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Quantitative Analysis of Aroma Compounds in Wheat and Rye Bread Crusts Using a Stable Isotope Dilution Assay

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A stable isotope dilution assay was developed for the quantitative analysis of acetylpyrazine, 2-methyl-3-ethylpyrazine, 5-methyl-5*H*-cyclopenta[*b*]pyrazine, and 2-acetyl-1-pyrroline in bread crusts. Model experiments showed that these compounds, which contribute significantly to the crust flavor, can be determined with high sensitivity and accuracy. The method revealed that the levels of the three pyrazines lie in similar concentration ranges in the wheat and rye crusts. In contrast, 2-acetyl-1-pyrroline appeared at an approximately 20-fold higher concentration in wheat crust than in rye. Comparison of two different processes for rye bread revealed that the level of the four compounds was twice as high in the three-stage sourdough than in the one-stage process.

During heating of a foodstuff a multiplicity of volatiles is formed in very low concentrations. The aroma compounds that are characteristic for the distinct flavor notes of a food are only small fractions of this volatile mixture. Therefore, they have to be enriched by several concentration steps for quantitative analysis, and the losses caused by these manipulations are corrected with internal standards. However, this method provides only accurate data when the stability and the physical properties of both the compound to be estimated and of the internal standard are very similar. The best internal standard would thus be the compound labeled with an appropriate isotope, provided that the label is not changed during the analytical procedure. The use of a stable isotope labeled internal standard for quantitative analysis was at first reported by Sweeley et al. (1966). After addition of deuteriated glucose as internal standard, they quantified this substance by determination of the ratio of the protium and deuterium forms with the aid of combined gas chromatography–mass spectrometry. In the meantime this method, known as the "isotope dilution assay", has been widely applied for the quantitative analysis of trace components.

We have used an isotope dilution assay for the quantitative analysis of acetylpyrazine (I), 2-methyl-3-ethylpyrazine (II), 5-methyl-5*H*-cyclopenta[*b*]pyrazine (III), and 2-acetyl-1-pyrroline (IV) in the crusts of wheat and rye breads since as shown previously these compounds contribute significantly to the crustlike aroma note of the bread flavors (Folkes and Gramshaw, 1981; Schieberle and Grosch, 1983, 1984, 1985; Sizer et al., 1975). The present paper deals with the development of the assay for the flavor compounds I–IV and its application on different types of wheat and rye bread.

EXPERIMENTAL SECTION

Breads. The wheat breads were prepared with and without the addition of 10% (w/w) of a dried sponge as described earlier (Schieberle and Grosch, 1985). One type of rye bread was prepared in a one-stage sourdough process with the aid of citric acid and the other type by the three-stage sourdough process of Seibel et al. (1978). The rye grist bread was prepared from coarse whole rye flour as described earlier (Schieberle and Grosch, 1983).

Chemicals. 2-Acetylpyrrole, 2,3-pentanedione, ethylenediamine, 3-methyl-1,2-cyclopentanedione, and rhodium on activated alumina were from Fluka (Buchs, Switzerland). Pyrazinamide and methanol-d_1 (99.9% isotopic purity) were from Sigma (Munich, Germany). Ethylenediamine-d_4 (98% isotopic purity) and iodomethane-d_3 (98% isotopic purity) were from MSD Isotopes (IC Chemicals, Munich, Germany). Deuterium gas (99.7% isotopic purity) and ethylpyrazine were from Alfa (Ventron GmbH, Karlsruhe, Germany). Palladium on charcoal (10% Pd) was from Merck (Darmstadt, Germany), and neutral alumina was from Woelm (Eschwege, Germany). The solvents were purified as done by Schieberle and Grosch (1983). Silica gel 60 (Merck, Darmstadt, Germany) was treated with HCl and deactivated with 7% (w/w) water according to Esterbauer (1968).

Synthesis. Unlabeled acetylpyrazine (I), 2-methyl-3-ethylpyrazine (II), 5-methyl-5*H*-cyclopenta[*b*]pyrazine (III), and 2-acetyl-1-pyrroline (IV) were prepared and purified according to Roberts (1968), Flament and Stoll (1967), Flament et al. (1973), and Buttery et al. (1983), respectively. The MS and ^{1}H NMR data agreed with those published by the authors. Synthesis of the corresponding deuteriated compounds required the following modifications of these procedures:

Deuteriated Acetylpyrazine (I-*d*). Cyanopyrazine (2.1 g, 20 mmol), which was prepared from pyrazinamide (Roberts, 1968), was dissolved in 30 mL of diethyl ether. During 20 min this solution was added dropwise to a stirred and cooled Grignard solution that contained 1.5 g of magnesium and 8.5 g of methyl-d_3 iodide in 60 mL of diethyl ether. The reaction mixture was poured on 400 g of ice, and pyrazine I-*d* was isolated and recrystallized as described by Roberts (1968) for unlabeled pyrazine I.

