

Modified Direct-Sampling GLC Method to Study Warmed-Over Flavor Related Volatiles

Seung-Yee Shin-Lee and Chong-Ouk Rhee*

Department of Food Science and Nutrition, University of Missouri-Columbia, Missouri, U.S.A.

**Department of Food Science and Technology, Chonnam National University, Kwangju, Korea*

Abstract

The modified direct GLC method was evaluated for analysis of volatile compounds associated with WOF of cooked meat. This modified method was pertaining to collection of volatiles from the samples that contain quantities of water. The modification was appropriate for the studies of low molecular weight saturated aldehydes (C5-C15), unsaturated aldehydes (C5-C9), and saturated alcohols (C5-C9).

Key words: gas chromatography, WOF, flavor, volatile compounds, direct-sampling method

Introduction

Lipid hydroperoxides, the primary products of autoxidation decompose to produce a very complex mixture of low molecular weight compounds, having a detrimental effects on flavor of many different types of foods⁽¹⁾. It has been reported that short chain aldehydes, alcohols and other decomposition products are produced as the results of lipid oxidation, are responsible for warmed-over flavor (WOF) of cooked meat⁽²⁾. WOF is an off-flavor associated with cooked meat and is a cause of the major problem in cooked meat. Relatively large quantities of aldehydes were associated with the development of WOF in cooked meat^(2,3).

The use of direct sampling and trapping on Tenax GC followed by capillary gas liquid chromatography (GLC) has been described by Suzuki and Bailey⁽⁴⁾, and Larick⁽⁵⁾ to determine the flavor constituents of lamb and beef. The direct sampling method, developed by Suzuki and Bailey⁽⁴⁾, was used to collect volatile compounds from heated animal fat. This method was extremely useful when the sample contained little or no water. However, for samples containing water, such as cooked or raw meat containing approximately 50-75% water, a modified procedure was necessary.

The major objective of this investigation was to evaluate modified direct sampling GLC to analyze volatiles related with WOF in the model system. Sa-

turated and unsaturated aldehydes, alcohols were employed.

Materials and Methods

GLC Analysis

Sample collection

Tenax GC (80/100 mesh) was used to trap the volatiles. The direct sampling procedure of Suzuki and Bailey⁽⁴⁾ was modified to collect volatiles from the meat sample. Due to the relatively large quantity of water in the cooked meat sample, which can produce artifacts when direct sampling was employed, the head space sampling technique was selected for trapping volatiles in this investigation.

Volatiles were collected from the system, containing volatile compounds and water, by heating in the apparatus diagrammed in Fig. 1. The volatiles were trapped on 250 mg of Tenax GC (80-100 mesh, Altech. Chem. Inc., USA) supported by volatile-free silanized glass wool in a Pyrex tube (9 × 90 mm) connected to the top of a condenser.

For head space sample collection, the mixture of aldehydes or alcohols dissolved in pure pentane along with internal standard 2-methyl-4-octanone was added into a 250 ml round bottom flask connected to the sampling tube with water condenser, and 25 ml of distilled water was added to the flask. The flask was heated for 30 min in a heating mantle (surface temperature 140°C) with a stream of nitrogen gas to desorb the volatiles dissolved in water. Bubbling with nitrogen gas while the sample was heated helped to remove more volatiles from

Corresponding author: Seung-Yee Shin-Lee, Department of Food Science and Nutrition, College of Agriculture, University of Missouri-Columbia, Missouri 65211, U.S.A.

