Factors Affecting Static Headspace-Gas Chromatographic Analysis of Lipid Oxidation in Precooked Meat

Y. Wu, F. Hamouz, and M. Schnepf*

Department of Nutritional Science and Dietetics, University of Nebraska—Lincoln, Lincoln, Nebraska 68583-0806

Sample equilibration time, the addition of butylated hydroxytoluene (BHT) during sample preparation, sample particle size, and sample phase fraction (Φ_S) were evaluated as factors affecting static headspace-gas chromatographic analysis of lipid oxidation in precooked meat. The addition of BHT during sample blending shortened the sample equilibration time from more than 40 min to 15 min and led to more reproducible and precise analytical results. The combination of smaller and homogeneous sample particles with $\Phi_S = 0.5$ resulted in an increased sensitivity and reproducibility of analysis.

Keywords: *Static headspace chromatography; lipid oxidation; equilibrium time; sample phase fraction; sample particle size*

INTRODUCTION

Lipid autoxidation is one of the major causes of deterioration of cooked meat quality during storage (Asghar et al., 1988) and leads to the formation of a variety of volatile products. Degradation products such as aldehydes and short-chain hydrocarbons are volatile and impart off-flavors, including warmed-over flavor (WOF) (Tims and Watts, 1958) in cooked, stored meat (Chang and Peterson, 1977).

Gas chromatographic measurements for tracing lipid oxidation and the development of WOF in cooked meat have been developed in recent years. One of these techniques is static headspace-gas chromatography (HS-GC) which is sometimes referred to as "direct headspace sampling" or "equilibrium HS-GC" (Wyllie et al., 1978; Ettre and Kolb, 1991). In this method, the establishment of the thermodynamic equilibrium distribution of volatile constituents between a sample and the gaseous mixture above the sample, the so-called "headspace", is essential for quantification. To achieve this equilibrium, a sample is placed in a closed system such as a vial, and thermostated and equilibrated. An aliquot of the equilibrated headspace is applied to the GC system and analyzed using a packed or capillary column. Automatic or semiautomatic instrumentation for introducing the equilibrated headspace onto the GC column has been improved greatly in recent years. The static HS-GC method has been used to determine the development of WOF in cooked chicken (Ang and Young, 1989; Su et al., 1991; Ang and Lyon, 1990; Ang and Huang, 1993), precooked pork chops (Hargens-Madsen et al., 1995; Handley et al., 1996), and precooked ground beef patties (Ma-Edmonds et al., 1995). A high correlation between chemical measurement values such as thiobarbituric acid reaction substances (TBARS) values and GC peak areas, or between sensory scores for WOF and GC peak areas were demonstrated in these studies. This simple and convenient method can be used where rapid analysis is necessary and major component analysis is satisfactory. For routine analysis, static HS-GC is the method of choice (Snyder et al., 1988). Problems with this method have been sensitivity and reproducibility. These two critical analytical features may be affected by the sample equilibration time and temperature, the difficulty of detecting low molecular weight volatiles (Carlat and Schnepf, 1990; Loliger, 1990), and the potential for oxidation to occur during sample preparation and analysis. Sample particle size as well as the volume of a sample placed in the headspace vial may also have an impact on the analysis (Ang and Young, 1989; Ettre and Kolb, 1991). Ettre and Kolb (1991) have theoretically illustrated that the sample phase fraction (Φ_S) rather than the absolute sample volume affected sensitivity and reproducibility of analysis. Sample phase fraction can be defined as $\Phi_S = V_S/V_V$ (where V_S = the volume of the original sample introduced into the sample vial; V_V = the volume of the sample vial). However, few applied research studies on these factors are available.

The objective of the present research was to determine the effect of the following factors on the static HS-GC analysis of WOF in a precooked meat system: addition of BHT during sample preparation, sample equilibrium time within the headspace autosampler, sample particle size, and sample phase fraction (Φ_S). Three independent experiments were conducted in this study.

