-ethyl-5-methylfuran	1014	EI: 110 (39), 109 (5), 96 (7), 95 (100), 82 (5), 81 (11), 67 (7), 65 (4), 55 (4), 53 (6), 51 (5), 43 (21), 41 (9), 39 (16)	standard
2,3,4-trimethylfuran	1076	EI: 110 (100), 109 (51), 95 (47), 81 (27), 67 (55), 53 (18)	Heyns et al. (1966)
5-methyl-2-*n*-propylfuran	1086	EI: 124 (35), 95 (100), 67 (10)	Heyns et al. (1966)
3(4)-methyl-2-*n*-propylfuran	1105	EI: 124 (44), 95 (100), 67 (13)	Heyns et al. (1966)
1-(2-furanyl)-3-pentanone	1701	EI: 153 (8), 152 (52), 124 (4), 123 (36), 96 (14), 95 (48), 94 (14), 82 (8), 81 (100), 68 (10), 67 (20), 66 (11), 57 (31), 53 (17), 52 (7), 50 (5), 41 (14), 39 (30)	
1-(5-methyl-2-furanyl)-4-pentanone	1769	EI: 166 (18), 109 (16), 95 (100), 57 (13), 43 (96)	
3-methyl-2-furfural	1467	EI: 110 (100), 109 (63), 53 (61)	standard
4-methyl-2-furfural	1537	EI: 110 (100), 109 (87), 53 (32)	
dimethyl-2-furfural	1594	EI: 124 (100), 123 (98), 95 (9), 67 (10)	
1-(5-methyl-2-furanyl)-1-propen-3-al	1855	EI: 137 (10), 136 (100), 135 (9), 108 (55), 107 (35), 82 (6), 81 (6), 80 (20), 79 (75), 78 (7), 77 (60), 68 (38), 65 (6), 63 (6), 51 (17), 50 (8), 43 (19), 39 (36)	
1-(5-methyl-2-furanyl)-1-buten-3-one	1990	EI: 151 (6), 150 (49), 136 (8), 135 (100), 134 (6), 107 (17), 79 (9), 78 (5), 77 (18), 67 (5), 65 (9), 63 (7), 53 (7), 52 (5), 51 (6), 43 (23), 39 (14)	Kadentsev et al. (1980)
4(3)-methyl-2-furfuryl alcohol	1587	EI: 112 (52), 111 (7), 97 (100), 95 (36), 94 (14), 84 (10), 69 (22), 67 (7), 66 (8), 65 (14), 43 (22), 41 (43), 39 (38)	
methyl 5-methylfuro-2-ate	1652	EI: 140 (43), 109 (100), 81 (9), 53 (11)	
2-(5-methyl-2-furfuryl)methylpyrazine	1652	EI: 188 (67), 145 (96), 95 (100), 67 (10), 65 (7), 53 (9), 51 (9), 43 (18), 39 (10)	
dimethyl-2-vinylpyrazine	1521	EI: 134 (63), 133 (100), 108 (2), 107 (3), 93 (3), 81 (4), 66 (9), 65 (7), 54 (24), 52 (6), 42 (54), 40 (12), 39 (15)	Buttery et al. (1971)
ethyl-2-vinylpyrazine	1528	EI: 134 (38), 133 (100), 119 (6), 108 (10), 107 (3), 92 (5), 81 (5), 79 (4), 78 (4), 56 (3), 54 (9), 52 (7), 41 (15), 39 (18)	Friedel et al. (1971)
ethylmethylvinylpyrazine	1573	EI: 148 (42), 147 (100), 133 (11), 122 (13), 107 (3), 106 (3), 80 (4), 79 (5), 68 (5), 66 (4), 54 (5), 52 (3)	
2,6-dimethyl-6,7-dihydro-5*H*-cyclopentapyrazine	1718	EI: 149 (7), 148 (64), 147 (100), 146 (9), 145 (6), 133 (49), 132 (9), 120 (6), 108 (9), 107 (12), 106 (6), 92 (6), 79 (8), 78 (7), 77 (7), 65 (12), 64 (6), 63 (6), 52 (7), 41 (5), 39 (16)	standard