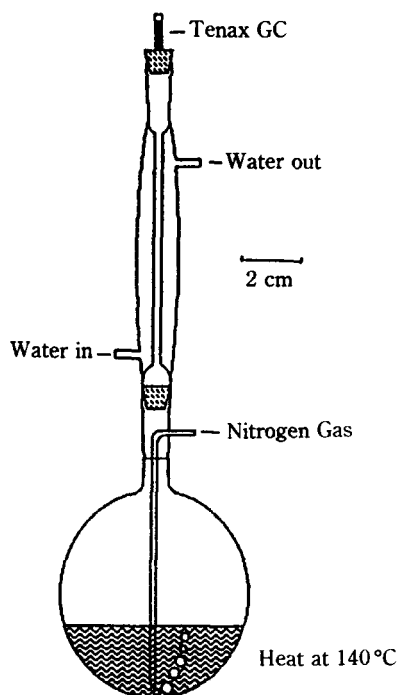


Fig. 1. Modified direct sampler used for trapping flavor volatiles from meat

the system when actual meat samples was analyzed. The flow rate of nitrogen gas was adjusted to 190 ml/min. The glass tube with Tenax GC-trapped volatile compounds was dried under nitrogen gas at the same flow rate for 5 min at room temperature.

Separation of volatiles

After collection of volatiles and drying of tube under nitrogen, the tube with the Tenax trap was immediately transferred to a direct sampler connected to a Perkin Elmer Sigma 2 gas chromatograph equipped with a flame ionization detector (FID). This sampler was connected to a modified injection port described by Suzuki and Bailey⁽⁴⁾ (Fig. 2). This was used as a sample splitter which in turn, was connected to a capillary column on the gas chromatograph. The transfer line was heated at 200°C by heating tape. The cap of the inlet port was screwed tightly on the six-port rotary valve in the "purge" position. The valve was then turned to an intermediate position between "purge" and "run", to eliminate the flow of gas through the sampler, and the quick connect Swagelok fitting was placed on the cap. The six-port rotary valve was im-

mediately turned to the "run" position and the GC temperature program was started. This manipulation was designed to prevent any possible loss in volatile components trapped in the Tenax GC tube.

A 50 meter fused silica capillary column (0.32 mm i.d.) coated with SE-54 (Hewlett Packard) was used to separate volatile components transferred to the column through the splitter injection port. The split ratio was adjusted to 1:26 with the carrier gas at 25 psi. The column temperature was maintained at 35°C for 5 min, programmed at 8°C/min to 220°C and then 2°C/min to 250°C. The column temperature (250°C) was maintained for 10 min to remove all of the remaining volatiles.

Quantitation of volatiles

Quantitation of the volatiles was accomplished by means of a Perkin Elmer laboratory integrator LCI-100. An internal standard (2-methyl 4-octanone), added to the sample before collecting the volatiles, was used to quantitate each volatile component. The response factors of the compounds, relative to the internal standard, were set at 1.0.

Precision of the system

The ultimate precision of the modified direct GLC method was determined by replicated analysis of standard aldehyde or alcohol solutions containing known amount of the internal standard, 2-methyl 4-octanone. Table 1 is the amount of standard saturated, unsaturated aldehydes and alcohols used for each run. The analysis of volatiles was followed by the method described in previous section.

Recovery of volatiles using a model system

Recovery rate of each volatile was determined by adding standard volatiles and internal standard (Table 1) in the presence of 5 g of freshly cooked meat sample. Ground pork meat was cooked in an 175°C convectional oven for 15 min until internal temperature reached to 70°C. The freshly cooked meat sample was mixed well and weighed into 250 ml round bottom flask. 25 ml of water along with 25 µl of standard aldehyde or alcohol mixture was added into the flask and the apparatus was immediately assembled. The volatiles were collected by the method described previously using the system shown in Fig. 1. Data were obtained from 6 independent trials.

