

increase in the percent nitrogen extracted as the concentration of sodium acetate was increased.

**Salts of the Third Type.** When the concentration of  $\text{Na}_2\text{EDTA}$  was increased from 0 to 1000 mg/L (Figure 4), nitrogen extractability dropped from 79.00 to 22.18%. The pH of the protein extracts (Figure 4) were also constantly dropping with the increase in salt concentration from an initial value of 6.52 to a minimum value of 5.55. This pattern was unique in comparison with those of other salts as it had only one dropping trend. It seems that the acidic effect of the salt had the predominant effect in inhibiting extractability by making the medium more acidic and thus closer to the pH range of minimum protein solubility. Therefore, other salts with some acidic effect would probably fit to the same type. However, the chelation of divalent cations with  $\text{Na}_2\text{EDTA}$  and its presumed effect on protein dispersibility was not evident under the experimental conditions used. Unfortunately, the experiments were not repeated at pH 7 or higher. Besides, the available literature on the effect of  $\text{Na}_2\text{EDTA}$  on protein extractability from legume flours is scarce.

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## Isolation and Identification of Volatile Compounds in Cooked Meat: Sukiyaki

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The sukuyaki volatiles obtained from beef, heated with vegetables and seasonings (sugar and soy sauce) under simulated cooking conditions, were isolated and identified by gas-liquid chromatography/mass spectrometry. Forty-four compounds were positively identified. This included furans, ketones, aldehydes, esters, acids, alcohols, pyrazines, hydrocarbons, and pyrroles. Dimethylnitrosamine was identified at a level of 0.02 ppb in the dichloromethane extract of the above sukuyaki broth by using a thermal energy analyzer (TEA) combined with a gas chromatograph.

There are many reports on the isolation and identification of cooked meat constituents (Persson and von Sydow, 1973; Mussinan and Walradt, 1974; Buttery et al., 1977). All studies have been based upon the analysis of a single meat sample. When meats are cooked, however, they are often heated with many other ingredients such as vegetables, cheese, seasonings, etc. The many volatile compounds in cooked foods may form from the reactions between the constituents of the various ingredients used.

Sukiyaki is one of the typical Japanese one-pot dishes. Thin-sliced beef is fried together with vegetables, usually (nitrate-rich) chinese cabbage or spinach (in a frying pan at the table). Fairly large amounts of sucrose and soy sauce are used as seasoning.

Soy sauce contains large amounts of various amino acids (Markley, 1951), and sucrose produces many carbonyl compounds by heat treatment; together (Hodge, 1967) they can form browning reaction products during the frying. The unique flavor of sukuyaki may come from the volatile browning reaction products or from secondary reactions

with other food components.

Recently, carcinogenic *N*-nitrosamines have been isolated and identified in a wide range of foodstuffs: canned meat (Sen et al., 1976), smoked beef (Stephany et al., 1976), sausages (Kann et al., 1976), and cooked bacon (Patterson and Mottram, 1974). Using sodium nitrite for curing meats is a major source for *N*-nitrosamine formation (Freimuth and Glaeser, 1970; Moehler and Mayrhofer, 1969; Sen, 1972).

*N*-Nitrosamines form when secondary amines react with nitrite. Since meats contain various secondary amines, for example, dimethylamine, diethylamine, and *n*-diisopropylamine (Golovnya et al., 1979), it seems likely that during the cooking process these amines can react with the nitrite in the cured meat. Small amounts of nitrite can also be generated from nitrate-rich vegetables when they are heated with other food ingredients (Hayano, 1976).

In this study, the volatile browning reaction products produced in sukuyaki, which was prepared under simulated home cooking conditions, were isolated and identified. The detection of *N*-nitrosamines in sukuyaki flavors was conducted using a Thermal Energy Analyzer.

