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Evaluation of *Sigumjang* Aroma by Stepwise Multiple Regression Analysis of Gas Chromatographic Profiles

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Abstract A linear correlation, by the stepwise multiple regression analysis, was found between the sensory test of *Sigumjang* aroma and the gas chromatographic data which were transformed with logarithm. GC data is the most objective method to evaluate *Sigumjang* aroma. A multiple correlation coefficient and a determination coefficient of more than 0.9 were obtained at the 9th and 13th steps, respectively. At step 31, the coefficient of determination level of 0.95 was attained. The accuracy of its estimation became higher as the number of the variables entered into the regression model increased. Over 90% of the *Sigumjang* aroma was explained by 13 compounds identified on GC. The contributing proportion of the peak 26 was the highest followed by peaks 57 (9.27%), 29 (7.51%), 54 (6.01%), 8 (5.99%), 49 (4.97%), and 13 (4.11%).

Key words: *Sigumjang*, stepwise multiple regression analysis, contributing proportion, aroma

The nutritional benefit of dietary fiber from different sources has been the subject for numerous studies [2, 3, 5]. Many conventional and non-conventional sources of fiber are available as commercial ingredients. Wheat, corn, and oats are among the common cereals of dietary fiber available commercially. Until now, barley bran has not been used extensively because comparatively small quantities of barley are milled and pearled to provide by-product bran.

Lupton and Robinson [11] investigated the accelerating effect of barley bran on the gastrointestinal transit time, and Lupton *et al.* [12] and Newman *et al.* [13] reported cholesterol-lowering properties of barley bran. In addition, Chaudhary and Weber [6] reported that barley bran flour outperformed other fiber ingredients in bread with a substantially higher content of dietary fiber, the highest loaf volume, and the highest quality score of the fiber-enriched bread in the study in which significant differences in both composition and quality of bread were observed when 15% of wheat flour was replaced by individual fiber ingredient.

Sigumjang made with barley bran flour is a traditional Korean food which is consumed as a dish mainly served with rice during winter. It is common knowledge that the fermentation speed of *Sigumjang* is the fastest among the fermented food, and it accelerates gastrointestinal transit time [9], and controls diverticulosis [7] and colonic cancer as well [7].

Chung *et al.* [9] reported the preparation method and characteristics of commercial *Sigumjang meju*. Choi *et al.* [7] analyzed components which participated in the taste of *Sigumjang* using a stepwise multiple regression model. Choi *et al.* [8] reported flavor components of commercial *Sigumjang mejus* and barley bran which were merchandised in the Kyeongsangbuk-do area. Unfortunately, the investigation on the aroma compound of *Sigumjang* was not reported.

As a part of the method to standardize *Sigumjang* properties, we found the compounds which contributed to the aroma of *Sigumjang*, using the stepwise multiple regression analysis.

MATERIALS AND METHODS

Materials and Sensory Test

Forty-two different brands of commercially available fermented *Sigumjang* filled in 1-l glass bottles were used. Each *Sigumjang* was evaluated and numbered according to the preference order by 16 well-trained members of the

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sensory panel at Yeungnam University. The total number of each sample of the order was used as the sensory score in this study.

Extraction and Identification of Aroma Compounds

An improved Nikerson and Nikens' simultaneous steam distillation and extraction apparatus was used to extract the aroma compounds of *Sigumjang* [14], using purified diethylether for extraction solvent. The process of extraction was as follows: First, place the sample and solvent in the sample and the solvent ports, respectively, and then extract the aroma compounds for more than two hours by increasing the temperature of the sample port to a boiling point after circulating the solvent preliminarily. Anhydrous Na_2SO_4 was added to the extracted fraction at 4°C to remove moisture. Then, the fraction was concentrated to a final 100 ml by using N_2 gas to obtain samples for GC analysis.

The mass spectrum of each of the aroma compounds was obtained by GC and GC-MS. The conditions of GC and GC-MS were as follows: Instrument, GC-Hewlett-Packard 5892, Mass-KRATOS Inc. CENCEPT SERIES-I (England); column, HP-FFAP 60 m×0.33 μm×0.2 mm; injector temperature, 230°C; detector temperature, 250°C; temperature program, 45°C for 2 min, 45-220°C (15°C/min) and then 220°C for 11.4 min; carrier gas, He (5 ml/min); electron voltage, 1,100 eV; split ratio, 10:1.