Deuteriated 2-Methyl-3-ethylpyrazine (II-*d*). 2,3-Pentanedione (400 mg, 4 mmol) in 5 mL of diethyl ether was dropped at 0 °C into a solution of ethylenediamine-d_4 (240 mg, 4 mmol) in 5 mL of diethyl ether under stirring.

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After 20 min the reaction mixture was refluxed for 5 min, then dried over Na_2SO_4, and concentrated to 1 mL in a stream of nitrogen. Each half of the concentrate was applied onto a column (20 × 1 cm) packed with neutral aluminum oxide (activity grade III) that was suspended in pentane–diethyl ether (95:5 v/v). Stepwise elution at 10–12 °C was then performed with 50 mL of 95:5 pentane–diethyl ether (v/v; fraction A), 50 mL of 75:25 pentane–diethyl ether (v/v; fraction B), and 60 mL of 1:1 pentane–diethyl ether (v/v; fraction C). Fraction C, which contains the deuteriated 2-methyl-3-ethyldihydropyrazine, was freed from the solvent in a stream of nitrogen. The residue was dissolved in 12 mL of octane and, after addition of 250 mg of palladium on charcoal, refluxed for 18 h. The reaction mixture was fractionated on aluminum oxide as described above. Pyrazine II-*d* appeared in fraction B. It was further purified by TLC on silica gel HF_{254} plates (0.5 mm thick) with the solvent system dichloromethane–ethanol (9:1 v/v).

Deuteriated 5-Methyl-5*H*-cyclopenta[*b*]pyrazine (III-*d*). Solutions of 5-methyl-1,2-pentanedione (500 mg, 4 mmol) and ethylenediamine-d_4 (240 mg, 4 mmol), each in 2 mL of methanol-*d*, were simultaneously dropped into a stirred solution of 500 mg of NaOH in 20 mL of methanol-*d*. The reaction mixture was refluxed for 7 h and cooled, and after addition of 2 g of Celite, the solvent methanol-*d* was evaporated under a reduced pressure of 150 mbar. The reaction products were extracted in a Soxhlet apparatus from the Celite with 50 mL of diethyl ether. After concentration to 4 mL in a stream of nitrogen, the reaction mixture was chromatographed on two columns with neutral aluminum oxide as described for compound II-*d*, this time eluting with 50 mL of 95:5 pentane–diethyl ether (v/v; fraction A), 50 mL of 75:25 pentane–diethyl ether (v/v; fraction B), 50 mL of 1:1 pentane–diethyl ether (v/v; fraction C), and 100 mL of diethyl ether (fraction D). From fraction D, which contains the deuteriated 5-methyldihydrocyclopentapyrazine, the solvent was removed at first by distillation until a volume of 20 mL was obtained and then in a stream of nitrogen. The residue was dissolved in 12 mL of xylene and, after addition of 300 mg of palladium on charcoal, refluxed for 18 h. The reaction mixture was separated by column chromatography as described above. Pyrazine III-*d* appeared in fractions B and C, and in addition, the unreacted 5-methyldihydrocyclopentapyrazine, in fraction D. Pyrazine III-*d* was further purified by TLC on silica gel HF_{254} plates (0.5 mm thick) with diethyl ether as solvent.

Deuteriated 2-Acetyl-1-pyrroline (IV-*d*). After addition of rhodium on alumina as catalyst (1.2 g), a solution of 2-acetylpyrrole (1.09 g, 10 mmol) in 25 mL of methanol-*d* was deuteriated in an autoclave for 24 h at 10 bar pressure with deuterium gas. The deuteriated 2-(1-hydroxyethyl)pyrrolidine obtained was freed from the solvent methanol and then dissolved in 80 mL of benzene (nitrogen saturated). After addition of silver carbonate on Celite as an oxidation catalyst (Fetizon and Golfier, 1968), the suspension was stirred and refluxed under nitrogen for 18 h. The solution was filtered over Celite and chromatographed in two portions on water-cooled columns (20 × 1 cm) packed with neutral aluminum oxide (activity grade IV), suspended in 95:5 pentane–diethyl ether (v/v). The benzene was eluted with 80 mL of 95:5 pentane–diethyl ether (v/v) and IV-*d* with 120 mL of 6:4 pentane–diethyl ether (v/v). The weakly bound, exchangeable deuterium atoms were immediately exchanged from pyrroline IV-*d* as follows: 50 mL of 2 M HCl was added under cooling and shaking to the combined fractions that contained IV-*d*, and after alkalization with 50 mL of 3 M NaOH, the organic layer was separated and dried over Na_2SO_4. The solvent was removed in a stream of nitrogen, and the residue was dissolved in 80 mL of benzene and rechromatographed on aluminum oxide as described above. The fractions containing IV-*d* were combined and concentrated to 100 mL on a Vigreux column.

