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Volatile Constituents of Green Tea, Gyokuro (*Camellia sinensis* L. var Yabukita)

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The volatile constituents of Gyokuro, which had not been studied prior to this report, have been investigated by gas chromatography/mass spectrometry. Seventy-nine compounds were positively identified and ten compounds were tentatively identified in the oil obtained from a methylene chloride extract of the steam distillate of the green tea leaf. The compounds reported here include 17 hydrocarbons, 17 alcohols, 16 aldehydes, 13 ketones, 8 esters, 2 ethers, 1 acid, and 5 others. Major constituents of this oil were identified as 2,6,6-trimethyl-2-hydroxycyclohexanone, linalool, geraniol, *cis*-jasmone, β -ionone, cyclohexanone, 5,6-epoxy- β -ionone, indole, and caffeine.

Green tea was introduced to Japan from China in 1191 and quickly became one of the most popular drinks. Domestic green tea production increased steadily, reaching 98 000 tons in 1979. Recently, green tea flavor has also been used in ice cream, soft drinks, etc.

Green tea flavor has been investigated by many researchers and over 100 volatile components have been identified (Yamanishi, 1975; Kiribuchi and Yamanishi, 1963). The compounds found range from low boiling point alcohols (e.g., 2-methylpropanol) to high boiling point acids (e.g., decanoic acid).

Gyokuro, one of the highest grades of green tea (annual production in 1979 = 494 tons), gives a fresh green aroma and has a mild taste. The characteristic taste of Gyokuro is due to the use of specially treated new tea leaves. The leaves are grown in the shade under nets made by rice straw for ~20 days. Nakagawa (1973) reported that the taste of Gyokuro depends upon the relative amounts of amino acids, caffeine, and tannin present. There are, however, no reports on the volatile constituents of Gyokuro. In this study, the aroma components of Gyokuro were isolated and identified by gas chromatography/mass spectrometry (GC/MS) techniques.

EXPERIMENTAL SECTION

Tea Sample. Gyokuro (*Camellia sinensis* L. var Yabukita) was obtained from The Agricultural Institute of Fukuoka Prefecture, Tea Branch, in May 1979.

Department of Environmental Toxicology, University of California, Davis, California 95616 (T.S.), and Ogawa & Co., Ltd., 6-32-9 Akabanenishi, Kita-Ku, Tokyo, Japan (K.Y.).

Isolation of Volatiles. Gyokuro (220 g) was made into powder by using a blender and placed in a 2-L round-bottomed flask. Deionized water (1 L) was added, and steam distillation was performed under reduced pressure (thermometer and pressure gauge reading = 50 °C and 40 mmHg). The steam distillate (800 mL) was gathered with the condensate (50 mL) obtained from the dry ice-acetone trap. The distillate was then extracted with 300 mL of methylene chloride for 20 h by using a liquid-liquid continuous extractor. The extract was dried over anhydrous sodium sulfate for 12 h, and solvent was removed by using a rotary flash evaporator to ~10 mL in volume. Further concentration was conducted with an N₂ stream in a micro test tube. Three batches of green tea samples were treated by the above method (total green tea used: 660 g). The concentrated extracts were combined and the composite was analyzed by the GC/MS technique described by Yamaguchi and Shibamoto (1979).

RESULTS AND DISCUSSION

The volatile compounds identified in green tea (*C. sinensis* L. var Yabukita) extract are listed in Table I. 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). Those peak areas (from the Carbowax 20M column) which had value of less than 0.1% are not listed. I_u designates retention indexes of unknowns. I_k represents the retention indexes of authentic samples. For some compounds, formulas were deduced from mass spectral data, but known compounds were not available. We listed those compounds as "tentatively" identified.

