icant differences in the contents of α-pinene, β-pinene, sabinene, myrsene, and 1,4-cineol.

The results display that the prostrate juniper bushes in particular, existing typically on the rocky islands in the Finnish archipelago, give good-quality berries with appropriate content of volatiles (International Standard, 1984).

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Volatile Compounds Formed from Thermal Interaction of 2,4-Decadienal with Cysteine and Glutathione

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Mixtures of 2,4-decadienal with either cysteine or glutathione were reacted in a closed sample cylinder in an aqueous medium. Each solution was adjusted to pH 7.5 and heated for 1 h at 180 °C, a representative frying temperature. The volatiles produced by degradations and interactions were isolated by simultaneous solvent–steam distillation and analyzed by gas chromatography and coupled gas chromatography–mass spectrometry. A total of 45 compounds was identified from the thermal interaction of 2,4-decadienal and cysteine with 2,4,6-trimethylperhydro-1,3,5-dithiazine as the major component. A total of 42 volatiles was determined from the thermal interaction of 2,4-decadienal and glutathione, and 2-pentylpyridine was the major component. Most of the identified products can be accounted for by well-known chemical pathways.

Volatiles generated from lipid–protein interaction in deep-fat fried foods have been reviewed by Ho et al. (1987). Due to the complexity of proteins and lipids found in foods, model systems were used for the study of these interactions. Amino acids, such as cysteine, valine, and lysine, were used in model systems by a few investigators (Lien and Nawar, 1974; Sims and Fioriti, 1975; Breitbart and Nawar, 1981; Henderson and Nawar, 1981). N-Substituted amides and nitriles were reported as the major products when amino acids were heated with short-chain triglycerides, while 2-pentylpyridine became the major product when valine was heated with linoleic acid and its esters.

In the present study, the thermal interaction between either 2,4-decadienal and cysteine or 2,4-decadienal and glutathione was examined. In an earlier publication, we reported the decomposition of cysteine and that of glutathione when heated separately under the same conditions

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(Zhang et al., 1988). 2,4-Decadienal was chosen because it is the major degradation product of linoleic acid, which is the main component of vegetable oils such as soybean oil and corn oil (Snyder et al., 1985; Patton et al., 1959). Both cysteine and glutathione are sulfur-containing components found in natural food materials. They were selected as models to simplify the lipid–protein interactions and to study the flavor component formations in deep-fat fried foods.

EXPERIMENTAL SECTION

Sample Preparation. A total of 600 mg (0.005 mol) of cysteine (98% free base, crystalline; Sigma Chemical Co., St. Louis, MO) or 1500 mg (0.005 mol) of glutathione (98–100%, reduced form, crystalline; Sigma) was dissolved in 100 mL of distilled water, the solution was adjusted to pH 7.5 with 1 N sodium hydroxide or 1 N hydrochloric acid, and 200 mg of 2,4-decadienal (0.001 mol) (reagent grade; Aldrich Chemical Co., Milwaukee, WI) was added. The mixture was transferred into a 0.3-L Hoke SS-DOT sample cylinder and sealed, followed by heating the cylinder at 180 °C in an oil bath for 1 h. The heated mixture of 2,4-decadienal and cysteine possessed a fresh onionlike sulfur note and a bloody and burnt beef aroma. The other heated mixture possessed a garliclike sulfur note. Next, the reaction mass was simultaneously solvent steam-distilled with use of diethyl ether in a Nickerson-Likens

Volatiles from Lipid–Protein Interactions

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Table I. Volatile Compounds Identified from Thermal Interaction of 2,4-Decadienal with either Cysteine or Glutathione