Concentrations of Deuteriated Compounds. After synthesis, the concentrations of compounds I-*d*, II-*d*, III-*d*, and IV-*d* were determined gas chromatographically with ethylpyrazine as internal standard. The correction factors for the internal standard were estimated by GC analysis of mixtures that consisted of known amounts of ethylpyrazine and of unlabeled compounds I–IV.

Analysis of Bread Crusts. After they were cut from the bread, the crusts were frozen in liquid nitrogen and ground (Schieberle and Grosch, 1983). Two methods were used for the isolation of the volatiles:

(*i*) *Vacuum Sublimation* (*VS*). The treatment of 1 kg of ground bread crust was carried out as described earlier (Schieberle and Grosch, 1983). Before concentration, the dichloromethane extract was spiked with a mixture consisting of I-*d* (40 μg), II-*d* (60 μg), III-*d* (30 μg), and IV-*d* (20 μg).

(*ii*) *Simultaneous Steam Distillation Extraction* (*SDE*). A mixture of I-*d* (20 μg), II-*d* (30 μg), III-*d* (15 μg), and IV-*d* (10 μg) in 5 mL of ethanol was added to a suspension of 350 g of ground bread crust in 2.3 L of water. The suspension was distilled and continuously extracted with 150 mL of diethyl ether in the apparatus according to Nickerson and Likens (1966). A second batch of 350 g of crust in 2.3 L of water (spiked with the mixture of internal standards) was extracted with the same 150 mL of diethyl ether. The extract obtained was washed twice with 150 mL of 0.5 M Na_2CO_3 and then with 150 mL of water, then dried over Na_2SO_4, and finally concentrated to 1 mL on a Vigreux column.

Column Chromatography. The extracts obtained by VS and SDE were fractionated at 10–12 °C on a water-cooled column (20 × 1 cm), packed with a slurry of silica gel in 95:5 pentane–diethyl ether (v/v). The elution was performed at 10–12 °C with 25 mL of 95:5 pentane–diethyl ether (v/v; fraction A), 50 mL of 85:15 pentane–diethyl ether (v/v; fraction B), 45 mL of 7:3 pentane–diethyl ether (v/v; fraction C), 25 mL of 1:1 pentane–diethyl ether (v/v; fraction D), 25 mL of 25:75 pentane–diethyl ether (v/v; fraction E), and 80 mL of diethyl ether (fraction F). Fractions D and E were combined and concentrated on a Vigreux column and by microdistillation (Bemelmans, 1979) to 100 μL. An aliquot was then analyzed by HRGC–MS for pyrroline IV.

HPLC. The combined fractions D–F were concentrated to 250 μL and then separated into five fractions by HPLC on a silica gel column with the solvent system 97:3 dichloromethane–ethanol (v/v) as described earlier (Schieberle and Grosch, 1983). The two fractions eluted between 48.7 and 60.9 mL (fraction a) and between 91.0 and 121.0 mL (fraction b) were pooled, and each fraction was concentrated to 150 μL by microdistillation. Fraction a, which contained pyrazines I and I-*d*, and fraction b, which contained pyrazines II, II-*d*, III, III-*d*, were analyzed by HRGC–MS.

Capillary Gas Chromatography (HRGC)–Mass Spectrometry (MS) Analysis. Mass spectrometry was performed on an MS 8230 (Finnigan, Bremen, Germany), in tandem with the GC capillaries described below. Mass spectra in the electron impact mode (MS-EI) were generated at 70 eV and in the chemical ionization mode

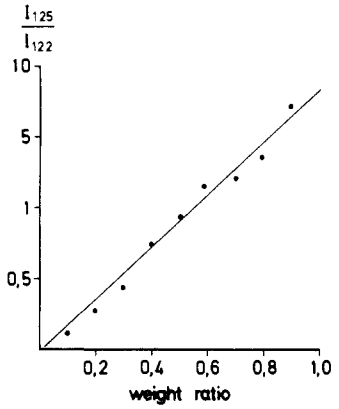

Figure 1. Standard curve for the determination of acetylpyrazine (I): intensity ratio of m/z 125 (I-d)/m/z 122 (I) vs. the weight ratio μg of I-d/(μg I-d + μg I).

