

Evaluation of Barley Bran Sauce Aroma by Multiple Regression Analysis

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Abstract The relationship between the gas chromatographic (GC) patterns of sauce made of barley bran and ranked order in sensory analysis was investigated by multiple regression analysis (MRA). Most of the 42 barley bran sauce samples comprised about 34 peaks, in which the content of 9,12-octadecanoic acid methyl ester was the highest, followed by those of 2-furanmethanol and 2-furancarboxaldehyde. It is difficult to estimate the aroma quality of barley bran sauce samples on the basis of only one peak. The 34 aroma compounds of the 42 samples were analyzed by an MRA model featuring six transformations. The most precise fit was calculated from the absolute value transformed with the root square of each peak, and the multiple determination coefficient showed that 91.6% of the variation in the sensory score could be explained on the basis of GC data.

Keywords: barley sauce, multiple regression analysis, aroma

Introduction

The flavor of processed foods is very complicated because it is derived from the integrated effects of many aromatic compounds which are produced by chemical and enzymatic reactions or bacterial effects. Despite great efforts, no positive relations have been observed between the organoleptic qualities of the flavor and the quantity of each peak on the gas chromatograms for most processed foods. Several attempts using multivariate analysis have been made to evaluate the flavor quality of foods on the basis of whole gas chromatograms (1-4).

Research has investigated barley bran, a useful source of dietary fiber. Lupton and Robinson (5) investigated the accelerating effect of barley bran on gastrointestinal transit time. Lupton *et al.* (6) and Newman *et al.* (7) reported on the cholesterol lowering properties of barley bran. Choi *et al.* (8) reported on the compounds which influence the taste of sigumjang made with barley bran.

As a research of sauce processing with barley bran, Kwon et al. (9) reported on the optimum conditions for the taste of sauce fermented with barley bran and Lee et al. (10) reported on the characteristics of sauce made with barley bran. Choi and Park (11) predicted that 93% of barley bran sauce taste was explained using multiple regression analysis (MRA) with taste compounds and sensory evaluation scores. However, there has been no report on the effective components to the aroma of barley bran sauce.

This report investigates the aroma compounds which form the aroma of barley bran sauce as a part of the standardization and optimization of barley bran sauce quality.

Materials and Methods

Material and sensory test Forty-two different barley

*Corresponding author: Tel: 82-53-819-8201; Fax: 82-53-819-8156 E-mail: cuk8272@hanmail.net Received July 9, 2005; accepted September 14, 2005 bran sauces made by the method of Kwon *et al.* (11) were used. Each barley bran sauce was evaluated and numbered on a 7-point scale according to preference order by 12 well-trained members of the sensory panel at Yeungnam University. The total number of each sample in the order was used as the sensory score in this study.

Extraction and identification of aroma compounds An improved Likens-Nickerson simultaneous steam distillation and extraction apparatus was used to extract the aroma compounds of barley bran sauce (12), using purified diethyl ether as the extraction solvent. The process of extraction was as follows. First, the sample and solvent were placed in the sample and solvent ports, respectively. Next, the aroma compounds were extracted for more than two hours by increasing the temperature of the sample port to boiling point after circulating the solvent preliminarily. Anhydrous Na₂SO₄ was added to the extracted fraction at 4°C to remove moisture. Finally, the fraction was concentrated to a final 100 mL by using N₂ gas to obtain samples for gas chromatographic (GC) analysis.

