Automated Dynamic Headspace/GC-MS Analyses Affects the Repeatability of Volatiles in Irradiated Turkey

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K. C. Nam, Postdoctoral research associate
J. Cordray, Professor, Animal Science Department
D. U. Ahn, Professor, Animal Science Department

Summary and Implications

The amounts of dimethyl disulfide and dimethyl trisulfide decreased as sample holding time in an autosampler (4 °C) before purge increased, whereas those of aldehdyes increased as holding time increased due to lipid oxidation. Helium flush of sample vials before sample loading on an autosampler retarded lipid oxidation and minimized the changes of sulfur volatiles in raw meat, but was not enough to prevent oxidative changes in cooked meat. Although DH/GC-MS is a powerful method for automatic analysis of volatiles in meat samples, the number of samples that can be loaded in an autosampler at a time should be limited within the range that can permit reasonable repeatabilities for target volatile compounds.

Introduction

Irradiation generates sulfur-containing volatiles, which play a major role in overall odor and flavor of many foods due to their high volatility and low odor threshold. The isolation and quantification of sulfur volatiles are difficult because they are highly volatile and susceptible to change under aerobic conditions. Thus, sample handling, exposure to environment, and sample holding time before purge are critical for the repeatability of sulfur-compounds. Although the DH/GC-MS is a convenient and reproducible method for volatile analysis, dramatic changes in the amounts of certain volatiles were observed when holding time increased.

The objective was to evaluate the effects of sample holding time before purge on the volatiles of irradiated raw and cooked turkey breast meat using an automated DH/GC-MS.

Materials and Methods

Turkey breast patties were individually vacuum-packaged in high oxygen-barrier bags and irradiated. The vacuum-packaged turkey patties were irradiated at 2.5 kGy using a Linear Acceleration Facility. After irradiation, samples were stored in a 4 °C cold room. For cooked meat, samples were cooked in bag in a water bath (90 °C), cooled with cold tap water, and then used for analysis.

Sample (3 g for raw meat and 2 g for cooked meat) was analyzed at a 40-min interval. Therefore, each sample (1^{st} to 8^{th} vial) has different holding time (0, 40, 80, 120, 160, 200, 240, and 280 min) in the autosampler (4 °C) before purge. Volatiles were Analysis by Automated Purge-and-Trap/GC-MS. 2-Thiobarbituric acid-reactive substances (TBARS). Lipid oxidation was determined using a TBARS method. Data were analyzed using the generalized linear model procedure of SAS software (*18*).

Results and Discussion

Irradiated raw turkey breast had various volatile compounds including hydrocarbons, alcohols, carbonyls (ketones and aldehydes), and sulfur-containing volatiles. Carbonyls and sulfur volatiles accounted for 26% and 56%, respectively, of the total volatiles in irradiated raw turkey breast, and dimethyl sulfide, dimethyl disulfide and 2-propanone were among the most predominant volatile compounds. The amounts of hydrocarbons, alcohols, and carbonyls of irradiated raw turkey were not much affected by holding time but sulfur compounds were significantly affected by sample holding time (**Figure 1**). The amount of dimethyl sulfide was significantly reduced after 120 min of sample holding in the 4 °C autosampler (4^{th} sample) and the amount at 280 min (8^{th} sample) was about 80% of the first sample. The production of dimethyl disulfide and dimethyl trisulfide was more sensitive to holding time in autosampler before purge than that of dimethyl sulfide. The amounts of dimethyl disulfide and dimethyl trisulfide began to decrease even from the second sample (40-min holding), and only 44% and 24% of first sample, respectively, were detected after 40 min of holding time. After 280 min of holding, the amount of dimethyl disulfide detected was only 9% of 0 min, and dimethyl trisulfide was not detected after 120 min of sample holding. Therefore, analysis of sulfur volatiles using an automated DH/GC-MS is highly dependent upon sample holding time, and their amounts can be severely underestimated if sample holding time is long. If sulfur compounds are the volatiles of interest, therefore, raw meat samples should be loaded one at a time.

In general, sulfur volatiles have high volatility and low odor threshold. The instabilities of sulfur volatiles during sample holding may be attributed to their high reactivity with headspace oxygen. Thus, to know the effect of headspace oxygen on the sulfur-volatiles, samples were flushed with helium before loading. The volatile composition of irradiated raw turkey breast flushed with helium was not much different from that of nonflushed sample at 0 holding time. Helium flushed samples had lower amounts of hexanal than nonflushed samples ($p < 0.05$), but helium flush was effective in improving the repeatability of sulfur volatiles during

sample holding time (**Figure 1**). The amounts of sulfur volatiles in helium-flushed samples were lower than those of non-flushed from 0 min indicating that some of the sulfur compounds were lost during helium flush. The amounts of dimethyl disulfide and dimethyl trisulfide decreased during the holding time as in non-flushed samples but to a less degree. After 280 min of sample holding, the amount of dimethyl disulfide left in the helium-flushed sample was 29% (non-flushed, 9%) of 0 min sample and dimethyl trisulfide was not detected after 200 min of sample holding (after 120 min in non-flushed sample). Therefore, helium flushing improved the stability of sulfur volatiles, but was not enough to prevent changes during sample holding time.