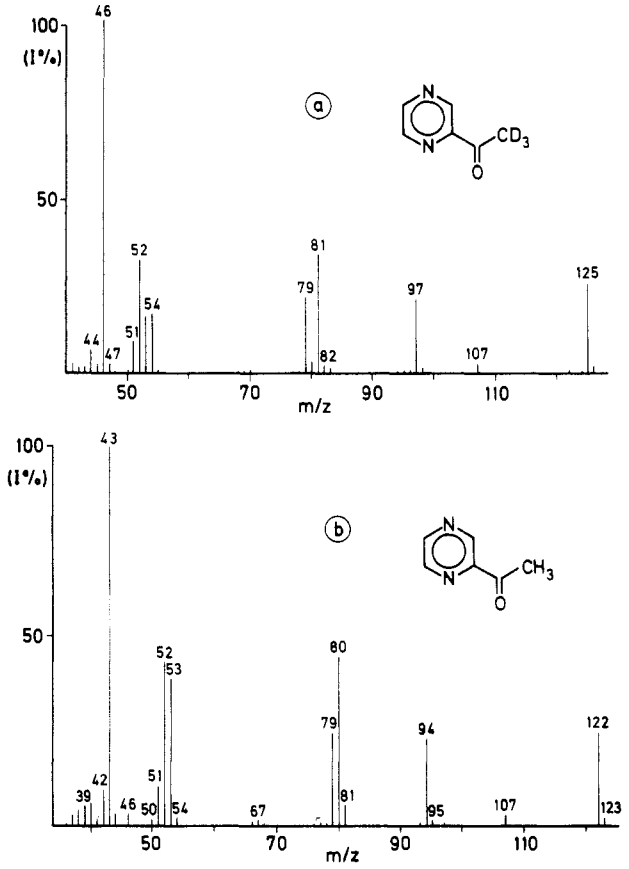

Figure 2. Mass spectra (MS-EI) of [$^2H_3$]acetylpyrazine (a) and acetylpyrazine (b).

(MS-CI) were obtained at 115 eV with isobutane as reagent gas. The MS were recorded in the scan ranges given in Table I with a scan time of 0.1 s/decade. The intensities of the ions shown in Table I were calculated by the MS data system. HRGC of compounds I, I-d, IV, IV-d were performed with a 30 m × 0.32 mm glass capillary coated with OV-1701. Compounds II, II-d, III, and III-d were chromatographed on a 30 m × 0.32 mm fused silica capillary (Supelcowax 10; Supelchem, Sulzbach, Germany). The samples (0.5 μL) were applied by the "on-column injection technique" at 35 °C, and the temperature of the capillaries was raised by 40 °C/min to 50 °C, 5 min isothermal, and then raised by 4 °C/min to 220 °C. The flow rate of the helium carrier gas was 2.0 mL/min.

Standard Curves. Standard curves were prepared for each of the four flavor compounds, here exemplified by

Table I. Selected Ions from the Mass Spectra for the Quantitative Analysis of the Crust Flavor Compounds

compound	ionizn mode	scan range (m/z)	selected ion (m/z)
acetylpyrazine (I)	EI	110–130	122
[^{2}H]acetylpyrazine (I-d)	EI	110–130	125
2-methyl-3-ethylpyrazine (II)	EI	110–140	121
[^{2}H]-2-methyl-3-ethylpyrazine (II-d)	EI	110–140	123, 124
5-methyl-5H-cyclopenta[b]pyrazine (III)	EI	110–140	119
[^{2}H]-5-methyl-5H-cyclopenta[b]-pyrazine (III-d)	EI	110–140	120
2-acetyl-1-pyrroline (IV)	CI	100–120	112
[^{2}H]-2-acetyl-1-pyrroline (IV-d)	CI	100–120	114–117[a]

[a] The sum of the intensities of the four ions was calculated.