Several probable reaction products from β -ionone (peak 133, OV-101) were found (represented by footnote *b* in

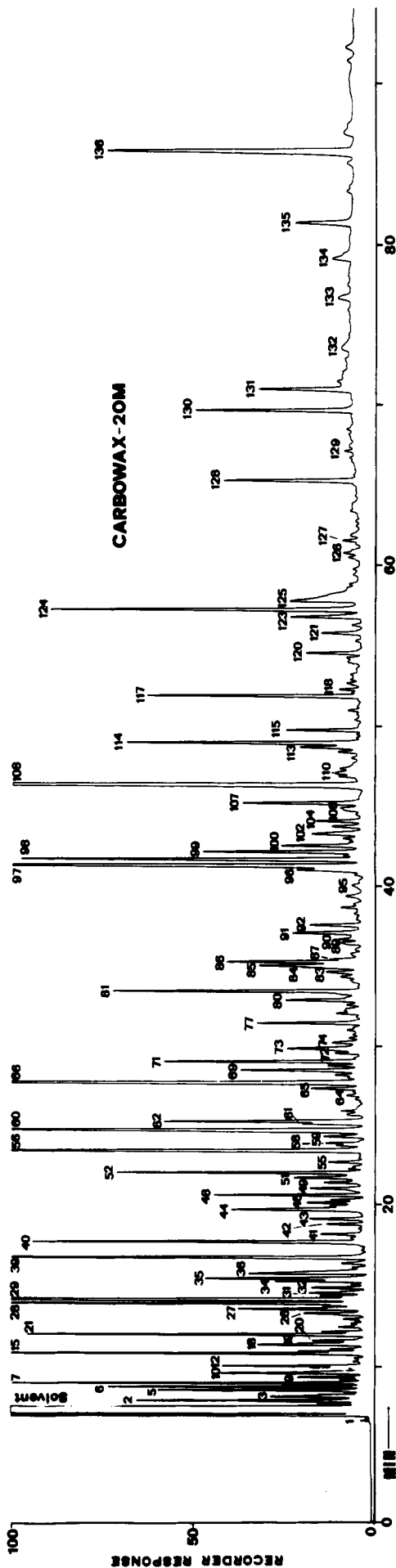


Figure 1. A typical gas chromatogram of the green tea extract. A Hewlett-Packard Model 5710A gas chromatograph equipped with a flame ionization detector and 50 m x 0.28 mm i.d. glass capillary column coated with Carbowax 20M was used. The column temperature was programmed from 80 to 220 °C at 2 °C/min. The nitrogen carrier gas flow rate was 0.6 mL/min. The injector and detector temperatures were 250 °C. See Table I for peak identification.

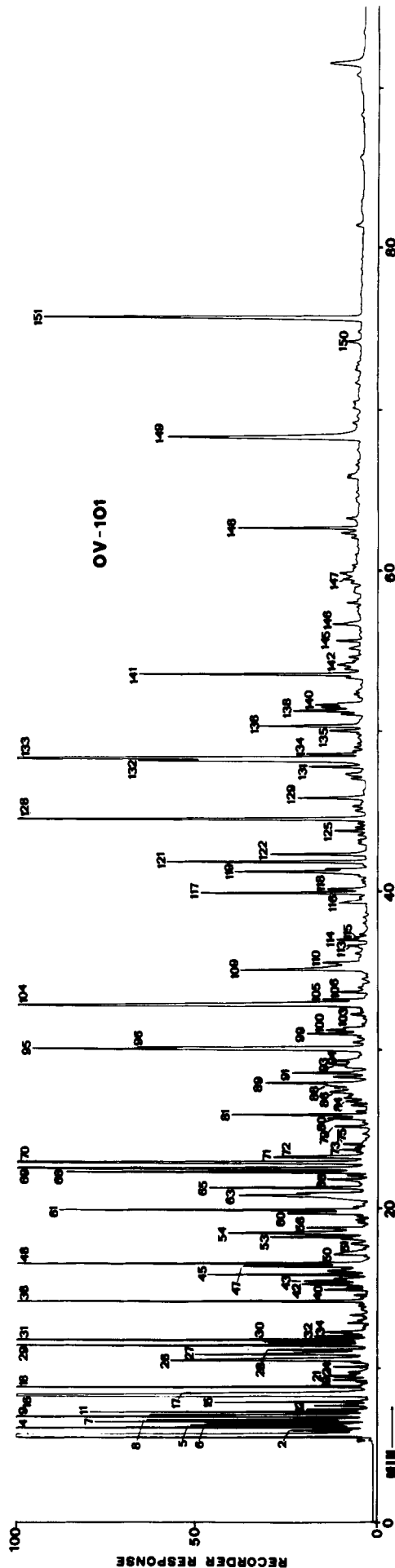


Figure 2. A typical gas chromatogram of the green tea extract on a 70 m x 0.28 mm i.d. glass capillary column coated with OV-101. The gas chromatographic conditions were the same as those described for Figure 1.

