

Optimization of Dynamic Headspace Purge Conditions for Concentration of Volatile Flavor Compounds of Roasted Perilla Seeds Oil by Response Surface Methodology

Suk-Ju Kim

Department of Food and Nutrition, Woosong Information College

반응표면 분석에 의한 볶음들기름의 향기성분 포집조건 최적화

김 석 주

우송정보대학 식품영양과

Abstract

Dynamic headspace purge conditions were investigated to obtain minimum loss of high volatile compounds by breakthrough and maximum recovery of low volatile components of roasted perilla seed oil (RPSO). A response surface methodology was applied to evaluate the effect of purge temperature, purge time, and sample weight on ℓn (total peak area), breakthrough ratio, and peak area of perilla ketone the least volatile component of RPSO. Sample weight was the most important factor on the ℓn (total peak area) but it did not affect peak area of perilla ketone. All process variables significantly influenced breakthrough ratio. The optimum condition was determined by superimposing contour plots at purge temperature of 48 °C for 12 min purge time at sample weight of 0.60 g. 2-Propanone, 2-butanone, acetic acid, 2-methyl propanal were main breakthrough compounds in RPSO flavor.

Key words: dynamic headspace system, response surface methodology, roasted perilla seed oil, breakthrough compounds.

INTRODUCTION

In the field of flavor research, dynamic headspace system (DHS) has been widely used for isolation and concentration of the volatile compounds of various food flavors¹⁻⁴ and lipid oxidation products⁵. Tenax-TA (2,6-diphenyl-*p*-phenylene oxide polymer) is commonly used as an adsorbent due to high boiling components trapping, low water adsorbing capacity, and high thermal stability suited to heat desorption⁶. When purging the volatile

flavor compounds of the foods by using DHS, small quantity of sample, short purge time, and ambient purge temperature or temperature of which mimic the release of the volatile compounds on the mouth during mastication are desirable. However, generally these mild purge conditions tend to low recovery of volatiles and result of lack of sensitivity⁷. Whereas more vigorous conditions, sometimes, bring to a reduction of recovery by breakthrough of high volatile components¹. Therefore, it is necessary for determining the optimum purge conditions

[†] Corresponding author : Suk-ju Kim, Department of Food and Nutrition, Woosong Information College, 226-2 Jayang-dong, Daejeon 300-715, Korea.

Tel : 82-42-629-6198, Fax : 82-42-629-6150, E-mail : sjkim@wsi.ac.kr

to avoid or minimize the drawbacks of DHS.

Simultaneous factor optimization approach is more useful for the optimization of purge and trap conditions than one-factor-at-a-time method because in most case the major handling factors (purging time, sample temperature, sample volume or weight) are interactive²⁾. Therefore, the random-centroid optimization method was used for determining the optimum purge condition. However, this method was difficult for the interpretation and prediction of the responses along with major factors. Morales and Aparicio⁸⁾ suggested that optimum trapping conditions could be evaluated by overall desirability function. It was composed of chromatographic balance (peak resolution), stripping quality (the total number of peaks), and standard recovery. Yet this concept is not generalized and it is easy to subjective. Talou *et al.*⁹⁾ optimized purge and trap conditions for the extraction of volatiles in black truffles by using response surface methodology (RSM). But they were restricted the response only total peak area, this does not always give the optimum condition if the sample has large amount of high volatiles.

RSM is effective for responses that are affected by many factors and their interaction. It can predict and visualize the effects of changing more than two interacting factors simultaneously on the responses clearly by mathematical models fitted to data using least square methods and three-dimensional response surface or contour plots¹⁰⁾. The objectives of the present study were to establish optimum purge conditions for obtaining minimum loss of high volatile compounds by breakthrough and maximum recovery of low volatile components of RPSO by RSM and identify the breakthrough flavor compounds of RPSO.

MATERIALS AND METHODS

1. Chemicals and Materials

Perilla seeds were roasted at 175°C for 3 min and oil was extracted according to the method previously described¹¹⁾. Standard chemicals for identification of volatiles in GC and MS were purchased from Aldrich Chemical Company (Milwaukee, WI), Sigma Chemical Co. (St. Louis, MO), and Fluka Chemie AG (Buchs, Switzerland).