MATERIALS AND METHODS

Meat Sample Preparation. Ground beef patties were used as a model in this study. Ground beef patties (20% fat or 80% lean) were processed by mixing and grinding 8 parts of lean and 2 parts of fat together in the Animal Science Department Meat Laboratory. U.S. utility grade cow rounds were used as the lean source and 50/50 trim as the fat source. Patties were grilled on each side for approximately 3 min on a flat Hobart griddle (Model HG-4, Troy, OH) at 171 °C to an internal temperature of 71 °C and immediately refrigerated

* Corresponding author (mail, Dr. Marilynn Schnepf, 316 Ruth Leverton Hall, University of Nebraska—Lincoln, Lincoln, NE 68583-0806; voice, (402) 472-3735; fax, (402) 472-1587; e-mail, hnfm001@unlvm.unl.edu).

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at 4 °C for 3 days. Refrigerated cooked ground beef patties (2 patties for each replicate in each experiment) were trimmed and cut into small cubes (about 1 cm^3) and treated depending on the experimental design described below.

HS-GC Analysis. Volatile analysis was performed with a Tekmar 7000 headspace autosampler (Teckmar, Cincinnati, OH) attached to a 5890 Hewlett-Packard gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a 30 m × 0.320 mm inner diameter, 1.00 μm film DB-5 fused silica capillary column (J & W Co., Folsom, CA,) and a flame ionization detector (FID).

The meat sample was sealed into a 22 mL headspace vial (Tekmar No. 14-4440-240, Cincinnati, OH) and equilibrated in the headspace autosampler at a platen temperature of 120 °C (the sample temperature was 120 °C when the equilibrium was reached). After thermal equilibration, samples were mixed for 2 min during the mix mode preprogramed in the Tekmar 7000 autosampler. The vial was shaken during this mode, which may reduce the mean diffusion path length of solutes as they migrate to the gas/sample interface within the vial. Samples were then stabilized for 1 min, pressurized for 0.5 min, and equilibrated for 1 min in the autosampler. After the loop was filled and equilibrated for 1 min, the carrier gas (helium) backflushed the loop and carried the volatiles through the heated transfer line (125 °C) into the GC. The released volatiles were automatically injected and trapped on the GC column. Column temperature was 120 °C, isothermal; injector temperature was 250 °C and FID was 270 °C. Flow rate of the helium carrier gas was 40 mL/min with an inlet pressure of 10 psi and a split injection ratio of 1:50. Air, hydrogen flows were adjusted to 400 and 40 mL/min, respectively. Blank vials were run between each sample to clean the column and prevent carry-over between samples.

Pentane and hexanal, major volatile products of linoleic acid oxidation (Boyd et al., 1992), were considered to be good indicators for evaluation of the oxidative state of meat lipids since both were found to correlate very well with rancid odor or WOF in either uncooked (Seo and Joel, 1980; Loliger, 1990) or precooked meat (Shahidi et al., 1987; Lamikanra and Duppy, 1990; Ang and Lyon, 1990; St. Angelo et al., 1987). Therefore, pentane, hexanal, and total volatiles were selected as markers for the development of WOF in Experiments I, II, and III. The degree of lipid oxidation was measured as the peak area counts for pentane, hexanal, and total volatiles by a Hewlett-Packard integrator (Model 3396A, Avondale, PA). Pentane and hexanal were identified by comparing the retention times with standards obtained from Sigma Chemical Co. (St. Louis, MO).

BHT Oil Solution Preparation. Butylated hydroxytoluene (BHT, Sigma Chemical Co., St. Louis, MO) was dissolved in corn oil to obtain concentrations of 1, 5, and 10% (w/v).

Experiment I: Use of BHT and Equilibration Time Evaluation. Refrigerated cooked ground beef cubes (60 g) were used for each treatment. They were blended in a Osterizer food processing blender (John Oster Mfg. Co. Milwaukee, WI) for 30 s at low speed (controlled by using the chop mode of the blender) with or without 3 mL of 10% BHT oil solution. A 6 g blended sample was placed into a 22 mL headspace vial with a sample phase fraction of $\Phi_S = 0.5$ and equilibrated in the headspace autosampler. The optimization program of the Tekmar autosampler was used to determine the most effective equilibration time. Headspace sampling occurred after 5, 10, 15, 20, 25, 30, 35, and 40 min equilibration.