#### EXPERIMENTAL SECTION

**Preparation of Sukuyaki Volatiles.** Beef (400 g), *Cryptotaenia japonica* Hassk (mitsuba, 60 g), *Brassica*

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Table I. Compounds Identified in Sukiyaki Volatiles and Their Occurrence

peak no. on CW 20M	compd	peak area, %	CW 20W		OV-101		peak no. on OV-101	occurrence in ingredients	ref
			$I_{un}^n$	$I_k^o$	$I_{un}$	$I_k$			
1	acetaldehyde	0.04	689	690	363	363	1	soy, sauce <sup>a</sup>	Stenhagen et al. (1974a, p 6)
2	2-methylpropanal	0.16	800	800	500	500	3	beef, <sup>d</sup> soy sauce <sup>a</sup>	Mussinan and Walradt (1974)
3	ethyl acetate	0.29	882	889	600	600	5	beef, <sup>d</sup> soy sauce, <sup>a</sup> sake <sup>b</sup>	Mussinan and Walradt (1974)
4	3-methylbutanal	0.95	917	921	643	645	8	beef, <sup>d</sup> soy sauce <sup>a</sup>	Mussinan and Walradt (1974)
5	2,3-butanedione	0.02	955	949	613	619	7	beef, <sup>d</sup> sake <sup>c</sup>	Mussinan and Walradt (1974)
6	1,1-dithoxybutane (tentative)	0.04	990						Stenhagen et al. (1974a, p 358)
7	2,3-pentanedione	0.10	1035	1036	674	675	10	beef <sup>d</sup>	van den Ouweland and Peer (1975)
8	2-methylpropanol	0.09	1060	1062	612	616	6	beef, <sup>c</sup> soy sauce, <sup>a</sup> sake <sup>b</sup>	Stenhagen et al. (1974a, p 28)
9	dimethyl disulfide	0.27	1069	1076	736	736	16	beef <sup>d</sup>	Walradt et al. (1971)
10	2-methyl-2-butenal (tentative)	0.02	1076						Kinlin et al. (1972)
11	3-methylbutanol	1.05	1174	1174	725	725	14	beef, <sup>c</sup> soy sauce <sup>a</sup>	Mussinan and Walradt (1974)
12	pyridine	0.10	1180	1181	736	733	16	beef <sup>d</sup>	Stenhagen et al. (1974a, p 35)
13	pyrazine	0.13	1200	1196	718	718	12	beef, <sup>g</sup> soybeans <sup>h</sup>	Mussinan and Walradt (1974)
14	2-methyltetrahydrofuran- 3-one (tentative)	3.56	1249		782		18	soy sauce <sup>a</sup>	Kinlin et al. (1972)
15	methylpyrazine	0.99	1254	1255	806	806	20	beef, <sup>g</sup> soy sauce <sup>a</sup>	Bondarovich et al. (1967)
16	acetoin	0.93	1264	1265	692	693	11	beef, <sup>c</sup> soy sauce, <sup>a</sup> sake <sup>c</sup>	Ferretti and Flanagan (1971a)
17	acetol	3.49	1277	1284	652	652	9		Ferretti and Flanagan (1971a)
18	acetol acetate (tentative)	0.12	1287		839		25		Ferretti and Flanagan (1971a)
19a	ethyl lactate	2.42	1309	1312	806	803	20	soy sauce <sup>a</sup>	Stenhagen et al. (1974a, p 174)
19b	2,5-dimethylpyrazine		1309	1309	887	891	30	beef <sup>g</sup>	Mussinan and Walradt (1974)
20	2,6-dimethylpyrazine (tentative)	1.02	1314					beef, <sup>g</sup> soy sauce <sup>a</sup>	Mussinan and Walradt (1974)
21	unknown	0.84	1312		837				Mussinan and Walradt (1974)
22	2,3-dimethylpyrazine + unknown	0.35	1334	1338	908	901	32	beef, <sup>g</sup> soy sauce <sup>a</sup>	
23	unknown	0.22	1343		846		26		Stoll et al. (1967)
24	1-hydroxy-2-one (tentative)	0.74	1348		751		17		
25	unknown	0.11	1372		846		26		
26	2-ethyl-6-methylpyra- zine (tentative)	0.13	1381		978				Mussinan and Walradt (1974)
27	2-ethyl-5-methylpyra- zine (tentative)	0.17	1390					beef, <sup>g</sup> soy sauce <sup>a</sup>	Bondarovich et al. (1967)
28	acetic acid	2.49	1400	1400	723	723	14	soy sauce <sup>a</sup>	Mussinan and Walradt (1974)
29	2-ethyl-3-methylpyra- zine (tentative)	0.57	1400					beef, <sup>g</sup> soy beans <sup>h</sup>	Mussinan and Walradt (1974)
30	trimethylpyrazine	trace	1401	1397	983	980	40	beef <sup>g</sup>	Mussinan and Walradt (1974)
31	unknown	0.24	1410		846		27		
32	2-methylmercapto- propionaldehyde (tentative)	0.11	1426		866		28		Stenhagen et al. (1974a, p 110)
33	furfural	21.47	1437	1437	820	812	22	beef, <sup>i</sup> soy sauce <sup>a</sup>	Stoll et al. (1967)
34	tetramethylpyrazine	0.01	1466	1467	1065	1068	51	beef, <sup>g</sup> soy sauce <sup>j</sup>	Stenhagen et al. (1974a, p 282)
35	2-methyl-5-vinylpyra- zine (tentative)	0.05	1468		994		42	beef <sup>g</sup>	Stenhagen et al. (1974a, p 180)