2. DHS /GC and GC-MS

Oil (0.10~1.10 g) was placed in a 100 ml round Pyrex vessel which had been flushed with nitrogen for 40 min and immersed in a water bath at experimental temperature. Four glass tubes (11.5 cm×4 mm) packed with Tenax-TA (200 mg each respectively, 60~80 mesh size, Supelco, Inc., Bellefonte, PA) were attached in series immediately. The tubes were conditioned prior to use by heating for 30 min at 250 °C with high purity nitrogen flow of 150 ml/min on the tube conditioner (Supelco Inc., Bellefonte, PA). After the sample vessel was preheated for 5 min without stirring, the headspace was purged with nitrogen (156 ml/min) coming from dynamic thermal stripper (Supelco Inc., Bellefonte, PA) for 6.6~23.4 min onto a thermal desorption tube with a magnetic stirring. The Tenax-TA tube temperature was maintained at 25 ±2°C. Breakthrough ratio was determined by the following equation:

Breakthrough ratio (%) =

$$(\text{sum of total peak area of the second, third, and fourth tubes} / \text{total peak area of the first tube}) \times 100$$

Using thermal tube desorber (Supelco Inc., Bellefonte, PA), the volatiles trapped on the Tenax-TA column were thermally desorbed at 250°C for 9 min and directly injected into GC, a HP 5890 series II plus (Hewlett-Packard Co., Palo Alto, CA) equipped with a flame ionization detector and a fused SE-54 silica capillary column (60 m, 0.32 mm id, 0.25 μm film thickness, Supelco Inc., Bellefonte, PA). The GC and GC-MS analysis conditions were the same as described previously¹¹⁾.

3. Experimental Design

Purge and trap conditions were optimized by RSM to obtain minimum loss of high volatile compounds and maximum recovery of low volatiles of RPSO. Natural log transformation of total peak area (TPA(ℓn), Y_1), breakthrough ratio (BTR, Y_2), and the peak area of perilla ketone (PAPK, Y_3) the least volatile component of RPSO¹¹⁾ were selected as a purging quality factors (dependent variables). The independent variables investigated were purge temperature (X_1), purge time (X_2), and

Table 1. Coded and actual levels of three variables used in experimental design

Variable	Symbol	Coded-variable levels				
		- 1.682	- 1	0	1	1.682
Purge temperature (°C)	X ₁	23.10	30	40	50	56.80
Purge time (min)	X ₂	6.60	10	15	20	23.40
Sample weight (g)	X ₃	0.10	0.30	0.60	0.90	1.10

Table 2. Experimental data for the total peak area, breakthrough ratio, and peak area of perilla ketone under different treatment conditions

Run ³⁾	Independent variables ¹⁾			Response variables ²⁾		
	Temp. (X ₁)	Time (X ₂)	Weight (X ₃)	Total peak area(μ n) ⁴⁾ (Y ₁)	Breakthrough ratio, % ⁴⁾ (Y ₂)	Peak area of perilla ketone ⁴⁾ (Y ₃)
1	- 1	- 1	- 1	12.184	1.63(1.63/0 /0) ⁵⁾	0
2	1	- 1	- 1	12.789	3.94(3.60/0.34/0)	11964.1
3	- 1	1	- 1	12.619	7.39(6.40/0.99/0)	4263
4	1	1	- 1	13.099	4.98(3.81/1.18/0)	27254.2
5	- 1	- 1	1	13.709	7.54(5.31/1.65/0.58)	2252
6	1	- 1	1	14.070	4.97(3.28/1.21/0.48)	13193.9
7	- 1	1	1	14.027	10.54(8.20/1.71/0.63)	4691.8
8	1	1	1	14.553	6.83(5.07/1.18/0.58)	28416
9	0	0	0	13.824	6.68(4.77/1.59/0.32)	11003
10	0	0	0	13.594	7.15(5.12/1.52/0.51)	12114.8
11	0	0	0	13.903	6.88(5.75/1.13/0)	10856.6
12	0	0	0	13.805	7.03(4.37/1.95/0.71)	13418.8
13	0	0	0	13.622	6.80(4.18/1.59/1.03)	11198.4
14	0	0	0	13.792	6.85(4.98/1.45/0.42)	10585.5
15	0	0	0	13.780	6.69(4.52/1.80/0.37)	10114.9
16	0	0	0	13.783	6.93(5.49/1.20/0.24)	10191.1
17	0	0	0	13.708	7.14(5.40/1.45/0.29)	10274.8
18	1.682	0	0	13.351	6.05(4.54/0.89/0.62)	33626.4
19	- 1.682	0	0	14.291	9.87(7.82/1.43/0.62)	0
20	0	1.682	0	13.471	6.84(5.16/1.45/0.23)	16862.6
21	0	- 1.682	0	13.941	2.92(1.91/0.55/0.46)	3739.1
22	0	0	1.682	13.028	5.68(4.50/0.82/0.36)	10700.4
23	0	0	- 1.682	14.284	3.67(3.44/0.23/0)	8333.9