compound	M_r	$I_k{}^a$	amt produced, mg/mol cysteine	amt produced, mg/mol gluta-thione	compound	M_r	$I_k{}^a$	amt produced, mg/mol cysteine	amt produced, mg/mol gluta-thione
Carbonyls									
2-pentanone	86	670	28.1		hexanoic acid	116	948		1.9
1-penten-3-one	84	753	10.5		2-octanone	128	968	10.4	62.7
2-hexanone	100	763	51.2	95.5	2-nonanone	142	1070	13.4	15.6
hexanal	100	772	278.6	45.4	1-(2-pyridinyl)pentanone	163	1297	5.1	6.8
3-*trans*-hepten-2-one	112	838		7.6	2-butyl-2-*trans*-octenal	182	1357	253.6	36.7
2-heptanone	114	877	39.8	106.7	2-butyl-2-*cis*-octenal	182	1369	5.2	
benzaldehyde	106	925	31.8	20.6	2-butyl-2-octenoic acid	198	1496	8.2	
Furans									
2-butylfuran	124	887	12.8	3.1	2-hexylfuran	152	1079	t	12.8
2-pentylfuran	138	973	6.4	t					
Thiophenes									
thiophene	84	646	3.5	7.2	methylbutylthiophene	154	1136		4.6
2-methylthiophene	98	745		34.4	2-pentylthiophene	154	1143	13.1	14.6
tetrahydrothiophen-3-one	102	906	10.5		methylpentylthiophene	168	1237	18.7	46.5
2-propylthiophene	126	937		2.3	methylpentylthiophene	168	1240	17.5	
methylpropylthiophene	140	1028		3.8	2-hexylthiophene	168	1246	42.0	87.8
2-butylthiophene	140	1041	57.2	56.6	2-heptylthiophene	182	1283	1.8	
3-methylthiophene-2-carbox-aldehyde	126	1045	29.8		3-(1-hexanoyl)thiophene	182	1446	9.3	60.8
					formylpentylthiophene	182	1477	15.6	21.0
Thiazoles									
thiazole	85	707	25.6	16.0	3-methylisothiazole	99	842	2.0	
2-methylthiazole	99	777		15.8	2-acetylthiazole	127	979	2.2	3.8
5-methylthiazole	99	814		14.5					
Other Sulfur-Containing Compounds									
butanethiol	90	699	6.2		1,2,5-trithiepane	152	1499	t	
2-methyl-1,3-dithiolane	120	965	5.0		3,5,7-trimethyl-1,2,4,6-tetrathiepane	212	1526		6.4
2,4,6-trimethylperhydro-1,3,5-thiadiazine	146	1062	828.5		3,5,7-trimethyl-1,2,4,6-tetrathiepane	212	1535		1.6
3,5-dimethyl-1,2,4-trithiolane	152	1101	122.8	162.1	2,4-dimethyl-6-pentylperhydro-1,3,5-dithiazine	219	1564	18.9	
3,5-dimethyl-1,2,4-trithiolane	152	1106	18.2	206.4	2-pentyl-4,6-dimethylperhydro-1,3,5-dithiazine	219	1571	28.7	
2,4,6-trimethylperhydro-1,3,5-dithiazine	163	1168	284.2		3-methyl-5-pentyl-1,2,4-trithiolane	208	1620	14.3	
3-methyl-1,2,4-trithiane	152	1214	42.5	1.6	4,7-dimethyl-1,2,3,5,6-pentathiepane	216	1626		6.7
3,6-dimethyl-1,2,4,5-tetrathiane	184	1343		59.2	4,7-dimethyl-1,2,3,5,6-pentathiepane	216	1628		6.3
3,6-dimethyl-1,2,4,5-tetrathiane	184	1347		1.1	2-propyl-4-methyl-6-pentylperhydro-1,3,5-dithiazine	247	1750	2.9	
4,6-dimethyl-1,2,3,5-tetrathiane	184	1390		76.1	3-methyl-6-pentyl-1,2,4,5-tetrathiane	240	1767		6.4
					3-methyl-6-pentyl-1,2,4,5-tetrathiane	240	1771		3.1
					2-methyl-4-butyl-6-pentylperhydro-1,3,5-dithiazine	261	1849	0.9	
Miscellaneous Compounds									
butylbenzene	140	1041	57.2	56.6	2-pentylpyridine	149	1174	501.5	1219.0

a Linear retention index.

apparatus. Internal standards of 666.7 ppm 4-heptanone (reagent grade; J. T. Baker Chemical Co., Phillipsburg, NJ) for the interaction mass of 2,4-decadienal and cysteine and of 666.7 ppm of ethyl heptanoate (98%; Aldrich) for the reaction mass of glutathione and 2,4-decadienal were added before the distillation. The distillates were dried over anhydrous sodium sulfate and concentrated with a Kuderna-Danish apparatus to a final volume of 0.5 mL. The concentrated samples were stored in a –40 °C freezer to minimize further reaction or decomposition.