36	2-acetylfuran	3.33	1482	1488	895	896	31	beef, <sup>i</sup> soy sauce <sup>a</sup>	Stoll et al. (1967) Mussinan and Walradt (1974)
37	2-furfuryl methyl ketone	0.33	1482	1488	-	-	-	-	-
38	propionic acid	0.39	1486	1492	790	781	20	beef <sup>e</sup>	Stoll et al. (1967)
39	benzaldehyde	0.51	1500	1509	951	947	39	beef <sup>d</sup>	Mussinan and Walradt (1974)
40	2-furfuryl acetate	0.04	1512	1514	969	967	40	soy sauce <sup>d</sup>	Stoll et al. (1967)
41	5-methylfurfural	11.44	1544	1551	987	984	39	beef <sup>f</sup>	Kinlin et al. (1972)
42	2-propionylfuran	1.85	1554	1551	987	984	41	beef, <sup>e</sup> soy sauce <sup>a</sup>	Stoll et al. (1967)
43	<i>n</i> -butanoic acid	0.49	1576	1581	828	834	23	beef, <sup>e</sup> soy sauce <sup>a</sup>	Stenhagen et al. (1974a, p 57)
44	unknown	1.03	1502	1014	1014	834	44	-	-
45	phenylacetaldehyde	4.64	1605	1613	1023	1019	45	beef, <sup>k</sup> soy sauce <sup>a</sup>	Mussinan and Walradt (1974)
46	furfuryl alcohol	7.78	1614	1617	857	856	27	beef, <sup>i</sup> soy sauce <sup>a</sup>	Mussinan and Walradt (1974)
47	3-methylbutanoic acid	0.80	1624	1626	910	900	33	-	Mussinan and Walradt (1974)
48	4-methyl-2-butenic acid $\gamma$ -lactone (tentative)	0.54	1642	1635	917	914	34	-	Ferretti and Flanagan (1971a)
49	$\beta$ -caryophyllene	1.85	1652	1642	1446	1442	59	essential oil <sup>l</sup>	Stenhagen et al. (1974b, p 1025)
50	5-methyl-2-furfuryl alcohol	0.33	1677	1678	-	-	-	-	Ferretti and Flanagan (1971b)
51	2,3-dimethylmaleic anhydride (tentative)	0.10	1680	-	996	-	43	-	Stoll et al. (1967)
52	$\delta$ -guaiene	0.57	1727	1729	1482	-	60	essential oil <sup>m</sup>	Stenhagen et al. (1974b, p 1026)
53	unknown (sesquiterpene)	1.26	1731	1492	1492	-	61	-	Stenhagen et al. (1974b, p 1028)
54	1-(2-furyl)-1,2-propanedione (tentative)	0.25	1746	-	1042	-	49	-	Stoll et al. (1967)
55	2-hydroxy-3-methyl-2-cyclopenten-1-one	0.82	1772	1777	1025	1021	46	-	Shigematsu et al. (1975)
56	2,5-dimethyl-4-hydroxy-3(2H)-furanone (tentative)	0.43	1784	-	-	-	-	-	-
57	<i>o</i> -methoxyphenol	0.12	1807	1808	1072	1077	53	soy sauce <sup>a</sup>	Stenhagen et al. (1974a, p 199)
58	benzyl alcohol	0.18	1821	1823	1039	1035	48	beef <sup>d</sup>	Stenhagen et al. (1974a, p 119)
59	1-(5-methyl-2-furyl)-1,2-propanedione (tentative)	0.31	1824	-	1155	-	56	-	Stoll et al. (1967)
60	phenylethyl alcohol	1.95	1861	1868	1102	1104	54	soy sauce, <sup>a</sup> sake <sup>b</sup>	Kinlin et al. (1972)
61	unknown	0.77	1879	-	1196	-	57	-	-
62	2-acetylpyrrole	1.38	1910	-	1064	-	50	beef, <sup>k</sup> soy sauce <sup>a</sup>	Kinlin et al. (1972)
63	pyrrole-2-carboxaldehyde (tentative)	0.24	1960	-	-	-	-	-	Kinlin et al. (1972)
64	3-methyl-2-pyridone (tentative)	0.60	2030	-	1131	-	55	-	Stenhagen et al. (1972b, p 122)
65	ethyl <i>n</i> -tetradecanoate	0.14	2057	2045	1800	1172	66	soy sauce <sup>a</sup>	Stenhagen et al. (1974b, p 1564)
66	unknown	3.65	2109	-	1664	-	67	-	-
67	unknown	0.23	2175	2235	1973	1981	68	sake <sup>b</sup>	Stenhagen et al. (1974c, p 1769)
68	ethyl <i>n</i> -hexadecanoate	1.30	2227	-	2002	-	68	-	-
69	unknown	1.18	2314	-	-	-	69	-	-