1-methylpyrrolo[1,2-*a*]pyrazine	1995	EI: 133 (11), 132 (100), 131 (55), 105 (8), 104 (19), 78 (9), 77 (8), 66 (12), 64 (7), 52 (8), 51 (8), 39 (11)	standard
4-methylpyrrolo[1,2-*a*]pyrazine	2093	EI: 132 (100), 131 (57), 105 (12), 104 (31); 79 (10), 78 (13), 66 (9), 64 (7), 63 (12), 52 (12), 51 (11), 39 (9)	standard
2,4-dimethylpyridine	1334	EI: 107 (100), 106 (39), 92 (15), 79 (22), 77 (11), 65 (12), 63 (4), 51 (12), 39 (12)	standard
2-ethyl-6-methylpyridine	1292	EI: 121 (55), 120 (100), 93 (30), 79 (17), 77 (18), 66 (14), 65 (13)	Stenhagen et al. (1974)
2-ethyl-5-methylpyridine	1385	EI: 121 (49), 120 (100), 106 (2), 93 (17), 91 (4), 79 (3), 77 (8), 66 (5), 65 (6), 51 (3), 39 (16)	
4-ethylpyridine	1387	EI: 107 (100), 106 (92), 92 (44), 79 (27), 77 (11), 65 (28), 52 (13), 51 (18)	standard
5-ethyl-2-methylpyridine	1408	EI: 121 (64), 120 (23), 106 (100), 93 (3), 91 (3), 79 (14), 77 (18), 65 (5), 52 (5), 51 (6), 39 (10)	standard
2-*n*-butyl-6-methylpyridine	1485	EI: 149 (2), 148 (10), 134 (18), 107 (100), 106 (11), 92 (15), 79 (8), 77 (6), 66 (3), 65 (7), 53 (4), 51 (5)	
2-methyl-5-*n*-propylpyridine	1494	EI: 135 (28), 120 (11), 106 (100), 93 (4), 91 (2), 79 (11), 77 (15), 65 (5), 52 (6), 51 (5), 41 (5), 39 (10)	
1-acetyl-2,5-dimethylpyrrole	1306	EI: 137 (57), 95 (49), 94 (100), 67 (16)	
2-acetyl-4(3)-methylpyrrole	2036	EI: 123 (83), 108 (100), 80 (37), 53 (43)	
2-(1-pyrrolyl)-1-ethanol	1935	EI: 112 (5), 111 (64), 81 (9), 80 (100), 78 (11), 68 (22), 67 (8), 53 (34), 51 (5), 41 (8), 39 (10)	Grünewald (1983)
methyl-*n*-butylpyrazine	1478	EI: 150 (13), 135 (13), 108 (100), 107 (16), 67 (7), 53 (5), 42 (9), 39 (10)	Buttery et al. (1971)
3,5(3,6)-dimethyl-2-*n*-propylpyrazine	1502	EI: 150 (38), 149 (41), 135 (20), 122 (100), 108 (3), 107 (6), 94 (2), 81 (3), 80 (5), 67 (6), 53 (8), 42 (20), 39 (16)	standard
3,6(3,5)-dimethyl-2-*n*-propylpyrazine	1525	EI: 150 (12), 149 (10), 135 (19), 122 (100), 108 (2), 107 (4), 81 (2), 80 (5), 66 (3), 53 (5), 42 (11), 39 (11)	standard
2,3-diethyl-5,6-dimethylpyrazine	1527	EI: 164 (100), 163 (54), 149 (87), 136 (8), 135 (7), 121 (4), 107 (2), 56 (21), 54 (13), 53 (12), 42 (4)	
2,6-diethyl-3,5-dimethylpyrazine	1536	EI: 164 (81), 163 (100), 149 (29), 136 (13), 135 (5), 121 (13), 108 (4), 107 (4), 94 (5), 80 (3), 67 (21), 66 (5), 56 (6), 53 (18), 41 (14), 39 (19)	