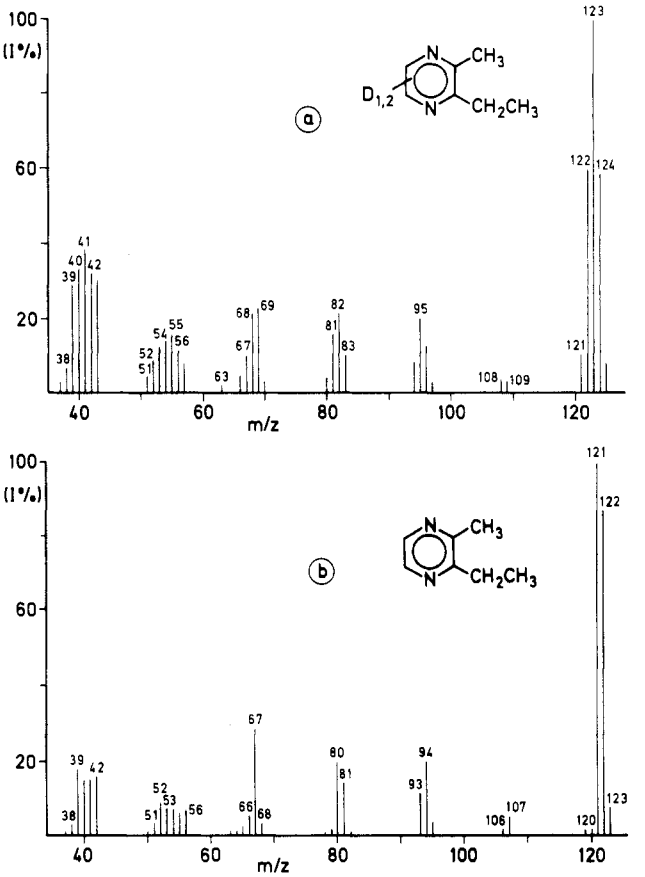

Figure 3. Mass spectra (MS-EI) of [^{2}H]-2-methyl-3-ethylpyrazine (a) and 2-methyl-3-ethylpyrazine (b).

pyrazine I, as follows: nine mixtures containing known amounts of I and I-d were analyzed by HRGC–MS. The ratio of the intensities of the ions selected for the quantification of pyrazine I and I-d (Table I) were plotted against the weight ratio of pyrazine I/pyrazine I-d as shown in Figure 1.

RESULTS

Comparison of MS Data. Figures 2–5 contrast the MS-EI of the deuteriated (I-d–IV-d) and of the corresponding unlabeled compounds.

Comparison of the MS data in parts a and b of Figure 2 indicates that the molecular ion of the deuteriated pyrazine I-d (m/z 125) was 3 mass units higher than that of unlabeled pyrazine I (m/z 122). This suggests the incorporation of three ^{2}H atoms in pyrazine I-d which, as the ion shift m/z 43–46 (Figure 2a) and the synthetic pathway indicate, occurs at the acetyl group. The molecular ions of pyrazines I and I-d (Table I) were selected for the

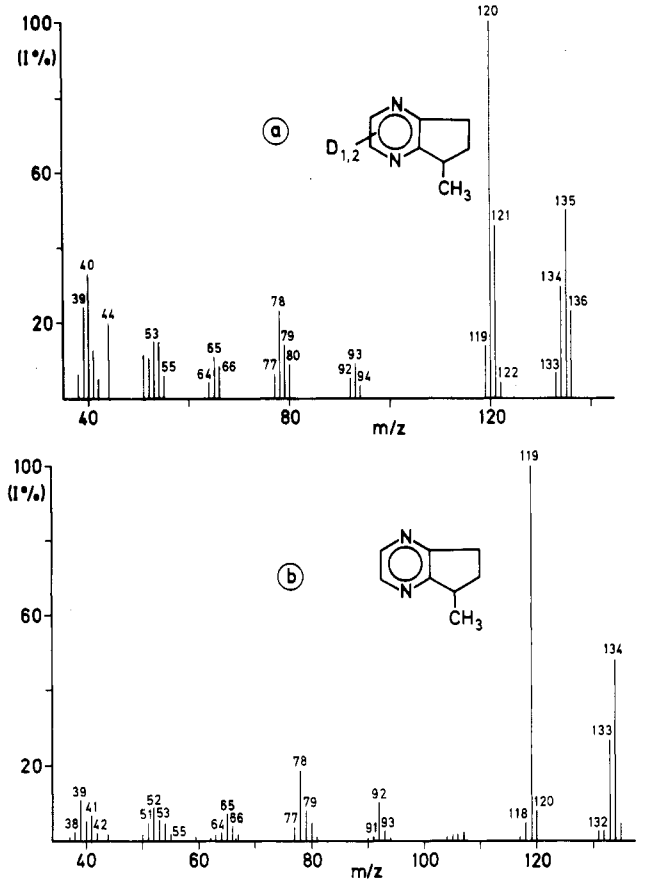

Figure 4. Mass spectra (MS-EI) of [^{2}H]-5-methyl-5*H*-cyclopenta[*b*]pyrazine (a) and 5-methyl-5*H*-cyclopenta[*b*]pyrazine (b).

quantitative analysis of this pyrazine in breads. Figure 6 shows a selected ion-monitoring chromatogram of a mixture of both compounds I and I-*d*. It is interesting to note that the deuteriated pyrazine I-*d* (*m*/*z* 125) was eluted at a shorter retention time than unlabeled pyrazine I (*m*/*z* 122).

The molecular ion (M^+; *m*/*z* 122) and the ion $M^+ - 1$ (*m*/*z* 121) showed the highest intensities in the MS of pyrazine II (Figure 3b). The latter ion was selected for quantitative measurements (Table I). The MS of labeled pyrazine II-*d* showed a cluster of intense ions in the range *m*/*z* 122 to *m*/*z* 124 (Figure 3a). Obviously a mixture of two components of which one contained one ^{2}H atom (M^+; *m*/*z* 123) and the other two ^{2}H atoms (M^+; *m*/*z* 124) appears during labeling. The sum of the intensities of the ions *m*/*z* 123 and *m*/*z* 124 of pyrazine II-*d* was used for the quantitative analysis (Table I).

