

## Analysis of Volatile Compounds in Bulgogi Prepared by Different Heating Procedures

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**Abstract** To compare volatile compounds in bulgogi cooked by different heating procedures, bulgogi was prepared by convection oven, electric pan and charcoal grill. A total of 61 volatile compounds, consisting of 4 pyrazines, 10 sulfur-containing compounds, 7 carbonyls, 7 alcohols, 7 aliphatic hydrocarbons, 25 terpene hydrocarbons, and 1 miscellaneous compound, were tentatively identified in bulgogi cooked by the three heating methods. Comparatively, the difference in volatile compounds identified in bulgogi using the three different heating methods was not significant, except for sulfur-containing compounds and carbonyls which were detected at higher levels in the bulgogi cooked by convection oven than in that cooked by the other two heating methods. On the other hand, some compounds, such as furfural, benzaldehyde, and (*E,E*)-2,4-decadienal, were detected only in the bulgogi cooked by charcoal.

**Keywords:** bulgogi, volatile compounds, meat flavor, heating methods, high vacuum sublimation

### Introduction

Although uncooked meat has little or no aroma, except for a blood-like taste, meat flavor is thermally formed by complex interactions between non-volatile compounds, such as proteins, carbohydrates, lipids and vitamins, in the lean and fatty tissues of meat during cooking (1). The primary reactions, during the cooking of meat, include decomposition of carbohydrates, pyrolysis of proteins and amino acids, and degradation of lipids. In addition, diverse interactions between two or more precursors, such as Strecker degradation, Maillard reaction, and various protein/lipid/carbohydrate interactions, can also contribute to the characteristic flavor of cooked meat (2). In general, the flavor of cooked meat is due to a mixture of compounds, including non-volatiles or water-soluble compounds with taste-tactile properties, enhancers and synergists, and volatiles (2). The desirable meat flavor, developed during heating, depends on the heating conditions, such as procedure and time, under which the meat is cooked (3). Some carbonyls, sulfides, pyrroles, and pyridines could be more important to roasted odor note compared to boiled or brothy qualities (4). Especially, a number of heterocyclic compounds, including 15 thiazoles, 1 thiazoline, 6 oxazoles, 11 pyrazines, 6 pyrroles, 2 piperidines, and 3 pyridines, were found in roasted beef (5). The formation of these heterocyclics, such as thiazoles, thiazolines, and pyrazines, was known to accelerate at higher temperatures (6).

Bulgogi, a Korean traditional food, is cooked beef with its unique sauce containing a variety of food ingredients, such as soy sauce, sugar, sesame oil, roasted sesame seeds, black pepper powder, pear juice, onion, welsh onion, garlic, and ginger (7). Many studies have been made on

volatile compounds of meat and various food ingredients used for bulgogi sauce (8-14). Our previous study on the volatiles in bulgogi cooked by convection oven identified a diverse class of volatile compounds, comprising 8 pyrazines, 9 sulfur-containing compounds, 11 carbonyls, 9 alcohols, 2 esters, 10 aliphatic hydrocarbons, 25 terpene hydrocarbons, and 1 miscellaneous compound (7). In the present study, volatile compounds in bulgogi prepared by three different heating methods, convection oven, electric pan, and charcoal grill, were isolated using high vacuum sublimation (HVS) and analyzed by gas chromatography-mass spectrometry (GC-MS) to compare the volatile components in bulgogi prepared by different heating methods.

### Materials and Methods

**Preparation of bulgogi** Fresh sliced Korean sirloin (grade A) was purchased from a local market in Seoul, Korea and stored at -20°C in freezer, then maintained at 4°C in a refrigerator for 1 day before use. Bulgogi was cooked according to the procedure of Cho et al. (7). For cooking the bulgogi, three different heating methods were used: convection oven, electric pan, and charcoal grill. A convection oven-broiler (Model 7091, Toastmaster Inc., Boonville, MO, USA) was preheated to 220°C, and the bulgogi sample was roasted at 190±3°C (internal oven temperature) on each side for 4 min. An electric pan (Model EHG-E13, Mahobing Co., Osaka, Japan) was preheated at 170°C for 10 min, and the bulgogi sample was heated until the bulgogi surface temperature reached 70±3°C. A grill (36 cm × 31 cm × 12 cm, Hunghwa Co., Daegu, Korea) was preheated by charcoal for 10 min, and the bulgogi sample was roasted until the bulgogi surface temperature reached 70±3°C.