Volatile Separation by Gas Chromatography. A Varian 3400 gas chromatograph equipped with a flame ionization detector and a nonpolar fused capillary column (60 M × 0.25 mm (i.d.); 0.25-μm thickness; DB-1; J & W Scientific) was used to analyze the volatile compounds isolated from the thermal reaction systems. For each sample, 0.2 μL was injected with a split ratio of 50:1. The GC was run with an injector temperature of 230 °C, a detector temperature of 260 °C, and a helium carrier flow rate of 1 mL/min. The temperature program included an initial column temperature of 40 °C, a temperature increase of 2 °C/min from 40 to 220 °C, and a 10-min isothermal period at the final column temperature.

Quantitative determination was accomplished by internal standards previously mentioned. The quantity of each component was finally converted into milligrams of the volatiles generated by either 1 mol of cysteine or 1 mol of glutathione. Linear retention indices for the volatile compounds were calculated versus *n*-paraffin standards (C_6–C_{22}; Alltech Associates) as references (Majlat et al., 1974).

GC–MS Analysis. The concentrated samples were analyzed by GC–MS using a Varian 3400 gas chromatograph coupled to a Finigan MAT 8230 high-resolution mass spectrometer equipped

with an open split interface. Mass spectra were obtained by electron ionization at 70 eV and a source temperature of 250 °C. The filament emission current was 1 mA, and spectra were recorded on a Finigan MAT SS 300 data system.

RESULTS AND DISCUSSION

Sixty-one compounds were identified in the two reaction systems by comparing their retention indices and mass spectral data with those of authentic compounds or previously published literature data (Boelens et al., 1974; Vitzthum and Werkhoff, 1974; Heller and Milne, 1980; Nixon et al., 1979; Ten Noever de Brauw et al., 1983; Hwang et al., 1986). Their molecular weights, retention indices, and quantitative data are compiled in Table I and grouped by different chemical classes.

The majority of the carbonyls are apparently derived from the thermal degradation of 2,4-decadienal. 2,4-Decadienal is susceptible to further degradation via classic Farmer autoxidation mechanism (Farmer et al., 1943; Matthews et al., 1971; Michalski and Hammond, 1972; Schieberle and Grosch, 1981; Josephson and Lindsay, 1987), leading to the formation of undesirable carbonyls. As proposed by Josephson and Lindsay (1987), under aqueous conditions 2,4-decadienal undergoes α,β-double-bond hydration and retro-aldol condensation to give hexanal and acetaldehyde as final aldehydes. The hexanal can then back-react with itself to give its aldol condensation products 2-butyl-2-*trans*-octenal and 2-butyl-2-*cis*-octenal,

Table II. Mass Spectra Data of Some Long-Chain Alkyl-Substituted Heterocyclic Compounds Identified in Both Systems