¹⁾ Coded symbols and levels of independent variables refer to Table 1.

²⁾ Mean of duplicate runs.

³⁾ Experimental runs were performed in random order.

⁴⁾ Coefficient of variations of Y₁, Y₂, and Y₃ at center point were 0.75, 2.33, and 9.84%, respectively.

⁵⁾ Breakthrough ratio of trap 2, 3, and 4.

sample weight (X_3) which had defined practical narrow range as a result of preliminary trials. A central composite rotatable design¹⁰⁾ with three-level and three-factor was used. The design was composed of 2^3 factorial points, 9 center point replicates, and 6 axial points. The actual values and coded values of three factors were Table 1. Experiments were performed in random order and done in duplicate. A second degree polynomial regression equation was used for predicting individual Y variable. The model proposed for each response of Y was

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \sum \beta_{ij} x_i x_j$$

where β_0 , β_i , β_{ii} , and β_{ij} are intercept, linear, quadratic, and interaction regression coefficient terms, respectively, and x_i and x_j are independent variables.

Statistical analysis was performed using the RSREG procedure of the Statistical Analysis System¹²⁾. The fitted quadratic polynomial equation was expressed graphically by means of the Response Surface Design of JMP¹³⁾ to create contour plots.

RESULTS AND DISCUSSION

1. Fitting the Models

The experimental design and data for three responses, Y_1 , Y_2 , and Y_3 were presented in Table 2. The coefficient of variation of Y_1 , Y_2 , and Y_3 at center point were 0.75, 2.33, and 9.84%, respectively indicated good reproducibility of dynamic headspace GC¹⁴⁾. The three regression models and effects of independent variables on response variables were tested for adequacy and fitness by analysis of variance (Table 3). The regression models for data were highly significant ($p < 0.001$) R^2 value of 0.881, 0.939, and 0.988, for Y_1 , Y_2 , and Y_3 , respectively. On the other hand, it revealed a significant lack of fit except for Y_3 . Sometimes such result was occurred when the pure error was too small by the uncertain experimental factors and it was recommended to add higher order terms in the model or transform the response variables more proper form¹⁰⁾. In this study, among the various transformations of the response variables, log transformation and the quadratic model gave the best results in the case of TPA and BTR, respectively. However they had a significant lack of fit yet, nevertheless, it could be used for a useful predictive tool of responses. The model for Y_1 results showed only the linear effect was highly significant ($p < 0.001$). The model for Y_2 and Y_3 were highly significant ($p < 0.001$) on the linear, quadratic and cross-

Table 3. Analysis of variance of the second order polynomial model showing the effect of treatment variable as a linear, quadratic term and interaction on the three response variables

Source	Degree of freedom	Sum of squares		
		Total peak area (ℓn) (Y_1)	Breakthrough ratio (Y_2)	Peak area of perilla ketone (Y_3)
Regression				
Linear	3	5.788 ^{***}	53.586 ^{***}	1.429×10^9 ^{***}
Quadratic	3	0.355	18.825 ^{***}	6.683×10^7 ^{***}
Cross - product	3	0.005	9.537 ^{**}	7.132×10^7 ^{***}
Total regression	9	6.149 ^{***}	81.947 ^{***}	1.567×10^8 ^{***}
Residual				
Lack of fit	5	0.750 ^{***}	5.051 ^{***}	9.727×10^6
Pure error	8	0.078	0.243	9.225×10^6
Total error	13	0.828	5.294	1.895×10^7
% Variability explained (R^2)		0.881	0.939	0.988

^{***} $p < 0.001$, ^{**} $p < 0.01$.