**Extraction of volatile compounds in bulgogi using high vacuum sublimation (HVS)** The cooked bulgogi was

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Received January 24, 2004; accepted May 23, 2005

placed in a stainless steel container, frozen in liquid nitrogen, and then ground in a blender (HMC-400T, Hanil Electric Co., Seoul, Korea). The ground bulgogi (80 g) was directly extracted with 150 mL of diethyl ether (Fisher Scientific Korea Ltd., Seoul, Korea) re-distilled before use. After 0.5 mL of tridecane (1000 ppm, 0.1 mg/100 mL in diethyl ether) was added as an internal standard compound, the ground sample suspended in diethyl ether was magnetically stirred at 350 rpm for 30 min and then filtered through Whatman No. 41 filter paper (Whatman Ltd., Maidstone, UK) under vacuum. The residue remaining on the filter paper was re-extracted with 30 mL of diethyl ether. All filtrates were combined and evaporated into 50 mL using a Vigreux column (50 cm × 3 cm) in a 40°C water bath. Volatiles of bulgogi were then separated from the non-volatile materials using a high vacuum pumping system (Model VPC-250F, ULVAC KIKO, Inc., Yokohama, Japan) connected with custom-made glassware (Chang Young Scientific, Seoul, Korea) modified from Sen *et al.* (15). The solvent extract (50 mL) was placed in an addition funnel and then added drop by drop into a 1-L round bottom flask when the operating vacuum level reached below  $3 \times 10^{-5}$  torr and the water bath temperature was at 40°C. Each sample droplet was dispersed in a round-bottom flask, and magnetically stirred at around 350 rpm. The distillate was collected in three cold traps immersed in liquid nitrogen. After all the extract was fed into the apparatus, the water bath temperature was increased to 50°C, and extraction continued for 2 hr. The final operating vacuum level was below  $2 \times 10^{-5}$  torr, typically. After the high vacuum sublimation was complete, the cold traps in the apparatus were warmed to room temperature. The resulting extract collected from the three cold traps was dehydrated over anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ), concentrated on a Vigreux column in a 40°C water bath, and then placed under a slow stream of nitrogen to obtain a final volume of 0.1 mL. The bulgogi extract obtained by HVS gave a similar odor note to the original bulgogi flavor. All sample preparations were carried out in triplicate.

**Analysis by GC-MS** GC-MS analysis was performed using an HP 5890A series II gas chromatograph-5972 mass selective detector (Hewlett-Packard Co., Palo Alto, CA, USA). A DB-5ms column (30 m length × 0.25 mm i.d. × 0.25 mm film thickness, J & W Scientific, Folsom, CA, USA) was used to separate the volatile compounds. The carrier gas was helium at a constant flow rate of 0.8 mL/min. One microliter of the extract was injected in the splitless mode. The oven temperature was held at 40°C for 5 min, then raised at 4°C/min to 200°C, and held at 200°C for 20 min. The injector and detector temperatures were 200 and 250°C, respectively. The mass detector was operated in electron impact mode with an ionization energy of 70 eV and a scanning range of 33-550 a.m.u.

#### Identification and quantification of volatile compounds

The tentative identification of the volatile compounds was based on comparison of their mass spectra with those of an on-computer library (Wiley 2751, 1995) (Hewlett-Packard Co., Palo Alto, CA, USA) and by manual interpretation. In addition, the linear retention indices (RI) of the

compounds, determined by using *n*-paraffins  $\text{C}_7\text{-C}_{22}$  as external references (16), were compared with those published in the literature (17-21). The relative amounts of volatile components in the bulgogi cooked by the three different heating methods were obtained by comparing their peak areas to that of the internal standard compound in the GC-MS total ion chromatogram.

#### Results and Discussion

We have previously reported the volatile compounds in bulgogi, using simultaneous steam distillation and solvent extraction (SDE) and solid phase microextraction (SPME) (7). Seventy five compounds were identified in the volatile compounds extracted from bulgogi in our previous study: 8 pyrazines, 9 sulfur-containing compounds, 11 carbonyls, 9 alcohols, 2 esters, 10 aliphatic hydrocarbons, 25 terpene hydrocarbons, and 1 miscellaneous compound (7). Most of these could be formed from thermal degradation of components and/or thermal interactions between them in meat and other ingredients used for bulgogi sauce. Terpene compounds, derived from various ingredients added in bulgogi sauce, were quantitatively major compounds in bulgogi flavor. On the other hand, the amounts of most heterocyclic compounds such as pyrazines, which could be formed *via* Maillard-type reaction, were not significant (7).