The differences in the masses of the molecular ions between the labeled (Figure 4a) and the unlabeled compound (Figure 4b) showed also that pyrazine III-*d* was a mixture of two components that contain one (*m*/*z* 135) and two deuterium atoms (*m*/*z* 136). The base peaks in the MS at *m*/*z* 119 (compound III) and *m*/*z* 120 (compound III-*d*), which were formed by elimination of the methyl group, were selected for quantitative analysis (Table I).

As the intensities of the molecular ions were small in the MS-EI of pyrrolines IV and IV-*d* (Figure 5 parts a and b), the MS-CI of these compounds were measured. Figure 5, parts c and d, shows that in contrast to the molecular ion of the unlabeled compound IV (*m*/*z* 112), that of the labeled pyrroline IV-*d* (Figure 5c) appeared as a cluster of ions with different ^{2}H content. For quantification, the intensities of the ions *m*/*z* 114, 115, 116, and 117 were summed up as indicated in Table I.

Table II. Recovery of the Flavor Compounds after Isolation by Extraction and Vacuum Sublimation (VS) and by Simultaneous Steam Distillation–Extraction (SDE)

		VS		SDE	
compound[a]	amt added, μg	amt measd, μg	rec, %	amt measd, μg	rec, %
acetylpyrazine (I)	40	31.6	79	1.6	4
2-methyl-3-ethylpyrazine (II)	60	43.2	72	41.4	69
5-methyl-5*H*-cyclopenta[*b*]-pyrazine (III)	30	16.8	56	14.4	48
2-acetyl-1-pyrroline (IV)	20	2.4	12	6.4	32

[a] The mixture of the four compounds dissolved in 5 mL of ethanol was added to 2.3 L of tap water or 150 mL of dichloromethane, respectively. The VS and SDE were performed as described in the Analysis of Bread Crusts. After concentration of the extracts to 100 μL, ethylpyrazine was added as internal standard for quantitative HRGC analysis. The data are mean values of duplicates.

Table III. Isotope Dilution Assay of a Model Mixture of the Flavor Compounds

		amt measd, μg	
compound[a]	amt added, μg	VS	SDE
acetylpyrazine (I)	40	43	44
2-methyl-3-ethylpyrazine (II)	60	57	58
5-methyl-5*H*-cyclopenta[*b*]pyrazine (III)	30	29	30
2-acetyl-1-pyrroline (IV)	20	21	21

[a] The model system dissolved in 2.3 L of water or 150 mL of dichloromethane, respectively, consisted of the four compounds I–IV and the labeled internal standards I-*d* (40 μg), II-*d* (60 μg), III-*d* (30 μg), and IV-*d* (20 μg). The volatiles were isolated from the model system by extraction and vacuum sublimation (VS) or simultaneous steam distillation–extraction (SDE). The extracts obtained were fractionated by column chromatography and HPLC as described in Analysis of Bread Crusts. The data are mean values of duplicates.

Bread crusts contain the three positional isomers of methylethylpyrazine of which only the 2-methyl-3-ethyl isomer contributes significantly to the crust flavor of breads (Schieberle and Grosch, 1984, 1985). Therefore, the HRGC of pyrazines II and II-*d* was performed on the Supelcowax polar capillary that separated the three isomers: 2-methyl-6-ethyl (RI 1372), 2-methyl-5-ethyl (RI 1388), and 2-methyl-3-ethyl (RI 1414).

Analysis of Model Mixtures. A model mixture containing the flavor compounds I–IV (Table II) was analyzed to determine the efficiency of the two isolation procedures VS and SDE. The data in Table II reveal that pyrazines II and III were obtained in the same yield by both methods. In contrast, the yield of pyrazine I was high only when the VS method was used. More than 95% of pyrazine I was lost by the SDE method. Pyrroline IV showed high losses in both isolation procedures. The best yield (32%) was found after isolation by the SDE method.