Irradiated cooked turkey breast produced greater number of aldehydes than irradiated raw meat. Hexanal was the most predominant among the aldehydes. The amounts of hydrocarbons, alcohols, and ketones were not much affected by holding time, while the amounts of sulfur volatiles and a few aldehydes changed significantly during holding time. The amounts of sulfur volatiles decreased as holding time increased and the changes were the most distinct in dimethyl disulfide and dimethyl trisulfide (**Figure 2**). However, the rates of decrease were not as steep as those in irradiated raw meat. The amount of dimethyl disulfide and dimethyl trisulfide detected after 280 min of sample holding was 58% and 60% of 0 min sample, respectively.

The changes of aldehydes in irradiated cooked meat were totally different from those of sulfur compounds (**Figure 3**). The amounts of propanal, pentanal, hexanal, and heptanal in cooked meat increased proportionally with holding time. The amounts of hexanal and pentanal, the representative lipid oxidation products, rapidly increased with holding time indicating that lipid oxidation in cooked meat is developing rapidly during sample holding due to oxygen in the headspace and posed a huge problem in the accuracy of the volatile analysis.

The stability of dimethyl disulfide and dimethyl trisulfide in helium-flushed cooked meat was more stable than nonflushed samples, but the absolute amounts of those volatiles were lower than those of non-flushed (**Figure 2**). The amounts of aldehydes in helium-flushed cooked meat also increased in proportion to the holding time, but their rates were slower than those in non-flushed ones (**Figure 3**). This indicated that helium flush alone was not effective enough to prevent lipid oxidation and changes in sulfur compounds in irradiated cooked meat during the holding time. When irradiated cooked meat is analyzed using a DH/GC-MS, therefore, aldehydes and sulfur volatiles are either overestimated or underestimated depending on the sample holding time in an autosampler. Therefore, the number of samples loaded in an autosampler at a time should be limited to one, or more efficient methods to prevent the changes of volatiles are needed if more than one sample is going to be loaded.

According to TBARS values (**Table 1**), irradiated cooked turkey breast developed lipid oxidation rapidly during the holding time, whereas lipid oxidation in irradiated raw meat was slow. Significant increase in TBARS values in irradiated cooked meat was observed after 200 to 240 min of sample holding, and was highly correlated with the amounts of hexanal detected by DH/GC-MS. The increases of propanal, pentanal, and hexanal in cooked meat were distinct during the short stay in an autosampler and they were more sensitive to the holding time than the TBARS values (**Figure 3**). This indicated that the amounts of aldehydes could be more informative for lipid oxidation in irradiated cooked meat than TBARS values.

When a few standard sulfur compounds were analyzed instead of irradiated meat samples, they were stable during the holding time (**Figure 4),** indicating that the sulfur compounds produced by irradiation in turkey meat were much less stable than standards probably due to the presence of many other reactive compounds from meat. Irradiation generates free radicals such as hydrated electrons, superoxide anions, and hydroxyl radicals. Therefore, the sulfur volatiles produced from meat are exposed to many other volatile compounds, aqueous electrons and free radicals and have greater chances to react with other compounds than standard alone.

Conclusion

Automated DH/GC-MS is a useful system to analyze volatile compounds in meat samples continuously. As the waiting time for a sample in an autosampler before purge increased, however, the profile and amounts of volatiles can be changed due to the instability of sulfur compounds and development of lipid oxidation. Therefore, the number of samples loaded in an autosampler at a time should to be limited depending on sample type and targeted volatile species. If multiple samples are going to be loaded, standard curves for the changes in certain volatiles over sample holding time may be needed for accurate measurements of sulfur compounds and aldehydes in irradiated raw and cooked meats.

