Volatile Flavor Components in Red Fermented Soybean (*Glycine max*) Curds

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Red fermented soybean (*Glycine max*) curds (FSC) were extracted with a simultaneous steam distillation and extraction (SDE) apparatus and analyzed by gas chromatography−mass spectrometry (GC-MS) and further evaluated by gas chromatography−mass spectrometry−flame ionization detector−olfactometry (GC-MS-FID-O). A combined total of 89 compounds were identified from three samples (A−C). The numbers of components identified (samples A−C) were 87, 85, and 81, respectively, which were divided into 10 compound classes. The quantity of 48 compounds was statistically significant ($p < 0.05$). Sample B generally had the lowest level, whereas sample C had the higher level of each common component among the samples. GC-MS-FID-O analyses showed that the majority of the fruity, diacetyl, cantaloupe-like odors were eluted early during the run (RI < 1100), followed by the meaty flavor, rosy, and prune-like aromas (RI > 1100). Ethyl 2-methylpropanoate, 2,3-butanedione, ethyl butanoate, ethyl 2-methylbutanoate, 3-(methylthio)propanal, benzeneacetaldehyde, and ethyl 3-phenylpropionate were found to be important common components contributing to the characteristic aroma of red FSCs.

Keywords: *Volatiles; soybean fermentation; red sufu; curd; Asia*

INTRODUCTION

Fermented soybean curds (FSC) are produced from soybean (*Glycine max*) and are divided into two major types according to the color of the products, namely, white and red ones (Steinkraus, 1983; Liu et al., 1988). Both products are produced by similar steps except the latter require the addition of *Monascus* koji in the final aging period (Chen and Ho, 1989; Hsu, 1994). Wang and Hesseltine (1970) indicated the blandness in taste of the freshly molded tofu and suggested the flavor and aroma of the products were developed during the brining and aging period. Liu et al. (1988) identified 52 and 40 compounds in the white and red FSCs, respectively. These workers suggested that the aromas of white FSCs were mainly attributable to by anethole, whereas the aromas of the red ones were contributed by esters and alcohols. Recently, Hwan and Chou (1999), investigating the effects of ethanol on the quantity and quality of volatile compounds produced in white FSCs, observed that most volatile compounds increased during aging, particularly esters and alcohols. They also reported the amount of esters determined was higher in the presence of ethanol, but no anethole was identified. Previous investigations determined the potent odorous components in FSCs based on the amount and the composition without organoleptic evaluation of the products. Therefore, the objectives of this study were (1) to further determine the common volatile components in steamed red FSCs and (2) to identify the odorous components in the products by a gas chromatography−olfactometry technique.

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MATERIALS AND METHODS

Samples. Three samples (A−C) of red FSC often available and consumed in Hong Kong were purchased three times in the summer of 1997 from local markets. Samples were transported to the Chinese University of Hong Kong in their own packages within 2 h of purchase. Original curds were in cubic shape, but of various sizes depending on brand. Before extraction, they were cut into smaller pieces (1 cm^3).

Simultaneous Steam Distillation and Extraction (SDE). A Likens and Nickerson (1964) type SDE apparatus (model 523010-000, Kontes, NJ) was used. Conditions for the extraction of samples were similar to those described by Chung (1999). Samples (60 g each) were loaded on a stainless steel mesh in a 5-L round-bottom sample flask. One milliliter of internal standard (IS), 2,4,6-trimethylpyridine (10 μg/mL), was added to the sample. Boiled, distilled water (400 mL) was added to the sample flask. Redistilled dichloromethane (50 mL) was used as the extraction solvent. Each extraction was carried out for 2 h. Extracts were initially concentrated by a gentle stream of nitrogen gas of 99.995% purity to 15 mL, dried with 2.3 g of anhydrous sodium sulfate, and further concentrated to 0.05 mL. Triplicate extractions were carried out for each sample. Extracts were kept in a freezer (−70 °C) until further analyzed.

Gas Chromatography−Mass Spectrometry (GC-MS). Five microliters of extract was injected at splitless mode into a Hewlett-Packard (HP) 6890 GC coupled with an HP 5973 mass selective detector (MSD), which was installed with a polar capillary column (Supelcowax 10, 60 m length × 0.25 mm. i.d. × 0.25 μm df, Supelco, Inc., Bellefonte, PA). GC oven conditions were initially at 35 °C, programmed at 2 °C/min until 195 °C, and held for 90 min. Injector temperature was 220 °C. Helium carrier gas flow was at 30 cm/s. MS conditions were as follows: ion source temperature, 230 °C; MS quadrupoles temperature, 106 °C; electron multiplier, 1160 V; and scan rate, 6.52 scans/s.

