Development of a Dynamic Headspace GC Method for Assessing the Influence of Heating End-Point Temperature on Volatiles of Chicken Breast Meat

Catharina Y. W. Ang*

Division of Chemistry, National Center for Toxicological Research, Department of Health and Human Services, Food and Drug Administration, 3900 NCTR Road, HFT-230, Jefferson, Arkansas 72079-9502

Feng Liu

Department of Food Science and Technology, University of Georgia, Athens, Georgia 30602

Tung Sun

Eastern Laboratory, Science Division, Food Safety and Inspection Service, U.S. Department of Agriculture, Athens, Georgia 30604

A dynamic headspace GC method was developed for the analysis of volatile compounds from frozen, stored chicken breast meat previously heat-treated to various end-point temperatures (EPTs) from 60 to 80 °C. Skinless, ground chicken breast meat was heated to various internal EPTs at 5 °C intervals in a water bath and then stored frozen. Samples were analyzed by a purge and trap dynamic headspace GC system with a HP-5 50 m × 0.32 mm × 1.05 μm column (cross-linked 5% phenyl methyl silicone). Optimum parameters of the dynamic headspace system were developed. Dependent on sample treatment, approximately 50 compounds were detectable by this method. Most of these volatile compounds increased in peak areas and peak heights as the EPT increased, particularly between 60 and 70 °C. The dynamic headspace GC profile is highly correlated to EPT of previously heated chicken breast meat under controlled conditions. Major volatiles identified included pentanal, hexanal, heptanal, propanol, 2-methylpropanal, 2,3-butanedione, 2-butanone, 3-methylbutanal, 1-penten-3-ol, dimethyl disulfide, heptanone, 7-octen-4-ol, and nonanal.

Keywords: *Headspace GC; heating temperature; poultry meat; volatiles*

INTRODUCTION

Raw meat and poultry are susceptible to microbiological contamination. For marketing as fully cooked items, these products must be heat-processed to adequate temperatures to destroy all pathogens. Uncured poultry meat is required to be cooked to a minimum internal end-point temperature (EPT) of 71.1 °C (USDA-FSIS, 1985).

A number of analytical methods have been reported for assessing the adequacy of heat treatment of animal tissues. A comprehensive review of published data was reported by Townsend and Blankenship (1989). Techniques being used by the U.S. Department of Agriculture Food Safety and Inspection Service (FSIS) include the protein coagulation test for beef and pork products heat-processed to temperatures below 65 °C (USDA-FSIS, 1986a), a residual acid phosphatase activity method to determine the required internal temperature (68.8 °C) of imported hams, picnics, and luncheon meat (USDA-FSIS, 1986b), and the bovine catalase test developed by Eye (1982) for the detection of under processing (below 62.8 °C) of rare beef and canned beef. The enzymatic activity or the protein solubility becomes too low at 71.1 °C, the required temperature for poultry meat, to be determined by the official methods. It was suggested by the FSIS that new approaches be explored, such as determining the increases of some chemical compounds as EPT increases (D. Ellis, Chemistry Division, USDA-FSIS, 1990).

During cooking of muscle food, many flavor compounds are generated (Wasserman, 1979; Ramaswamy and Richards, 1982; Ang, 1991; Ang and Lyon, 1990). Gas chromatographic methods (GC) have been used in studies concerning poultry flavors (Ramaswamy and Richards, 1982; Dupuy et al., 1987) and volatile compounds as affected by heating treatments (Wu and Sheldon, 1988; Smith et al., 1987; Su et al., 1991). A rapid, static headspace GC method using a packed column was developed for studying oxidative changes of cooked chicken meat (Ang and Young, 1989) and the influence of EPT and packaging methods on volatiles from cooked broiler leg patties (Ang and Huang, 1993). Ang and Liu (1994) analyzed the headspace volatiles of cooked chicken meat using a capillary column and a static headspace GC system. High correlations between EPTs and several compounds were obtained.