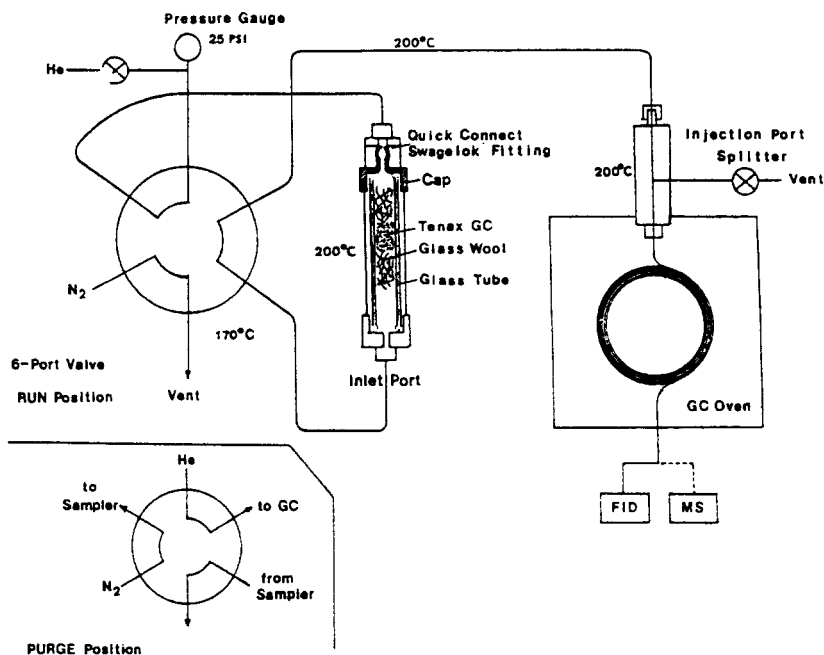


Fig. 2. Modified injection port of direct-sampling GLC analysis used by Suzuki and Bailey⁽⁴⁾

Table 1. The concentrations of standard aldehydes and alcohols

Compound	Concentration (μg)	Compound	Concentration (μg)
Saturated aldehydes			
Pentanal	0.809	Hexanal	1.020
Heptanal	0.920	Octanal	1.083
Nonanal	1.238	Decanal	1.550
Undecanal	1.473	Dodecanal	1.492
Tetradecanal	1.172	Benzaldehyde	2.032
I.S. ^{a)}	1.380		
Unsaturated aldehydes			
2-Pentenal	1.164	2-Hexenal	1.307
2-Heptenal	1.224	2-Octenal	1.342
2-Nonenal	1.404		
I.S. ^{a)}	1.380		
Saturated alcohols			
Butanol	0.890	Pentanol	1.150
Hexanol	1.300	Heptanol	1.320
Octanol	1.190	Nonanol	1.260
I.S. ^{a)}	1.580		

^{a)} I.S.: Internal standard, 2-methyl 4-octanone

Qualitative analysis of WOF by GLC

The sampling procedure used for analysis of volatiles by GLC was the same as that described above, except that actual meat samples were run to

collect volatile. Freshly cooked pork and 3 day stored pork samples were analyzed for volatiles.

Results and Discussion

Table 2 represents the precision of the GLC system when standard aldehydes were used. Standard deviation (SD) of volatile aldehydes ranged from as low as 0.003 for hexanal to 0.0818 for tetradecanal. Bailey *et al.*⁽²⁾ reported that hexanal was one of the most important volatiles produced during WOF development in cooked meat. The relative standard deviation (RSD) for each volatile aldehyde ranged from 0.308% to 6.979%. The RSDs for relatively high molecular weight aldehydes, dodecanal and tetradecanal, were higher than those for low molecular weight volatile aldehydes. Aldehydes with medium molecular weights (C6-C12) were best resolved by this modified GLC system compared with values obtained for C5, C12 and C14 aldehydes. Possibly this was due to the handling and technique involved in the desorption of volatiles from the system. Collection of volatiles involved refluxing, drying and injection of volatiles at 250°C. The average SD and RSD of the 14 aldehydes were 0.1955 and 1.63%, respectively (Table 2). The data indicate

Table 2. Standard deviation and relative standard deviation of selected volatile aldehydes analyzed by modified direct sampling GLC