compound	mass spectral data, m/z (rel intens)
2-pentylthiophene	97 (100), 98 (23), 154 (19), 53 (8), 99 (7), 69 (4); M_r 154
methylpentylthiophene	111 (100), 81 (28), 168 (25), 67 (18), 112 (16), 95 (14), 137 (10), 152 (7); M_r 168
methylpentylthiophene	111 (100), 168 (33), 82 (28), 112 (16), 67 (12), 113 (10), 53 (7); M_r 168
2-hexylthiophene	97 (100), 98 (85), 168 (60), 53 (24), 99 (22), 111 (18), 84 (14), 139 (60), 125 (5); M_r 168
2-heptylthiophene	97 (100), 55 (21), 69 (17), 98 (11), 53 (10), 71 (9), 83 (9), 111 (7), 182 (4); M_r 182
hexanoylthiophene	111 (100), 126 (91), 83 (13), 139 (12), 182 (9), 97 (6); M_r 182
formyl, pentylthiophene	125 (100), 97 (80), 126 (68), 182 (57), 53 (16), 127 (11), 111 (10), 139 (8); M_r 182
3-methyl-5-pentyl-1,2,4-trithiolane	143 (100), 60 (80), 92 (75), 55 (71), 59 (57), 87 (45), 64 (36); M_r 208
2,4-dimethyl-6-pentylperhydro-1,3,5-dithiazine	126 (100), 100 (73), 70 (42), 60 (39), 219 (34), 59 (28), 127 (22), 112 (16); M_r 219
2-pentyl-4,6-dimethylperhydro-1,3,5-dithiazine	71 (100), 70 (81), 103 (57), 60 (50), 219 (30), 57 (28), 97 (24), 59 (23); M_r 219
3-methyl-5-pentyl-1,2,4,5-tetrathiane	59 (100), 60 (99), 115 (90), 45 (57), 41 (50), 55 (44), 73 (30), 87 (29), 81 (24), 180 (18), 175 (16), 240 (16), 242 (4); M_r 240
3-methyl-5-pentyl-1,2,4,5-tetrathiane	60 (100), 59 (92), 115 (62), 41 (60), 45 (52), 43 (51), 55 (48), 73 (35), 87 (29), 124 (29), 175 (15), 180 (14); M_r 240
2-propyl-4-methyl-6-pentylperhydro-1,3,5-dithiazine	126 (100), 55 (75), 60 (62), 98 (58), 70 (54), 72 (46), 100 (42), 247 (25); M_r 247
2-methyl-4-butyl-6-pentylperhydro-1,3,5-dithiazine	168 (100), 55 (64), 60 (44), 67 (33), 82 (30), 95 (26), 112 (25), 261 (12); M_r 261
2-pentylpyridine	93 (100), 106 (70), 120 (68), 65 (33), 51 (28), 78 (27), 94 (26), 79 (19); M_r 149

two of the other major carbonyls in the reaction systems. The aldol condensation can be catalyzed by primary amino groups of amino acids or proteins (Pokorny et al., 1987). In this case, in the presence of cysteine or glutathione, hexanal reacts with a primary amino group to form a Schiff base and dimerizes through aldol condensation and abstraction of amine. Hexanal and its aldol condensation dimers are further oxidized to their related acids. Acetaldehyde was not detected in either system. Either it is too volatile to be extracted with diethyl ether or it could be completely involved in the formation of other volatiles such as 3,5-dimethyl-1,2,4-trithiolane, or 2,4,6-trimethylperhydro-1,3,5-dithiazine. Benzaldehyde, the other major component of the carbonyls, is a typical thermal degradation product of 2,4-decadienal. The quantity rapidly increased, in accordance with temperature increases, from 150 to 200 °C under neutral aqueous conditions. We proposed a formation mechanism involving the condensation of alkyl aldehydes and 2,4-decadienal followed by the cyclization via pericyclic reaction and cleavage of the secondary alkyl group at the position where the formyl group is attached (Bruechert et al., 1988).

A considerably large number of long-chain alkyl-substituted heterocyclic compounds including thiophene, pyridine, thialdine, and other sulfur-containing compounds were detected in the interaction systems. Their mass spectral data are provided in Table II. The position of the alkyl groups in some of the thiophene compounds remains unknown. According to the formation mechanism of heterocyclic compounds from a model system of aldehydes, hydrogen sulfide, and ammonia (Vernin and Parkanyi, 1982; Hwang et al., 1986), aldehydes such as acetaldehyde, butanal, and hexanal must have been involved in the formation of heterocyclic compounds identified in this study. It is worth noting that hexanal was active enough to participate in the formation of five out of six dithiazines identified. It also can be integrated into cyclic polysulfides such as 3-methyl-6-pentyl-1,2,4,5-tetrathiane and 3-methyl-5-pentyl-1,2,4-trithiolane. It is interesting to note that there was no long-chain-substituted thiadiazine formed in either system, although the amount of 2,4,6-trimethylperhydro-1,3,5-thiadiazine was much higher than that of 2,4,6-trimethylperhydro-1,3,5-dithiazine. It was also found that 2,4,6-trimethylperhydro-1,3,5-thiadiazine was unstable and that it disappeared from the gas chromatogram after 2 weeks, even when the sample was stored in a −40 °C freezer. The observed absence of long-chain-substituted thiadiazines suggests that the long-chain substituents may make these compounds even more unstable.