Trace amounts of volatile organic compounds can be detected by dynamic headspace analysis, and it is more sensitive than the static headspace method. The increased sensitivity is probably due to the continuous purging of volatiles from samples and the concentration of the volatiles in a "trap" before analysis. Kirk (1987) and Kirk and Lehan (1989) reported the use of a dynamic headspace GC method in the analysis of dairy

* e-mail CANG @FDANT.NCTR.FDA.GOV.

† C.Y.W.A. was with the Richard B. Russell Agricultural Research Center, Agricultural Research Service, U.S. Department of Agriculture, Athens, GA, while the experimental phase of the research was completed.

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and meat products, respectively. The technique has been adopted successfully in the analysis of organic compounds in drinking water (EPA, 1988).

The objective of the present study was to develop a dynamic headspace GC method for analyzing volatiles from chicken meat cooked to EPT of 60–80 °C and stored frozen. The correlations between volatile compounds and EPTs were to be established. The study aimed to evaluate the dynamic headspace profiles of previously cooked poultry meat as influenced by cooking EPT.

MATERIALS AND METHODS

Materials and Heating Treatments. Fresh (unfrozen) broiler breast halves were purchased from a local supermarket. Skin, bones, and fatty tissues were removed and discarded. The muscle tissue was ground once through a 0.45 cm plate using a meat grinder. Fifteen grams of the ground tissue was weighed into a glass test tube (180 × 250 mm). A type T thermocouple (copper/constantan, type PT-6, Physitemp Instruments Inc., Clifton, NJ) was inserted into the geometric center of the sample in the test tube. All tubes were kept at −20 °C overnight.

Sample tubes were tempered in an ice–water bath until the internal temperature reached between 0 and 1 °C. Heating treatments were performed in a water bath (Precision Scientific Group, Chicago, IL) at 1 °C above the target EPTs of samples. Two tubes were cooked at a time. The target EPTs were 60, 65, 70, 75, and 80 °C. The temperatures were monitored by connecting the thermocouples to a digital thermometer (Model BAT-12, Physitemp Instruments). Immediately after the specific target temperature was reached, the tubes were cooled to below 15 °C in an ice–water bath to prevent any further heating effect.

Sample Preparation. The cooked tissue was blended in a small food processor (Model HC-20, Handy Chopper, Black & Decker (U.S.) Inc., Shelton, CT) for 30 s under nitrogen. The container with sample was flushed with a stream of nitrogen prior to blending. An aliquot of 0.5 g of prepared muscle sample was weighed into a test tube (19 mm × 150 mm). The tube was flushed with nitrogen for 10 s, tightly covered with Parafilm (American Can Co., Greenwich, CT), and stored at −20 °C overnight. (The reason for frozen storage of samples prior to analysis was to simulate industrial processing practice for fully cooked meat products. The majority of commercially processed, cooked meat, other than canned products, are immediately stored frozen. Subsequently, samples of frozen, stored products can be tested for EPT compliance in a regulatory laboratory.) Each tube was analyzed in duplicate. The entire experiment was repeated once.

Dynamic Headspace GC Method. A Tekmar 2000 purge and trap concentrator (Tekmar Co., Cincinnati, OH) was interfaced with the GC (Sigma 2000 gas chromatograph, Perkin-Elmer Corp., Norwalk, CT) for the dynamic headspace analysis. The analytical column used was a fused silica capillary column (50 m × 0.32 mm × 1.05 μm film thickness) with cross-linked 5% phenyl methyl silicone (HP-5, Hewlett-Packard, Kennett Square, PA). The injector and the flame ionization detector temperatures were 110 and 290 °C, respectively. The GC response was monitored by an integrator (LCI-100, Perkin-Elmer).