Experiment II: Levels of BHT. Four BHT oil solutions (0, 1, 5, and 10% BHT) were used in this experiment. One milliliter of oil solution was added to 20 g of cooked ground beef cubes before blending for 30 s at low speed. The amount of BHT per gram meat was equivalent to 0.0005, 0.0025, and 0.005 g, respectively, when 1%, 5%, and 10% BHT oil solution were used. For the 0% BHT treatment, 1 mL of corn oil was added to the sample before blending. An additional sample was blended without the addition of oil solution. For volatile analysis, the amount of blended sample per vial was the same as that in Experiment I. On the basis of results from Experiment I, samples were equilibrated at 120 °C for 15 min.

Experiment III: Sample Blending Time and Sample Phase Fraction (Φ_S). Cooked ground beef cubes (60 g) were blended with 10% BHT at low speed for 30 or 15 s which produced two kinds of sample particle size, small (approximately 1.5−2.5 mm^3) vs large (approximately 2−5 mm^3). Twenty-two milliliter headspace vials were used in this experiment. The vial volume was adjusted to 30, 50, and 70% by adding distilled water in the amount of 6.6, 11, and 15.4, respectively, and the high of water was then marked before the experiment. Blended samples (3.6, 6, and 8.4 g, repectively) were loosely placed into 22 mL headspace vials to occupy the vial volume under the mark line. When loosely placed a 1 g sample has a volume of 1.83 mL. Therefore, the ratio of sample to vial volume was adjusted to 30, 50, and 70%, respectively, which was equivalent to a sample phase fraction of $\Phi_S = 0.3$, 0.5, and 0.7, respectively.

Statistical Analysis. Data from Experiment I were analyzed as a 2 × 8 factorial with replicates as blocks. Data from Experiment II were analyzed as a random complete block design with five treatments and blocked by replicates. Data from Experiment III were analyzed as a 2 × 3 factorial model with replicates as blocks. Three replicates were done in each experiment. All data collected were subjected to analysis of variance (ANOVA). The General Linear Model Procedure developed by Statistical Analysis System (SAS Institute, Inc., 1989) was used to compute means, standard deviations, coefficients of variation (CVs) of each selected peak area, regression analysis, and contract analysis. Fisher's protected least significant differences (Fisher's protected LSD) and probability of differences (PDIFF) were calculated to indicate differences among mean values. Significance was accepted at a level of $P < 0.05$.

RESULTS AND DISCUSSION

Experiment I. The response of peak area to the equilibration time for all selected volatiles was different between the control and the BHT treatment (Figure 1). As the equilibration time increased, peak areas of pentane (Figure 1a), hexanal (Figure 1b), and total volatiles (Figure 1c) from the BHT treatment significantly increased at the beginning and then reached the equilibrium in 15, 10, and 15 min, respectively. Peak areas from the control increased gradually and did not reach the equilibrium within the time period evaluated. Statistical analysis revealed that the regression relationship between the peak areas of all selected volatiles and the equilibration time for the control and the BHT treatment were different ($P < 0.01$). Linear and quadratic effects of equilibration time were evident for the control while only a quadratic response was indicated for the BHT treatments. Apparently, blending with BHT facilitated a much shorter sample equilibration time for the HS-GC analysis. A critical heating procedure was involved in the HS-GC analysis, which was necessary for releasing volatile compounds from samples during equilibrium. This heating, in the presence of air, may promote sample autoxidation and lead to artificially high analytical values. When samples were prepared by blending, aeration could also lead to the further oxidation of meat samples. These may contribute to the much longer equilibration times for the sample blended without BHT. The addition of BHT during sample preparation can control this continuous autoxidation and thus shorten sample equilibration time. Results from this experiment (Figure 1) show that the equilibration time for pentane and hexanal were somewhat different (15 and 10 min), suggesting that the equilibration time for different volatile compounds may