In this study, volatile compounds in bulgogi prepared by three different heating methods were isolated using HVS and analyzed by GC-MS for comparison. HVS was employed as an isolation technique, mainly due to its high extraction efficiency and mild extraction condition. The apparatus utilizes the principle of vaporization of volatiles from heated, non-volatile materials at  $40 \pm 20^\circ\text{C}$  under ultra high vacuum. The distillate is collected in a series of traps cryogenically cooled with liquid nitrogen. Since this method is performed under mild temperatures, thermal degradation of volatiles can be avoided during the extraction procedure.

Table 1 lists the volatile compounds identified in the bulgogi cooked by the three different heating methods, their relative peak areas, and RI values on the DB-5ms column. Sixty one volatile compounds were detected in this study: 4 pyrazines, 10 sulfur-containing compounds, 7 carbonyls, 7 alcohols, 7 aliphatic hydrocarbons, 25 terpene hydrocarbons, and 1 miscellaneous compound. In general, more aliphatic sulfur-containing compounds, such as allyl methyl disulfide and dipropyl trisulfide, were found in bulgogi volatiles extracted by HVS in this study compared to those found by SDE and SPME in our previous study. In particular, an extensively heat-labile compound, dipropyl trisulfide, could be detected only in HVS extracts. It was also interesting to note that the amount of diallyl disulfide ( $33.98 \pm 7.75\%$ , peak area of diallyl disulfide/total peak area × 100), possibly derived from garlic during cooking, was tremendous in the HVS extract. In addition, 3-hydroxy-2-butanone and furfural, which had not been found in the volatile isolated by the three SPME fibers, were detected by HVS.

A total of 55, 50, and 47 volatile compounds were found in the bulgogi cooked by convection oven, electric pan, and charcoal grill, respectively. The difference in