Volatiles from breast tissue were analyzed according to two methods. In method 1, the sample tube was allowed to thaw at room temperature for 30 min followed by conditioning in a water bath for 4 min at 35 °C before the purging step. The sample was purged at 35 °C for 10 min with helium. The volatiles were concentrated on a Tenax trap (Anspec Co., Ann Arbor, MI) at room temperature (25 °C). Subsequently, the trap was heated to 175 °C and compounds were thermally desorbed for 4 min at 180 °C from the trap onto the capillary column. The column temperature was held at 45 °C for 4 min during desorption, programmed to 90 °C at 5 °C/min, to 170 °C at 8 °C/min, and to 240 °C at 15 °C/min and held at 240 °C for 17 min. In method 2, the conditioning temperature and purge temperature were both at 50 °C and the column was held at 0 °C for 4 min during desorption, programmed to 4 °C at 1 °C/min, to 190 °C at 6 °C/min, and to 250 °C at 20 °C/min and held at 250 °C for 8 min. Other conditions were the same as in method 1. The linear velocity of carrier gas (helium) was 21 cm/s.

Individual standard solutions as well as a mixture of all standards were prepared in hexadecane in small vials flushed with nitrogen and then stored frozen (−20 °C). All chemicals were of the highest purity grade purchased from Aldrich Chemical Co. (Milwaukee, WI).

Table 1. Reproducibility of Dynamic Headspace GC Analysis (Method 1) of Standard Solution (n = 8)

compound	level (ppb)	peak height unit (mv) $\bar{X}$[a]	SD[b]	CV%[c]
butyraldehyde	10.0	8.94	0.45	5.07
2-methylbutanal	2.5	1.84	0.27	14.74
1-penten-3-ol	5.0	17.18	1.42	8.24
pentanal	10.0	56.27	5.09	9.05
trans-2-pentenal	0.5	3.25	0.31	9.52
hexanal	100.0	580.48	47.94	8.26
2-heptanone	0.5	13.01	0.72	5.56
heptanal	6.0	45.75	2.77	6.05
trans-2-heptanal	0.5	6.81	0.48	7.08
benzaldehyde	3.0	44.23	2.53	5.72
1-octen-3-ol	3.0	74.98	3.09	4.13
octanal	2.0	41.66	2.65	6.37
trans-2-octanal	0.5	7.08	0.55	7.78
nonanal	5.0	107.47	11.92	11.09
trans-2-nonenal	0.5	4.61	0.49	10.68
decylaldehyde	2.0	36.85	8.21	22.29
trans,trans-2,4-nonadienal	0.5	3.25	0.31	9.52

[a] $\bar{X}$ = mean. [b] SD, standard deviation. [c] CV, coefficient of variation; CV% = (SD/$\bar{X}$) × 100%.

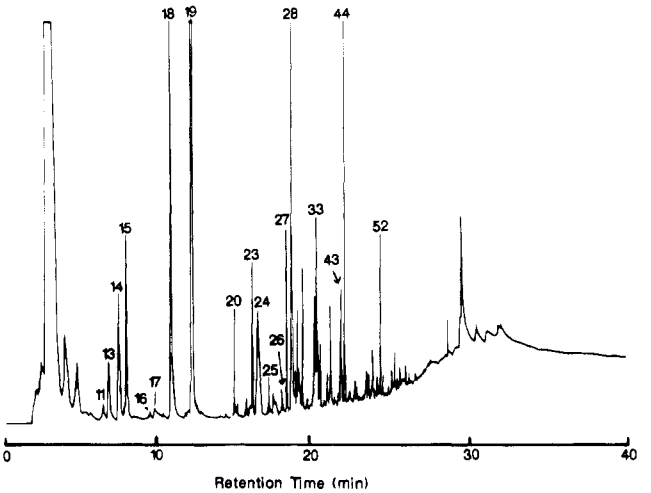

Figure 1. Dynamic headspace GC chromatograms of volatiles from chicken breast meat previously heated to end-point temperature of 70 °C (method 1, conditions are described in the text).