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

Multiple regression analysis (MRA) MRA was carried out by the method of Aishima and Nobuhara (3). The relation between the sensory evaluation score and the content of aroma components is shown in Fig. 1. The number of sensory scores corresponding to each of the barley bran sauce samples could be described as $Y = (y_1, y_2, ..., y_i, ..., y_n)$, 1 < i < n, as shown in matrix A. If the gas chromatogram of each flavor concentrate shows m peaks, each gas chromatogram could be described as $(x_{i1}, x_{i2}, ..., x_{ij}, ..., x_{im})$, 1 < j < m, and consequently the gas chromatogram

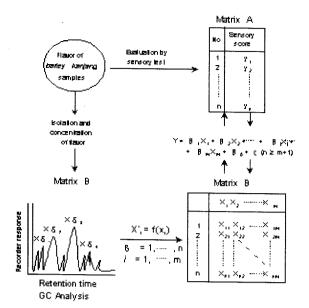


Fig. 1. Scheme of the relationship between sensory test and aroma components of the barley bran sauce.

for the entire samples could be described by the matrix shown in B, where i=1 to 42, j=1 to 34, in this study. The MRA model is generally shown as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_j X_j + \dots + \beta_m X_m + \epsilon$$
(1)

Y: dependent variable (sensory score)

X_j: independent variable (aroma compounds)

 β_0 : constant

 β_{i} : partial regression coefficient

 ε : random error

where β_0 and β_j are computed by the linear least squares method, and the equation is solvable for n>m. The correlation coefficient between the real Y and an estimated Y obtained from the computed MRA model was designated as multiple correlation (R). R^2 , which represents the ratio of variance of Y explained by the MRA model, is called the coefficient of multiple determination. When the MRA model for quality tests is used, smaller numbers of independent variables are desirable to simplify the estimation of Y. Among the many different types of multivariate analysis methods, stepwise regression analysis (SRA) was the most adequate method for selecting a subset of variables.

In this study, data of the aroma components was analyzed after transformation process (Table 1). The constant 1.0 was added to the original data in the logarithmic transformation to obtain 0 by transformation when analyzing non-existing samples, while 1×10^{-10} was added to the original data in the transformation with root square to avoid calculating the root square of 0.

Results and Discussion

Sensory test and GC analysis The sensory evaluation was undertaken by 12 well-trained members of the sensory

Table 1. Transformation of independent variables

	Absolute values	Relative values
1	X_{i}	$X_i = X_i / \Sigma X_j \times 100$
2	$ln(X_i + 1.0)$	$ln(X'_i + 1.0)$
3	$\sqrt{(X_i + 10^{-10})}$	$\sqrt{(X'_i + 10^{-10})}$

panel of Yeungnam University, and from the results the most favorable and the most unfavorable samples scored 72 and 29, respectively. One sample received more than 72, 6 samples 48-60, 22 samples 36-48, and 9 samples 24-36. The mean value of these samples was 43.3 and the standard deviation was 10.5.

Most of the barley bran sauce samples consisted of about 34 peaks, in which the content of 9,12-octadecanoic acid, methyl ester was the highest, followed by those of 2-furanmethanol and 2-furancarboxaldehyde, as shown Table 2 and 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 3. The negative correlation coefficients in each peak indicate that its peak gives a reverse contribution to the quantity, while the positive correlation coefficients show that its peak gives a preferable contribution. Many peaks showed a significant relationship with the sensory score, including peaks 9 (methyl furfural), 12 (phenylacetaldehyde), 21 (tetradecanoic acid), 29 (9,12,15-octadecatrienoic acid, methyl ester) and 31 (9,12-octadecadienoic acid). Among them, the correlation coefficients of peaks 9 (methyl furfural) and 29 (9,12,15-octadecatrienoic acid, methyl ester) were significant at the 1% level at every transformation.

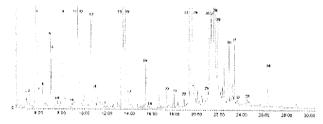


Fig. 2. Total ion chromatogram of aroma components in barley bran sauce.

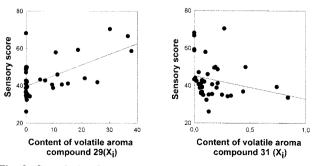


Fig. 3. Correlation between sensory scores and the content of component numbers 29 (9,12,15-octadecatrienoic acid, methyl ester) and 31 (9,12-octadecadienoic acid) calculated from the absolute value (X_i) .