Table 1. Volatile compounds in bulgogi cooked by three different heating methods

No.	Possible compounds	RI <sup>1)</sup>	P.A. <sup>2)</sup>			ID <sup>6)</sup>	Ref <sup>7)</sup>
			oven <sup>3)</sup>	pan <sup>4)</sup>	charcoal <sup>5)</sup>		
Pyrazines							
1	2-methylpyrazine	828	0.02±0.01	0.11±0.01	0.04±0.01	MS/RI	3
2	2,5-dimethylpyrazine	922	0.05±0.04	0.08±0.01	0.03±0.01	MS/RI	2
3	2,6-dimethylpyrazine	925	0.09±0.06	0.08±0.01	0.04±0.02	MS/RI	1,2
4	2-ethyl-6-methylpyrazine	1002	0.05±0.03	0.05±0.00	- <sup>8)</sup>	MS	
Sulfur-containing compounds							
5	allyl methyl disulfide	916	0.30±0.21	0.20±0.07	0.09±0.02	MS/RI	1,5
6	diallyl disulfide	1088	7.73±1.14	8.23±4.35	2.30±0.53	MS/RI	4
7	allyl propyl disulfide	1097	0.51±0.08	0.08±0.00	-	MS	
8	dipropyl disulfide	1111	0.63±0.10	0.13±0.77	0.20±0.07	MS/RI	1
9	( <i>E</i> )-1-propenyl propyl disulfide	1121	0.17±0.02	0.30±0.04	0.04±0.00	MS	
10	methyl allyl trisulfide	1135	0.03±0.00	-	-	MS	
11	3-vinyl-4 <i>H</i> -1,2-dithiin	1192	0.25±0.09	0.20±0.13	0.06±0.01	MS	
12	2-vinyl-4 <i>H</i> -1,3-dithiin	1220	0.51±0.17	0.48±0.33	0.11±0.02	MS	
13	diallyl trisulfide	1305	0.33±0.10	-	-	MS/RI	1,4
14	dipropyl trisulfide	1330	0.07±0.02	0.02±0.00	-	MS	
Carbonyls							
15	3-hydroxy-2-butanone	713	0.51±0.19	0.56±0.25	0.42±0.15	MS/RI	1,3
16	furfural	837	-	-	0.16±0.11	MS/RI	1,2
17	benzaldehyde	968	-	-	0.01±0.01	MS/RI	2
18	benzeneacetaldehyde	1052	0.07±0.04	0.16±0.00	0.11±0.05	MS/RI	2
19	neral	1244	0.06±0.02	0.52±0.00	-	MS/RI	2
20	geranial	1272	0.14±0.07	0.47±0.00	0.06±0.04	MS/RI	1,2
21	( <i>E,E</i> )-2,4-decadienal	1327	-	-	0.04±0.01	MS/RI	2,3
Alcohols							
22	furfuryl alcohol	871	0.23±0.07	0.56±0.16	0.50±0.16	MS/RI	1,2
23	2-methoxy phenol (guaiacol)	1093	0.05±0.02	0.09±0.00	0.11±0.00	MS/RI	2,3
24	linalool	1105	0.11±0.04	0.13±0.00	0.04±0.00	MS/RI	2,3
25	borneol	1178	0.03±0.01	0.09±0.03	-	MS/RI	2
26	4-terpinenol	1184	0.05±0.02	0.05±0.00	-	MS/RI	1,2
27	4-vinyl-2-methoxy phenol	1323	-	0.10±0.10	-	MS	
28	spathulenol	1632	0.01±0.00	0.03±0.01	-	MS/RI	1
Terpene Hydrocarbons							
29	$\alpha$ -pinene	931	0.31±0.07	0.54±0.15	0.20±0.10	MS/RI	2,3
30	camphene	948	0.33±0.06	0.93±0.37	0.15±0.05	MS/RI	1,2
31	sabinene	972	0.13±0.03	0.18±0.06	0.11±0.07	MS/RI	1,2
32	$\beta$ -pinene	975	0.15±0.02	0.31±0.15	0.09±0.03	MS/RI	1,2
33	myrcene	994	0.07±0.01	0.07±0.00	-	MS/RI	1,2
34	$\delta$ -3-carene	1010	0.37±0.14	0.48±0.10	0.24±0.00	MS/RI	1
35	limonene	1030	-	-	0.18±0.01	MS/RI	2,3
36	$\beta$ -phellandrene	1032	1.33±0.16	2.59±1.35	0.17±0.13	MS/RI	1,2
37	1,8-cineole	1035	-	0.37±0.13	0.06±0.02	MS/RI	2
38	$\delta$ -elemene	1337	0.24±0.07	0.52±0.00	0.20±0.00	MS/RI	1,2
39	$\alpha$ -cubebene	1348	0.06±0.02	0.24±0.14	0.04±0.02	MS/RI	1,2
40	$\alpha$ -copaene	1379	0.76±0.21	2.48±0.80	0.48±0.22	MS/RI	2
41	$\beta$ -elemene	1391	0.17±0.07	0.52±0.18	0.06±0.01	MS/RI	2
42	$\beta$ -caryophyllene	1430	5.70±1.69	12.82±10.31	3.85±1.72	MS/RI	1
43	( <i>E</i> )-caryophyllene	1433	0.03±0.02	0.10±0.01	0.02±0.01	MS/RI	2
44	$\alpha$ -guaiene	1437	0.05±0.02	0.16±0.05	0.03±0.02	MS/RI	2
45	( <i>E</i> )- $\beta$ -farnesene	1451	0.02±0.00	-	-	MS/RI	1,2
46	$\alpha$ -humulene	1459	0.22±0.07	1.62±0.05	0.13±0.05	MS/RI	2
47	ar-curcumene	1482	0.07±0.03	0.27±0.13	0.03±0.01	MS/RI	2

Table 1. (continue)

No.	Possible compounds	RI <sup>1)</sup>	P.A. <sup>2)</sup>			ID <sup>6)</sup>	Ref <sup>7)</sup>
			oven <sup>3)</sup>	pan <sup>4)</sup>	charcoal <sup>5)</sup>		
48	$\beta$ -selinene	1491	0.16±0.05	0.52±0.17	0.12±0.04	MS/RI	2
49	zingiberene	1496	0.37±0.15	1.67±1.19	0.09±0.05	MS	
50	( <i>E,E</i> )- $\alpha$ -farnesene	1503	0.06±0.03	0.14±0.01	0.01±0.00	MS/RI	2
51	$\beta$ -bisabolene	1508	0.06±0.02	0.21±0.11	0.02±0.01	MS/RI	2
52	$\delta$ -cadiene	1519	0.08±0.03	0.25±0.05	0.04±0.01	MS/RI	2
53	$\beta$ -sesquiphellandrene	1524	0.09±0.03	0.35±0.22	0.03±0.01	MS/RI	2
Aliphatic Hydrocarbons							
54	decane	1000	0.09±0.08	-	0.01±0.00	MS/RI	3
55	1-dodecene	1190	0.21±0.00	-	-	MS/RI	2
56	dodecane	1200	0.03±0.01	0.15±0.07	0.08±0.04	MS/RI	2,3
57	1-tetradecene	1395	0.04±0.00	-	0.04±0.01	MS/RI	2
58	tetradecane	1400	0.03±0.02	-	0.03±0.01	MS/RI	2,3
59	hexadecane	1600	0.01±0.00	0.03±0.01	-	MS/RI	2,3
60	heptadecane	1700	0.01±0.00	0.03±0.00	0.01±0.00	MS/RI	2
Miscellaneous							
61	caryophyllene oxide	1585	0.04±0.02	0.10±0.03	0.02±0.00	MS/RI	2