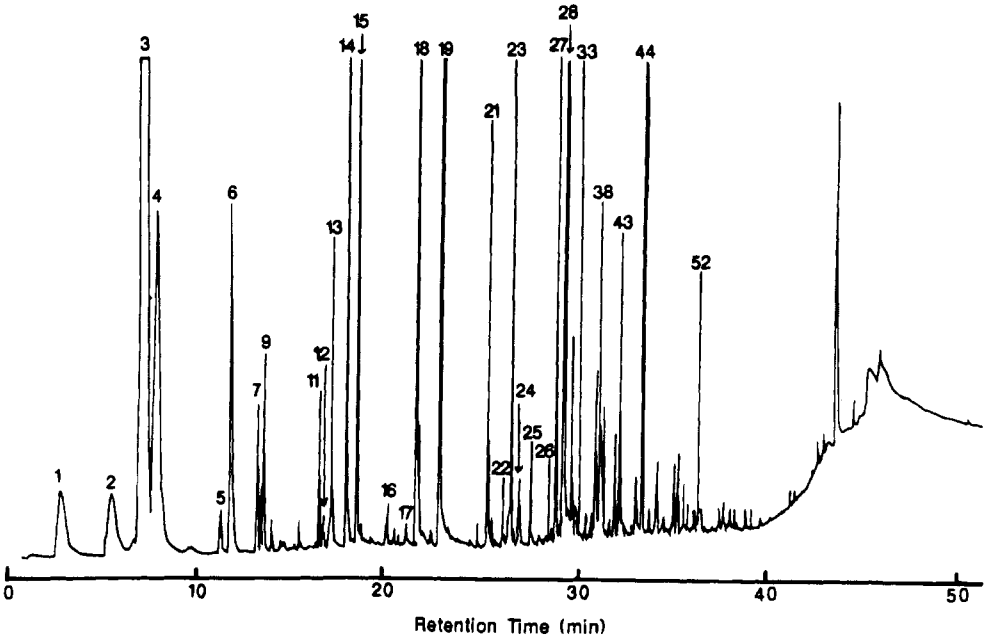

Figure 2. Dynamic headspace GC chromatograms of volatiles from chicken breast meat previously heated to end-point temperature of 70 °C (method 2, conditions are described in the text).

The reproducibility of the dynamic headspace technique was tested daily by analyzing a mixture of standard solutions at levels from 0.5 to 100 ppb, comparable to those levels from the chicken samples. Five microliters of stock standard solution in hexadecane was added to 5 mL of water for the dynamic headspace analysis. This trace amount (5 μL) of hexadecane was dispersed in water with sparging. Substituting ethanol or methanol for hexadecane was not satisfactory because these alcohols showed large peaks and masked other volatile compounds. For qualitative identification, major peaks were first identified by matching the retention times of the unknown peaks with those of the reference standards and by adding standards to the sample before the analysis. The identity of these compounds was further confirmed by the GC–MS system (Finnigan MAT SSQ 710, San Jose, CA) interfaced with the dynamic headspace concentrator. For quantitative estimation, the peak heights of a sample were calculated against the peak heights of reference standards with known amounts.

Statistical Analysis. The Statistical Analysis System (SAS, 1985) was used for all computations including the mean, standard deviation, and coefficient of variation (CV). The General Linear Model (GLM) procedure was used to determine the regression curves of each volatile over EPT, and the Correlation procedures were used for searching the correlations between peak height and EPT. *R*-square and Stepwise procedures were used for establishing the multiple correlations and prediction models.

RESULTS AND DISCUSSION

Development of Dynamic Headspace GC Method. Optimum parameters for analysis of volatiles by dynamic GC methods were developed regarding factors such as the sample weight, sample conditioning time and temperature, and purge time and temperature. Sample weights at 0.05, 0.1, 0.2, 0.4, 0.5, and 1.0 g were tested using method 1 with the purge temperature at 35 °C. When the sample size was less than 0.5 g, the peak area responded to the sample weight linearly for most peaks. However, when the sample weight increased to 1 g, the levels of volatiles released from the sample were not in linear proportion to the weight. Thus, it was concluded that a sample weight of 0.5 g would be used for all tests.