A second model experiment was performed to test whether a deuterium/protium exchange takes place during the analytical procedure used for the investigation of bread crusts. The model mixture consisted of flavor compounds I–IV and the corresponding deuteriated internal standards (I-d–IV-d). The data listed in Table III indicate that the differences between the theoretical values and the values measured amounted to not more than 10%, which was found for pyrazine I. This suggests that the deuterium/protium exchange proceeds, if at all, very slowly and does not hinder the isotope dilution assay of the four flavor compounds. The methods used for the isolation of the flavor compounds did not affect these results (Table III). Even the low yields of pyrazine I and the pyrroline IV,

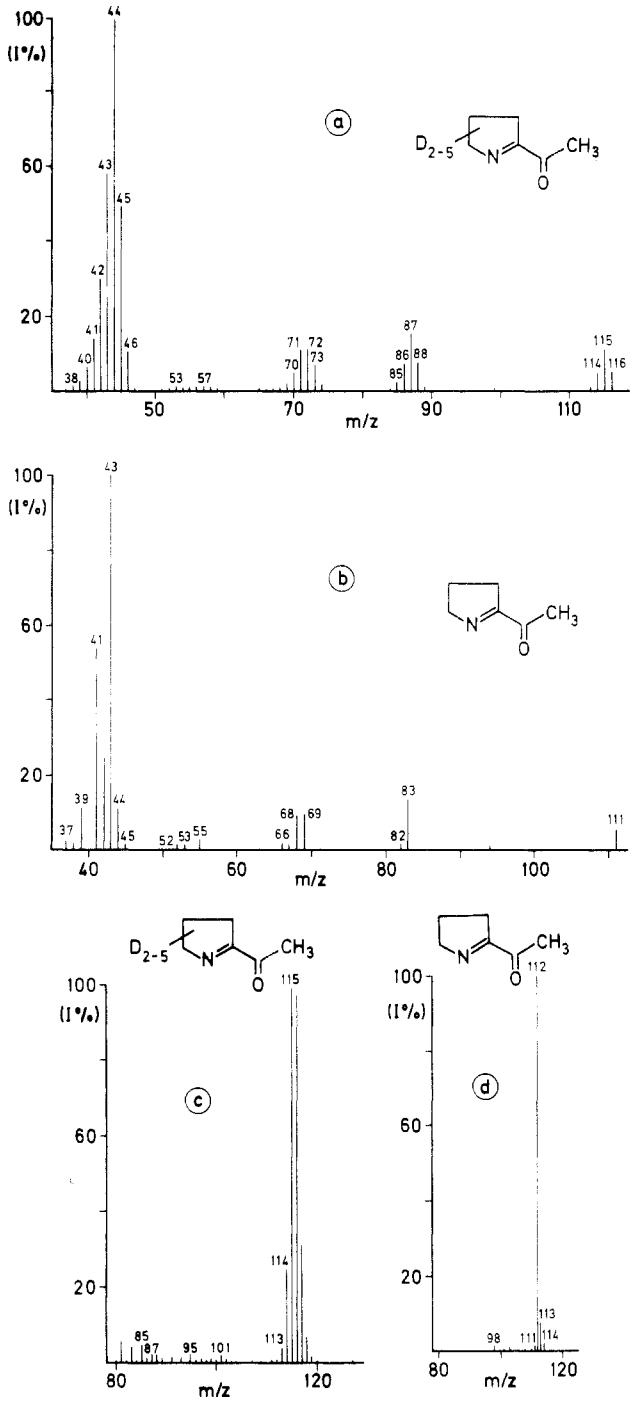

Figure 5. Mass spectra of (a) [^{2}H]-2-acetyl-1-pyrroline (MS-EI), (b) 2-acetyl-1-pyrroline (MS-EI), (c) [^{2}H]-2-acetyl-1-pyrroline (MS-CI), and (d) 2-acetyl-1-pyrroline (MS-CI).

which were obtained with the SDE or the VS method, respectively (Table II), allowed an accurate determination of these compounds in the model mixture. However, the isolation procedure with the highest yields was used for the quantitative analysis of pyrazine I and pyrroline IV in the bread crusts, since now the great number of background signals interfered in the quantification of the ions when their signals were too low.

Analysis of Bread Crusts. The concentrations of the three pyrazines and of the pyrroline that were found in the crusts of wheat and rye breads are listed in Table IV. The high level of pyrroline IV in the crusts of the wheat breads was the most striking difference between the wheat and rye breads. A comparison of the wheat breads showed that the pyrazines increased, when the bread was prepared

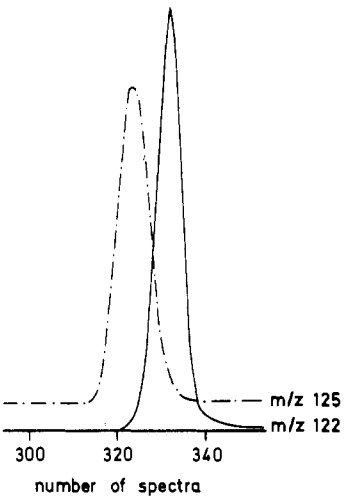

Figure 6. Selected ion-monitoring chromatogram of a 1:1 mixture of [$^2H_3$]acetylpyrazine (m/z 125) and acetylpyrazine (m/z 122).