Compound Identification and Quantification. Positive identification of a component was performed by comparison of its retention time or retention index (RI) and mass spectrum

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Table 1. Proximate Analyses (Wet Percent) of Three Commercial Red Fermented Bean (*G. max*) Curds (Samples A−C)[a]

sample	moisture	protein	fat	ash	carbohydrate[b]
A	63.620 ± 0.132	11.2 ± 0.1	8.037 ± 0.040	13.94 ± 0.01	3.3
B	64.193 ± 0.049	8.5 ± 0.1	7.163 ± 0.065	12.84 ± 0.00	7.3
C	61.832 ± 0.106	11.5 ± 0.1	5.856 ± 0.028	10.67 ± 0.02	10.1

[a] Data are expressed as mean ± standard deviation of $n = 3$. [b] Carbohydrate % = 100% − (moisture + protein + fat + ash)% (Merril and Watt, 1973).

with that of the authentic compound as described in Chung (1999). Retention indices were calculated according to the method of van den Dool and Kratz (1963). Tentatively identified compounds were uniquely identified on the basis of the mass spectra from the Wiley library of mass spectral database (Hewlett-Packard Co., 1995). For compound quantification, a prominent fragment of each compound was chosen and used to calculate its quantity. The concentration of each compound was calculated from the standard curve derived from the area ratio and the concentration ratio of individual compound. Relative concentration of tentatively identified compounds was estimated from the ratio of the relative area of a specific fragment of the tentatively identified compound to that of internal standard. Quantities of the compounds in the three samples of red FSCs were evaluated by one-way analysis of variance (ANOVA) and compared by the Tukey test at $p \leq 0.05$ level of significance (Ott, 1988).

Gas Chromatography−Mass Spectrometry−Flame Ionization Detection−Olfactometry (GC-MS-FID-O). The GC injector helium gas flow was split into two identical capillary columns (Supelcowax 10, 60 m length × 0.25 mm. i.d. × 0.25 μm d_f, Supelco, Inc.) by a short piece of precolumn (20 cm length, 0.25 mm. i.d.) from the injector and a Y Vu-connector (Restek, Bellefonte, PA). One column was directed to an MS, whereas the other one was split equally to an HP FID and to an olfactometer using a Y presstight splitter (Restek) and two 40-cm, 0.25 mm. i.d. postcolumns. GC-O analysis was carried out according to the method described by Chung and Cadwallader (1994) with some modifications. The GC temperature ramp rate was set at 6 °C/min with both initial and final temperature of 35 and 195 °C for 5 and 45 min, respectively. The same subsamples were pooled and rediluted to 0.5 mL before evaluation. Two microliters of extract was injected at splitless mode into the GC. Each pooled sample was evaluated by two panelists with a total of four evaluations. Identification of odorous components was carried out by comparing the retention time or index, odor property, and spectrum of unknown components with those of the authentic standards.

Proximate Analysis. Moisture, protein, fat, and ash analyses were determined according to the AOAC official methods (AOAC, 1980).

RESULTS AND DISCUSSION

Table 1 shows the results from the proximate analysis of samples A−C. For moisture content, the red FSCs (range = 61.8−64.2%) are lower than the white FSCs (range = 70.3−78.5%) (Chung, 1999). In general, much higher levels of protein, ash, and carbohydrate are found in the red FSCs. A total of 91 compounds of volatiles were found, and 89 were identified from the three samples (A−C). They belonged to 10 classes of compounds including acid (1), aldehydes (7), esters (22), miscellaneous compounds (8), other nitrogen-containing compounds (8), pyrazines (4), other oxygen-containing compounds (5), alcohols (24), ketones (8), and sulfur-containing compounds (3). Eighty percent of these compounds were previously reported in soybeans or soybean processed products (Ames and Macleod, 1984; Chung, 1999; Hwan and Chou, 1999). Seventy percent of them were identified in the white FSCs (Chung, 1999). Seventy-eight compounds were found in all three samples, but their quantitative levels varied (Table 2). Forty-eight components were statistically significant ($p < 0.05$). Similar to the white FSCs, both esters and alcohols had the highest number of components (Liu et al., 1988; Chung, 1999; Hwan and Chou, 1999). Sample C had the highest concentration levels of the common components among the three samples. Sample B generally had lower mean concentration levels compared with sample A, although statistically they were similar ($p > 0.05$).

Of the 111 compounds identified in our previous white FSCs, the same 55 compounds were found in the present red FSCs. When additional components from the works of Hwan and Chou (1999) were considered and compared, a total of 62 common compounds were found. In aldehydes, benzeneacetaldehyde, (*E*)- and (*Z*)-2-phenyl-2-butenal, 4-methoxybenzaldehyde, cinnamaldehyde, and 5-methyl-2-phenyl-2-hexenal were found only in red FSCs. Ames and Macleod (1984) reported the presence of benzeneacetaldehyde and (*E*)- and (*Z*)-2-phenyl-2-butenal in the unflavored textured soy protein. The former has a harsh, hawthorn aroma when diluted (Fenaroli, 1995). The latter was reported as an odorous component in black tea and *Phallus impudicus* (Fenaroli, 1995). 4-Methoxybenzaldehyde has a sweet mimosa and hawthorn odor and is often found in essential oils together with anethole (Bauer and Garbe, 1985). (*E*)-Cinnamaldehyde is found in Ceylon cinnamon bark oil having a spicy, cinnamon-like aroma (Bauer and Garbe, 1985).