Aldehydes	Concentration (μg) ^{a)}	SD ^{b)}	RSD ^{c)} (%)
Pentanal	0.809	0.032	3.960
Hexanal	1.033	0.003	0.308
Heptanal	0.910	0.004	0.467
Octanal	1.083	0.018	1.687
Nonanal	1.238	0.010	0.814
Decanal	1.550	0.010	0.645
Undecanal	1.473	0.013	0.859
Dodecanal	1.492	0.034	2.281
Tetradecanal	1.172	0.082	6.979
Benzaldehyde	2.032	0.015	0.738
2-Pentenal	1.164	0.029	2.483
2-Hexenal	1.307	0.007	0.565
2-Heptenal	1.224	0.009	0.699
2-Octenal	1.342	0.016	1.186
2-Nonenal	1.404	0.011	0.801

^{a)} Average concentration added and detected

^{b)} SD denotes standard deviation of 6 replications

^{c)} RSD denotes relative standard deviation

that the modified direct sampling GLC system was an excellent method to determine the quantities of volatile aldehydes.

Table 3 contains data obtained when standard alcohols were used. Essentially, the procedure was the same as for the study of standard aldehydes. Values were from 6 replication. RSD ranged from 2.24% to 8.79% when alcohols were injected on Tenax GC and desorbed thermally on the modified injector. The average RSD was 4.30%. There was more deviation associated with alcohols than with aldehydes (Table 2). This may have been due to the column employed. The SE-54 column is nonpolar and does not provide good separation of polar compounds. However, this technique proved to be suitable for quantitating low molecular weight alcohols.

Tables 4 and 5 are summaries of results obtained from recovery studies of volatiles associated with WOF in cooked meat.

Fifteen aldehydes were used for recovery studies. Known concentrations of these aldehydes were added into the system that containing the freshly cooked meat. These aldehydes were pentanal, 2-pentenal, hexanal, 2-hexenal, heptanal, 2-heptenal, benzaldehyde, octanal, 2-octenal, nonanal, 2-nonenal, decanal, undecanal, dodecanal, and tetra-

Table 3. Standard deviation and relative standard deviation of selected standard alcohols analyzed by modified direct sampling GLC

Compounds	Concentration (μg) ^{a)}	SD ^{b)}	RSD ^{c)} (%)
Butanol	0.89	0.08	8.79
Pentanol	1.15	0.05	4.62
Hexanol	1.30	0.06	4.30
Heptanol	1.32	0.03	2.26
Octanol	1.19	0.03	2.24
Nonanol	1.26	0.05	3.61

^{a)} Average concentration added and detected

^{b)} SD denotes standard deviation of 6 replications

^{c)} RSD denotes relative standard deviation

decanal. Data in Table 3 represent the amounts (μg) added to the system, amounts recovered, SD and RSD when aldehydes were trapped in the presence of water.

When the standard volatile aldehydes were refluxed for 30 min at 100 °C, the SD for each volatile ranged from 0.0096 to 0.0953. Percent RSD for the standard aldehydes ranged from 0.72% to 7.5%. Among 14 standard aldehydes, nonanal and heptanal were the only volatiles with RSDs greater than 5%. This indicates that the modified trapping technique is a reliable method. Since the low molecular weight aldehydes were the major volatile compounds responsible for WOF in cooked meat⁽²⁾, this procedure is suitable for studying WOF.

Except for a few high molecular weight aldehydes, most volatiles were recovered at a high percentage (Table 4). As the molecular weight of the aldehydes increased, they became more difficult to be trapped in the Tenax GC system. This phenomenon can be explained by the high molecular weight and low volatility of these compounds compared to that of low molecular weight aldehydes. Some of these high molecular weight aldehydes may have been condensed by the refluxing system used to prevent water trapping by Tenax GC.

Also, low molecular weight aldehydes were lost during trapping. This might have been due to the long heating time required to collect volatiles with the positive flow of nitrogen gas. The recovery rate of these aldehydes ranged from 85% to 100.7%. The average percent recovery for the 14 aldehydes was 98.27 (Table 4).