Table I. Compounds Identified in the Green Tea Extract and Their Kovats Indexes

peak no. on CW 20M	compound	peak area, %	Kovats indexes				peak no. on OV-101
			Carbowax 20M		OV-101		
			I_u	I_k	I_u	I_k	
1	acetaldehyde		690	692	363	358	1
2	unknown	0.27	943		700		11
3	toluene		997	1001	764	765	16 ^c
5	<i>n</i> -hexanal	0.10	1036	1041	782	779	18 ^c
6 ^c	4-methyl-3-penten-2-one (tentative)	0.45	1055		782		18 ^c
6 ^c	ethylbenzene		1055	1063	857	855	27 ^c
7	1-penten-3-ol	0.75	1072	1080	649	656	9
8	<i>m</i> - or <i>p</i> -xylene ^a		1087	1191	864	864	28 ^c
9	<i>n</i> -undecane	0.10	1100	1100	1100	1100	71 ^c
10	unknown	0.32	1144		586		8
12	unknown	0.23	1132		525		5
15	<i>n</i> -heptanal	1.84	1173	1171	882	883	31
17	<i>o</i> -xylene ^a		1183	1178	887	887	32
18	unknown	0.18	1194		811		20
19	limonene		1202	1200	1029	1030	56
20	<i>trans</i> -2-hexenal	0.11	1208	1205	832	830	24
21	<i>n</i> -amyl alcohol	0.97	1220	1214	764	759	16 ^c
26 ^c	<i>p</i> -cymene		1263	1270	1016	1019	52
26 ^c	acetoin	0.11	1263	1270	714	710	12
27	<i>n</i> -octanal	0.25	1270	1277	988	987	48 ^c
28	cyclohexanone ^a	2.34	1280	1281	874	872	29
29	<i>cis</i> -2-penten-1-ol (tentative)	3.70	1288		764		16 ^c
30	perillene (tentative)	0.18	1292		1099		71 ^c
31	<i>n</i> -tridecane	0.15	1300	1300	1300	1300	114 ^c
32	2,6,6-trimethylcyclohexanone ^{a, b}	0.14	1307	1303	1019	1022	53
34	unknown	0.38	1317		878		30
35	6-methyl-5-hepten-2-one	0.96	1322	1318	964	968	42
36	<i>n</i> -hexanol	0.54	1339	1332	857	858	27
39	<i>cis</i> -3-hexen-1-ol	2.02	1354	1351	846	847	26
40	<i>n</i> -nonanal	1.95	1374	1380	1086	1088	68
41	2,6,6-trimethyl-2-cyclohexenone ^{a, b}	0.19	1384	1386	1046	1054	60 ^c
42	tetradecane		1400	1400	1400	1400	125
43	unknown		1403		1112		75
44 ^c	linalool oxide, <i>cis</i> -furanoid	0.35	1414	1410	1066	1066	64
45 ^c	<i>m</i> -dichlorobenzene	0.22	1414	1409	1000	997	50 ^c
46	furfural	0.29	1425	1432	818	815	21
48	<i>trans</i> -2, <i>cis</i> -4-heptadienal	0.36	1435	1430	976	975	45
49	linalool oxide, <i>trans</i> -furanoid	0.28	1444	1443	1079	1081	66
51	<i>o</i> -dichlorobenzene	0.38	1459	1455	1024	1019	54 ^c
52	<i>trans</i> -2, <i>trans</i> -4-heptadienal	0.79	1463	1458	988	989	48 ^c
55	<i>n</i> -decanal	0.25	1474	1481	1185	1187	93
56 ^c	<i>trans</i> -3, <i>cis</i> -5-octadien-2-one (tentative) ^a	2.61	1488		1050		61
56 ^c	benzaldehyde		1488	1482	941	946	38
58	<i>n</i> -pentadecane	0.12	1500	1500	1500	1500	136
59	unknown	0.20	1510		1141		81
60	linalool	3.40	1514	1518	1089	1088	69
61	<i>n</i> -octanol	0.26	1521	1519	1055	1060	63
62	<i>trans</i> -3, <i>trans</i> -5-octadien-2-one (tentative)	1.09	1524		1072		65
65	unknown	0.19	1558		1102		72
66	2,6,6-trimethyl-2-hydroxycyclohexanone ^{a, b}	4.08	1565	1561	1093	1093	70
69	diethylene glycol monoethyl ether	1.31	1579	1577	985	985	47
71	β -cyclocitral ^{a, b}	1.22	1586	1584	1200	1200	96
72	phenylacetaldehyde		1592	1585	1028	1022	55
73 ^c	acetophenone		1600	1606	1063	1058	62
73 ^c	<i>n</i> -hexadecane	0.38	1600	1600	1600	1600	146
77	<i>cis</i> -3-hexenyl hexanoate	1.35	1631	1638	1364	1370	119
80	α -terpineol	0.55	1656	1655	1179	1179	91
81	unknown	1.30	1666		1200		95
83	2,5-dimethylbenzaldehyde ^a	0.12	1683	1680	1154	1159	84
84	naphthalene ^a	0.28	1688	1688	1170	1172	89
85	benzyl acetate	0.40	1690	1689	1138	1138	80
86 ^c	neryl acetate		1700	1699	1337	1344	116
86 ^c	linalool oxide, <i>cis</i> -pyranoid	1.22	1700	1684	1162	1160	86
87	<i>cis</i> -3-hexenyl <i>trans</i> -2-hexenoate		1703	1706	1401	1405	126
89	α -muurolene (tentative)		1717		1378		122 ^c
90	linalool oxide, <i>trans</i> -pyranoid		1722	1716	1168	1164	88
91 ^c	methyl salicylate		1727	1730	1177	1181	90
91 ^c	β -sesquiphellandrene (tentative)		1727		1406		127
92 ^c	unknown	0.37	1735		1220		100
92 ^c	α -cadinene (tentative)		1735		1410		128 ^c
95	nerol		1781	1774	1222	1219	101
96	2-methylnaphthalene ^a	0.58	1793	1794	1279	1281	110
97 ^c	geraniol	5.88	1800	1800	1247	1244	104
97 ^c	calamenene (tentative)		1800		1460		131