Table I (Continued)

peak no. on CW 20M	compd	peak area, %	CW 20W		OV-101		peak no. on OV-101	occurrence in ingredients	ref
			$I_{un}^n$	$I_k^o$	$I_{un}$	$I_k$			
70	unknown	0.24	2333	2410	1747	1270	65		
71	5-(hydroxymethyl) furfural (tentative)	0.71	2392	2442	1267	2180	58		Ferretti and Flanagan (1971a)
72	ethyl <i>n</i> -octadecanoate	0.17	2429	2455	2171	2149	70	sake <sup>b</sup>	Stenhagen et al. (1974c, p 1945)
73	ethyl <i>cis</i> -9-octadecanoate	0.70	2446		2142	2149	71		Stenhagen et al. (1974c, p 1934)

<sup>a</sup> Numomura et al. (1976). <sup>b</sup> Yoshizawa (1966, p 481). <sup>c</sup> Yoshizawa (1966, p 585). <sup>d</sup> Persson and von Sydow (1973). <sup>e</sup> Hirai et al. (1973). <sup>f</sup> Chang and Peterson (1977).  
<sup>g</sup> Mussinan et al. (1973). <sup>h</sup> Wilkens and Lin (1970). <sup>i</sup> Chang et al. (1977). <sup>j</sup> Kosuge et al. (1971). <sup>k</sup> Liebich et al. (1972). <sup>l</sup> Furia and Bellanca (1971, p 326). <sup>m</sup> Furia and Bellanca (1971, p 416). <sup>n</sup> Kovats index of unknown. <sup>o</sup> Kovats index of authentic sample.