Standard alcohols in WOF were also studied to

Table 4. Standard deviation and recovery of selected volatile aldehydes using modified direct sampling GLC

Compound	Amount added (μg)	Amount found (μg)	SD ^{a)} (μg)	RSD ^{b)} (%)	Recovery ^{c)} (%)
Pentanal	0.809	0.752	0.014	1.876	92.89
Hexanal	1.033	1.009	0.020	1.942	97.70
Heptanal	0.910	0.932	0.048	5.162	102.45
Octanal	1.083	1.166	0.030	2.582	107.61
Nonanal	1.238	1.275	0.095	7.476	102.98
Decanal	1.550	1.513	0.035	2.301	97.57
Undecanal	1.473	1.446	0.019	1.301	98.13
Dodecanal	1.492	1.533	0.010	0.720	89.31
Tridecanal	1.172	1.005	0.047	4.698	85.75
Benzaldehyde	2.032	2.035	0.060	2.963	100.18
2-Pentenal	1.164	1.094	0.027	2.504	94.00
2-Hexenal	1.307	1.303	0.041	3.170	99.66
2-Heptenal	1.224	1.260	0.044	3.509	102.92
2-Octenal	1.343	1.366	0.042	3.020	101.74
2-Nonenal	1.404	1.344	0.048	3.557	95.75

^{a)}SD denotes standard deviation of 6 replications

^{b)}RSD denotes relative standard deviation

^{c)}Recovery values were obtained by the following equation:

$$\% \text{ Recovery} = \frac{\mu\text{g (Meat + standard)} - \mu\text{g (Meat)}}{\mu\text{g (Standard)}} \times 100$$

Table 5. Standard deviation and recovery of selected volatile alcohols using modified direct sampling GLC

Compound	Amount added (μg)	Amount found (μg)	SD ^{a)}	RSD ^{b)} (%)	Recovery ^{c)} (%)
Butanol	0.886	0.971	0.014	1.453	109.52
Pentanol	1.150	1.296	0.027	2.115	112.63
Hexanol	1.300	1.213	0.020	1.615	93.35
Heptanol	1.321	1.371	0.041	3.013	103.74
Octanol	1.188	1.360	0.048	3.538	114.44
Nonanol	1.263	1.253	0.044	3.528	99.22

^{a)}SD denotes standard deviation of 6 replications

^{b)}RSD denotes relative standard deviation

^{c)}Recovery values were obtained by the following equation:

$$\% \text{ Recovery} = \frac{\mu\text{g (Meat + Standard)} - \mu\text{g (Meat)}}{\mu\text{g (Standard)}} \times 100$$

determine the precision of these analyses. Table 5 is a summary of the results of the recovery study. These results represented values obtained when the refluxing system, described for aldehydes, was used to collect alcohols. SD for alcohols ranged from 0.0141 to 0.0442. RSD ranged from 1.45% to 3.54%. As with aldehydes, high molecular weight alcohols were difficult to be trapped on the Tenax GC, but, Tenax GC was effective for trapping C4-C9 alcohols (Table 5). The recovery values ranged from 92% to 114%.

Fig. 3 represent chromatograms of volatiles from ground and freshly cooked pork and ground-cooked pork stored for 3 days at 4 °C. More than 58 compounds were resolved as volatiles related with WOF. The major peak separated from the 3-day stored pork volatiles were hexanal, heptanal, octanal, 2,3-octandione, 1-pentanol and 2-heptenal, and 2-octenal according to retention time. This was further identified by GLC/MS (will be published). Table 6 is the list of major volatile compound identified from the cooked pork meat samples stored for