Except for both diethyl succinate and ethyl cinnamate, all components in the esters were previously found in the white FSCs. The former is described as having a faint, pleasant odor, and the latter as having a sweet, honey, balsamic, cinnamon, and plum-like aroma (Fenaroli, 1995). Quantitatively, esters with high molecular weight fatty acid moieties have the highest concentration (>7000 μg/kg dry basis). These components at low concentration probably would not have strong aroma impact, but at high concentration, ethyl palmitate has a mild waxy odor and a recognition threshold value of 2.45 wt % (Fazzalari, 1978; Aldrich, 1998).

Among the eight compounds found in the miscellaneous class, only linalool, (*E*)-anethole, eugenol, and coumarin were present in the red FSCs. Linalool is a major constituent in coriander oil (Bauer and Garbe, 1985). It has a refreshing, floral, citrus, and sweet fragrance (Fenaroli, 1995). (*E*)-Anethole has an anise-like odor and sweet taste. It is a major component in anise oil and is often used in the alcoholic beverage industry (Bauer and Garbe, 1985). With a spicy clove-like odor, eugenol is found in clove leaf oil and cinnamon leaf oil (Bauer and Garbe, 1985). Coumarin has a hay-like, spicy odor and is distributed widely in nature. Both naphthalene and eugenol were previously identified in the white FSCs.

1*H*-Pyrrole, 2-methyl-1*H*-pyrrole, and 2-acetylpyrrole identified here were not detected among the nitrogen-containing compounds in the white FSCs. Pyrroles can be produced by the Maillard reaction (Fors, 1983). 1*H*-

Table 2. Volatile Components in Three Commercial Red Fermented Soybean (*G. max*) Curds (A−C)