Table I (Continued)

peak no. on CW 20M	compound	peak area, %	Kovats indexes				peak no. no. OV-101
			Carbowax 20M		OV-101		
			I_u	I_k	I_u	I_k	
97 ^c	octadecane		1800	1800	1800	1800	149 ^c
98 ^c	α -ionone ^b	2.03	1805	1811	1410	1412	128 ^c
98 ^c	benzyl alcohol		1805	1806	1046	1039	60 ^c
99	geranyl acetone	1.65	1811	1816	1431	1434	129
100	unknown	1.13	1824		1348		118
101	1-methylnaphthalene ^a		1831	1828	1305	1298	115
102 ^c	unknown	0.31	1837		1167		87 ^c
102 ^c	<i>cis</i> -3-hexenyl octanoate	0.36	1837	1838	1562	1564	142
103	2-phenylethyl alcohol	0.28	1846	1845	1107	1105	73
104	unknown	0.65	1853		1167		87 ^c
106	benzothiazole	0.32	1867	1873	1207	1202	97
107	butylated hydroxytoluene ^e	0.97	1873	1870	1500	1501	136 ^c
108 ^c	<i>cis</i> -jasmone	4.16	1891	1886	1372	1368	121
108 ^c	β -ionone ^b	2.26	1891	1896	1467	1469	133
110	phenol	0.51	1909	1915	1002	1002	50 ^c
113	unknown		1934		1214		99 ^c
114	5,6-epoxy- β -ionone ^b	2.30	1940	1934	1465	1467	132
115	diphenyl ether ^a		1965	1958	1378	1380	122 ^c
117	nerolidol	1.23	2000	2000	1552	1551	141
120	unknown	0.85	2051		1460		131 ^c
121	2-phenoxyethyl alcohol ^a	0.57	2072	2069	1214	1210	99 ^c
123	1,5,5-trimethyl-9-oxabicyclo[4.3]non-6-en-3-one (tentative)	0.65		2090	1500		136 ^c
124	eugenol	2.18	2100	2103	1347	1347	117
125	nonanoic acid	0.44	2115	2110	1300	1302	114 ^c
128	dihydroactinidiolide ^b	1.65	2260	2257	1513	1508	138
129	diethyl phthalate ^f	0.34	2309	2303	1568	1565	143
130	indole	3.30	2351	2358	1274	1273	109
131	unknown	0.92	2381		1700		148
133	<i>n</i> -pentacosane		2500	2500	2500	2500	
135	unknown	1.26	2575		1523		140
136	dibutyl phthalate ^f	9.32	2631	2630	1919	1922	151
	caffeine ^d	7.87			1800	1802	149 ^c

