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Volatile Sulfur Compounds in Irradiated Precooked Turkey Breast Analyzed with Pulsed Flame Photometric Detection

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Ionizing radiation is an effective processing technology for pathogen inactivation on various foods. However, the generation of off-odor is a concern for some irradiated meats. This study was conducted to investigate volatile sulfur compounds of precooked ready-to-eat turkey breast as functions of radiation dose and subsequent storage. Precooked turkey breast was exposed to 0, 1, 2, 3, 4, and 5 kGy of gamma radiation and stored for 14 days at 5 °C. Volatile sulfur compounds were extracted using solid phase microextraction (SPME), followed by gas chromatographic separation and pulsed flame photometric detection. Irradiation dramatically increased concentrations of hydrogen sulfide, sulfur dioxide, methanethiol, and dimethyl disulfide. The rate of increase was higher at low doses (0−2 kGy) than at higher doses of 3−5 kGy. Carbon disulfide was the only volatile sulfur compound that was reduced by irradiation. Concentrations of all volatile sulfur compounds decreased in both irradiated and nonirradiated samples stored at 5 °C.

KEYWORDS: Irradiation; sulfur compounds; off-odor; turkey breast; PFPD

INTRODUCTION

Food-borne pathogens cause numerous illnesses, hospitalizations, and even deaths every year in the United States. The FDA has proposed application of processing or other treatments that achieve a 5-log reduction in the number of harmful microbes. Irradiation has been demonstrated to be a very effective processing technology for pathogen inactivation for both raw and cooked meats (*1*, *2*). To achieve a 5-log reduction of most common pathogens on meats, doses of 2.45−3.6 kGy are required (*1*, *3*). However, meat may develop an unpleasant odor when irradiated (*4*, *5*). The off-odor has been called "irradiation" odor, and has been described as "wet dog", "sulfide", "metallic", "wet grain", "goaty", or "burnt" (*4*). The cause of the off-flavor and off-odor is primarily due to the formation of volatile compounds. The major volatile compounds associated with irradiated meats are generated from lipids and include hydrocarbons, alcohols, and aldehydes (*6*, *7*). However, a typical irradiation odor was not observed when a lipid fraction of meat was irradiated, although irradiation of the aqueous-soluble phase of meat resulted in the typical off-odor (*8*), indicating that compounds derived from protein are involved in the development of the off-odor. Reineccius (*4*), however, noted that irradiation odor was not observed when either a lipid or a protein fraction of meat was irradiated separately. Nevertheless, volatile sulfur compounds have been suggested to be the main source of the off-odor (*8*−*10*), but the nature of these sulfur compounds was not completely clear, partially due to difficulties associated with the detection of volatile sulfur compounds.

The concentrations of volatile sulfur compounds in various foods are low, but most sulfur compounds have very low odor thresholds (in the ppb range) and possess a pungent, unpleasant odor at sufficient concentrations (*11*). Because of the very low amounts of volatile sulfur compounds in foods, selective and accurate detection of these compounds has been a challenge for researchers (*12*). Traditional analysis of sulfur compounds has employed dynamic headspace sampling followed by gas chromatographic (GC) separation and detection. There are several conventional detectors available for sulfur detection such as mass spectrometry (MS), flame photometric detection (FPD), sulfur chemiluminescence detection (SCD), and atomic emission detection (AED) (*12*). However, all of these detectors have some drawbacks, cost, lack of sensitivity, stability, and reliability are among the major weaknesses (*12*).

Pulsed flame photometric detection (PFPD) is a relatively new technique and offers several advantages over the other detectors for sulfur compounds, such as high sensitivity, selectivity, and repeatability (*13*). PFPD has been used for analyzing volatile sulfur compounds in beer (*14*) and for several other applications (*15*, *16*). In the present study, we extracted volatile sulfur compounds from precooked ready-to-eat turkey breast using solid-phase microextraction (SPME) (*17*). SPME has been used for extraction of sulfur compounds in several foods (*18*, *19*). The sulfur compounds were then separated and detected using a GC/PFPD.

Irradiation odor may be a greater concern for ready-to-eat meat than raw meat because ready-to-eats are consumed without further cooking. Turkey muscle is the most sensitive animal protein food to irradiation in terms of off-flavor development

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(*20*). The objective of this study was to investigate the effect of irradiation and subsequent storage on the concentrations of sulfur compounds at radiation doses used for pathogen inactivation in precooked turkey breast.