no.[a]	compound[b]	ref[c]	CAS Registry No.[d]	RI[e]	fragment used[f] (*m*/*z*)	sig[g]	sample A concn[h] (μg/kg)	sample A SD[i]	sample B concn[h] (μg/kg)	sample B SD[i]	sample C concn[h] (μg/kg)	sample C SD[i]
	acid (1)											
40	acetic acid	2, 5, 6ab	64-19-7	1472	60	*	560[a]	260	67[b]	58	660[a]	180
	aldehydes (7)											
10	*n*-hexanal	1, 2, 3, 4, 6ab	66-25-1	1026	44	*	260[a]	110	210[a]	190	880[b]	300
48	benaldehyde	1, 2, 3, 6b	100-52-7	1530	106	*	360[a]	200	200[a]	180	920[b]	110
57	benzeneacetaldehyde	3	122-78-1	1652	91	*	3300[a]	910	1100[b]	990	6100[c]	310
70	(*E*)- and (*Z*)-2-phenyl-2-butenal[j]	3, 5	4411-89-6	1939	115	---	150	110	62	53	220	2
75	4-methoxybenzaldehyde		123-11-5	2035	136	---	62	59	nd[k]	------[l]	12	0.33
78	cinnamaldehyde		104-55-2	2049	131	---	44	30	27	24	nd	------
80	5-methyl-2-phenyl-2-hexenal[j]		21834-92-4	2083	117	---	82	89	64	57	69	34
	esters (22)											
2	ethyl 2-methylpropanoate		97-62-1	1000>	71	---	43	40	26	45	390	620
5	ethyl butanoate	1, 2, 3, 5	105-54-4	1039	71	*	3400[ab]	60	1700[a]	1700	4900[b]	690
7	ethyl 2-methylbutanoate	1, 2, 5	7452-79-1	1055	57	*	270[a]	240	310[a]	270	2000[b]	760
9	ethyl 3-methylbutanoate	1	108-64-5	1072	88	*	160[ab]	170	120[a]	110	520[b]	150
13	isoamyl acetate	1	123-92-2	1126	43	---	210	310	7.7	8.5	210	210
15	ethyl pentanoate	1	539-82-2	1139	88	---	300	380	99	89	140	130
18	ethy 2-butenoate	1, 2	10544-63-5	1165	69	---	47	19	67	65	68	6.9
23	ethyl hexanoate	1, 2, 5	123-66-0	1238	99	*	2200[ab]	370	1500[a]	1300	3900[b]	680
32	ethyl heptanoate	2	106-30-9	1334	88	*	68[a]	19	39[a]	35	230[b]	17
33	ethyl lactate	1, 2, 5	97-64-3	1347	45	*	2300[a]	1900	670[a]	580	5600[b]	130
38	ethyl octanoate	1, 2, 5	106-32-1	1440	88	---	1300	840	830	730	2500	280
47	ethyl 3-hydroxybutanoate	1, 5	5405-41-4	1524	43	*	41[a]	22	31[a]	27	420[b]	29
59	ethyl benzoate	2, 5	93-89-0	1675	105	---	330	140	70	610	550	41
60	diethyl succinate[j]	5	123-25-1	1681	101	*	200[a]	120	130[a]	120	580[b]	14
64	ethylphenyl acetate	1, 5	101-97-3	1793	91	*	490[ab]	170	220[a]	200	680[b]	68
68	ethyl 3-phenylpropionate	1, 2, 5	2021-28-5	1892	104	*	310[a]	140	220[a]	190	2500[b]	190
79	ethyl myristate	2, 5	124-06-1	2055	88	---	1800	1600	590	520	1400	260
82	ethyl cinnamate		103-36-6	2139	131	---	78	37	85	75	nd	------
86	ethyl palmitate	2, 5	628-97-7	2256	88	---	42000	27000	13000	11000	37000	6400
90	ethyl oleate	1, 2, 5	111-62-6	2480	310	*	33000[ab]	14000	9300[a]	8000	45000[b]	8500
91	ethyl linoleate	1, 2, 5	544-35-4	2530	308	*	75000[a]	15000	21000[b]	18000	98000[b]	12000
92	ethyl linolenate	1	1191-41-9	2596	261	*	16000[ab]	2100	7100[b]	6200	23000[b]	4500
	miscellaneous compounds (8)											
50	linalool		78-70-6	1551	93	---	150	140	62	53	nd	------
54	unknown		--------	1591	96	*	54[a]	38	31[a]	27	150[b]	11
61	unknown		--------	1687	112	*	41[a]	35	25[a]	21	200[b]	53
63	naphthalene	1, 3, 6ab	91-20-3	1748	128	---	69	33	100	97	22	1
65	(*E*)-anethole		4180-23-8	1835	148	---	1000	910	260	230	nd	------
83	eugenol[j]		97-53-0	2176	164	*	2200[a]	650	780[b]	670	5.1[b]	0.49
88	1*H*-indole	1	120-72-9	2444	117	*	70[a]	36	nd[a]	------	470[b]	39
89	coumarin		91-64-5	2450	118	---	250	260	130	110	nd	------
	other N-containing compounds (9)											
19	pyridine	1	110-86-1	1179	79	---	33	28	26	26	62	4.6
22	2-pentylfuran	1, 6ab	3777-69-3	1236	81	*	82[a]	13	88[a]	82	440[b]	90
34	2,4,6-trimethylpyridine (IS)		108-75-8	1365	121	---	nd	------	nd	------	nd	------
42	2-furancarboxaldehyde	1, 3	98-01-1	1472	96	---	1000	1000	1000	930	1900	1700
45	1-(2-furanyl)ethanone	1	1192-62-7	1511	95	*	110[ab]	76	72[a]	63	430[b]	220
46	1*H*-pyrrole		109-97-7	1524	67	*	41[ab]	23	20[a]	21	87[b]	8.9
51	2-methyl-1*H*-pyrrole[j]	3	636-41-9	1563	80	---	44	14	32	28	14	0.37
72	2-acetylpyrrole		1072-83-9	1978	94	---	370	250	130	110	4300	6800
74	1*H*-pyrrole-2-carboxaldehyde	1	1003-29-8	2032	95	---	32	16	29	25	66	29
	pyrazines (4)											
26	2-methylpyrazine	1, 3, 6ab	109-08-0	1266	94	*	74[a]	18	64[a]	58	380[b]	25
30	2,5-dimethypyrazine	1,2,3,6ab	123-32-0	1322	108	*	90[a]	11	36[a]	43	310[b]	27
31	2,6-dimethypyrazine	1, 2, 6ab	108-50-9	1328	108	*	290[a]	79	72[a]	76	950[b]	140
43	tetramethylpyrazine		1124-11-4	1475	136	---	nd	------	nd	------	580	40
	other O-containing compounds (5)											
53	5-methylfurfural		620-02-0	1580	110	---	190	97	150	130	460	380
55	dihydro-5-methyl-2(3*H*)-furanone	1, 6a	108-29-2	1619	56	*	180[a]	120	8.4[b]	7.5	nd	------
56	dihydro-2(3*H*)-furanone	6ab	96-48-0	1636	42	*	120[a]	47	65[a]	56	880[b]	100
58	2-furanmethanol	1, 5	98-00-0	1666	98	*	1200[ab]	150	550[a]	480	1700[b]	150
76	dihydro-5-pentyl-2(3*H*)-furanone	1	104-61-0	2038	114	---	530	380	260	220	500	78