Table 4. Regression coefficient of predicted quadratic polynomial model for three response variables

Coefficients()	Total peak area (ℓn) (Y ₁)	Breakthrough ratio (Y ₂)	Peak area of perilla ketone (Y ₃)
Linear			
β_0	13.764	6.901	11101
β_1	0.260**	- 0.938***	9238***
β_2	0.178	1.336***	4340***
β_3	0.570***	1.122***	662
Quadratic			
β_{11}	- 0.047	0.415	1864***
β_{22}	- 0.097	- 0.647***	- 437
β_{33}	- 0.105	- 0.746***	- 714
Interaction			
β_{12}	0.005	- 0.733**	2796***
β_{13}	- 0.025	- 0.773**	- 36
β_{23}	0.007	- 0.243	- 236

*** $p < 0.001$, ** $p < 0.01$.

product effects except for Y₂ on cross product ($p < 0.01$).

The regression coefficients of three factors on responses are shown in Table 4. Results showed that Y₁ was linearly affected by X₁ (purge temperature), X₂ (purge time), and X₃ (sample weight). Among these factors the sample weight was the most important one influencing TPA. Whereas, Y₂ was linearly and quadratically affected by X₂ and X₃. On the other hand, their interaction was not significant. Y₃ was linearly affected by X₁ and X₂, but quadratic effect was significant on X₁ and X₃. Interaction of X₁ and X₂ was highly significant ($p < 0.001$).

The overall effect of the three independent variables on response variables was analyzed (Table 5). X₃ was the most important factor on the Y₁, whereas, it could not any affect on Y₃. All process variables significantly influenced Y₂. Results indicated the purge factors affected the responses (GC chromatogram qualities) very selectively and characteristically.

2. Optimum Condition

In order to investigate the effect of independent variables on the responses and determine the optimum

Table 5. Analysis of variance for the overall effect of the independent variables on the response variables

Independent variable	Degree of freedom	Sum of squares		
		Total peak area (ℓn) (Y ₁)	Breakthrough ratio (Y ₂)	Peak area of perilla ketone (Y ₃)
Purge temperature (°C)	4	0.965	23.809***	1.269×10 ⁹ ***
Purge time (min)	4	0.583	36.373***	3.390×10 ⁸ ***
Sample weight (g)	4	4.611***	31.282***	1.219×10 ⁷

*** $p < 0.001$.

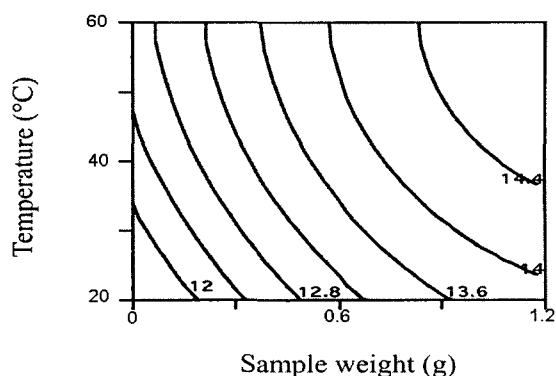


Fig. 1. Contour plot of total peak area (ℓn) as a function of purge temperature and sample weight at constant purge time of 15 min.

point, contour plots were generated as a function of two variables, while the third variable was fixed at middle level. Fig. 1 shows the effect of sample weight and temperature on the TPA (ℓn) at middle levels of purge time. TPA (ℓn) increased with increased level of sample weight and temperature, and the first was more effective. Talou *et al.*⁹⁾ also reported an increase of total peak area with increased sample amount and scavenging time. Buckholz *et al.*¹⁾ observed an increase of total volatile components with increased purge time. However, in our experiment, purge time did not affect TPA (ℓn) (Table 5). This inconsistency probably may be resulted from higher purge gas flow rate (156 ml/min), such a case the lowest level of purge time (6 min) was enough to sweep the most volatiles up to the highest level of sample weight and temperature condition.