*rapa L. var. Chinesis Kitamura* (taisai, 300 g), welsh onion (150 g), bean curd (tofu, 180 g), granulated sugar (150 g), rice wine (sake, 150 mL), and soy sauce (150 mL) were minced together in a blender. The blended samples were placed into two 1-L Kjeldhal flasks. The flasks were flame-sealed and placed in an oven at 100 °C for 12 h. The reaction product was filtered through a sheet of gauze. The volatiles were isolated by a modified method of Likens and Nickerson (1964), using a simultaneous distillation and extraction apparatus (SDE) (Schultz et al., 1977). The steam distillation-extraction (water/dichloromethane = 1500 mL/200 mL) was continued for 12 h. The extract was dried over anhydrous sodium sulfate for 12 h, and the solvent was removed by using a Kuderna-Danish evaporative concentrator. The dark brown oily residue (~0.5 g) was subjected to instrumental analysis.

**Gas-Liquid Chromatography (GC).** A Hewlett-Packard Model 5710A chromatograph equipped with a flame ionization detector was used for routine GC analysis. Two types of wall coated open tubular (WCOT) glass capillary columns were used in the experiments: 50 m × 0.28 mm i.d. coated with Carbowax 20M and 80 m × 0.28 mm i.d. coated with OV-101. For the Carbowax 20M column, the oven temperature was programmed to increase 2 °C/min from 80 to 200 °C, and the nitrogen carrier gas flow rate was 13 cm/s. For the OV-101 column, the oven temperature was programmed to increase 1 °C/min from 80 to 200 °C and the nitrogen carrier gas flow rate was 17 cm/s. The injector and detector temperatures were 250 °C. A Hewlett-Packard Model 3385-A reporting integrator was used to determine the peak area (reported in Table I).

The nitrogen- and sulfur-containing compounds were analyzed by using a gas chromatograph (Hitachi Model 163) fitted with a flame thermionic detector (Hitachi FTD) and a 50 m × 0.28 mm i.d. glass capillary column coated with Carbowax 20M. The oven temperature was programmed to increase from 80 to 200 °C at 2 °C/min. The flow rates of carrier gas He, H<sub>2</sub>, and air were 6, 12, and 100 mL/min, respectively.

**Gas Chromatography-Mass Spectrometry (GC-MS).** A Hitachi Model RMU-6M combination mass spectrometer-gas chromatograph equipped with a Hitachi Model 002B Datalyzer was used for mass spectral identification of the gas chromatographic components under the following conditions: ion source, 200 °C; ion acceleration, 3.1 kV; electron energy, 70 eV.

Identification of the volatile constituents of sukiyaki flavors was made by comparison of their MS and Kovats indices to those of authentic compounds. The compounds analyzed by MS fragmentation alone because of the lack of authentic samples are listed as "tentatively" identified in Table I.

**Thermal Energy Analyzer (TEA).** Samples were analyzed for *N*-nitrosamines by GC combined with a Thermal Energy Analyzer (Thermo Electron Corp. Model 502/LC). The gas chromatograph was equipped with a glass column (1 m × 2.8 mm i.d.) packed with 5% Carbowax 20M on Chromosorb G (80/100, AW/DMCS). The oven temperature was programmed to increase 2 °C/min from 80 to 210 °C. The argon carrier gas flow was 20 mL/min.

## RESULTS AND DISCUSSION

Table I shows the compounds identified from the sukiyaki volatiles. Peak numbers on the left side show the elution order on the Carbowax 20M column (Figure 1), peak numbers on the right side show the elution order on the OV-101 column (Figure 2).  $I_{un}$  designates retention