Table 2. (Continued)

no.[a]	compound[b]	ref[c]	CAS Registry No.[d]	RI[e]	fragment used[f] (*m*/*z*)	sig[g]	sample A concn[h] (μg/kg)	sample A SD[i]	sample B concn[h] (μg/kg)	sample B SD[i]	sample C concn[h] (μg/kg)	sample C SD[i]
	alcohols (24)											
1	ethanol	1, 2, 4, 5, 6ab	64-17-5	1000>	45	- - -	3.7×10^5	1.2×10^5	1.9×10^4	1.8×10^4	3.5×10^6	4.5×10^6
4	2-butanol	1, 2, 6ab	78-92-2	1027	45	*	170[a]	120	61[a]	56	450[b]	62
6	1-propanol	1, 2, 5, 6ab	71-23-8	1041	59	*	4200[a]	4000	590[a]	560	12000[b]	1000
11	2-methyl-1-propanol	1, 2, 5, 6ab	78-83-1	1094	43	*	1000[a]	970	570[a]	520	13000[b]	1600
12	2-pentanol	1, 2, 3, 6a	6032-29-7	1120	45	- - -	82	110	56	59	23	3.6
16	1-butanol	1, 2, 5, 6ab	71-36-3	1144	56	*	210[a]	140	260[a]	240	720[b]	83
17	1-penten-3-ol	1, 4, 6ab	616-25-1	1160	57	*	59[ab]	36	37[a]	36	120[b]	13
21	3-methyl-1-butanol	5, 6ab	123-51-3	1209	55	*	2400[a]	1200	1500[a]	1300	25000[b]	1500
24	1-pentanol	1, 3, 4, 5, 6ab	71-41-0	1250	55	*	280[a]	87	180[a]	170	680[b]	61
29	2-heptanol	1, 6ab	543-49-7	1321	45	- - -	25	8.4	58	50	59	1.2
35	3-ethoxy-1-propanol	1, 5	111-35-3	1377	59	*	15[a]	23	17[a]	14	860[b]	83
37	2-butoxyethanol	1	111-76-2	1404	45	- - -	610	670	nd	- - - - - -	180	130
39	1-octen-3-ol	1, 3, 4, 6ab	3391-86-4	1454	57	- - -	480	97	160	140	480	260
44	2-ethyl-1-hexanol	2, 5	104-76-7	1493	57	- - -	360	430	75	69	nd	- - - - - -
49	2,3-butanediol	1	513-85-9	1543	45	*	nd	- - - - - -	78[a]	67	1400[b]	110
66	2-methoxyphenol	1	90-05-1	1868	109	- - -	110	54	110	96	61	0.95
67	benzenemethanol	1, 2, 5	100-51-6	1882	108	- - -	100	41	64	56	98	10
69	benzeneethanol	1, 2, 3, 5	60-12-8	1917	91	*	1100[a]	600	660[a]	580	11000[b]	1300
73	phenol	1, 2, 5	108-95-2	2015	94	*	1200[a]	330	120[b]	110	13000[c]	550
77	4-ethyl-2-methoxyphenol	1	2785-89-9	2038	137	- - -	310	220	390	340	53	2.8
81	4-methylphenol	1	106-44-5	2091	107	*	33[a]	14	23[a]	20	200[b]	12
84	4-ethylphenol	1	123-07-9	2183	107	- - -	270	170	400	350	160	6
85	2-methoxy-4-vinylphenol[j]	1, 3	7786-61-0	2203	150	- - -	680	270	410	360	550	56
87	3-phenyl-2-propenol[j]		104-54-1	2294	92	- - -	92	68	70	64	nd	- - - - - -
	ketones (9)											
3	2,3-butanedione	3, 6b	431-03-8	1000>	86	- - -	20	17	52	75	100	120
8	2,3-pentanedione	4, 6b	600-14-6	1066	43	- - -	14	12	10	9.3	130	170
14	3-penten-2-one		3102-33-8	1128	69	- - -	560	140	480	450	1100	100
20	2-heptanone	1, 3, 4, 6b	110-43-0	1185	43	*	350[a]	84	150[a]	140	730[b]	150
25	3-octanone	1, 3, 4, 5, 6b	106-68-3	1258	43	- - -	26	24	8.9	9.5	40	20
27	3-hydroxy-2-butanone	1, 5	513-86-0	1288	45	*	230[a]	110	290[a]	300	860[b]	37
28	1-hydroxy-2-propanone		116-09-6	1305	43	*	2900[a]	1900	1800[a]	1700	8300[b]	1200
36	2-nonanone	2, 3, 5	821-55-6	1394	58	- - -	180	77	51	46	230	130
71	3-hydroxy-2-methyl-4-pyrone	5	118-71-8	1969	126	- - -	1800	970	1300	1100	98	22
	S-containing compounds (3)											
41	3-(methylthio)propanal		3268-49-3	1463	48	*	2200[a]	1200	870[a]	750	4800[b]	430
52	ethyl 3-(methylthio)propanoate[j]		13327-56-5	1569	74	*	16[a]	4.6	8.4[a]	7.2	32[b]	1.6
62	3-(methylthio)propanol	1	505-10-2	1721	106	*	59[a]	23	60[a]	52	160[b]	16