^a Has not been found in green tea prior to this report. ^b Probable reaction products from β -ionone. ^c More than two compounds in one peak. ^d Not found in the Carbowax 20M chromatogram. ^e Probable added antioxidant. ^f Probable contaminants from plastic container.

Table I). β -Ionone is formed from β -carotene by oxidative degradation (Sanderson et al., 1971). β -Cyclocitral (peak 71, Carbowax 20M) possesses a sweet odor (Lloyd et al., 1976) and has been found in black tea (Renold et al., 1974) but not, until this study, in green tea. 2,6,6-Trimethylcyclohexanone, too, had been found in black tea (Bondarovich et al., 1967) but not in green tea prior to this study. The mass spectra of peak 66 (Figure 1) is identical with that of the 2,6,6-trimethyl-2-hydroxycyclohexanone reported by Muggler-Chavan et al. (1969) in black tea. This compound was synthesized in our laboratory from 2,6,6-trimethylcyclohexanone (Aldrich) by the method reported by de Botton (1966).

Cyclohexanone was isolated from Gyokuro in fairly large quantities (gas chromatographic peak area percent is 2.34). This compound was also found in cellulose cigarette smoke (Sakuma et al., 1980) and may be formed from a fiber in the tea leaf.

Many aromatic hydrocarbons were identified in Gyokuro. The condensate in the dry ice-acetone trap gave an alkylbenzene-like odor and contained *o*-, *m*-, and *p*-xylene and *p*-cymene. These alkylbenzenes may come from β -carotene upon heat degradation (Kawashima and Yamanishi, 1973). 2,5-Dimethylbenzaldehyde might be regarded as a degradation product of polyphenolic substances (Renold et al., 1974). Naphthalene and 1-methyl- and 2-methylnaphthalene were identified in Gyokuro. They are also regarded as heat degradation products of fiber (Sakuma et al., 1980).

Aliphatic compounds were found in the low boiling point range on the gas chromatograms. Kobayashi et al. (1965)

reported that *cis*-2-penten-1-ol (peak 29, Carbowax 20M) possesses a fruity note and is found in green tea. The isomers *trans*-2,*cis*-4-heptadienal (peak 48) and *trans*-2,*trans*-4-heptadienal (peak 52) have strong oily green notes and have also been isolated from green tea (Hara and Kubota, 1973). *trans*-3,*trans*-5-Octadien-2-one (peak 56 or 62, isomers) has been found in green tea (Yamanishi et al., 1970). The *trans*-*cis* form generally has a shorter retention time than the *trans*-*trans* form as shown in the case of 2,4-heptadienal. Peaks 56 and 62 were, therefore, tentatively identified as *trans*-3,*cis*-5-octadien-2-one and *trans*-3,*trans*-5-octadien-2-one, respectively.