Table IV (Continued)

compound	retention index	MS	references
2,5-diethyl-3,6-dimethylpyrazine	1539	EI: 164 (70), 163 (53), 149 (100), 136 (9), 121 (7), 108 (3), 94 (4), 67 (20), 66 (4), 56 (5), 53 (12), 41 (12), 39 (14)	
ethylmethyl-*n*-propylpyrazine	1553	EI: 164 (24), 149 (31), 136 (100), 135 (27), 121 (5), 108 (7), 107 (4), 94 (2), 80 (3), 67 (4), 56 (4), 54 (4), 53 (7), 43 (8), 41 (9), 39 (10)	
triethylmethylpyrazine	1560	EI: 178 (100), 177 (57), 163 (94), 149 (11), 135 (17), 121 (12), 94 (5), 93 (5), 81 (5), 80 (7), 68 (10), 67 (22), 66 (9), 56 (10), 54 (8), 53 (15)	
3-methyl-2-vinylpyrazine	1476	EI: 121 (7), 120 (70), 119 (100), 79 (20), 78 (7), 67 (40), 54 (13), 52 (11), 42 (10), 41 (6), 39 (20)	Mussinan et al. (1974)
3-*n*-butylpyridine	1566	EI: 136 (8), 135 (77), 134 (7), 106 (20), 93 (98), 92 (100), 91 (6), 78 (11), 77 (9), 66 (7), 65 (17), 51 (13), 50 (7), 43 (15), 41 (13), 39 (20)	standard
2-isopropyl-4(5)-methyloxazole	1203	EI: 125 (44), 124 (7), 110 (100), 82 (45)	
2-isopropyl-4,5-dimethyloxazole	1261	EI: 139 (32), 138 (7), 124 (100), 96 (38), 70 (15), 55 (23), 54 (7), 43 (24), 42 (18), 41 (11), 39 (11)	standard
2-isopropylethyloxazole	1267	EI: 139 (30), 138 (5), 124 (100), 96 (22)	Ho et al. (1983)
4-*n*-propyl-2,5-dimethyloxazole	1279	EI: 139 (19), 124 (21), 111 (56), 110 (100)	
2-isopropylethylmethyloxazole	1283	EI: 153 (31), 138 (100), 110 (25), 96 (32), 84 (10), 69 (21), 56 (11), 55 (15)	Ho et al. (1983)
4-*n*-butyl-2(5)-methyloxazole	1291	EI: 139 (15), 138 (6), 124 (17), 97 (100), 96 (40), 82 (12)	Ho et al. (1981, 1983)
4-*n*-propylethylmethyloxazole	1331	EI: 153 (24), 138 (20), 125 (51), 124 (100), 96 (35)	Ho et al. (1983)
4-*n*-butyl-2,5-dimethyl- or -ethyloxazole	1342	EI: 153 (24), 138 (20), 124 (9), 111 (100), 110 (65), 55 (25)	
5-*n*-butylethylmethyloxazole	1366	EI: 167 (25), 152 (9), 138 (10), 125 (23), 124 (100)	Ho et al. (1983)

roast aroma products of serine and threonine obviously do not require a Maillard reaction or Strecker degradation for their formation. On the contrary, the pyrolytic degradation and subsequent reaction of the decomposition products seem to be more important. Because of the high molecular weight in the pyrazine fraction of up to 192, the formation of these compounds cannot be a simple dimerization of decarboxylated serine and threonine!

Roasting of Coffee. As mentioned before, we were interested in a possible contribution of serine and threonine to coffee aroma. The composition of coffee aroma is very well investigated; an excellent overview was given by Vitzthum (1976). To compare our results with the aroma components of coffee, we have roasted a specimen of ground green Arabica Brazil coffee in the roast tube already described. The volatiles collected were first preseparated by chromatography on Al_2O_3 and then separated by capillary GC/MS. The mass spectra obtained were compared with the spectra already stored in the data system from our previous model experiments. More than 220 compounds were identified. Beside a greater number of furans that might have been formed from carbohydrates, most of the compounds belong predominantly to the classes of pyrazines and pyridines. Only a few compounds that were formed by the model reactions were not found in the coffee volatiles. On the other hand, the suggestion that serine and threonine are the precursors of oxazoles in coffee aroma does not seem to hold (Vitzthum and Werkhoff, 1974). In our model experiment, we have only found 9 oxazoles while by roasting coffee 21 oxazoles were formed. From our experiments it appears that the reaction pathway to pyrazines is preferred. The ion intensities of the oxazoles were only about 4% of the values of the alkylpyrazines.