Table 2. Volatile Compounds of Chicken Breast Meat Previously Heated to 70 °C, Analyzed by Dynamic Headspace GC Methods and Identified by GC/MS

peak no.	retention time (min) method 1	retention time (min) method 2	compound
1		3.884	H_2S, CO_2
2		5.441	methanol
3		7.980	propanal and acetone
4		8.696	2-propanol and thiobismethane
5		11.982	2-methylpropanal
6		12.428	1-propanol
7		13.866	2,3-butanedione
8		14.056	hexane
9		14.161	2-butanone
11	7.374	16.905	3-methylbutanal
13	7.714	17.500	2-methylbutanal
14	8.338	18.329	1-penten-3-ol
15	8.796	18.862	pentanal
18	11.558	21.806	dimethyl disulfide
19	12.820	23.042	hexanal
21	15.462	25.505	1-hexanol
22		26.298	2-heptanone
23	16.566	26.678	heptanal
26		28.565	1-heptanol
27	18.686	28.886	benzaldehyde
28	18.981	29.221	7-octen-4-ol
33	20.526	30.014	octanal
38		30.966	*d*-limonene
43	22.069	32.885	undecane
44	22.270	33.092	nonanal
52	24.472	35.549	dodecane

The effect of sample conditioning time (preheating time in water bath prior to purge) on the results of peak areas was tested. The water bath temperature was preset at 50 °C, and the time periods tested were 4, 9, and 14 min. As the conditioning time in the water bath increased, the peak areas and peak heights also increased. However, the increases did not vary linearly with time. To avoid any undesirable changes, a 4-min conditioning time was selected for all further tests.

Table 3. Reproducibility of Volatile Compound Data from Chicken Breast Muscle Heated to Three End-Point Temperatures As Analyzed by Dynamic Headspace GC Method

peak no.	retention time (min)	65 °C $\bar{X}$[a]	65 °C CV%[b]	70 °C $\bar{X}$	70 °C CV%	75 °C $\bar{X}$	75 °C CV%	av CV%
		Method 1, Peak Height Unit (mv), (n = 8)						
14	8.357	5.60	44.1	11.4	21.5	11.1	12.1	25.9
15	8.805	9.06	43.8	22.2	22.6	29.4	13.2	26.5
18	11.538	27.6	16.8	36.6	39.0	32.5	14.8	23.5
19	12.805	203	25.3	430	28.7	396	13.2	22.4
23	16.550	15.4	20.1	19.4	23.7	20.1	9.06	17.6
28	18.964	32.5	21.2	47.8	16.7	44.2	8.01	15.3
32	19.638	10.7	23.0	16.6	19.8	17.1	6.85	16.8
44	22.756	42.8	13.5	51.2	17.7	52.8	9.43	13.5
		Method 2, Peak Height Unit (mv), (n = 4)						
14	18.270	21.6	12.6	64.2	10.8	56.9	4.23	9.21
15	18.794	43.9	6.25	141	9.13	155	3.59	6.32
18	21.765	79.1	8.89	194	11.8	153	8.62	9.77
19	22.961	540	13.5	150 × 10	8.13	134 × 10	3.07	8.23
22	26.250	2.76	1.68	5.27	15.0	4.47	1.04	5.91
23	26.630	29.9	7.59	62.6	6.85	56.2	7.16	7.20
28	29.196	48.3	10.9	125	10.2	116	9.66	10.2
33	29.981	17.5	6.68	44.1	4.82	43.5	4.87	5.46
44	33.058	6.26	9.58	115	4.62	111	6.86	7.02

[a] $\bar{X}$, mean. [b] CV%, coefficient of variation, %.