<sup>1)</sup>Retention indices were determined using *n*-paraffins C<sub>7</sub>-C<sub>22</sub> as external references.

<sup>2)</sup>Average of relative ratio of peak area to that of the internal standard compound in GC-MS total ion chromatogram (*n*=3) ± standard deviation.

<sup>3)</sup>Convection oven.

<sup>4)</sup>Electric pan.

<sup>5)</sup>Charcoal grill.

<sup>6)</sup>Tentative identification was performed as follows: MS/RI, mass spectrum was identical with that of Wiley mass spectral database (1995) (Hewlett-Packard Co., Palo Alto, CA, USA), and retention index was consistent with that of the literature [1, Kondjoyan and Berdague (1996); 2, Adams (2002); 3, Acree and Arn (1997); 4, Kubec et al. (1997); 5, Ansorena et al. (2001)]; MS, mass spectrum was consistent with that of Wiley mass spectrum database.

<sup>7)</sup>References.

<sup>8)</sup>not detected.

volatile compounds using the three heating methods was not significant, except for sulfur-containing compounds and carbonyls, when analyzed by GC-MS. The amounts of sulfur-containing compounds detected (0.03±0.00-7.73±1.14%) were larger in the bulgogi cooked by convection oven than by the other two heating methods. Especially, the formation of sulfur-containing compounds was lowest in the bulgogi cooked by charcoal. Some sulfur-containing compounds, allyl propyl disulfide, methyl allyl trisulfide, diallyl trisulfide, and dipropyl trisulfide, were not detected in the bulgogi prepared by charcoal grill. These compounds might be easily degraded by direct heating treatment, such as charcoal. However, more diverse kinds of carbonyls were found in the bulgogi cooked by charcoal grill. Some compounds, such as furfural, benzaldehyde, and (*E,E*)-2,4-decadienal, were detected only in the bulgogi cooked by charcoal grill. The formation of these compounds could be enhanced by high heating method, such as charcoal. On the other hand, 2-ethyl-6-methyl pyrazine, allyl propyl disulfide, neral, borneol, 4-terpinenol, 4-vinyl-2-methoxy phenol, and myrcene were found in the bulgogi cooked by convection oven and electric pan, but not in the bulgogi cooked by charcoal. Four pyrazines, 2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, and 2-ethyl-6-methylpyrazine, were found in the bulgogi cooked by all three heating procedures. Among these pyrazines, 2-ethyl-6-methylpyrazine, which was not detected in the bulgogi cooked by charcoal, was regarded as more important in bulgogi volatiles, because ethyl-substituted pyrazines, in general, have much lower threshold values than methyl-substituted pyrazines (1).

Methyl- and ethyl-substituted pyrazines identified in bulgogi could have been formed from thermal interactions between components from diverse sources which are added during bulgogi preparation, as well as from the meat itself.

There was not a considerable difference in volatile compounds of bulgogi cooked using the different heating methods, except for some sulfur-containing compounds and carbonyls, when analyzed by GC-MS. More sulfur-containing compounds, such as allyl propyl disulfide, methyl allyl trisulfide, and diallyl trisulfide, were found in the bulgogi cooked by convection oven, whereas some carbonyls, such as furfural, benzaldehyde, and (*E,E*)-2,4-decadienal, were detected only in the bulgogi grilled by charcoal. Some other compounds, present at low levels and not analyzed by GC-MS in this study, may also be important to the bulgogi flavor if they had low threshold values and characteristic odor notes. Further studies investigating these trace components related to bulgogi flavor and correlating instrumental data with sensory evaluation are required.

### Acknowledgments

This work was supported by the Agricultural Research Promotion Program under the Ministry of Agricultural and Forestry, Korea.

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