[a] Compound number. [b] Compounds in order of their elution sequences. [c] Articles in which the compounds were reported: 1, Chung, 1999; 2, Hwan and Chou, 1999; 3, Ames and Macleod, 1984; 4, Wilkens and Lin, 1970; 5, Liu et al., 1988; 6, del Rosario et al., 1984a, raw soybean, 1984b, heated soybean. [d] Chemical Abstracts Service Registry No., provided by the author. [e] Retention indices calculated according to the method of van den Dool and Kratz (1963). [f] Specific fragment used for calculation of concentration of a specific compound. [g] *, statistically significant difference at $p < 0.05$; - - -, statistically insignificant difference at $p > 0.05$. Values of the amount in the same row with different superscripts are significantly different (Tukey, $p < 0.05$). [h] Mean concentration from three replicates on a dry weight basis. [i] Standard deviation. [j] Tentatively identified compound. [k] nd, not determined. [l] - - - - - -, not calculated.

Pyrrole has a nutty, sweet, ethereal odor; 2-methyl-1*H*-pyrrole has a smoky, woody, herbaceous odor when diluted, whereas 2-acetylpyrrole has a bread, walnut, and licorice-like odor (Fenaroli, 1995; Aldrich 1998). The concentration of 2-furancarboxaldehye is relatively high among most compounds in the same class. Tetramethylpyrazine was identified only in sample C, which has a sweet, musty, chocolate, cocoa, lard, and burnt note (Aldrich, 1998). Other pyrazines including 2-methylpyrazine, 2,5-dimethylpyrazine, and 2,6-dimethylpyrazine were found in the white FSCs.

Among the other oxygen-containing compounds, 5-methylfurfural, dihydro-2(3*H*)-furanone, and 3-hydroxy-2-methyl-4-pyrone were not detected in our previous white FSCs (Chung, 1999). However, Liu et al. (1988) reported the presence of 3-hydroxy-2-methyl-4-pyrone in red FSCs. 5-Methylfurfural has a sweet, warm, caramel, spicy aroma, dihydro-2(3*H*)-furanone has a faint, sweet, caramel flavor, and 3-hydroxy-2-methyl-4-pyrone has a malt, toasted sensation (Fenaroli, 1995; Aldrich 1998).

Chung (1999) reported that 32 alcohols were identified in the white FSCs. In the present studies, 24 alcohols were found in the red FSCs. Except for 3-methyl-1-butanol and 3-phenyl-2-propenol, the rest of the components were previously identified in the white FSCs. The former has fusel oil, whiskey-like odors; the latter has sweet, balsamic, hyacinth-like odors (Fenaroli, 1995; Aldrich, 1998). 2-Ethyl-1-hexanol was reported in the sample of Hwan and Chou (1999). The compound has a mild, oily, sweet, slight rosy aroma (Aldrich, 1998). Although the number of ketones found was nine, more than half of them were found only in the red FSC including 2,3-butanedione, 2,3-pentanedione, 3-penten-2-one, 2-nonanone, and 1-hydroxy-2-propanone. 1-Hydroxy-2-propanone has the highest concentration in the same class of compounds. Three sulfur-containing compounds were identified in the red FSCs, whereas seven were found previously in the white ones. Both 3-(methylthio)propanal and ethyl 3-(methylthio)-propanoate were found solely in the red FSCs. Within this class of the same sample, the former has the highest

Table 3. GC-MS-FID-O Evaluations of Red Fermented Soybean (*G. max*) Curd Samples A−C

no.[a]	compound[b]	RI[c]	sample A descriptor(s)	RT[d]	sample B descriptor(s)	RT[d]	sample C descriptor(s)	RT[d]
2	ethyl 2-methylpropanoate	1000>	fruity	12.80	fruity	12.90	fruity	12.90
3	2,3-butanedione	1000>	diacetyl	13.10	diacetyl	13.10	diactyl	13.20
5	ethyl butanoate	1039	fruity, cantaloupe	14.70	fruity, cantaloupe	14.80	fruity	14.80
7	ethyl 2-methylbutanoate	1055	fruity	15.30	fruity, cantaloupe	15.30	fruity, cantaloupe	15.30
9	ethyl 3-methylbutanoate	1072	fruity	15.70	fruity	15.70		
15	ethyl pentanoate	1139	fruity	17.60	fruity	17.60		
20	2-heptanone	1185	unknown	18.90				
28	1-hydroxy-2-propanone	1305	unknown	22.10			mushroom	22.00
32	ethyl heptanoate	1334					fruity	19.00
41	3-(methylthio)propanal	1463	meaty, salty	25.20	salty, moldy	25.70	salty	25.60
57	benzeneacetaldehyde	1652	rosy	30.00	rosy	29.80	rosy	29.80
68	ethyl 3-phenylpropionate	1892	cool, prune	34.80	prune	34.40	prune	34.60
70	(*E*) and (*Z*)−2-phenyl-2-butenal[e]	1939	prune	35.60			floral	35.50
72	2-acetylpyrrole	1978					floral	36.20
76	dihydro-5-pentyl-2(3*H*)-furanone	2038	prune	38.30	beer-like	38.50		
80	5-methyl-2-phenyl-2-hexenal[e]	2083	prune	38.60				
86	ethyl palmitate	2256	unknown	45.60				
87	3-phenyl-2-propenol[e]	2294	prune	52.00				
90	ethyl oleate	2480	coconut	61.50	coconut, sweet	63.00		
91	ethyl linoleate	2530	sweet	68.00	creamy, mild	68.00		