Table 2. Composition of aroma compounds and sensory evaluation values in barley bran sauce

No	Aroma components	Mean	S.D. ¹⁾	Max.2)	Min.3)
X_1	3-Furanaldehyde	6.70	16.93	69.39	0
X_2	2-Furanmethanol	8.75	16.02	57.24	0
X_3	2-Furancarboxaldehyde	8.67	18.61	61.16	0
X_4	4-Pentynoic acid	2.39	10.64	54.26	0
X_5	7-Cyclopropane	0.38	1.78	11.63	0
X_6	Trimethylphosphine	0.12	0.11	0.43	0
X_7	2-Ethyl-5-methylfuran	0.03	0.09	0.48	0
X_8	5-Methyl-2-(1-methylethyl) cyclohexane	0.06	0.08	0.45	0
X_9	Methyl furfural	3.81	4.89	29.65	0
X_{10}	4-Acetyl heptanenitrile	0.45	2.86	18.51	0
X_{11}	Benzeneacetic acid	2.23	4.85	22.96	0
X_{12}	Phenyl acetaldehyde	4.18	4.94	20.11	0
X_{13}	4-Methoxypheol	0.11	0.09	0.52	0
X_{14}	Phenylethyl alcohol	0.39	0.38	1.44	0
X_{15}	4-Mercaptophenol	0.23	0.48	2.05	0
X_{16}	4-Vinyl-2-methoxy-phenol	2.60	3.19	18.57	0
X_{17}	2-Methoxy-4-(1-propenyl)phenol	0.04	0.14	0.89	0
X_{18}	2,6-Bis(1,1-dimethylethyl)-4-methyl phenol	0.80	1.28	7.52	0
X_{19}	1-Furfuryl-2-formyl pyrrole	0.18	0.44	2.83	0
X_{20}	Tetradecanoic acid ethyl ester	0.14	0.40	2.30	0
X_{21}	Tetradecanoic acid	0.05	0.05	0.21	0
X_{22}	Hexadecanoic acid, methyl ester	0.05	0.05	0.23	0
X_{23}	Hexadecanoic acid, ethyl ester	1.77	3.33	13.92	0
X_{24}	Hexadecanoic acid	7.73	6.48	27.47	0
X_{25}	1,2-Benzenedicarboxylic acid, dibutyl ester	0.11	0.29	1.82	0
X_{26}	9-Otadecenoic acid, ethyl ester	2.45	4.30	19.06	0
X_{27}	9,12-Octadecadienoic acid, methyl ester	18.74	23.65	75.23	0
X_{28}	9,12-Octadecadienoic acid, ethyl ester	18.73	21.06	78.84	0
X_{29}	9,12,15-Octadecatrienoic acid, methyl ester	6.11	10.45	37.90	0
X_{30}	9-Octadecenoic acid, 9-octadecenyl ester	0.06	0.11	0.52	0
X_{31}	9,12-Octadecadienoic acid	0.15	0.18	0.84	0
X_{32}	Unknown	0.07	0.14	0.81	0
X_{33}	Octadecanoic acid, phenyl methyl ester	0.08	0.13	0.70	0
X_{34}	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl)ester	0.25	0.77	4.56	0
	S.E. V. ⁴)	43.26	10.48	72.00	29.00

DS.D.: standard deviation

²⁾Max.: maximum value ³⁾Min.: minimum value

⁴⁾S.E.V.: sensory evaluation values indicate the sum of the score in the range from 1 (dislike extremely) to 7 (like extremely) that were recorded by the panel of 12 sensors.

In the case of peaks 26 (9-octadecadienoic acid, ethyl ester), 27 (9,12-octadecadienoic acid, methyl ester), 28 (9, 12-octadecadienoic acid, ethyl ester) and 31 (9,12-octadecadienoic acid), although there was no significant relationship calculated with the absolute value, high correlations were nevertheless found when calculated with the absolute value transformed with the root square. This result implied that the statistical analysis of barley bran sauce aroma was affected by transformation.