Stepwise Regression Analysis

Multiple regression analysis was carried out by the method of Aishima and Nobuhara [1]. The relation between the

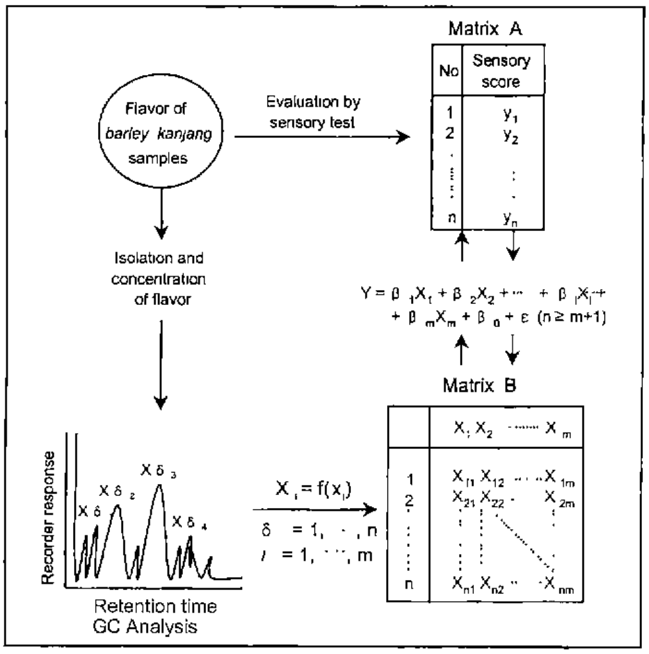

Fig. 1. Scheme for the relation between sensory test and aroma components of the traditional *Sigumjang*.

sensory evaluation score and the content of aroma components is shown in Fig. 1. The number of sensory scores corresponding to each of the *Sigumjang* samples could be described as $Y=(y_1, y_2 \cdots\cdots y_i \cdots\cdots, y_n)$, $1 \leq i \leq n$, as shown in matrix A. If the gas chromatogram of each flavor concentrate shows m peaks, each gas chromatogram could be described as $(x_{i1}, x_{i2}, \cdots\cdots, x_{ij}, \cdots\cdots x_{im})$, and consequently the gas chromatograms for the entire samples could be shown as the matrix shown in B, where i=1 to 42, j=1 to 31 in this study.

The multiple regression model is generally shown as follows:

$$Y=\alpha_1X_1+\alpha_2X_2+\cdots\cdots+\alpha_jX_j+\cdots\cdots+\alpha_mX_m+\beta+\varepsilon \cdots\cdots \quad (1)$$

α_j=regression coefficient
β=intercept
ε=random error
Y=dependent variable
X_j=independent variable

where α_j and β are computed by the linear least squares methods, and the equation is solvable for n>m. The correlation coefficient between the real Y and an estimated Y obtained from the computed multiple regression model was designated as multiple correlation (R). R^2, which represents the ratio of variance of Y explained by the regression model, is called the coefficient of multiple determination.

When the multiple regression model for quality tests is used, the smaller numbers of independent variables should be desirable for making an easier estimation of Y. Recently, many different types of multivariate analysis methods have been developed, but stepwise regression analysis (SRA) has been considered to be the most adequate method for selecting a subset of variables. According to Draper and Smith [10], among several different algorithms have been developed for selecting the variables in SRA, but the increasing and decreasing method is regarded as the most appropriate way, by taking into consideration into the calculation of the efficiency and the accuracy of the analysis. Therefore, the relationships between the GC patterns and the sensory scores were analyzed by the increasing and decreasing SRA method in this study. The SRA is performed by trial and error peak selection for obtaining the most suitable subset of the variables on the basis of the F-value from the analysis of variance on each independent variable at each step, since the degree of significance of an

Table 1. Transformation of independent variables.