[a] Compound number. [b] Compounds in order of their elution sequences. [c] Retention indices from Table 1. [d] Typical retention time from four replicated evaluations by two panelists; GC oven ramp rate at 6 °C/min. [e] Tentatively identified.

concentration, but the latter has the lowest concentration. 3-(Methylthio)propanal was reported to have a meaty, soy sauce-like flavor and ethyl 3-(methylthio)-propanoate a pineapple, citrus-like aroma (Chung and Cadwallader, 1994; Fenaroli, 1995).

For GC-MS-FID-O analysis of samples A−C, desirable aromas were detected at the beginning of the evaluation, at retention indices (RI) of <1100 (Table 3). There were five compounds detected in samples A, five in sample B, and four in sample C within this RI range. This was dominated by fruity, diacetyl, cantaloupe-like aromas. Four common components were identified in all three samples including ethyl 2-methylpropanoate, 2,3-butanedione, ethyl butanoate, and ethyl 2-methylbutanoate. Ethyl 3-methylbutanoate was found only in samples A and B, representing the unique characteristic of these samples.

Between RI values of 1100 and 2000, seven components were detected in each of the samples A and C, whereas four components were detected in sample B. Five common components were found in samples A and C including 1-hydroxy-2-propanone, (*E*)- and (*Z*)-2-phenyl-2-butenal, 3-(methythio)propanal, benzeneacetaldehyde, and ethyl 3-phenylpropionate. Only the latter three components were recognized in all three samples. 1-Hydroxy-2-propanone with a mushroom note was recognized in sample C but was detected in sample A by panelists. (*E*)- and (*Z*)-2-phenyl-2-butenal at RI 1939 was described as prune, floral-like aromas. Meaty, salty aromas were noted at RI 1463 for all three samples corresponding to the compound 3-(methythio)propanal. A prominent rosy odor was also detected and was later identified as benzeneacetadehyde at RI 1652. A dry prune-like aroma was contributed by ethyl 3-phenylpropionate at RI 1892. Except for 2-heptanone detected at RI 1185 in sample A, the rest of the components were recognized to carry the fruity/floral aroma. These compounds include ethyl pentanoate in samples A and B and ethyl heptanoate and 2-acetylpyrrole in sample C.

With RI >2000, three common recognizable compounds were found in both samples A and B including dihydro-5-pentyl-2(3*H*)-furanone, ethyl oleate, and ethyl linoleate at RIs 2038, 2480, and 2530, respectively. No detectable aroma was found in sample C. Among all six compounds identified in this RI range, three ethyl esters were high in molecular mass. Both ethyl oleate and ethyl linoleate had coconut and sweet aromas, respectively, in samples A and B. Ethyl palmitate, found only in sample A, did not have a recognizable flavor. Prune-like aroma was recognized in dihydro-5-pentyl-2(3*H*)-furanone, 5-methyl-2-phenyl-2-hexenal, and 3-phenyl-2-propenol.

Each pooled sample was evaluated by two panelists familiar with the samples, and a total of four evaluations were made. When both odor and retention time of an odorous compound agreed in all four evaluations, the results were considered to be positive. These steps made the GC-MS-FID-O evaluation highly conservative and the data more reliable. However, this contributed to the low number of odorous compounds found as shown in Table 3. When the three samples of red FSCs were compared, seven common components were found including ethyl 2-methylpropanoate, 2,3-butanedione, ethyl butanoate, ethyl 2-methylbutanoate, 3-(methythio)-propanal, benzeneacetaldehyde, and ethyl 3-phenylpropionate. Other odorous components found might contribute to the individual unique subtle flavor in each sample. Four of these seven common components belonged to the ethyl esters, suggesting the desirable fruity, prune-like aromas were dominating the background aroma of all the red FSCs. This apparently agreed well with the results of Su (1986) and Ho et al. (1989) on the importance of esters in FSCs. Although the GC-MS analyses showed the alcohols had very high numbers of components, the GC-MS-FID-O analyses showed their contributions to the flavor of red FSC were low.