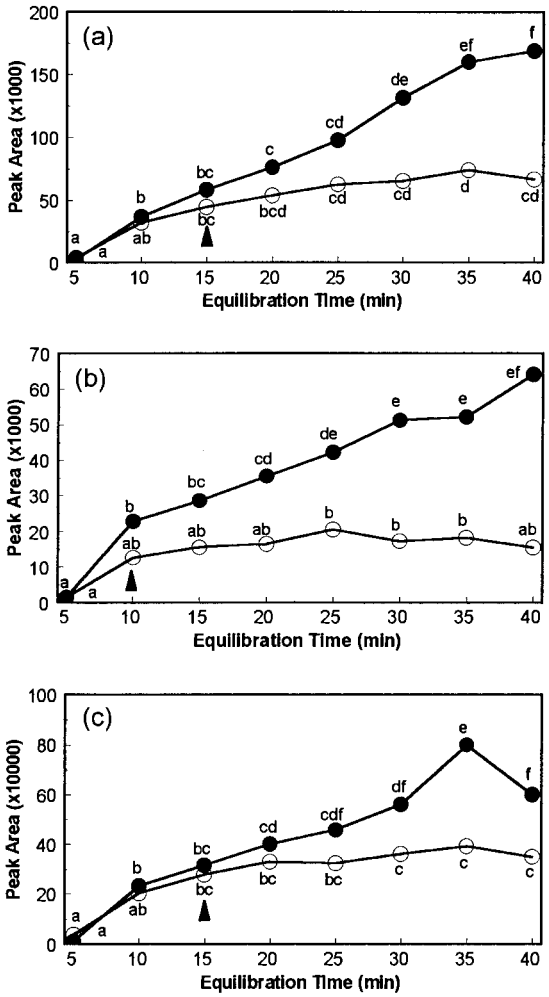

Figure 1. Sample equilibration time for the selected volatiles when two different sample preparation methods were used in Experiment I: (a) equilibration time for pentane, (b) equilibration time for hexanal, and (c) equilibration time for total volatiles. Methods: ○, BHT treatment; ●, control. The letters a−f indicate peak area means within time are significantly different ($P < 0.05$). Arrows signify the equilibration time of the BHT treatment for pentane, hexanal, and total volatiles.

be different under certain equilibration conditions. Ang and Young (1989) used a static HS-GC method to study WOF in cooked chicken meat and reported that the ideal equilibration for all solutes present in the sample under one time−temperature condition appeared to be unattainable. However, by using major volatile compounds with similar equilibrium times under certain equilibration conditions as meat oxidation markers, the static HS-GC method can be successfully applied to meat samples to follow the changes of volatile profiles (Ang and Huang, 1993) and trace the oxidative states of the meat samples. On the basis of these data obtained in this experiment, 15 min was chosen as the optimal sample equilibrium time for the analysis of WOF in precooked ground beef patties.

Experiment II. To determine the amount of BHT needed to reduce variation in analysis, different levels of BHT were compared. For all selected volatile compounds, blending with BHT treatments resulted in significantly lower means of peak area ($P < 0.05$) and much lower coefficients of variation (CVs) for these means than the control (blending alone) (Table 1). Sample autoxidation during sample preparation and sample equilibration may result in the production of

Table 1. Comparison of Peak Area Variation of Selected Volatile Compounds from Cooked Ground Beef Patties When Different Sample Preparation Methods Were Used in Experiment II

	peak area (×1000)					
	pentane		hexanal		total	
preparation method	mean	CV %[a]	mean	CV %	mean	CV %
blending	48.0[a,c]	20.3	26.7[a]	15.6	302.2[b]	20.8
blending/0% BHT[b]	33.9[b]	13.6	14.7[b]	14.7	231.7[c]	15.1
blending/1% BHT	31.2[b]	6.2	16.9[b]	3.5	226.5[c]	4.1
blending/5% BHT	31.9[b]	8.4	15.3[b]	3.2	237.9[c]	5.1
blending/10% BHT	32.7[b]	5.9	15.2[b]	2.0	236.4[c]	1.7

[a] The sample-to-sample coefficient of variation. [b] Sample + oil only. [c] Means within a column with different letters are significantly different ($P < 0.05$).