	Absolute values	Relative values
1	X_i	$X'_i=X_i/\Sigma X_i\times100$
2	$\ln(X_i+1.0)$	$\ln(X'_i+1.0)$
3	$\sqrt{(X_i+10^{-10})}$	$\sqrt{(X'_i+10^{-10})}$

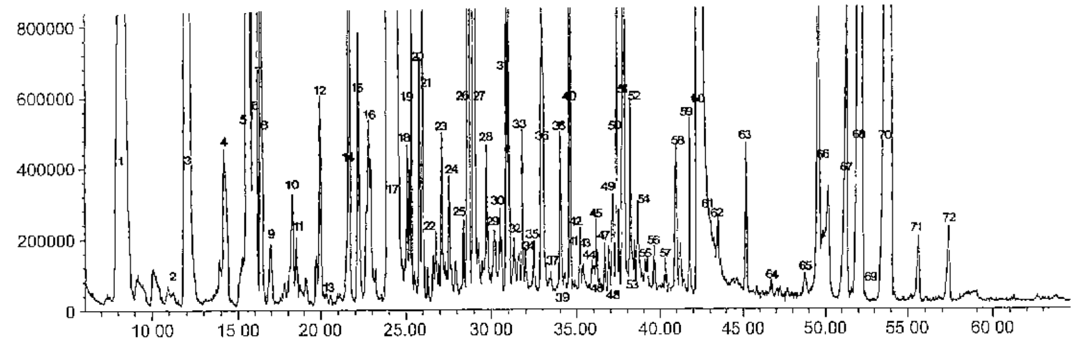

Fig. 2. Gas chromatogram of *Sigumjang*.
1, 3-methylbutanal; 5, limonene, 6, unknown 1; 7, 2-pentylfuran, 8, β-phallendrenal: 9, sabinene, 10, methylpyrazine; 11, 2-octanone: 13, 2,3-dimethylpyrazine, 14, dimethyltrisulfide; 17, tetramethylpyrazine; 23, 5-methyl-2-furancarboxaldehyde; 25, pentanoic acid· 26, 2-furancarboxaldehyde: 29, 1,2-dimethoxybenzene: 32, tetramethylnaphthalene; 37, 2-methoxyphenol, 39, benzylalcohol: 41, unknown 2; 42, phenylethylalcohol, 45, 2-methoxy-4-methylphenol, 48, phenol: 49, hexadecanal; 54 octanoic acid; 55, dihydro-5-pentyl-2(3H)-furanone, 57, nonanoic acid; 58, 4-ethylphenol: 59, hexadecanoic acid; 67, 10,13-octadecanoic acid, methyl ester; 70, linoleic acid, ethyl ester, 72, unknown 3

entered variable changes by entry of another variable. Therefore, an entry of a variable which is not yet included on the regression, and the removal of variables which are already included in the regression, are determined from the settled conditions of the F-value on each variable.

The settled conditions are as follows. The variable having the highest F-value among the variables which are not yet included in the regression is selected for entry. In a case where the F-value of the variables included already in the regression are under 0.005, the variables are removed from the regression model. If the F-value of all the variables not included are under 0.1, the SRA will be discontinued at that step. The maximum number of the step is settled at 70. The SRA was carried out using the SPSS 7.5 program. In this report, GC data was analyzed after transformation, as shown in Table 1.

Table 2. Correlation coefficients (r) between sensory scores and the content of taste components.

Peak No.	r	Peak No.	r	Peak No.	r
1	0.431**	23	-0.521**	49	0.189
5	0.226	25	-0.366**	54	-0.232
6	0.214	26	-0.311*	55	-0.277
7	-0.294	29	-0.343*	57	-0.428**
8	0.449**	32	0.178	58	-0.393**
9	0.186	37	-0.186	59	-0.331*
10	0.349*	39	-0.434**	67	0.193
11	0.395**	41	0.434**	70	0.260
13	-0.491**	42	-0.225	72	0.165
14	0.173	45	-0.200		
17	0.280	48	-0.267		

*: p<0.05, **: p<0.01.
1, 3-methylbutanal; 5, limonene, 6, unknown 1: 7, 2-pentylfuran, 8, β-phallendrenal· 9, sabinene; 10, methylpyrazine; 11, 2-octanone, 13, 2,3-dimethylpyrazine; 14, dimethyltrisulfide; 17, tetramethylpyrazine; 23, 5-methyl-2-furancarboxaldehyde: 25, pentanoic acid; 26, 2-furancarboxaldehyde; 29, 1,2-dimethoxybenzene, 32, tetramethylnaphthalene; 37, 2-methoxyphenol, 39, benzylalcohol: 41, unknown 2; 42, phenylethylalcohol: 45, 2-methoxy-4-methylphenol; 48, phenol, 49, hexadecanal; 54, octanoic acid; 55, dihydro-5-pentyl-2(3H)-furanone; 57, nonanoic acid: 58, 4-ethylphenol: 59, hexadecanoic acid, 67, 10,13-octadecanoic acid, methyl ester; 70, linoleic acid, ethyl ester: 72, unknown 3

Contributing Proportion

Contributing proportion (P_i), the relative importance of each PC for aroma quality, is calculated by Barylko-Pikielena's method [4], shown as follows:

$$P_i = \frac{|S_i r_{iy} \beta_i|}{\Sigma |S_i r_{iy} \beta_i|} \times 100R^2 \qquad (2)$$

where S_i and r_{iy} represent the standard deviation of each PC and the correlation coefficient between each variable and sensory score. This analysis was performed by using a SPSS 7.5 program.