By examination of Vitzthum's list of coffee volatiles, we realized that 53 compounds of our coffee roasting have not yet been described as coffee aroma compounds. These are listed in Table IV. With the exception of some pyridines, all these compounds were found in trace amounts. But now it seems to be clear that pyridines, well-known as aroma ingredients of coffee, are formed not only by trigonellin pyrolysis but also from serine and threonine. Another way of pyridine formation under roast conditions was shown by Suyama and Adachi (1986). Pyrrolylethanol, furfurylpyrazines, and pyrrolopyrazines have not been described to be present in coffee aroma up to now.

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Registry No. Sucrose, 57-50-1; L-serine, 56-45-1; L-threonine, 72-19-5; 3-(1-pyrrolyl)propan-2-ol, 104815-66-9; 2-(1-pyrrolyl)-ethan-1-ol, 6719-02-4; 3-(dimethyl-1-pyrrolyl)propan-1-ol, 107054-23-9; 2-(dimethyl-1-pyrrolyl)ethan-1-ol, 107054-24-0; 2-ethyl-5-methylfuran, 1703-52-2; 2,3,4-trimethylfuran, 10599-57-2; 5-methyl-2-*n*-propylfuran, 1456-16-2; 3(4)-methyl-2-*n*-propylfuran, 106100-52-1; 1-(2-furanyl)-3-pentanone, 69978-21-8; 1-(5-methyl-2-furanyl)-4-pentanone, 106060-94-0; 3-ethyl-2-furfural, 33342-48-2; 4-methyl-2-furfural, 33342-49-3; dimethyl-2-furfural, 106100-49-6; 1-(5-methyl-2-furanyl)-1-propen-3-al, 5555-90-8; 1-(5-methyl-2-furanyl)-1-buten-3-one, 23120-57-2; 4(3)-methyl-2-furfuryl alcohol, 106188-67-4; methyl 5-methyl-2-furoate, 2527-96-0; 2-(5-methyl-2-furfuryl)methylpyrazine, 107082-33-7; dimethyl-2-vinylpyrazine, 81352-79-6; ethyl-2-vinylpyrazine, 59094-70-1; ethylmethylvinylpyrazine, 106100-51-0; 2,6-dimethyl-6,7-dihydro-5*H*-cyclopentapyrazine, 61928-63-0; 1-methylpyrrolo[1,2-*a*]pyrazine, 64608-59-9; 4-methylpyrrolo[1,2-*a*]pyrazine, 64608-60-2; 2,4-dimethylpyridine, 108-47-4; 2-ethyl-6-methylpyridine, 1122-69-6; 2-ethyl-5-methylpyridine, 18113-81-0; 4-ethylpyridine, 536-75-4; 5-ethyl-2-methylpyridine, 104-90-5; 2-*n*-butyl-6-methylpyridine, 5335-76-2; 2-methyl-5-*n*-propylpyridine, 874-75-9; 1-acetyl-2,5-dimethylpyrrole, 5044-31-5; 2-acetyl-4(3)-methylpyrrole, 106100-50-9; methyl-*n*-butylpyrazine, 106100-48-5; 3,5-dimethyl-2-*n*-propylpyrazine, 32350-16-6; 3,6-dimethyl-2-*n*-propylpyrazine, 18433-97-1; 2,3-diethyl-5,6-dimethylpyrazine, 106060-96-2; 2,6-diethyl-3,5-dimethylpyrazine, 18940-74-4; 2,5-diethyl-3,6-dimethylpyrazine, 18903-30-5; ethylmethyl-*n*-propylpyrazine, 107054-27-3; triethylmethylpyrazine, 106073-56-7; 3-methyl-2-vinylpyrazine, 25058-19-9; 3-*n*-butylpyridine, 539-32-2; 2-isopropyl-4(5)-methyloxazole, 106100-40-7; 2-isopropyl-4,5-dimethyloxazole, 19519-45-0; 2-isopropylethyloxazole, 106100-39-4; 4-*n*-propyl-2,5-dimethyloxazole, 30674-60-3; 2-isopropylethylmethyloxazole, 106100-44-1; 4-*n*-butyl-2(5)-methyloxazole, 106100-45-2; 4-*n*-propylethylmethyloxazole, 106123-28-8; 4-*n*-butyl-2,5-dimethyloxazole, 30408-62-9; 4-*n*-butylethyloxazole, 107054-28-4; 5-*n*-butylethylmethyloxazole, 106100-43-0; hydroxymethylfurfural, 25376-49-2.