MATERIALS AND METHODS

Samples. Sliced, cooked, ready-to-eat turkey breast was purchased from a local supermarket. The meat was then diced, and 7.5-g samples were placed into 40-mL glass vials. The vials were tightly sealed using Teflon-lined septa and screw caps. The vials containing the turkey breast were stored at 5 °C overnight, and then irradiated at doses of 0 (control), 1, 2, 3, 4, and 5 kGy gamma radiation at 5 °C. For the study of irradiation dose effect, volatile sulfur compounds were analyzed immediately after irradiation. To study the effects of storage, the turkey breast was stored in the sealed vials at 5 °C. Volatile sulfur compounds from samples irradiated at 0, 2, and 4 kGy were measured after 7 and 14 days of storage.

SPME. The vials containing the 7.5 g of turkey breast were incubated at 30 °C for 20 min on the Multi-block heater (Lab Line Instruments, Melrose Park, IL) before the SPME fiber was inserted into the vials. An 85-μm carboxen/poly(dimethylsiloxane) (Supelco, Bellefonte, PA) was the SPME fiber used in this study. We tested several types of fibers and found this fiber to be the most effective for extracting sulfur compounds, which is in agreement with a previous report (*21*). Only one fiber was used in the entire experiment to eliminate variation caused by individual fibers. The fiber was conditioned at 250 °C for 5 min by inserting the fiber in the GC injection port before each extraction and used immediately to prevent contamination. To extract volatile sulfur compounds from the samples, the stainless steel needle in which the fiber was housed was pierced through the vial septum. Once inside the vial, the fiber was pushed out of the housing and exposed to the headspace above the meat sample for 15 min at 30 °C. Then the fiber was pulled back into the housing, and the SPME device was removed from the vial and immediately inserted into the injection port of a GC system for thermal desorption at 250 °C for 5 min.

Standard Curves in Water. First, sulfur compounds were dissolved in ethanol to obtain approximately 1 mg mL^{-1} stock solutions. Different concentrations of sulfur compounds were then prepared by diluting the stock solutions in 7.5 mL of water in 40-mL vials. Microliter syringes (Hamilton, Reno, NV) were used to dispense the sulfur compounds and to prepare dilutions. For compounds that are gases at ambient temperature, dilution was made first in dilution bottles, and then the diluted compounds in air were further diluted into 40-mL vials containing 7.5 mL water. The vials were vigorously shaken for 30 min using a VXR-S10 shaker at a setting of 400 cycles/min (Tekmar, Columbus, OH) before being incubated at 30 °C for 20 min. Sulfur compounds were then extracted using the SPME fiber.

Separation and Identification. Volatile compounds were separated with Agilent 6890 GC (Agilent Technologies, Palo Alto, CA) equipped with a DB-5MS column (30 m × 0.32 mm i.d., 1 μm film thickness) operated in the splitless mode, and detected using the PFPD (OI Analytical, College Station, TX) at optimized sulfur detection conditions. A specially designed 0.8-mm SPME injector liner (Supelco, Bellefonte, PA) was used to prevent peak broadening. The temperature of the GC oven was set at 40 °C for 3 min, increased to 150 °C at 20 °C min^{-1}, then increased to 250 °C at 50 °C min^{-1}, and held for 2 min at the final temperature. Helium was the carrier gas with a linear flow rate of 20.7 cm s^{-1}. The PFPD was operated in the sulfur mode with a 2-mm combuster sleeve and a B-12 filter. Voltage of the R1925 photomultiplier tube was 600 V. The signal collection gate was from 6 to 24 ms, and trigger level was 150 mV. Ignitor current was 2.8 A. A model 5380 detector controller was used to collect signal and manually control gas flow to the PFPD. The gas flow rate to the detector was set to be 11.5 mL min^{-1} for hydrogen, 10 mL min^{-1} for air 1, and 15 mL min^{-1} for air 2. Fine adjustment of gas flow rate was made to optimize the sulfur signal. The detector signal and operation of the detector were facilitated by the use of WinPulse software package (OI Analytical, College Station, TX).

Compounds were identified by comparison of retention times of the sample compounds with those of standards. Standards were purchased from Aldrich (Milwaukee, WI). These sulfur standards included hydrogen sulfide, sulfur dioxide, dimethyl disulfide, methyl sulfide, carbon disulfide, bis(methylthio) methane, dimethyl trisulfide, methanethiol, ethanethiol, (methylthio) acetic acid, methional, (dimethylthio) methane, ethyl methyl sulfide, and ethyl sulfide.

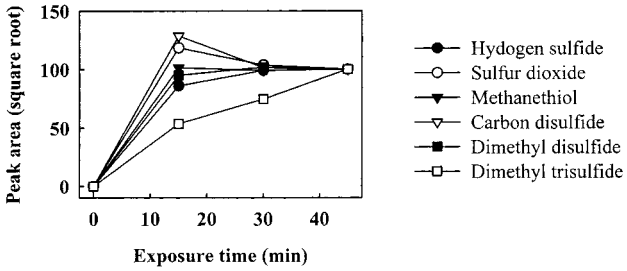

Figure 1. Effect of SPME fiber exposure time on the extraction efficiency of sulfur compounds in cooked turkey breast. Turkey breast sealed in 40-mL vials was irradiated with 5 kGy gamma rays at 5 °C. The vials containing 7.5 g of meat were incubated at 30 °C for 20 min, and then the SPME fiber was inserted into the headspace of vials. The exposure times of fiber were 0, 15, 30, and 45 min. Sulfur compounds were then analyzed. The data were the averages of two replicates. The square root of peak area was normalized to 45 min.