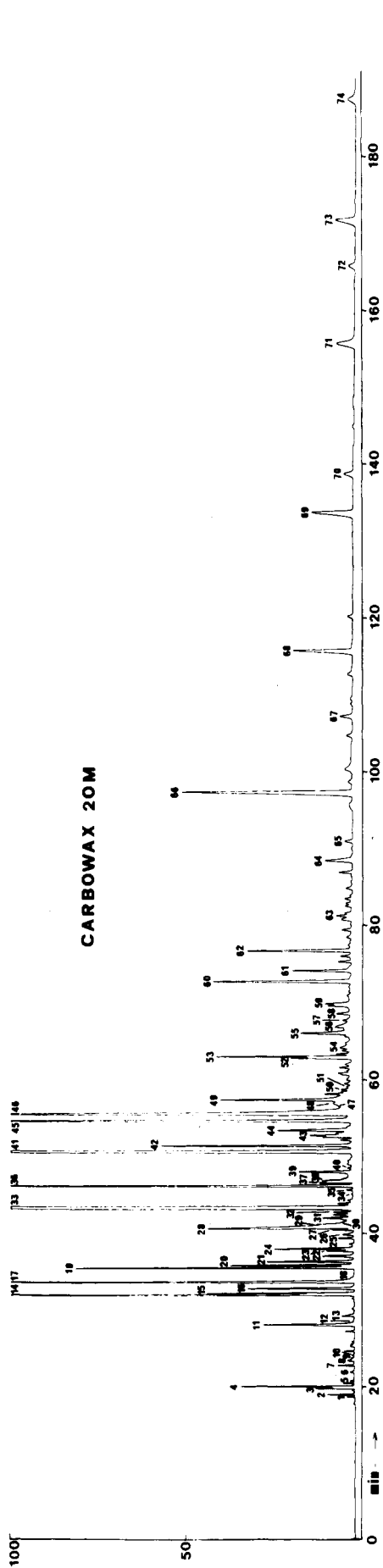


Figure 1. Gas chromatogram of sukiyaki volatiles, on a 50 m x 0.28 i.d. WCOT glass capillary column coated with Carbowax 20 M. See Table I for peak identification.

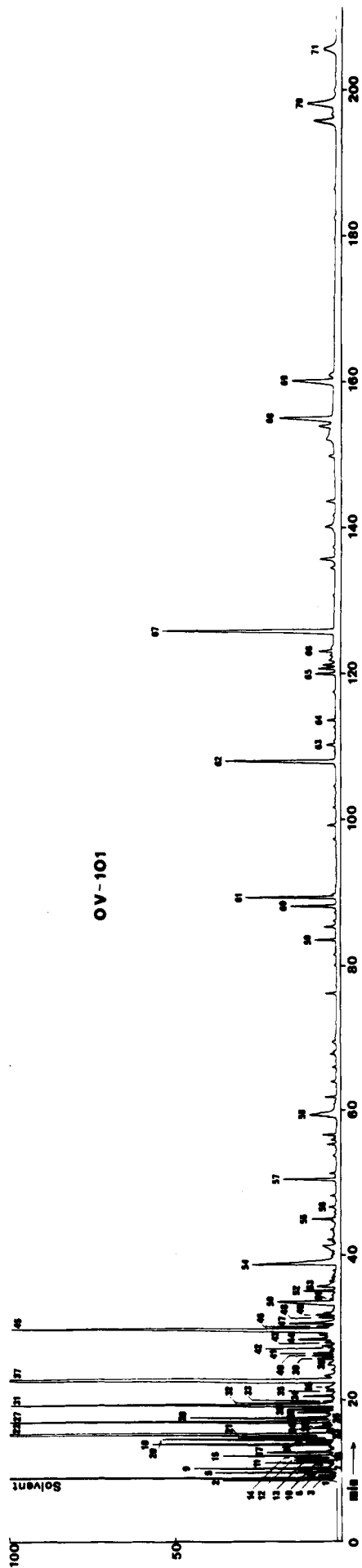


Figure 2. Gas chromatogram of sukiyaki volatiles, on a 80 m x 0.28 i.d. WCOT glass capillary column coated with methyl silicon OV-101. See Table I for peak identification.