The gas chromatogram of green tea extract shows the presence of over 300 chemicals. Further study is necessary to identify all the constituents of green tea.

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Pectic Substance Content of Detergent-Extracted Dietary Fibers

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Acid and neutral detergent fibers (ADF and NDF) prepared from apple, pear, tomato, carrot, onion, potato, wheat bran, and orange albedo were analyzed for total pectic substances (PS). Total PS content and the relative proportion of the original total PS recovered as dietary fiber (DF) were higher in ADF than in NDF, suggesting more PS were solubilized and extracted by neutral detergent. About 50 and 38% of total PS in orange albedo were recovered in ADF and NDF, respectively. In fruits, vegetable, and wheat bran, total PS recovered as ADF and NDF ranged from 3.0 to 15.0% and 0.6 to 7.0%, respectively. For potato, the proportion of total PS recovered in extracts and washings during ADF and NDF analysis was 94.8 and 99.8%, respectively. These data emphasize that the detergent methods which are increasingly used to report dietary fiber values in foods underestimate PS, a component of dietary fiber complex with important nutritional and physiological effects. More attention should be directed toward separate analysis of pectin or modifications of existing methods to reflect pectin more accurately.

Ever since the epidemiological demonstrations of the beneficial effect of what is now called dietary fiber (DF) on the etiology of certain "Western" diseases by Burkitt (1973) and Trowell (1972), a great deal of effort has been directed toward development of simple routine methods to assay the dietary fiber content of food. The acid detergent fiber (ADF) and neutral detergent fiber (NDF) methods (Van Soest, 1963; Van Soest and Wine, 1967) are at present considered the most convenient analytical methods for fiber determination and have largely replaced the crude fiber method (AOAC, 1975). Compared to the enzymatic procedure by Hellendoorn et al. (1975) and to the fractionation method of Southgate (1969, 1976), they are simpler, less expensive, more rapid, and practical for routine analysis of large number of samples. The use of these methods is increasing. The ADF method is listed as an official first action method of the Association of Official Analytical Chemists (AOAC, 1975) while a modified NDF is an official method of the American Association of Cereal Chemists (AACC, 1978).

Due to the complex nature of DF in food materials, the development of a method that will encompass all of the components in the DF complex has presented a difficulty and challenge to workers in this subject. For instance, the ADF and NDF methods are designed to measure insoluble components and therefore exclude most of the water-soluble polysaccharides and other polymers.

Pectic substances (PS) or pectins along with cellulose, hemicellulose, and lignin are the major components of the

plant cell wall, and collectively they are part of what is now called DF (Southgate, (1979)). PS had been reported to have important physiological and nutritional effects such as hypocholesterolemic effect, increased excretion of fecal sterols and lipids, binding of bile salts, binding of polyvalent cations (Story and Kritchevsky, 1969; Kay and Truswell, 1977; Kay et al., 1978; Kelsay, 1978), and increased requirement for vitamin B₁₂ (Cullen and Oace, 1978). With the growing interest in its biological effects, it is desirable to determine this DF component more accurately in foods. Due to their relative water solubility, some PS are solubilized during analysis and therefore not included in the total fiber value (Bailey and Ulyatt, 1970; Bailey et al., 1978). However, some insoluble PS may remain with the fiber (Baker et al., 1979). Bailey et al. (1978) determined the solubility of pectins from a number of common plant foods, indicating that a considerable portion of pectin is lost to commonly used analytical procedures. However, PS analyses of actual detergent fiber residues do not appear to have been reported in the literature. This information is especially important with the increased usage of ADF and NDF in reporting fiber values. For these reasons, PS content of selected foods and their detergent-extracted DF were investigated, and results are discussed in this report.

MATERIALS AND METHODS

Sample and Sample Preparation. Food samples used in this study were potato, carrot, tomato, apple, pear, white onion, orange albedo, and wheat bran. Except for wheat bran, all samples were bought from a local grocery store. Wheat bran was AACC grade obtained from Dr. G. Briggs' laboratory (Department of Nutritional Sciences, University of California, Berkeley). Fruits and vegetable samples were washed and dried. Dried outer skins of onions were re-

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