Table III. Quantitation (mg/mol) of Some Major Flavor Components[a]

	A	B
total volatiles	2627.3	2511.0
hexanal	278.6	45.4
3,5-dimethyl-1,2,4-trithiolane	140.9	368.5
2,4,6-trimethylperhydro-1,3,5-thiadiazine	828.5	
2,4,6-trimethylperhydro-1,3,5-dithiazine	284.2	
2-pentylpyridine	501.5	1219.0
sum	2033.7	1632.9
% total volatiles	77.4	65.0

[a] A, 2,4-decadienal with cysteine; B, 2,4-decadienal with glutathione.

Another interesting group of heterocyclics is thiophene and its derivatives. These have been recognized as important food flavors for several years (Maga, 1975). Of the fifteen thiophenes identified, eight appeared as alkyl- and methyl alkyl-substituted pairs. They seemed to be derived from a series of carbonyl analogues, but the formation mechanism deserves more detailed studies.

A total of 45 volatile compounds was identified in the interaction system of 2,4-decadienal and cysteine and 42 compounds in the system of 2,4-decadienal and glutathione. The quantity of total volatiles generated for both systems was almost the same (Table III). However, as we reported before, cysteine degradation in an aqueous medium produces 4 times as many volatiles as glutathione degradation (Zhang et al., 1988). Therefore, glutathione becomes much more active in the presence of 2,4-decadienal, and the thermal interaction between these two components generates a larger amount of volatile compounds. The degradation of glutathione releases hydrogen sulfide rapidly when it is heated (Ohloff et al., 1985), and ammonia is released too slowly to produce enough nitrogen-containing compounds (Zhang et al., 1988). As a result, a greater number of polysulfur rather than nitrogen-containing sulfur compounds was observed in the reaction of 2,4-decadienal and glutathione. The nitrogen-containing sulfur compounds were only about 2% of the total volatiles by weight identified from this system. On the other hand, the interaction of 2,4-decadienal and cysteine generated a great number of nitrogen-containing sulfur compounds, which accounted for 45% of the total volatiles. Some of them were substituted with one or two long-chain alkyl groups.

The quantities of five major volatile components generated for these two systems are listed in Table III. The greater amount of 2-pentylpyridine and smaller amount
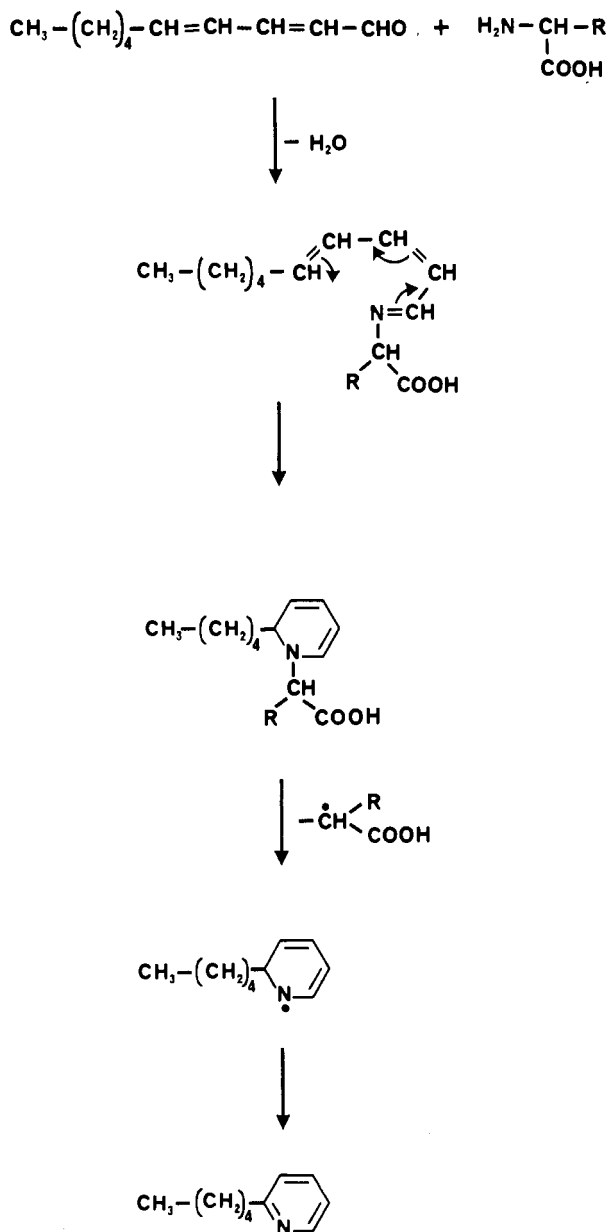
Figure 1. Mechanism for the formation of 2-pentylpyridine.