Table IV. Concentrations (μg/kg) of the Pyrazines and the Pyrroline in the Crusts of Wheat and Rye Breads

	wheat bread		rye bread[a] sourdough process		
compound	without dried sponge	with dried sponge	one-stage	three-stage	rye grist bread[b]
acetylpyrazine[c] (I)	19	34	51	80	7
2-methyl-3-ethyl-pyrazine[c] (II)	66	75	41	94	18
5-methyl-5*H*-cyclopenta-[*b*]pyrazine[c] (III)	15	29	17	37	10
2-acetyl-1-pyrroline[c] (IV)	78	72	1	4	nd

[a] Prepared from rye flour. [b] Prepared from coarse whole rye flour. [c] Extraction and vacuum sublimation (VS). [d] Simultaneous steam distillation–extraction (SDE) was used for the isolation of the flavor compounds. nd = not detectable. The data are mean values of duplicates.

by addition of the "dried sponge" as a baking helper.

The aroma of the rye bread was more intense when the sourdough was prepared by a three-stage instead of a one-stage process. Our results show that this possibly arises from the difference in the concentrations of the flavor compounds I–IV (Table IV). In particular pyrazines II and III were doubled and also pyrroline IV increased very strongly in the bread prepared by the three-stage sourdough process.

The levels of pyrazines I–III were low, and pyrroline IV was not detected in the crust of the rye grist bread. These results agree also with the subjective odor impression, since this type of rye bread showed the weakest crust flavor of all breads investigated.

DISCUSSION

The results indicate that an isotope dilution assay is suitable for the quantitative analysis of specific flavor compounds in a complex mixture of volatiles. Several steps of enrichment of the compounds can be performed without losses in accuracy provided that the initial ratio between the compound and its labeled analogue remains unchanged during the entire procedure.

The method was applied to flavor compounds that contribute significantly to the crust flavor note of breads. Because of both its low odor threshold of 0.1 ppb in water (Buttery et al., 1983) and its high concentration (see above), 2-acetyl-1-pyrroline is the most important flavor compound of the white bread crust. The compound was at first found in cooked rice aroma (Buttery and Ling,

1982). During cooking of very aromatic rice varieties, even more 2-acetyl-1-pyrroline is formed (Buttery et al., 1986) than in the crust of the wheat breads. The reason for the high concentration of this pyrroline derivative in wheat in comparison to rye bread is yet to be clarified. According to Tressl et al. (1985), it originates from a reaction of proline with monosaccharides.

In rye bread, on the other hand, the pyrazines predominated. However, the 2-acetyl-1-pyrroline may still play a role in the crust flavor of these breads because of its low odor threshold (see above) compared to those of the pyrazines: I = 62 ppb (Teranishi et al., 1975); II = 30 ppb (Fors, 1985); III, between 15 and 25 ppb (Schieberle and Grosch, unpublished results).

The results also indicate that a change in the sourdough process influences the concentrations of the four flavor compounds in the crusts of rye bread. Approximately twice as much pyrazine and also much more pyrroline were formed, when the sourdough was prepared in a three-stage process (Table IV). Obviously the longer fermentation time during this process leads to an increase in the precursors from which these flavor compounds are formed.

This study also shows that differences in the concentrations of important flavor compounds caused by changes in food processing can be accurately quantified by an isotope dilution assay.

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Fractionation and HPLC Determination of Grape Phenolics

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An improved analytical method was developed for the determination of phenolic compounds in grapes. Phenolic compounds were fractionated into acidic and neutral groups by passing deproteinated grape juice through a preconditioned C18 SEP-PAK cartridge, and then the fractions were sequentially injected into a HPLC column. The separation was made on a C18 Radial-PAK column using 5% acetic acid for acidic phenolics and 40% acetonitrile for neutral phenolics. This fractionation technique showed a high recovery and resolution, and it was effective in quantitation of major phenolic compounds in grapes.

Hydroxycinnamic acid tartrates and catechins and procyanidins are important phenolic compounds in grapes and wine. They contribute to the sensory quality and browning of the products (Singleton and Esau, 1969; Lea et al., 1979). One of the major problems involved in separation of these phenolic compounds is their similarity in chemical characteristics. Many traditional separation techniques such as paper, thin-layer, and column chromatography are being replaced by high-performance liquid chromatography (HPLC). Recently, HPLC using reversed-phase columns has been used to separate the various hydroxycinnamic acid tartrates and procyanidins in grapes and wines (Lea et al., 1979; Nagel et al., 1979; Ong and Nagel, 1978; Wulf and Nagel, 1976). However, the quantitation of phenolic compounds by HPLC has not yet been perfected, because many phenolics show similar ultraviolet absorption spectra with maxima in a narrow range

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