In general, the lower boiling point compounds tend to breakthrough the trap more easily. In a restricted and defined experimental region of these experiments (from -1 to 1 of independent variables), breakthrough was inevitable because of high content of most volatile components of RPSO (Fig. 2). Breakthrough occurred when the maximum retention volume of the trap was exceeded due to long purge times, large sample weights, and high temperatures (Table 5). To get a precise information of breakthrough behavior, contour plots for breakthrough ratio as a function of three variables were presented (Fig. 3). It was increased with increase of purge time and sample weight, and had a minimum purge time of 10~12 min and the sample weight of 0.3~0.45 g, and maximum near the time of 20 min and sample weight of

0.80 g at constant medium level of purge temperature (Fig. 3-a). Whereas, keeping constant purge time while varying purge temperature and sample weight, as well as varying purge temperature and time while maintaining a constant sample weight gave a typical saddle point in contour plots (Fig. 3-b and c). The minimum BTR was obtained at temperature of 42 °C and sample weight of 0.3 g, and temperature of 40~45 °C at time of 10 min in the case of keeping constant purge time of 15 min and sample weight of 0.60 g, respectively, in Fig. 3-b and c. Similar results were obtained at constant purge time of 10 and 20 min, and at constant sample weight of 0.3 and 0.9 g (not shown). From these results, minimum BTR region was existed in the lower sample weight < 0.45 g, and time < 12 min (Fig. 3-b and c). If the time and sample weight were below minimum BTR region, effect of purge temperature on the BTR was a little, on the other hand, low BTR region (sample weight > 0.60 g, purge time > 15 min), the temperature had a significant effect on the BTR because of relatively low portions of breakthrough of high volatile compound compared to total volatiles entrapped in Tenax-TA trap 1. In this study, the grand average of breakthrough ratio was 6.30% and had the range of 1.63~10.54 %. At center point, the average ratio and ratio of trap 2, 3, and 4 were 6.91, 4.95, 1.52, and 0.43 %, respectively (Table 2). Buckholz *et al.*¹⁾ reported that acetaldehyde, acetone, isobutyraldehyde, isovaleraldehyde, 2-methyl butanal, and pentanal were breakthrough compounds in roasted peanut flavor. Bartley¹⁵⁾ observed breakthrough of acetaldehyde in kiwi fruit was about 10 % by using Tenax-GC. In our results, 2-propanone, 2-butanone, acetic acid, 2-methyl propanal were main breakthrough compounds (Table 6).

Although DHS is most suitable method to analyze volatile component of food flavors, it has some limitations to be resolved. One of these is poor recovery of less volatile compounds by inefficient trapping compare to other extraction methods like steam distillation extraction¹⁶⁾. To maximize recovery of less volatile compounds, perilla ketone was selected as an index of low volatile component (Fig. 2). A contour plot was generated for a constant sample weight by changing temperature and purge time (Fig. 4). The PAPK (Y_3) was

Table 6. Compounds of breakthrough trap 2 in roasted perilla seed oil

Peak No. ¹⁾	Compound	ID ²⁾
1	2-Propanone	MS, GC
2	Acetic acid methyl ester	MS
3	2-Methyl propanal	MS
4	2-Butanone	MS, GC
6	Acetic acid	MS, GC

¹⁾ The peak numbers correspond to numbers in Fig. 2.

²⁾ MS, tentatively identified by mass spectrometer; GC, GC retention time consistent with that of authentic compounds.