Purge times were tested for 5, 10, 15, and 20 min at a purge temperature of 50 °C and a flow rate of 40 mL/min. As the purge time increased from 5 to 10 min, most peak areas increased. However, further extension of the purging time reduced some peak areas and peak heights, due to the fact that some low boiling point compounds were also removed from the Tenax column during the purging step as a breakthrough phenomenon. Thus, the 10-min purge time was selected for further experiments.

The effect of purge temperature on the response of peak area was monitored. Two purge temperatures, 35 and 50 °C, were tested. For either column program, purge temperature at 50 °C revealed more peaks (approximately 20–30 more peaks depending on the EPT) and significantly larger peaks than those obtained from the purge temperature of 35 °C, possibly due to the impact of purge temperature on vapor pressure. At the same purge temperature, peaks separated by column program 2 were consistently larger than those obtained by column program 1, possibly due to the effect of subambient column trapping. The resolution of column program 2 was also better than that of column program 1. Since column program 2 with 50 °C purge temperature revealed the largest number of peaks having the largest peak areas, this method (designated dynamic headspace GC method 2) was chosen for further tests. On the other hand, the present study was focused on developing a headspace GC method to determine the cooking EPT regardless of the number of peaks detectable by the method. Thus, column program 1 with 35 °C purge temperature (dynamic headspace method 1) was also tested. If suitable, this procedure (method 1) would have the advantage of not using a subambient temperature oven and a low purge temperature to avoid any heating-related effect during the purge period.

The reproducibility of the chromatograms was checked daily with standard reference solutions. The results of eight repeated analyses of standard solutions by the dynamic headspace GC method 1 indicated that variations of most of the peaks were within 15% (Table 1).

Volatile Profiles by Dynamic Headspace GC. Typical chromatograms obtained by dynamic headspace analysis of heated chicken breast meat are shown in Figures 1 and 2 for methods 1 and 2, respectively. Since there were many peaks observed in the chromatograms by dynamic headspace GC methods, the minimum integratable area was set at 500. Thus, only those peaks with areas larger than this level were integrated.

For cooked chicken breast meat analyzed by method 1, there were 26–40 peaks integrated for all temperatures and the mean numbers of peaks were 34, 33, 34, 33, and 31 for EPT 60, 65, 70, 75, and 80 °C, respectively. The chicken breast meat analyzed by method 2 had 41–59 peaks integrated and the mean numbers of peaks were 46, 45, 50, 53, and 51 for EPT 60, 65, 70, 75, and 80 °C, respectively. Thus, the number of peaks integrated was irrelevant to EPT.

The compounds identified and confirmed by GC–MS method are shown in Table 2. By calibrations with standards, the quantities were estimated to be between 0.46 (*trans*-2-nonenal) and 22.1 ppb (pentanal) for most volatiles and 227 ppb for hexanal for chicken breast meat heated to 70 °C. Since volatiles were not completely released from the sample, the amount estimated

Table 4. Characteristics of Volatile Peaks from Heated Chicken Breast Muscle As Analyzed by Dynamic Headspace GC Methods

group no.	relationship with end-point temperature	peak no. method 1	peak no. method 2
1	linear; increased from 60 to 80 °C	11, 15	5, 7, 9, 15, 29, 41
2	quadratic; increased from 60 to 70 °C or from 60 to 75 °C, stable or slightly decreased from 70 or 75 to 80 °C	14, 18, 19, 23, 28, 32, 44	1, 3, 6, 11, 14, 16, 18, 19, 21, 22, 23, 28, 33, 42, 44
3	cubic; peaks were at their height at 70 °C, then decreased significantly with temperature	13	13, 27, 28, 37, 38
4	no significant correlations (P > 0.05)	21, 31, 33, 34, 52	2, 4, 16, 17, 31, 40, 43, 46