Results and Discussion

Sensory Test and GC Analysis

The sensory evaluation was made by 16 well-trained members of the sensory panel of Yeungnam University.

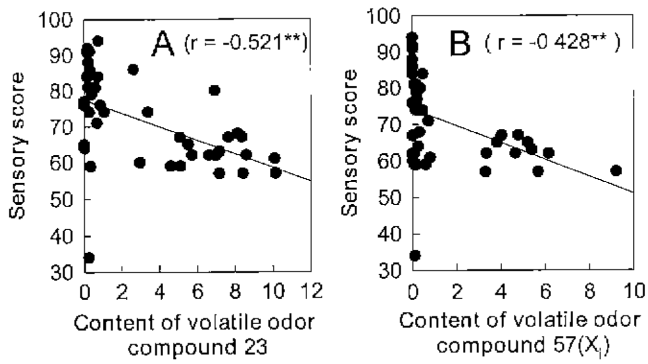

Fig. 3. Sensory scores vs. content of peak numbers 23 (5-methyl-2-furancarboxaldehyde) and 57 (nonanoic acid).
Peak 23 and peak 57 were entered into the regression models at the first step and the seventh step, respectively. **: p<0.01.

Table 3. Selection order and correlation matrix of variables entered into the regression model at step 10.

Selection order	1	2	3	4	5	6	7	8	9	10
Peak No.	23	13	8	41	39	1	57	11	58	25
23	1.000	-0.083	-0.244	-0.289	0.471	-0.261	0.573**	-0.313*	0.452	-0.149
13		1.000	-0.053	-0.075	-0.052	-0.018	-0.077	-0.086	-0.097	0.795**
8			1.000	0.570	-0.167	0.416**	-0.203	0.602**	-0.178	-0.077
41				1.000	-0.206	0.236	-0.205	0.277	-0.183	-0.079
39					1.000	-0.236	0.506**	-0.022	0.540**	0.131
1						1.000	-0.256	-0.024	-0.240	-0.035
57							1.000	-0.199	0.913**	-0.141
11								1.000	-0.180	-0.078
58									1.000	-0.134
25										1.000

and the result showed that the most favorable and the most unfavorable samples were 94 and 34, respectively. Four samples received more than 90, 8 samples 80–89, 8 samples 70–79, 15 samples 60–69, and 7 samples less than 70. The mean value of these samples was 70.9 and the standard deviation was 12.7.

Most of all *Sigumjang* samples consisted of about 75 peaks, but the ratio among the peaks was highly variable. Among these peaks, 31 peaks in which the correlation coefficient was more than 0.1 were selected as variable, as shown in Fig. 2.

Correlation Between Sensory Scores and the Content of Aroma Compounds

The correlation coefficients between the sensory score of each sample and the quantity of each peak are shown in Table 2. The negative correlation coefficients in each peak means that its peak gives reverse contribution as the quantity, and for the positive correlation coefficients its peak gives the preferable contribution. There are many peaks such as peaks 1, 8, 10, 11, 13, 23, 25, 26, 29, 39, 41, 57, 58, 59, and 70 which show a significant relationship with the sensory score. The correlation coefficient of peak 23 shows a value higher than 0.52.

In Fig. 3, the quantities of peaks 23 and 57 were plotted against the sensory scores, however, it is difficult to estimate the aroma quality of *Sigumjang* samples on the basis of only one peak, in spite of the highly significant relationship between the quantity of the peak and the sensory score.

Multiple Regression Analysis

Each of the 31 aroma compound samples were analyzed by a multiple regression model, and some of the peaks which are not significant were removed. Only 15 peaks were selected for variables.

The correlation matrix for the variables in the regression model at step 10 is shown in Table 3. Two important conditions are presented for the purpose in this report. First, there exist several groups of peaks which are similar to each other, therefore, each of these groups should be represented by one typical peak. Secondly, there exist several peaks with practically zero values of the contributing proportion, therefore, they should be removed from the regression analysis. Thus, the computing of the multiple regression model was carried out by using a portion of 15 independent variables, with the expectation that this would not lower the accuracy of the estimation.