In Fig. 3, the quantities of peaks 29 (9,12,15-octade-catrienoic acid, methyl ester) and 31 (9,12-octadecadienoic acid) were plotted against the sensory scores. It was difficult to estimate the aroma quality of barley bran sauce samples on the basis of only one peak, despite the highly significant relationship between the quantity of the peak

and the sensory score.

Aishima (1) found that the most negative and positive correlations between the GC peaks and sensory data of soy sauce were shown by trans-2-hexen-1-ol, contributing to a preferable aroma, and iso-butyric acid, concerning to an unpleasant smell, respectively. Ji *et al.* (13) reported that the component which emitted a disagreeable odor was identified as 3-methyl-1-butanol, while the components which emitted a soy sauce-like odor were identified as dimethyl trisulfide benzeneacetaldehyde and benzenemethanol.

Multiple regression analysis (MRA) The 34 aroma compounds of the 42 samples were analyzed by an MRA model. The correlation matrix for the variables in the

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Table 3. Correlation coefficients (r) between sensory scores and contents of aroma compounds

No		r			
	X_{i}	$ln(X_i+1.0)$	$(X_i + 10^{-10})$		
X_1	-0.04	-0.04	-0.05		
X_2	0.12	0.13	0.12		
X_3	0.01	-0.05	0.04		
X_4	0.15	0.13	0.14		
X_5	-0.13	-0.20	-0.25		
X_6	0.32^{*}	0.32^{*}	0.22		
X_7	0.30	0.29	0.24		
X_8	-0.05	-0.04	0.01		
X_9	0.42**	0.40^{**}	0.41**		
X_{10}	0.01	0.01	0.01		
X_{11}	-0.15	-0.21	-0.21		
X_{12}	0.33^{*}	0.33*	0.33^{*}		
X_{13}	0.22	0.24	0.23		
X_{14}	-0.21	-0.25	-0.30		
X_{15}	0.12	0.13	0.12		
X_{16}	0.15	0.16	0.15		
X_{17}	-0.01	-0.01	0.03		
X_{18}	-0.20	-0.20	-0.20		
X_{19}	-0.46	-0.08	-0.09		
X_{20}	-0.14	-0.12	-0.11		
X_{21}	0.36^{*}	0.35^{*}	0.31^{*}		
X_{22}	0.05	0.05	0.02		
X_{23}	-0.08	-0.23	-0.23		
X ₂₄	0.20	0.14	0.16		
X_{25}	-0.09	-0.13	-0.24		
X_{26}	-0.19	-0.33*	-0.31*		
X_{27}	-0.27	-0.39*	-0.35*		
X_{28}	-0.29	-0.46**	-0.41**		
X_{29}	0.57**	0.49**	0.51**		
X_{30}	-0.15	-0.17	-0.25		
X_{31}	-0.30	-0.32*	-0.46**		
X_{32}	0.06	-0.08	0.19		
X_{33}	0.06	0.09	0.18		
X_{34}	0.09	0.12	0.14		

*p<0.05, **p<0.01

MRA model at step 10 is shown in Table 4. Two important conditions were presented for the purpose in this report. First, as there was a strong similarity between several of

Table 5. Multiple correlation coefficient (R) and coefficient of multiple determination of multiple regression models of aroma computed from the values which were transformed with variables

	Absolute values				
	X_{i}	$ln(X_i+1.0)$	$(X_i + 10^{-10})$		
$R^{(1)}$	0.947	0.903	0.957		
$R^2 \times 100^{2)}$	89.7	81.6	91.6		
S.E. ³⁾	8.14	10.87	7.36		
F	1.794	0.914	2.232		
Accuracy order	2	3	1		

(1)2)3): See the legend in Fig. 4.

the groups of peaks, each of these groups should be represented by one typical and representative peak. Second, as there were several peaks with practically zero values of the contributing proportion, they should be removed from MRA. Thus, the MAR model was computed using independent variables, and this was not expected to lower the accuracy of the estimation.