Figure 1. The production of sulfur volatiles from irradiated raw turkey breast during sample holding time in an autosampler (4 °C) before purge

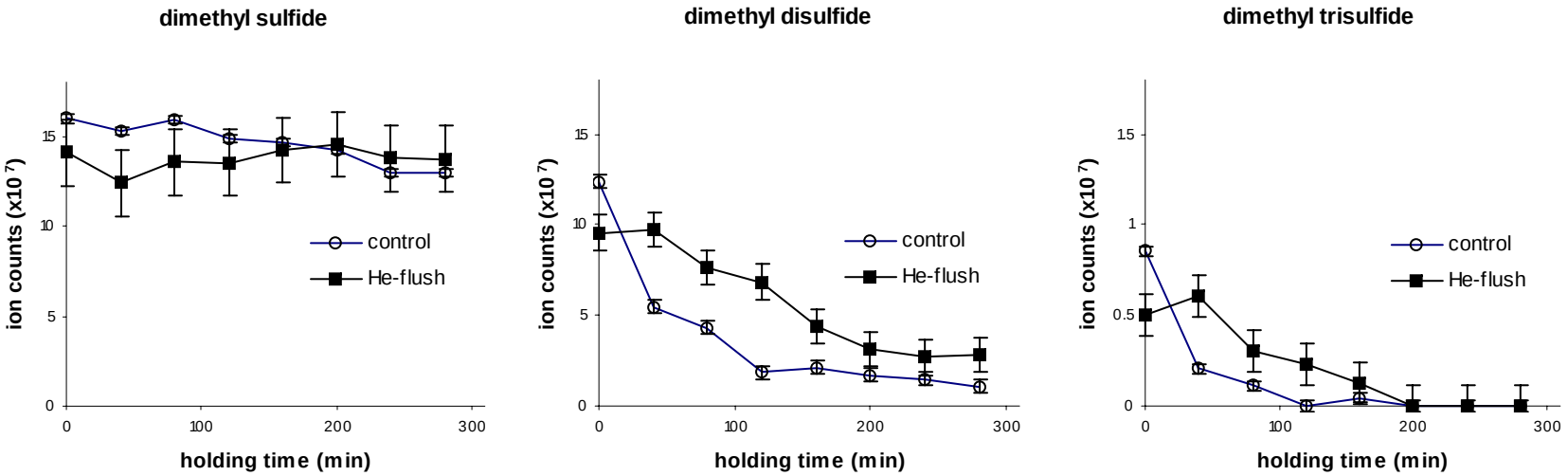

Figure 2. The production of sulfur compounds from irradiated cooked turkey breast during sample holding time in an autosampler (4 °C) before purge

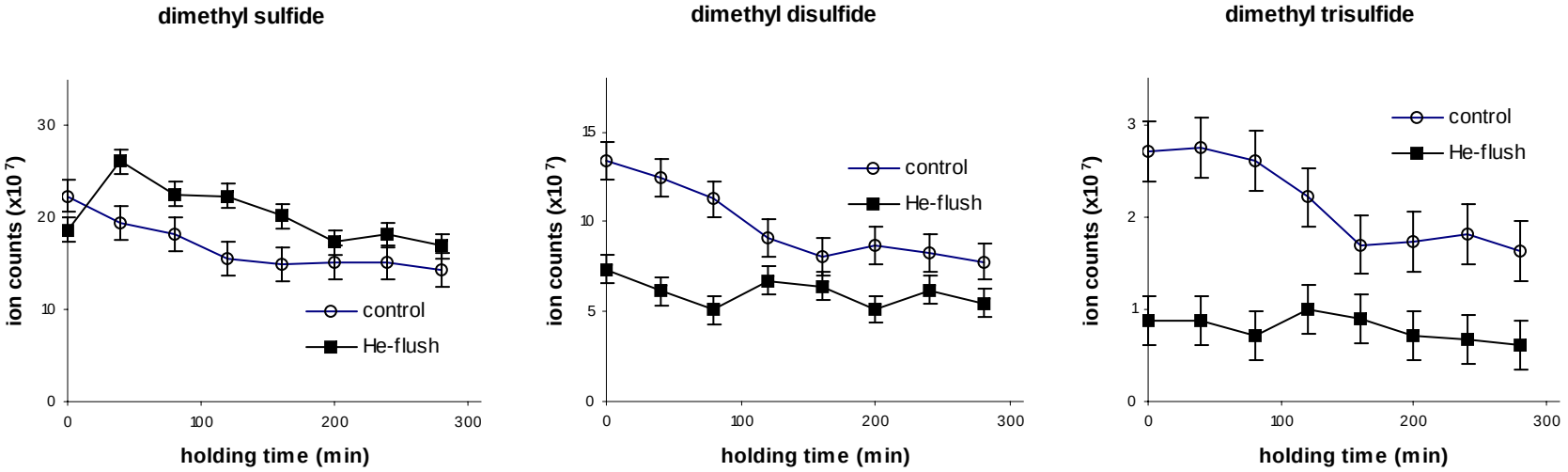

Figure 3. The production of aldehdyes from irradiated cooked turkey breast during sample holding time in an autosampler (4 °C) before purge