Table 4. Multiple correlation coefficient (R) and coefficient of multiple determination of multiple regression models computed from the values which are transformed with variables.

	Absolute values			Relative values		
	X_i	$\ln(X_i+1.0)$	$\sqrt{(X_i+10^{-10})}$	X'_i	$\ln(X'_i+1.0)$	$\sqrt{(X'_i+10^{-10})}$
R[a]	0.975	0.970	0.961	0.977	0.964	0.965
$R^2 \times 100$[b]	95.1	94.1	92.4	95.5	92.9	93.2
S.E.[c]	5.66	6.23	7.04	5.17	6.81	6.68
F	6.293	5.130	3.942	7.805	4.235	4.416
P<	0.002	0.005	0.013	0.000	0.010	0.009
Accuracy order	2	3	6	1	5	4

[a]Multiple correlation coefficient

[b]Coefficient of multiple determination.

[c]Standard error.

A) Step 9

$$Y = -4.124X_{13} - 1.066X_{57} - 0.474X_{23} + 3.990X_{8} - 0.219X_{59} - 0.689X_{54} + 2.450X_{67}$$

(−5.719**) (−4.216**) (−5.330**) (1.012−) (−2.338*) (−2.546*) (2.909**)

$$- 0.581X_{26} + 0.399X_{5} + 83.176$$

(−2.195*) (2.062*) (39.354**)

$R = 0.910$, $R^2 = 0.829$, $F = 17.228^{**}$, S.E. = 5.92

B) Step 15.

$$Y = -3.444X_{13} - 0.556X_{57} - 0.357X_{23} - 3.257X_{8} - 0.125X_{59} - 0.720X_{54} + 3.852X_{67}$$

(−4.670**) (−1.715−) (−4.000**) (−0.781−) (−1.652−) (−3.119**) (4.679**)

$$- 1.455X_{26} + 0.378X_{5} + 0.145X_{17} + 0.593X_{6} - 0.154X_{58} - 3.532X_{29} - 1.432X_{37}$$

(−3.533**) (2.137*) (3.618**) (2.634*) (−1.915−) (−1.603−) (−1.757−)

$$+ 0.452X_{7} + 79.996$$

(1.286−) (30.595**)

$R = 0.959$, $R^2 = 0.919$, $F = 19.685^{**}$, S.E. = 4.52

Fig. 4. Multiple regression models computed at steps 9 and 15 from the relative value.
*p<0.05. **p<0.01 The numbers in parentheses show the t-value for each entered variable

Effect of Transformation

In order to compare the fitness on a linear equation model, the multiple correlation, the coefficient of the multiple determination, standard error, and F-value of GC data were all transformed into six different forms, and are shown in Table 4. The most precise fit was calculated from a relative value of each peak, and the multiple determination coefficient showed 95.5% of variation in the sensory score that could be explained on the basis of GC data. Therefore, it might be appropriate to state that the relative value was effective in linearizing the GC data of the sensory score.

Stepwise Multiple Regression Analysis

Two regression models computed at the 9th and 15th steps from a relative value are shown in Fig. 4. According to the Figure, the significance of the regression coefficients is examined on the basis of the F-value, and the regression coefficient is significant for every variable at step 9 (A). At step 15 (B), it is significant for 8 variables such as 13, 23, 54, 67, 26, 5, 17, and 6.

The F-value of these two multiple regression models was significant at the 1% level. In spite of the increase in variables, the F-value was 19.685 at step 15, which was higher than 17.728 at step 9. The standard error of the estimate was 4.52 at step 15, which was smaller than 5.92 at step 9. The standard error of estimation and multiple regression coefficient were to show the rise of detailed estimation.

The multiple correlation (R), the coefficient of the multiple determination (R^2) and the increase in absolute value R, and relative values at each step are shown in Fig. 5. In the case of absolute value, the R-value increases with the increase of the step number, and it exceeds 0.9 by entry of peak 5 at step 9. The coefficient of determination was 0.9 at step 13, indicating that 90% of the variance of the sensory score can be explained by 13 peaks. The R-value of 0.975 at step 31 and the coefficient of determination of 0.951 indicated that over 95% of the variance of the sensory score can be explained by the regression model. The results derived from the relative values indicated a similar tendency.