artifacts and analytical variation. High peak area means and the relatively higher CVs from the control sample for all selected volatile compounds may be the results of continuous sample oxidation. Higher CVs for hexanal from the control samples may also result from the further oxidation of hexanal itself since hexanal has the potential to react further (Loliger, 1990) and may be oxidized to hexanoic acid (Shahidi, 1994). In studies on the control of WOF in pork chops where samples were blended without adding antioxidant, Handley (1993) also reported higher CVs for the peak area means of pentane and total volatiles. In automated HS-GC, only small sample amounts are analyzed. Therefore, the reproducibility of the sample preparation often limits the precision of the results (Vieths, 1992). In this experiment, the addition of BHT might protect samples from further oxidation thus providing reproducible and precise analytical results.

Data from this experiment showed that the addition of 0% BHT (corn oil only) to the meat sample reduced the levels of the volatiles (pentane, hexanal, and total) drastically in comparison to levels of the volatiles in control samples. This may be due to the natural antioxidants in the oil. A subsequent HPLC analysis using the methodology of Ueda and Igarashi (1987) showed that the oil contained 390 μg/mL of α-tocopherol and 1074 μg/mL of γ-tocopherol, the total of 1464 μg/mL tocopherols. Hence, when 1 mL of oil was added into 20 g meat sample cubes (20% fat) the quantity of tocopherols per gram of fat in the meat sample was 0.000366 g, which was 0.0366% of fat. This concentration may be high enough to inhibit oxidation of fat in the meat sample. When crackers, pastry, and potato chips were prepared with lard treated with 0.01−0.1% tocopherol, either alone or in combination with BHA (0.01%), antioxidant-treated samples showed appreciably more resistant to rancidity than control samples (Chipault, 1962; Dugan, Jr., and Kraybill, 1956).

The addition of BHT did not show significant difference from the 0% BHT (corn oil only) treatment. This may be due to the too high concentration of antioxidants. The concentration of 1, 5, and 10% BHT, which was 0.25, 1.25, and 2.5% of fat, respectively, and the concentration of tocopherols in the corn oil together may be over the optimum concentration to reduce oxidation. Kraybill et al. (1949) reported that the antioxidant activity of BHA increased with increases in concentration up to 0.02% and remained approximately constant at higher levels.

However, BHT treatments resulted in lower CVs than either the control or 0% BHT (corn oil only) treatment,

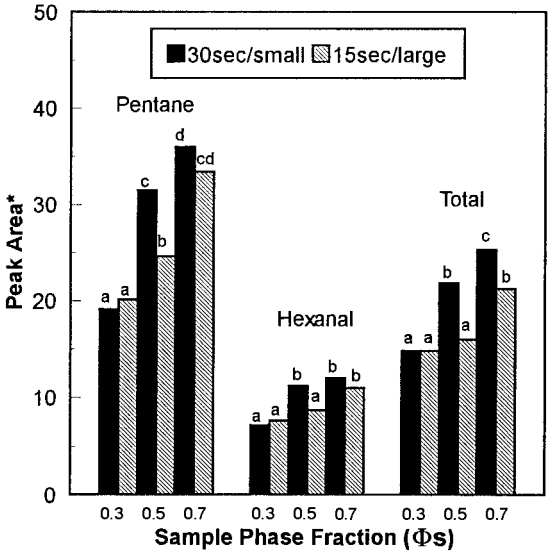

Figure 2. Effect of sample blending time/sample particle size and sample phase fraction (Φ_S) on the peak areas of selected volatile compounds from cooked ground beef patties in Experiment III. *Peak area for pentane and hexanal = data × 1000; peak area for total volatiles = data × 10 000. Letters a−d indicate peak area means within Φ_S are significantly different ($P < 0.05$).

still indicating the effectiveness of BHT in reducing variability in the analysis.

Although no significant difference in peak areas was found among BHT treatments, the treatment with 10% BHT gave rise to lower CVs of the peak area means than those with 1% and 5% BHT, indicating that the 10% BHT may be more effective in reducing variability. Pikul et al. (1983) added BHT into lipid samples in the thiobarbituric acid (TBA) assay and reported that sample autoxidation could be reduced which resulted in more precise analysis. To prevent sample oxidation and consequently low analytical reproducibility, antioxidant protection during sample preparation is also necessary for HS-GC analysis.