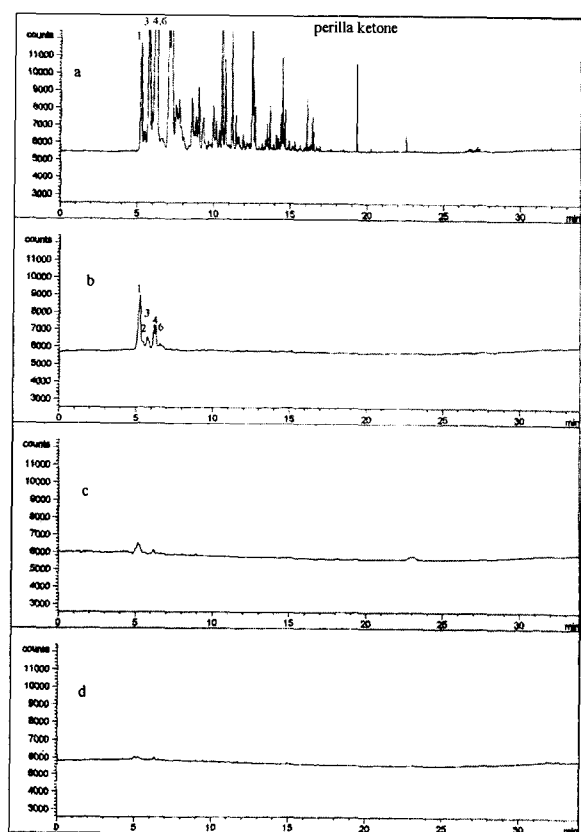


Fig. 2. A comparison of GC profiles of the four traps in series showing the trap 1 (a), 2 (b), 3 (c), and 4 (d). Compounds are identified by numbers shown in Table 6.

sharply increased as the temperature was increased and the purge temperature was more significant than purge time (Table 5) because volatility was a function of temperature¹⁷⁾.

Optimum condition was defined previously as the point

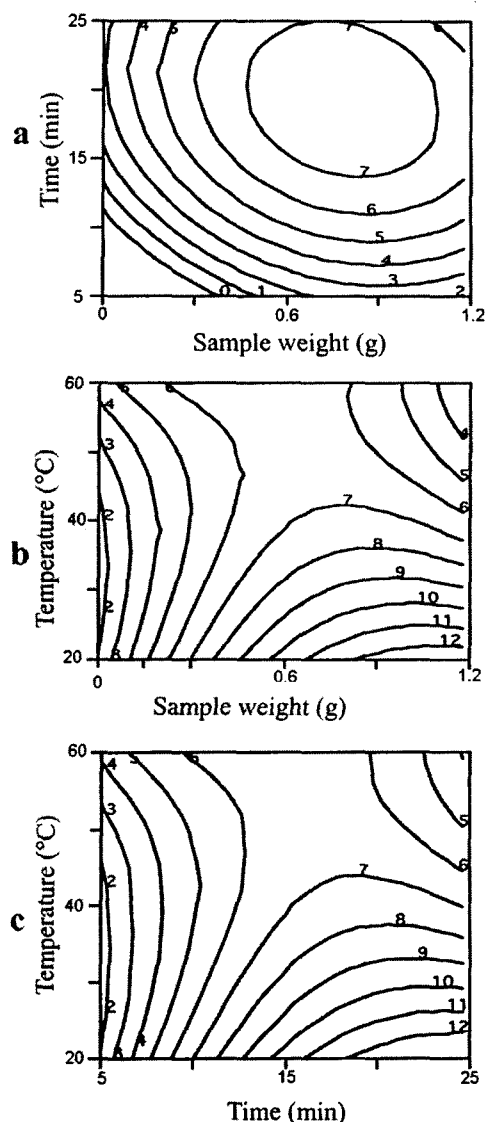


Fig. 3. Contour plots of breakthrough ratio as a function of purge time and sample weight (a), temperature and sample weight (b), and temperature and time (c) at constant temperature, time, and sample weight, respectively.

which the minimum loss of high volatile compounds and maximum recovery of low volatiles of RPSO, that is a point of maximum Y_1 and Y_3 and minimum Y_2 simultaneously within the experimental region possible. This region was obtained by superimposing the contour plots. Since sample weight did not affect Y_3 (Table 5), the optimum region was determined by superimposing the contour plots of Y_1 (not shown), Y_2 (Fig. 3-c) and Y_3 (Fig. 4) obtained at constant sample weight of 0.60 g. The same procedure was also performed to obtain other