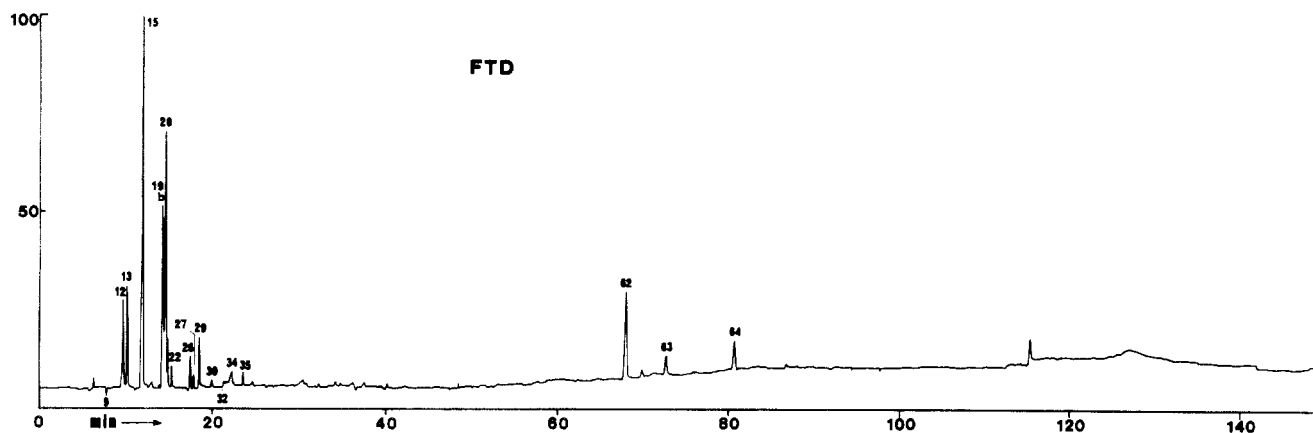


Figure 3. Gas chromatogram of sukiyaki volatiles detected with a flame thermionic detector (FTD). See Table I for peak identification.

indices of unknowns.  $I_k$  represents the retention indices of authentic samples. Figure 3 shows a gas chromatogram of sukiyaki volatiles detected with a flame thermionic detector. In this chromatogram, the positive peaks are nitrogen compounds, e.g., pyrazines and pyrroles, whereas the negative peaks represent sulfur compounds like dimethyl disulfide and 2-methylmercaptopyrrolinaldehyde. Peak no. 19 in Figure 2 contains both ethyl lactate (no. 19a) and 2,5-dimethylpyrazine (no. 19b), but no. 19 in Figure 3 contains only the latter (no. 19b).

The chemicals identified include furans, ketones, esters, aldehydes, acids, alcohols, pyrazines, hydrocarbons, and pyrroles. Most of these compounds seem to be formed from the degradation or caramelization of sugar from Maillard browning reactions (Hodge, 1967; Reynolds, 1970; Self et al., 1963). Furans are the main constituents of the sukiyaki volatiles (47.6% of total peak area) and also have been found in a wide variety of food systems (Maga, 1979).

Thirty-one of the sukiyaki volatiles identified have been found in beef (Table I). They are hydrocarbons, alcohols, aldehydes, ketones, acids, lactones, and esters, which may be formed from the oxidation of fat (Forss, 1972; Sink, 1973; Macleod and Coppock, 1976) or from sugar degradation (Hodge, 1967). We could, however, only identify relatively few fat oxidation products; this is probably due to the low fat content of the beef used.

Twenty-five of the chemicals identified in sukiyaki volatiles in this study also have been found in soy sauce. Soy sauce consists of a mixture of amino acids, peptides, polypeptides, peptones, simple proteins, purines, and carbohydrates, which are typical precursors of the Maillard-type reactions (Markley, 1951). Soy sauce, therefore, appears to be one of the most important contributors to sukiyaki flavor. The high content of protein (50%) and fat (30%) in bean curd also may contribute to the formation of browning reaction products.

The sesquiterpenes ( $\beta$ -caryophyllene and  $\delta$ -guaiene) identified probably were derived from the vegetables.

The result of thermal energy analysis (TEA) indicates that about 0.02 ppb of dimethylnitrosamine is present in a dichloromethane extract of sukiyaki broth.

The dichloromethane extracts obtained from individual sukiyaki ingredients were analyzed by TEA. The results indicate that the ingredients used for this experiment do not form *N*-nitrosamines when they are heated separately alone.

Dimethylamine in sukiyaki can originate from two sources. One is the heated beef (Golovnya et al., 1979) and the other is the browning reaction products. The beef seems to be the primary source, since alkyl secondary amines have not been found in any browning reaction as

yet. Various browning reaction products, however, contain large quantities of tertiary amines (pyrazines, pyrroles, and imidazoles) which may form *N*-nitrosamines after dealkylation (Lijinsky et al., 1972), although this has not been conclusively demonstrated.