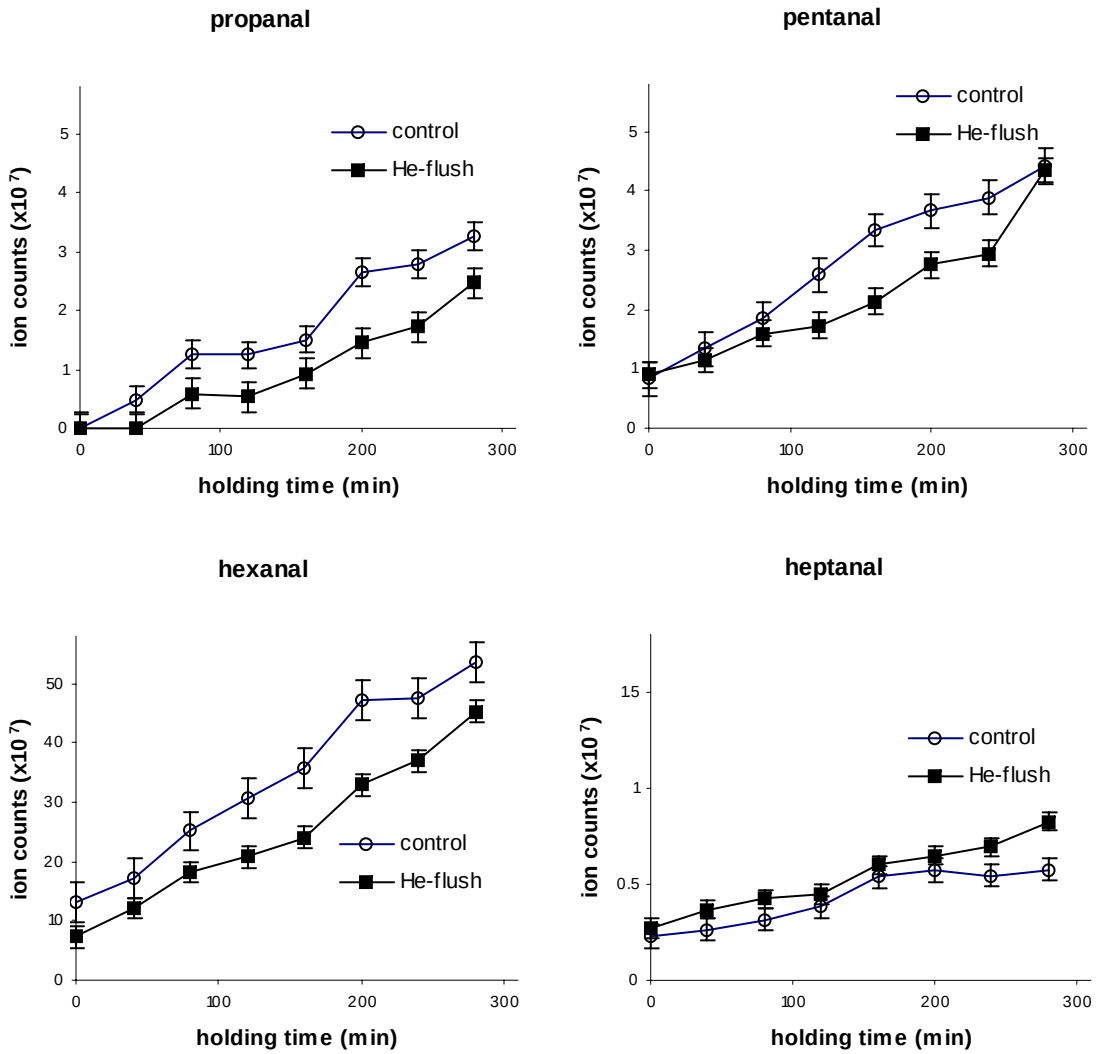

Table 1. **TBARS Values of Irradiated Raw and Cooked Turkey Breast Affected by Helium Flush during Holding Time in an Autosampler (4°C) before Purge**[a]

	Sample holding time (min)								
	0	40	80	120	160	200	240	280	SEM
raw meat									
control	0.31	0.34	0.33	0.35	0.35	0.34	0.33	0.37	0.02
He flush	0.30	0.35	0.34	0.34	0.35	0.38	0.35	0.33	0.02
SEM	0.01	0.02	0.03	0.02	0.02	0.01	0.02	0.01	
cooked meat									
control	1.49d	1.62cd	1.51d	1.74cd	1.77cdx	1.86bcx	2.01b	2.39ax	0.07
He flush	1.42b	1.43b	1.48b	1.61b	1.66by	1.65by	1.89a	1.87ay	0.06
SEM	0.03	0.11	0.05	0.06	0.02	0.03	0.05	0.08	

[a] $n = 4$, mg MDA/kg meat. Different letters (a-d) within a row indicate significant differences ($P < 0.05$). Different letters (x, y) in a column indicate significant difference ($P < 0.05$). SEM, standard error of the means.

Figure 4. The repeatability of standard sulfur-volatile compounds without and with helium flushing during the sample holding time in autosampler (4°C) before purge