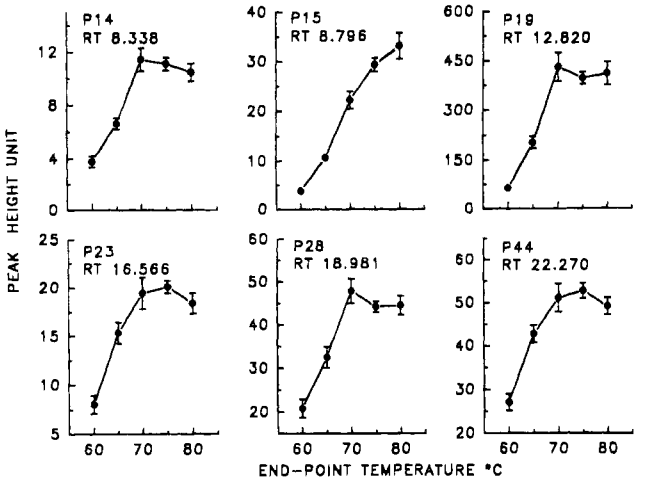

Figure 3. Influence of end-point temperature on selected volatiles of chicken breast meat as analyzed by the dynamic headspace GC method, method 1 (mean and standard errors; $n = 8$).

was only the amount of released volatiles from the sample under the experimental conditions.

The precision of dynamic headspace analysis of chicken breast meat was evaluated (Table 3). The range of average CV of selected peaks obtained by method 1 was from 13.5% to 26.5% for samples heated to 65, 70, and 75 °C. For method 2, the range of average CV was 5.46–10.2%. Results from method 2 showed fewer variations than method 1 results.

Peak areas from dynamic headspace GC chromatograms of chicken breast meat heated to various temperatures were analyzed. Peaks were categorized into four groups based on their changes with the EPT (Table 4). The characteristics of these four groups were similar to those obtained by the static headspace GC method (Ang and Liu, 1994). The most important peaks from the chromatograms for the purpose of estimating EPT were in groups 1 and 2. The changes of selected peaks with EPT are shown in Figures 3 and 4 as determined by dynamic headspace methods 1 and 2, respectively. Simple correlations between peak heights and EPT of selected volatiles are shown in Table 5. A number of compounds were highly correlated with the temperature. Peaks with significant correlations with EPT were tested for multiple correlations as described under Materials and Methods. Examples of the best fit prediction models with their multiple correlation coefficients (R^2) are as follows:

Method 1: $R^2 = 0.9229$

$$\text{EPT} = 57.035 + 1.708\ \text{P11} - 0.993\ \text{P14} + 0.751\ \text{P15} - 0.026\ \text{P19} + 0.334\ \text{P28}$$

Method 2: $R^2 = 0.9806$

$$\text{EPT} = 58.891 + 1.503\ \text{P1} + 0.072\ \text{P2} - 0.854\ \text{P9} + 1.118\ \text{P22}$$

Method 2, with a higher correlation coefficient, was a better choice than method 1. Method 2 developed in this study provided a substantial improvement over the static headspace GC method (Ang and Liu, 1994). More compounds were detected and higher correlations between volatiles and EPTs were obtained by method 2 dynamic headspace GC than by static headspace GC.