It has not been conclusively demonstrated, however, that *N*-nitrosamines form from the tertiary amines found in the browning reaction. It is, therefore, more reasonable to propose that dimethylnitrosamine formed from dimethylamine in the raw beef [ $\sim 5.6$  ppb, Golovnya et al. (1979)] in combination with nitrite generated from the nitrate-rich vegetables in sukiyaki.

The amount of dimethylnitrosamine found in sukiyaki volatiles was not as high as the amounts found in cured meat (Ender and Ceh, 1967; Freimuth and Glaeser, 1970; Fazio et al., 1971). But this study demonstrates that *N*-nitrosamine can be formed during the cooking of beef together with other food ingredients even when sodium nitrite is not present as a preservative.

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## Isolation of *l*-Epicatechin and *d*-Catechin by Column Chromatography

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A simple method for the isolation of large quantities of *d*-catechin and *l*-epicatechin is given. Polyamide chromatography of a grape extract was found to separate these flavanols from other classes of phenolic metabolites. The flavanols themselves were separated by using Sephadex LH-20 chromatography, giving good enough resolution to allow for the crystallization of individual compounds. The combination of these two chromatographic separations is likely to be applicable to the isolation of large quantities of many other phenolic metabolites.

The flavanols *d*-catechin and *l*-epicatechin (3,3',4',5,7-pentahydroxyflavan) are common constituents of grapes and have been implicated in the aging of red wines (Timberlake and Bridle, 1976). In a series of ongoing experiments in our laboratory investigating the mechanism of the aging process, a need arose for a relatively inexpensive source of the pure compounds. This paper reports a procedure for the quick, large-scale isolation of *d*-catechin and *l*-epicatechin from grapes.

### MATERIALS AND METHODS

**Apparatus.** A Waters Associates (Milford, MA) high-performance liquid chromatograph (LC) was used for the identification of the compounds and in the determination of their purity. This included a Model 6000A pump and a U6K injector. Detection at 280 nm for the catechins was accomplished by using a Micromeritics Model 785 chromometer detector (Norcross, GA), coupled to an Omniscribe recorder (Houston Instruments, Austin, TX). The solvent used was acetonitrile-2% acetic acid (1:9), pumped at 2 mL/min. All solvents were distilled and filtered through a 0.45- $\mu$ m Millipore filter. Solvents were degassed immediately prior to use. The column used was a Zorbax ODS (25 cm  $\times$  4.6 mm i.d.; Du Pont, Inc., Wilmington, DE)

reverse-phase column preceded by a 4 cm  $\times$  4.6 mm i.d. precolumn packed with Lichrosorb ODS.

**Sample Preparation.** White grapes were chosen over red grapes for the catechin isolations to alleviate the problems encountered in the separation of the red grape anthocyanins from other phenolic compounds. In other fruits, it has been shown that the catechin levels are highest in the immature stages, decreasing during ripening, so that relatively immature fruit is favored as a phenolic source (Mosel and Herrmann, 1974; Stöhr and Herrmann, 1975). Seibel 9110 (Verdelet) grapes were obtained at  $\sim$ 10 °Brix, from the Irrigated Agriculture Research and Extension Center, Prosser, WA. To 500 g of destemmed grapes in a Waring blender was added 200 mL of 95% ethanol adjusted to 1000 ppm of SO<sub>2</sub>, added as NaHSO<sub>3</sub>. After the mixture was blended for 2 min, the resultant slurry was filtered through four layers of cheese cloth. The filtrates from 5 kg of grapes were combined and centrifuged at 5000g for 15 min. The supernatant was decanted and evaporated under vacuum at 30 °C to  $\sim$ 1 L. This was extracted 3 times with an equal volume of ethyl acetate. The ethyl acetate extracts were combined and evaporated to dryness under vacuum. The resultant residue was resuspended in  $\sim$ 60 mL of distilled water prior to column separation.

**Column Chromatography.** Chromatographic separation of the catechins was accomplished by using two suc-

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