Irradiation and Dosimetry. Irradiation was conducted using a self-contained, Lockheed Corporation ^{137}Cs gamma radiation source (Marietta, GA). The unit has 23 ^{137}Cs pencils placed in an annular array around a 63.5-cm-high stainless steel cylindrical chamber with a 22.9-cm internal diameter. The source strength at the time of this study was ca. 109 000 Ci (4.0 PBq) with a dose rate of 0.1 kGy min^{-1}. The dose rate was established using alanine transfer dosimeters from the National Institute of Standards and Technology (Gaithersburg, MD). Corrections for source decay were made monthly. Variations in radiation dose absorption were minimized by placing the samples within a uniform area of the radiation field and by irradiating them within a polypropylene container (4-mm wall) to absorb Compton electrons. The same geometry was employed for sample irradiation during the entire study. Routine dosimetry was performed using 5-mm-diameter alanine dosimeters (Bruker Instruments, Rjeomstettem, Germany), and the free-radical signal was measured using a Bruker EMS 104 EPR analyzer (*22*). The dosimeters were placed into 1.2-mL cryogenic vials (Nalgene, Rochester, NY), and the cryogenic vials were taped onto the tubes containing the 7.5-g samples prior to irradiation. Temperature in the radiation chamber was maintained by introducing the gas phase from liquid nitrogen into the radiation chamber.

Statistical Analysis. There were four replicate vials per dose. Data were subjected to statistical analysis using the SAS version 7 procedure (SAS Institute, Cary, NC). Mean separation was achieved by the least significant difference (LSD) analysis of the general linear model. In some of the figures, mean standard deviations are presented. Differences between means that exceed the standard deviations were always significant when analyzed using the LSD procedure at $P < 0.05$ level.

RESULTS AND DISCUSSION

In a test of the uniformity of the Carboxen/PDMS fibers for sulfur extraction, we found that there was considerable variation among the same type of fibers (data not shown). Therefore, only one fiber was used for the entire study to reduce experimental errors. We also studied the optimum exposure time of the SPME fiber in the headspace of vials. Our results showed that an exposure time of 15 min was sufficient for most volatile sulfur compounds of precooked turkey breast (**Figure 1**). For compounds such as dimethyl trisulfide, which has a higher boiling point, a longer exposure time was required. In the present experiment, we chose the exposure time of 15 min to accommodate the number of samples we had to analyze within 1 day for the dose effect experiment.

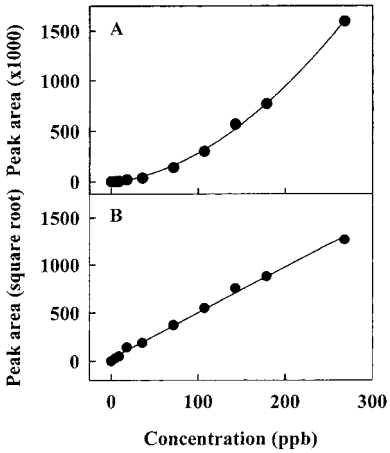

Figure 2. Calibration curve for methanethiol in water. Sulfur signal was expressed as either peak area (A) or the square root of peak area (B).

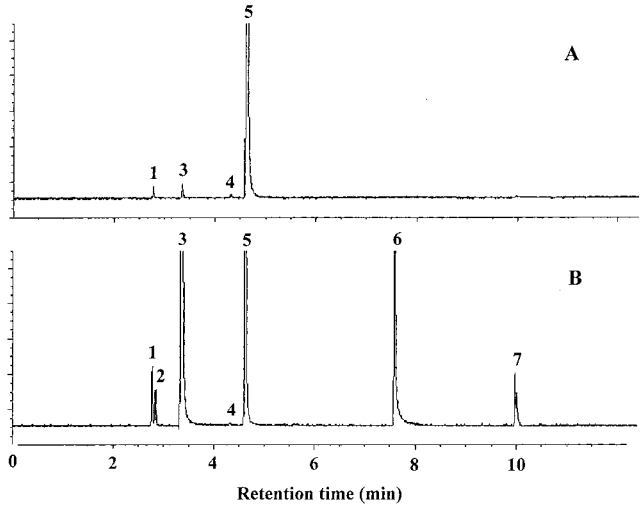

Figure 3. Sulfur compound profiles of nonirradiated turkey breast (A) and those irradiated at 3 kGy (B). The *Y*-scale was arbitrary units in the same scale.