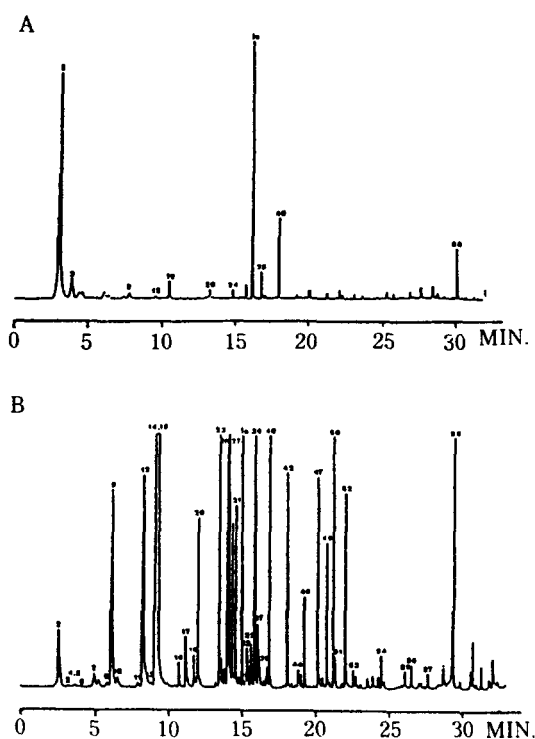


Fig. 3. Chromatograms of volatiles from ground pork freshly cooked (A) and 3-day stored at 4°C (B)

3 days at 4°C. Most of these compounds were saturated and unsaturated aldehydes with alcohols and some ketone compounds.

Many volatiles, including aldehydes and other ketones are produced during the process of lipid oxidation through the break-down of hydroperoxides as secondary products⁽⁹⁾. These volatile compounds have been successfully used by many investigators as an indication of flavor deterioration^(7,8,10).

This investigation leads to a conclusion that modified direct-sampling GLC method is an useful way to analyze volatiles produced during the development of WOF. The major volatiles related with WOF are mainly saturated and unsaturated aldehydes along with some alcohols and ketone compounds. The further study will be needed to identify the volatile compound that are responsible for this particular flavor defect-WOF. Since WOF is a subjective flavor, evaluation of WOF by sensory analysis along with an objective GLC-MS analysis will be essential.

Table 6. Volatile compounds identified in cooked and stored (4°C) pork analyzed by modified direct sampling GLC

Peak #	Retention time	Compound name
2	2.61	Pentane
4	3.65	2,3-Butandione
5	3.70	2-Methyl propanal
8	5.91	2-Methyl butanal
9	6.36	Pentanal
12	8.63	1-Pentanol
14	9.78 ^{a)}	Octane
15	9.78	Hexanal
16	11.04	2-Hexenal
17	11.80	1-Hexanol
20	12.81	Heptanal
23	13.77	2-Heptenal
25	14.10	1-Heptanol
26	14.44	1-Hepten 3-ol
27	14.67	2,3-Octandione
29	14.80	2-Pentyl furan
31	15.22	Octanal
36	16.56	2-Octenal
37	16.70	2-Octen 1-ol
40	17.73	Nonanal
42	18.56	2-Nonenal
45	19.33	Decanal
46	19.56	2,4-Nonadienal
47	20.44	2-Decenal
50	21.70	2,4-Decadienal (E,E)
52	22.56	2-Undecenal
53	23.10	Dodecanal
54	24.89	Tridecanal
56	26.56	Tetradecanal
57	28.22	Pentadecanal
58	29.80	Hexadecanal

^{a)} Resolved with hexanal peak

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揮發性 Warmed-Over Flavor의 研究를 위한 變形된 Direct Sampling GLC法

신승이 · 이종욱*

미국 미주리주립대학교 식품영양학과, *전남대학교 식품공학과

調理 肉類의 WOF와 關聯된 揮發性 成分을 分析하기 위하여 變形된 Direct GLC 方法을 利用하였다. 이 變形된 方法은 既存의 다른 方法과는 달리 水分含量이 많은 試料로부터 揮發性 成分을 分離하는데 有用하였으며 특

히 低分子量的 飽和 aldehyde(C₆-C₁₅)類, 不飽和 aldehyde(C₅-C₉)類, 그리고 飽和 alcohol(C₅-C₉)類의 研究에 적절한 것으로 判明되었다.