Results from this experiment also suggest that further studies on searching for the optimum concentration of antioxidant and using antioxidant stripped vegetable oil or other solvent as the matrix for the dispersion of BHT into the meat sample are necessary.

Experiment III. Meat sample particle size/sample blending time and the sample phase fraction (Φ_S) were hypothesized to be factors affecting the sensitivity and reproducibility of HS-GC analysis in this experiment. Statistical analysis showed that the interaction between Φ_S and the sample particle size/sample blending time was significant ($P < 0.05$), indicating that the response of peak areas to Φ_S was dependent on the sample particle size/sample blending time or vice versa. Data in Figure 2 show that the interaction was mainly a difference in the magnitude of response. When blending samples for 30 s which resulted in small and more homogeneous sample particles, peak areas increased as Φ_S increased. Shorter blending time, resulted in large and nonhomogeneous sample particles and peak areas also showed an increasing trend as Φ_S increased but with a different pattern, particularly in the peak areas of hexanal and total volatiles. However, peak area of all selected volatiles per gram sample showed a decreasing trend as Φ_S increased while for samples with large particle size this decreasing trend was significant

Table 2. Effect of Sample Blending Time/Sample Particle Size and Sample Phase Fraction (Φ_S) on the Peak Area of Selected Volatiles per Gram Sample

	peak area per gram sample		
treatments	pentane[a]	hexanal[a]	total[b]
$\Phi_S = 0.3$, 30 s/small	5.31[a c]	2.01[a]	4.11[a]
$\Phi_S = 0.3$, 15 s/large	5.37[a]	2.11[a]	4.11[a]
$\Phi_S = 0.5$, 30 s/small	5.25[a]	1.87[a]	3.63[a]
$\Phi_S = 0.5$, 15 s/large	4.11[b]	1.45[b]	2.67[b]
$\Phi_S = 0.7$, 30 s/small	4.29[ab]	1.43[b]	3.01[a]
$\Phi_S = 0.7$, 15 s/large	3.98[b]	1.31[b]	2.50[b]

[a] Peak area/g sample = data × 1000. [b] Peak area/g sample = data × 10 000. [c] Means within a column with different letters are significantly different ($P < 0.05$).

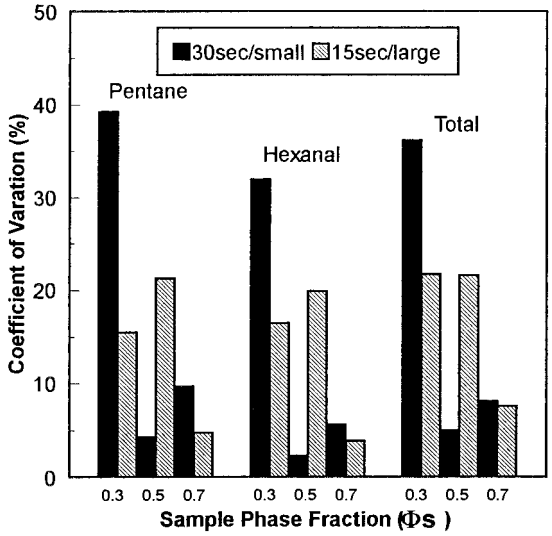

Figure 3. Effect of sample blending time/sample particle size and sample phase fraction (Φ_S) on the peak area variation of selected volatile compounds from cooked ground beef patties in Experiment III.

($P < 0.05$) as Φ_S increased from 0.3 to 0.5 and 0.7 (Table 2). Data in Figure 2 and Table 2 also showed that no significant difference ($P < 0.05$) in either peak area or peak area per gram sample was found between samples with small and large particle sizes for all selected volatiles when $\Phi_S = 0.3$. However, when $\Phi_S = 0.5$, small particle sizes resulted in higher peak areas ($P < 0.05$) and peak area per gram sample than large particle sizes for all selected volatiles. Similar results were also observed for total volatiles when $\Phi_S = 0.7$. These results suggested that both Φ_S and sample particle size influenced the release of the volatile compounds and may be related to the surface area of the sample particle and the length of the path for volatiles to be released. Ang and Young (1989) reported that finer sample particle size increased the surface area and facilitated the release of volatile compounds. Data from this experiment also suggested that the effect of sample particle size on the sensitivity of the analysis may be more important for samples with higher Φ_S than for those with low Φ_S.