of hexanal were observed in the system of glutathione, suggesting that 2,4-decadienal was involved in forming a Schiff base with the amino group in glutathione directly and thus left less free 2,4-decadienal to go to autoxidation or retro-aldolization. According to the pathway proposed by Henderson and Nawar (1981), 2-pentylpyridine, which accounted for almost half of the total volatiles identified in the reaction of glutathione and 2,4-decadienal, can be formed via the Schiff base intermediate from the reaction of 2,4-decadienal with ammonia (Henderson and Nawar, 1981). It is known that the formation of dithiazine and thiadiazine requires the presence of free ammonia (Boelens et al., 1974). Since the absence of dithiazine or thiadiazine formed in 2,4-decadienal/glutathione indicates that no free ammonia is available, this suggests that free ammonia may not be necessary for the formation of 2-pentylpyridine. It is possible that the amino group from amino acids or peptides condenses directly with the aldehydic group of 2,4-decadienal and is then followed by an electrocyclic reaction and aromatization to form 2-pentylpyridine (Figure 1). Cysteine is easy to degrade into acetaldehyde, ammonia, and hydrogen sulfide under aqueous conditions (Shu et al., 1985); therefore, less 2-pentylpyridine was

formed and the quantity of thiadiazine and dithiazines increased.

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Selective Purge-and-Trap Method for the Analysis of Volatile Pyrazines

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A simple and selective purge-and-trap method for isolation, concentration, and fractionation of volatile pyrazines from a matrix with complex volatiles was developed. A dilute HCl aqueous solution was used to selectively trap pyrazines from total headspace components from a model system generated product and potato chips. After being titrated to pH 13, the acid-trapped pyrazines were then recovered by the headspace technique and analyzed by GC and GC–MS. Not only can the method quantify minor or trace pyrazines from a very small amount of sample with complex volatiles but also it is much less laborious and time-consuming compared with other traditional isolation, fractionation methods.

Alkylpyrazines have been considered important, characteristic, and essential trace flavor components present in many cooked, deep-fat fried, roasted, and toasted foods (Maga, 1982). Food products generally contain a large number of volatiles in various concentrations. In some cases, a direct determination of pyrazines of interest is possible. In most cases, the quantitative analysis of the pyrazines, which often are minor in quantity, is interfered with by the major constituents or by compounds with the same chromatographic properties. Physical or chemical fractionation after isolation is therefore required. Although there are no universal methods for isolation and fractionation, selective separation of volatile compounds based on functional groups is an approach that has been used for a long time. Pyrazines as well as other organic bases such as pyridines, pyrroles, and thiazoles can be separated from the solvent by extraction with dilute aqueous HCl. This is followed by transformation of the hydrochloride salts into free bases with 10% aqueous KOH (or NaOH) and

extraction with diethyl ether. However, this method normally requires a larger amount of material, and it is also quite laborious and time-consuming (Peyron, 1982).

Headspace analysis, by purge-and-trap on a porous polymer adsorbent, followed by thermal desorption or solvent elution, has been regarded as a simple and useful isolation method. The volatiles collected have an aroma note very close to that of the original sample (Chen et al., 1982). Maarse and Schaefer (1978) developed methods for collection and quantitative analysis of specific groups of compounds, such as pyrazines, phenols, acids, and carbonyls, in vapors above food products and in gases emitted during the processing of food. Pyrazines in the emission gases of a cocoa factory were collected by a trap with 10 mL of 1 N H_2SO_4. The collected pyrazines were then recovered by diethyl ether extraction after titrating the acid solution to pH 9. This method allowed the quantitative analysis of pyrazines occurring in low concentration, when the total chromatogram contained many interfering compounds. However, the method mentioned above still involved many procedures and was also laborious.

In this study, a simple and selective purge-and-trap method, using dilute HCl as a trap, was developed in order to isolate, concentrate, and fractionate pyrazines from samples containing complex volatiles. The method was applied for the determination of pyrazines generated from

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