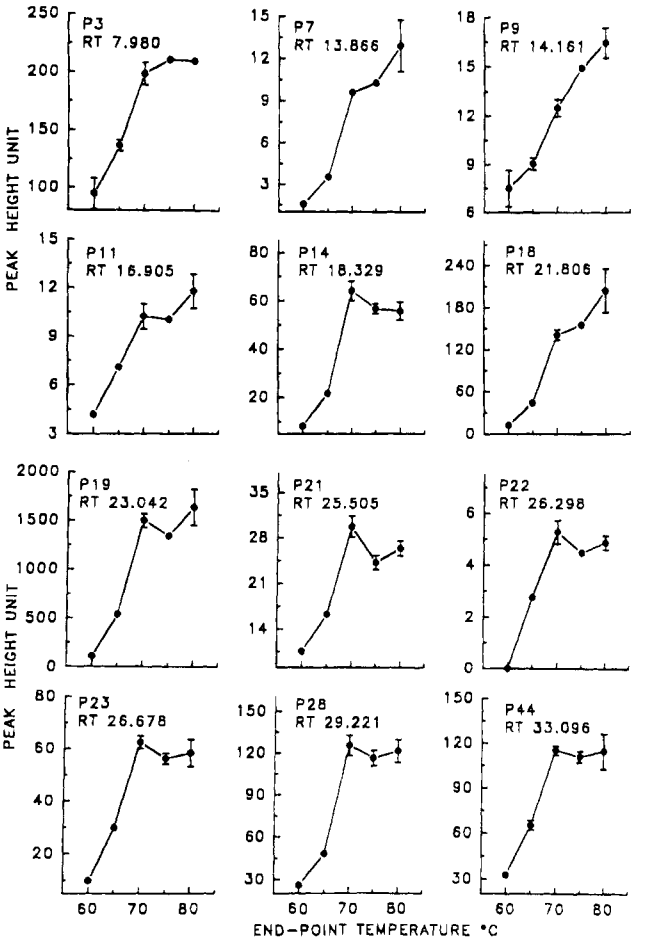

Figure 4. Influence of end-point temperature on selected volatiles of chicken breast meat as analyzed by the dynamic headspace GC method, method 2 (mean and standard errors; $n = 8$).

Table 5. Correlation Coefficients (r)[a] between End-Point Temperature and Peak Height of Selected Volatiles from Heated Broiler Breast Meat As Analyzed by Dynamic Headspace GC Method

method 1 ($n = 20$)	method 2 ($n = 18$)
peak 11, 0.7581; peak 14, 0.7456; peak 15, 0.9249; peak 19, 0.7867; peak 28, 0.7892; peak 32, 0.8067; peak 44, 0.8564	peak 1, 0.8793; peak 3, 0.9068; peak 5, 0.9418; peak 6, 0.8327; peak 7, 0.9418; peak 9, 0.9374; peak 11, 0.9147; peak 14, 0.8478; peak 15, 0.9461; peak 18, 0.8336; peak 19, 0.9101; peak 21, 0.8077; peak 22, 0.8467; peak 23, 0.8665; peak 28, 0.8786; peak 33, 0.9119; peak 41, 0.9049; peak 44, 0.8867

[a] All coefficients are significant ($P < 0.01$).

Variability of Dynamic Headspace Analysis. Headspace analysis of solid samples, especially for those nonhomogeneous matrices such as meat tissues, presents a great challenge to the analyst. Previous work of Kirk (1987) and Kirk and Lehan (1989) only presented qualitative data of flavor compounds from beef, pork, and dairy products. No information was given regarding the quantity of each compound, the history of the samples, or the reproducibility of their analytical methods. The present study investigated not only the quantitative aspect but also the effect of heating treatment on the dynamic headspace GC profile. The chicken meat samples were precisely cooked to target EPTs and stored frozen prior to analysis. Results indicated the changes of dynamic headspace volatiles as a function of cooking EPT.

Quantitative determination of the released volatiles by using capillary column and dynamic headspace analysis is a greater challenge than the qualitative analysis. The levels of many of these volatiles may also be affected by factors other than temperature, such as air, time of exposure during preparation, and storage conditions. Even though the present study was not intended to extract 100% of each volatile, precise control is still required during sample preparation and analytical steps. The length of time for each step was precisely controlled, including preheating preparation, during heating and storage. The regression equations developed in this study for chicken breast meat should not be applied directly to other products with different compositions, formulations, and/or processing conditions.

The dynamic headspace GC method using a capillary column may not be a practical method for routine quality control procedure in all food-processing plants because variables may not be easily controlled in such an environment. However, in a well-equipped laboratory with well-trained operators and when product formulations and processing techniques are unchanged, the dynamic headspace sampling and analytical techniques of either method 1 or method 2 may be used as a potential means for determining the extent of heat treatment of previously cooked meat products.

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