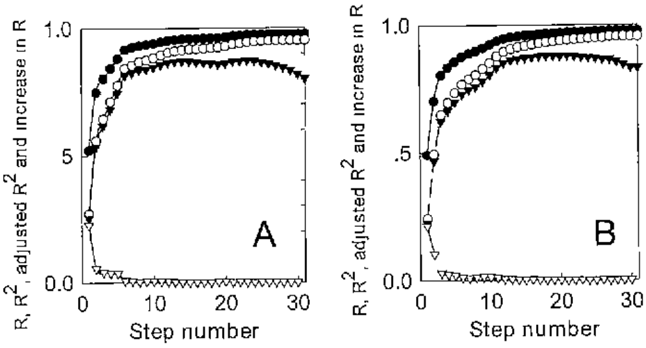

Fig. 5. Multiple correlation coefficient (R), coefficient of multiple determination (R^2), adjusted R^2, and increase in R at each step.
A: Absolute value (X_i). B: Relative value transformed with logarithm ($\ln(X'_i+1.0)$); -●- multiple correlation coefficient (R); -○- coefficient of multiple determination (R^2); -▽- adjusted R^2; -△- increase in R.

Standard Error of Estimates

The change in the standard error of estimates on each step is shown in Fig. 6. The standard error of the estimates is defined as the standard deviation of residuals (e_u) expressed in the equation (3).

$$e_u = Y_{estimated} - Y_{observed} \quad (3)$$

Accuracy of estimation becomes higher with an increase in the number of the variables entered into the regression model. Consequently, it is assumed that the increase in the number of efficient peaks in the regression models results in the decrease in the standard error of the estimate with accompanying great increase in R. On the contrary, the standard error of the estimate increases despite the increase in R when inefficient peaks enter. Therefore, the selection

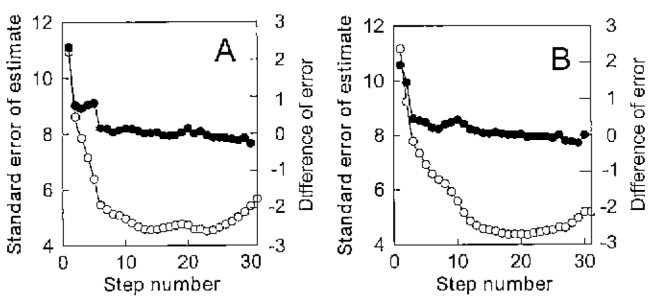

Fig. 6. Changes in standard error of estimate computed for each step.
A absolute value (X_i), B relative value transformed with logarithm ($\ln(X'_i+1.0)$). -●- standard error of estimate, -○- difference of error.

Table 5. Contributing proportion (P_i%) of each peak calculated from relative value.

Peak No.	P_i(%)	Peak No.	P_i(%)	Peak No.	P_i(%)
1	0.82	23	2.86	49	4.97
5	1.87	25	4.07	54	6.01
6	1.23	26	9.95	55	2.01
7	3.37	29	7.51	57	9.27
8	5.99	32	0.04	58	3.81
9	1.86	37	1.89	59	0.21
10	2.58	39	0.24	67	2.87
11	0.38	41	2.27	70	3.54
13	4.11	42	1.91	72	1.87
14	0.65	45	0.02		
17	1.20	48	2.65		

of adequate peaks as independent variables is very important for the accuracy of the estimation.

Every information for statistical analysis should be utilized in order to search for the most appropriate aroma compound, because it is the most important subject in the analysis of aroma compounds of fermented food. The accuracy of the stepwise multiple regression model was affected by the content of aroma compounds, the method of analysis, and the accuracy of sensory evaluation, etc. In particular, inaccuracy of the olfactory sensation is a strong limitating factor in obtaining accuracy of the regression analysis, because olfactory sensation is extremely complicated and the perception mechanism of the aroma may not be perfect. In this report, however, over 90% of the *Sigumjang* aroma was detected in 13 compounds identified on GC.

Contributing Proportion

In contributing proportion of each peak on GC to aroma of *Sigumjang* is shown in Table 5. It was found that the contributing proportion of peak 26 was the highest among the peaks, followed by 57 (9.27%), 29 (7.51%), 54 (6.01%), 8 (5.99%), 49 (4.97%), and 13 (4.11%). On the other hand, the peaks 1, 11, 14, 32, 39, 45, and 59 did not contribute to the aroma of *Sigumjang*.

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