The results of absolute value and absolute value transformed with root square among the three different forms of MRA models are expressed in Fig. 4. Not all the marks of each biased regression coefficient were in accordance with the mark of the correlation coefficient because a correlation existed in the increase and decrease areas between the independent variables.

The multiple correlation coefficient, F-value and statistical significance of these MRA models revealed a linear correlation between the sensory scores and aroma compounds.

Effect of transformation In order to compare the fitness of the linear equation model, the multiple correlation, coefficient of the multiple determination, standard error, and F-value of the GC data were all transformed into six different forms, as shown in Table 5. The most precise fit was calculated from the absolute value transformed with the root square of each peak, for which the multiple determination coefficient showed 91.6% of the variation in the sensory score that could be explained on the basis of the GC data. Therefore, it was confirmed that the absolute value transformed with root square was effective in linearizing the GC data of the sensory scores.

Table 4. Selection order and correlation matrix of variables (aroma components) entered into the regression model at step 10

Selection order	1	2	3	4	5	6	7	8	9	10
Aroma components	X ₂₉	X ₇	X_6	X_2	X ₈	X ₂₄	X ₂₈	X ₁₆	X_{19}	X_{18}
\overline{X}_{29}	1.00	-0.21	0.26	-0.24	-0.20	0.06	-0.20	0.16	0.15	-0.17
X_7		1.00	-0.20	0.26	-0.63	0.22	0.07	0.03	-0.04	-0.14
X_6			1.00	-0.09	0.50^{**}	-0.16	-0.20	0.09	-0.05	0.07
\mathbf{X}_2				1.00	0.74	-0.01	-0.02	0.04	0.03	-0.02
X_8					1.00	0.12	-0.16	0.49^{**}	0.51**	-0.22
X_{24}						1.00	0.06	0.10	0.22	-0.01
X_{28}							1.00	-0.15	-0.20	0.46^{**}
X_{16}^{-1}								1.00	0.83**	0.03
X_{19}^{-1}									1.00	-0.15
X_{18}										1.00