Table 4 lists the published threshold values of selected odorous compounds from the GC-MS-FID-O analyses as well as their calculated odor activity values (OAVs) (Guadagni et al., 1966). 3-(Methylthio)propanal had the highest OAVs in all samples, followed by ethyl 2-methylbutanoate, benzeneacetaldehyde, and ethyl butanoate. OAVs of ethyl 2-methylpropanoate in both samples A and C were higher than that of 2,3-butanedione, but the order of the two compounds was reversed for sample B. Components with OAVs of <1 included 2-heptanone, 2-acetylpyrrole, ethyl palmitate, ethyl

Table 4. Threshold Values and Calculated OAVs of Selected Odorous Components Found in Red Fermented Soybean (*G. max*) Curd Samples A−C

no.	compound	retention index	threshold value (μg/kg)	calcd OAV[c]		
				sample A	sample B	sample C
2	ethyl 2-methylpropanoate[a]	1000>	3.9	1.1×10^1	6.7	9.9×10^1
3	2,3-butanedione[b]	1000>	5.4	3.7	9.6	1.9×10^1
5	ethyl butanoate[b]	1039	1.5×10^1	2.2×10^2	1.1×10^2	3.2×10^2
7	ethyl 2-methylbutanoate[b]	1055	1.5×10^{-1}	1.8×10^3	2.1×10^3	1.3×10^4
9	ethyl 3-methylbutanoate[a]	1072	2.0×10^{-1}	7.9×10^2	6.0×10^2	2.6×10^3
15	ethyl pentanoate[a]	1139	1.4×10^{-1}	2.1×10^3	7.1×10^2	9.7×10^2
20	2-heptanone[b]	1185	3.0×10^4	1.2×10^{-2}	5.1×10^{-3}	2.4×10^{-2}
32	ethyl heptanoate[a]	1334	1.8×10^{-1}	3.8×10^2	2.2×10^2	1.3×10^3
41	3-(methylthio)propanal[a]	1463	2.0×10^{-3}	1.1×10^6	4.4×10^5	2.4×10^6
57	benzenacetaldehyde[b]	1652	4.0	8.3×10^2	2.9×10^2	1.5×10^3
72	2-acetylpyrrole[b]	1978	2.0×10^5	1.8×10^{-3}	6.6×10^{-4}	2.2×10^{-2}
76	dihydro-5-pentyl-2(3*H*)-furanone[b]	2038	1.0×10^1	5.3×10^1	2.6×10^1	5.0×10^1
86	ethyl palmitate[b]	2256	2.5×10^7	1.7×10^{-3}	5.2×10^{-4}	1.5×10^{-3}
90	ethyl oleate[b]	2480	1.3×10^5	2.5×10^{-1}	7.1×10^{-2}	3.4×10^{-1}
92	ethyl linoleate[b]	2596	3.4×10^6	2.2×10^{-2}	6.2×10^{-3}	2.9×10^{-2}

[a] Reference threshold values from Devos et al. (1990). [b] Reference threshold values from Fazzalari (1978). [c] OAV = concentration of a compound/threshold of the same compound (Guadagni et al., 1966).

oleate, and ethyl linoleate. These compounds contributed weakly to the overall odor of these samples on the basis of their low OAVs.

Comparison of the data between the red FSCs in the GC-MS-FID-O analyses and those of the white FSCs in the GC-MS analyses showed that only ethyl butanoate, ethyl 2-methylbutanoate, and ethyl 3-phenylpropionate were found in both types of FSCs (Chung, 1999). Except for ethyl 3-phenylpropionate, the average concentration of the other two compounds in the red FSCs was higher than that in the white ones. This suggested that the red FSCs might have a much stronger fruity, cantaloupe-like aroma in the samples, whereas the white ones have stronger prune-like aroma. The other four odorous components that were not found in the white FSCs might be critical to the characteristic aroma of the red FSCs.

Among the 89 components identified by GC-MS, 19 of them were reported here in red FSCs for the first time, but not in the white ones (Chung, 1999). Most classes of compounds had one or two new components except miscellaneous compounds and aldehydes, which had four and three compounds, respectively. Eleven of these 19 components were found in all three samples, suggesting the components might have similar origins and contribute to the common background flavor of all red FSC samples. Because the preparation of red FSCs required the addition of *Monascus* koji to the fermenting soybean curds, this step might contribute these additional aromatic components to the final products. However, further investigation into the contribution of *Monascus* koji to the volatile flavor of the red FSCs may be necessary to clarify their contributions.

In conclusion, results from both GC-MS and GC-MS-FID-O analyses on the common components in the steamed red FSC samples have confirmed that esters remained as the dominating class of compounds contributing to the aroma of the samples, whereas alcohols do not. These common esters, including ethyl 2-methylbutanoate, ethyl butanoate, ethyl 2-methylpropanoate, and ethyl 3-phenylpropionate, provided fruity, cantaloupe-like, and prune-like aromas to the final products. Besides, several components including 2,3-butanedione, benzeneacetaldehyde, and 3-(methylthio)propanal were also important to the overall common characteristic flavor of red FSCs.

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