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Volatile Nitrosamines in Cured Meats Packaged in Elastic Rubber Nettings

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An investigation was carried out to determine the levels of volatile nitrosamines in elastic rubber nettings and cured meats packaged in such nettings. Most unused nettings analyzed contained only traces of nitrosamines (mainly *N*-nitrosodiethylamine and *N*-nitrosodi-*n*-butylamine) but the used nettings contained extremely high levels (up to 504 ppb) of the same nitrosamines. The homogenized meats from such packages also contained significant levels of *N*-nitrosodi-*n*-butylamine (up to 29 ppb) and traces of *N*-nitrosodiethylamine. There was also a definite concentration gradient of these two nitrosamines along the cross section of the meat. Cured meats packaged in plastic wrappings or cotton nettings were negative. The above two nitrosamines detected in the meats and used nettings most likely originated as a result of the interaction of the amine precursors (e.g., dithiocarbamates) in the rubber and nitrite additive commonly used for the preparation of cured meat products.

Man is exposed to *N*-nitrosamines through a variety of sources such as cigarette smoke, cosmetics, foods (e.g., cured meats, fried bacon, fish) and beverages (e.g., beer and ale, whiskies), industrial and agricultural chemicals (e.g., cutting fluids, pesticides), and rubber products (Fine et al., 1980). Since most *N*-nitrosamines are potent carcinogens in laboratory animals, their occurrence or formation in the above items has been a matter of concern (Preussmann and Stewart, 1984). Research during the past 10 years has shown that various rubber products used by man can contribute significantly toward his/her total body burden of nitrosamines (Fine et al., 1980; Spiegelhelder and Preussman, 1983).

The first indication that rubber products may contain traces of nitrosamines originated from the studies by Fajen et al. (1979), who detected varying levels of *N*-nitrosodimethylamine (NDMA), *N*-nitrosomorpholine (NMOR), and *N*-nitrosodiphenylamine (NDPhA) in the air from several rubber industry factories. Soon after, Fine et al. (1980) reported the occurrence of fairly high levels of nitrosamines (mainly NDMA and NMOR) in rubber tires and in the interior air of new automobiles. Working independently, Ireland et al. (1980) detected traces of volatile nitrosamines in a variety of household rubber products such as baby bottle rubber nipples, rubber gloves, and natural rubber condoms. This latter group of workers also suggested that most of the nitrosamines in rubber originated via nitrosation of various dialkylamino compounds (e.g., dialkyldithiocarbamates) used as vulcanization accelerators in the rubber industry. The nature of the amine accelerator used determined which nitrosamine would be found in the rubber.

The main nitrosating agents responsible for the formation of nitrosamines in rubber are believed to be NDPhA, a retarder used in the vulcanization of rubber and a good transnitrosating agent, and nitrogen oxide gases present in the factory air (Spiegelhalder and Preussmann, 1983). Several preventive measures can be taken to reduce nitrosamine levels in rubber. These include (a) the use of alternative accelerators that do not form nitrosamines or form only noncarcinogenic nitrosamines, (b) the replace-

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