* p<0.05, **p<0.01

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X.
  Y = -70.060 + 1.443X_{29} - 14.434X_7 + 7.702X_6 + 1.035X_2 - 121.774X_8 + 1.217X_{24} + 1.162X_{28} + 3.430X_{16} - 23.438X_{19} + 1.035X_{19} + 1.035X_{
                                  (-0.891:-)(1.618:-)(-0.273:-)(0.167:-)(1.255:-)(-1.105:-)(1.455:-)(1.444:-)(1.208:-)(-0.640:-)
                         -4.514X_{18}-1.937X_{14}+0.456X_4+2.966X_{12}+23.644X_{31}-106.760X_{30}\\+13.047X_{34}+1.118X_{27}+1.278X_{9}
                               (-1.476:-) (-0.146:-) (0.502:-) (2.032:-) (0.835:-) (-1.273:-) (1.429:-) (1.430:-) (1.354:-)
                            +\ 1.375X_{11}+99.931X_{13}+10.066X_{25}-2.288X_{32}+24.956X_{33}-73.627X_{21}+1.516X_{10}-0.450X_{17}+1.325X_{20}
                                      (1.212:-) (0.876:-) (1.127:-) (-0.044:-) (0.590:-) (-1.034:-) (0.181:-) (-0.009:-) (0.223:-)
                       +0.883X_3 + 0.979X_5 + 0.865X_1 + 0.662X_{23} + 0.686X_{26} - 2.042X_{15} - 3.133X_{22}
                                  (1.135:-) (0.742:-) (1.072:-) (0.470:-) (0.565:-) (-0.175:-) (-0.078:-)
                         ^{1)}R = 0.947 ^{2)}R<sup>2</sup> = 0.897 ^{3)}S.E = 8.13 F = 1.794
(X_i+1.0^{-10})^{-2}
  Y = 144.645 - 4.564X_{29} + 39.149X_7 - 1.228X_9 - 7.643X_2 - 21.535X_{19} - 7.853X_{28} - 8.633X_{16} - 2.086X_{24} + 1.204X_{26} - 18.209X_{34} + 1.204X_{35} - 1.204X_{35} + 1.204X_{35} +
                         (4.193:^*)(-1.871:-) (2.208:-) (-0.398:-) (-2.901:^*) (-2.398:^*) (-3.433:^*) (-1.164:-) (-1.084:-) (0.486:-) (-1.751:-)
                       +51.253X_{30} - 9.027X_3 - 20.275X_{25} - 63.279X_{31} - 6.142X_4 + 43.905X_{22} + 34.084X_{32} - 5.443X_{27} - 8.807X_1 + 0.085X_{10} + 20.085X_{10} + 20
                                  -7.986X_{23} + 28.156X_{14} - 9.945X_{12} + 55.144X_{13} - 1.701X_{18} + 25.353X_{33} - 9.573X_{20} - 7.910X_{11} + 41.523X_8 + 4.894X_5 + 4.
                              (-1.877:-) (2.504:^*) (-2.063:-) (1.561:-) (-0.171:-) (1.441:-) (-1.299:-) (-1.483:-) (1.359:-) (0.881:-)
                             -7.615X_{15} - 7.684X_{17} + 8.936X_{21} + 6.431X_{6}
                                 (-0.894:-) (0.499:-) (0.366:-) (0.337:-)
                                                                                              R^2 = 0.916 S.E = 7.36
                               R = 0.957
                                                                                                                                                                                                                                                        F = 2.232
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Fig. 4. Multiple regression models of aroma computed from the absolute value (X_i) and absolute value transformed with root square [(X_i +1.0⁻¹⁰)-²]. X_1 ; 3-furanaldehyde, X_2 ; 2-furanmethanol, X_3 ; 2-furancarboxaldehyde, X_4 ; 4-pentynoic acid, X_5 ; 7-cyclopropane, X_6 ; trimethylphosphine, X_7 ; 2-ethyl-5-methylfuran, X_8 ; 5-methyl-2-(1-methylethyl) cyclohexane, X_9 ; methyl furfural, X_{10} ; 4-acetyl heptanenitrile, X_{11} ; benzeneacetic acid, X_{12} ; phenyl acetaldehyde, X_{13} ; 4-methoxypheol, X_{14} ; phenylethyl alcohol, X_{15} ; 4-metrophylphenol, X_{16} ; 4-vinyl-2-methoxy-phenol, X_{17} ; 2-methoxy-4-(1-propenyl)phenol, X_{18} ; 2,6-bis(1,1-dimethylethyl)-4-methyl phenol, X_{19} ; 1-furfuryl-2-formyl pyrrole, X_{20} ; tetradecanoic acid ethyl ester, X_{21} ; tetradecanoic acid, X_{22} ; hexadecanoic acid, methyl ester, X_{23} ; hexadecanoic acid, ethyl ester, X_{24} ; hexadecanoic acid, X_{25} ; 1,2-benzenedicarboxylic acid, dibutyl ester, X_{26} ; 9-otadecenoic acid, ethyl ester, X_{26} ; 9-12-octadecadienoic acid, methyl ester, X_{28} ; 9,12-octadecadienoic acid, ethyl ester, X_{29} ; 9,12,15-octadecatrienoic acid, methyl ester, X_{30} ; 9-octadecenoic acid, 9-octadecenyl ester, X_{31} ; 9,12-octadecadienoic acid, X_{32} ; unknown, X_{33} ; octadecanoic acid, phenyl methyl ester, X_{34} ; 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl)ester. ¹⁾Multiple correlation, ²⁾Coefficient of multiple determination, ³⁾Standard error of estimation, *: P<0.05, **: P<0.01. Numbers in parentheses show t-value for each of the entered variables.

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