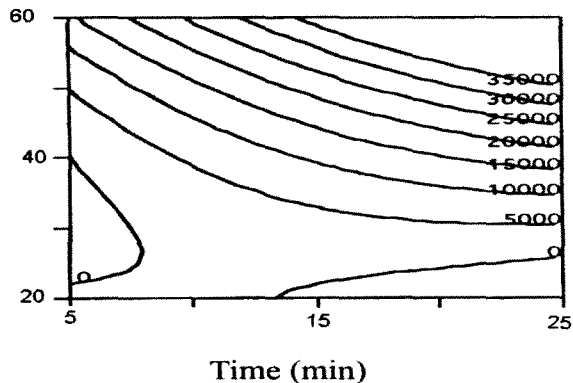


Fig. 4. Contour plot of peak area of perilla ketone as a function of purge temperature and time at constant sample weight of 0.60 g.

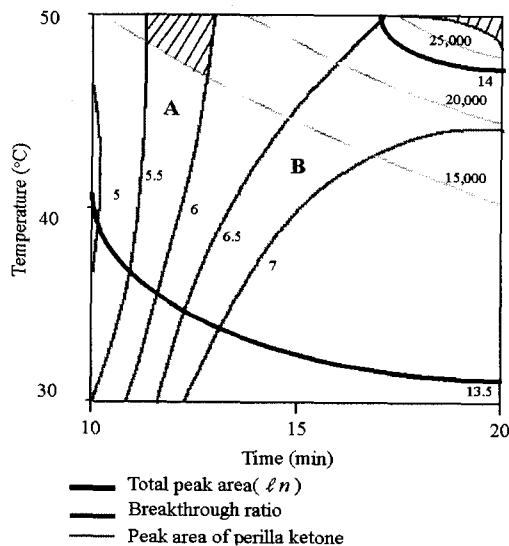


Fig. 5. Optimum regions superimposing contour plots of total peak area(ℓn), breakthrough ratio, and peak area of perilla ketone as a function of purge temperature and time at constant sample weight of 0.60 g. A and B was represents low and high breakthrough ratio region. Optimum condition was selected in low breakthrough region (shaded area).

optimum region by superimposing Y_1 and Y_2 , and Y_3 as a function of purge temperature and sample weight at constant purge times of 10, 12.5, 15, 17.5, and 20 min. The results of two procedures were very similar. As pointed out earlier, the experimental region was contained the saddle point, generally they had two characteristic areas depend on BTR. Minimum BTR region had low TPA(ℓn) and PAPK, on the other hand, low BTR region had high TPA(ℓn) and PAPK. For the rapid sampling and good separation of volatiles, the optimum purge

condition was selected in minimum BTR region at a purge temperature of 48 °C for 12 min purge time at sample weight of 0.60 g (Fig. 5), which resulted in 5.73 % BTR, 13.80 TPA(ℓn), and 15580 PAPK. This condition was satisfied the requirement of minimal loss of high volatile compounds and maximum recovery of low volatiles reasonably. Adequacy of the model was tested experimentally at the optimum purge condition. The experimental results of TPA(ℓn) were 13.71~13.84 (mean 13.78), BTR 5.54~5.88 % (mean 5.71 %), and PAPK 15414~15992 (mean 15703). These values were reasonably close to the predicted value, indicating that the model was adequate for dynamic headspace purge process of the RPSO in narrow experimental region.

요 약

Tenax-TA와 동적 headspace 향 포집방법을 이용하여 볶음들기름의 향기성분을 농축하였다. 이때 반응표면분석법을 이용하여 Tenax-TA에 결합한 향기성분의 탈착에 의한 손실을 최소화 하고 휘발성이 낮은 화합물의 포집 효율을 극대화 시키는 최적조건을 결정하였다. 독립변수로 향 포집 온도와 시간 및 시료량을 설정하였고 총 peak 면적, 탈착율, 휘발성이 가장 낮은 화합물인 perilla ketone의 면적을 종속변수로 하였다. 등고선 그림을 겹친 결과 0.6 g의 볶음들기름을 48 °C에서 12분 동안 포집시키는 것이 최적조건이었으며 Tenax-TA에서 탈착되는 주요한 휘발성 화합물은 2-propanone, 2-butanone, acetic acid 및 2-methyl propanal이었다.

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