The effect of sample particle size/sample blending time and Φ_S on the reproducibility of the analysis was also investigated in this experiment and the results are shown in Figure 3. With a small particle size, the sample-to-sample coefficient of variation (CVs) dramatically decreased from 39.2% to 4.3%, 31.9% to 2.3%, and 36.1 to 5% for pentane, hexanal, and total volatiles, respectively as sample phase fraction increased from Φ_S

$= 0.3$ to $\Phi_S = 0.5$. Larger sample particle size resulted in higher CVs (between15% and 22%) for all selected volatiles when $\Phi_S = 0.3$ and 0.5. With either a small or large sample particle size, however, $\Phi_S = 0.7$ resulted in much lower CVs for all selected volatiles. They were all lower than 10%. These results indicated that both sensitivity and reproducibility of the analysis were influenced by the change in Φ_S and the blending time or sample particle size. Sample homogeneity may be another factor affecting the analysis.

In addition, peak area means for pentane and hexanal had different response patterns to Φ_S as shown in Figure 2. This difference may be related to the volatility difference between these two compounds (pentane is a highly volatile compound with a boiling point of 37 °C while hexanal is less volatile with a boiling point of 134 °C), suggesting that volatility may also be an important factor to consider in the static HS-GC analysis. As shown in Figure 2, results for total volatiles from small and more homogeneous samples had the same pattern as that for pentane since the majority of the volatile compounds in the sample used were low molecular weight and highly volatile compounds, which were revealed by the GC profile obtained in this study. A similar GC profile was also obtained by Ma-Edmonds (1994) for ground beef patties. Snyder et al. (1988) reported that static HS-GC method favored the relative proportion of low molecular weight compounds.

The importance of the Φ_S and the volatility of some specific volatile compounds in the static HS-GC analysis has been addressed by Ettre and Kolb (1991). The following equation developed by Ettre and Kolb (1991) expressed that the equilibrium concentration of a specific volatile in the sample vial's headspace (c_G^*) was a function of the original volatile sample concentration (c_S), the distribution coefficient (K), which reflects the volatility of the volatile and the sample phase fraction (Φ_S):

$$c_G^* = \frac{c_S}{K + \dfrac{1 - \Phi_S}{\Phi_S}}$$

According to the equation, the influence of the Φ_S on the sensitivity and reproducibility of the analysis was significant for a highly volatile compound but negligible for a less volatile compound. The results found in this experiment showed that both Φ_S and sample volatility play important roles in static HS-GC analysis. However, the CVs for peak area means of hexanal changed as Φ_S changed in this experiment, indicating that the influence of the Φ_S on the reproducibility of the analysis was also important for a less volatile compound. These results imply that the volatility of a specific volatile compound may be influenced by other factors due to the complexity of the sample matrix. Bohnenstengel et al. (1993) concluded that in HS-GC analysis, influencing factors, such as volatility, polarity, and solubility of the analytes in the sample matrix were difficult to estimate. Data from this experiment suggested that sample particle size and sample homogeniety, which may affect the release of volatiles, may also play an important role in the analysis and need to be more specifically studied.

CONCLUSIONS

On the basis of the work reported in this paper, the following conclusions can be made in the static HS-GC analysis of lipid oxidation in precooked meat: the addition of BHT during sample preparation is necessary for a shorter sample equilibrium time (15 min) and for more reproducible and precise analytical results. Sample phase fraction value of $\Phi_S = 0.5$ as well as a smaller and homogeneous sample particle size are recommended to increase sensitivity and reproducibility of analysis. These factors should be controlled to increase reproducibility of results when using static HS-GC analysis of volatiles.

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