Figure 2 shows the standard curve of methanethiol in water. The plot became linear after ($R^2 = 0.999$) conversion to square root of peak area. Our results support earlier claims that the sulfur response of the PFPD is purely quadratic (*13*, *23*). Ideally, standards would have been prepared in sample matrix, but we were unable to establish standard curves for sulfur compounds using turkey breast because of the complexity of the sample matrix. Standard curves have been prepared in liquid foods and beer (*14*, *21*), but the extraction efficiency of SMPE fiber was shown to be influenced by the amount of salt and ethanol in wine, and different kinds of beers. Owing to the impurity of some of the standards and the instability of sulfur compounds, as well as their reaction with the SPME fiber coating, we could not accurately establish standard curves for all of the compounds even in water. Therefore, in this report, the amounts of the sulfur compounds are presented and discussed as square root of peak area. We believe that this expression is suitable for our objective which was to study the effect of irradiation dose and storage on the *relative* concentration of volatile sulfur compounds.

The profile of sulfur compounds differed substantially between nonirradiated and irradiated samples (**Figure 3**). There were six major peaks in irradiated samples, whereas only 3 peaks appeared in the nonirradiated samples. We tried to identify these compounds using a GC−MS, but because of the low concentrations of the sulfur compounds and the presence of other non-

Table 1. Volatile Sulfur Compounds Identified in Cooked Turkey Breast

peak	retention time (min)	compound
1	2.78	hydrogen sulfide
2	2.84	sulfur dioxide
3	3.34	methanethiol
4	4.30	methyl sulfide
5	4.61	carbon disulfide
6	7.58	dimethyl disulfide
7	9.98	dimethyl trisulfide/(methylthio) acetic acid

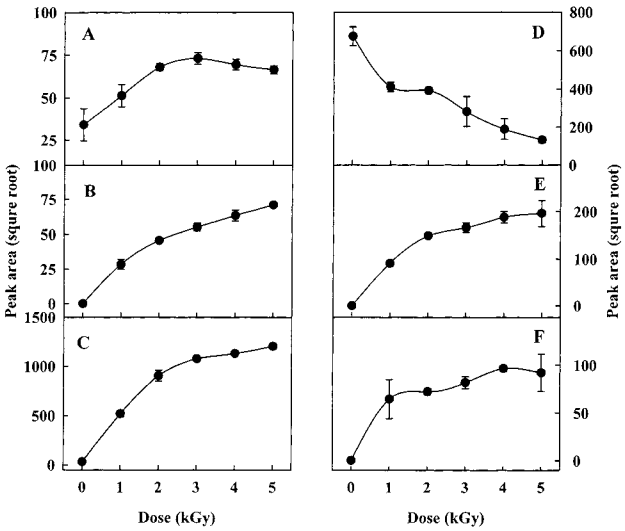

Figure 4. Effect of irradiation dose on the concentrations of hydrogen sulfide (A), sulfur dioxide (B), methanethiol (C), carbon disulfide (D), dimethyl disulfide (E), and dimethyl trisulfide/(methanethio) acetic acid (F). Concentrations of sulfur compounds were expressed as square root of peak area. Vertical bars represents standard deviations of means.

sulfur compounds at high concentrations, we could identify only one sulfur compound (dimethyl disulfide) using MS. Therefore, most of these peaks were identified by retention time comparison with those of standards using the GC−PFPD (**Table 1**). The identified sulfur compounds were hydrogen sulfide, sulfur dioxide, methanethiol, carbon disulfide, and dimethyl disulfide. Peak 7 was identified as either dimethyl trisulfide or (methylthio) acetic acid. Because these two compounds have very close retention times, we could not confidently distinguish them. Optimization of GC column and oven temperature may help to resolve the two compounds. Another compound, methyl sulfide, was also identified, but the peak area of the compounds was very small, and it appears that methyl sulfide was not affected by irradiation. Our unpublished data show that methyl sulfide is one of the major sulfur compounds in irradiated raw meat, but becomes a minor peak in cooked meat.

Sulfur dioxide, dimethyl disulfide, and dimethyl trisulfide and/or (methylthio) acetic acid were not observed in nonirradiated samples (**Figure 4**). Irradiation increased concentrations of hydrogen sulfide, sulfur dioxide, methanethiol, dimethyl disulfide, and dimethyl trisulfide/(methylthio) acetic acid based on the square root of peak area relative to those of untreated controls (**Figure 4**). Methanethiol increased the most in terms of peak area. The increase in hydrogen sulfide grew linearly between 0 and 2 kGy, but leveled off after 3 kGy. For sulfur dioxide, methanethiol, dimethyl disulfide, and dimethyl trisulfide/(methylthio) acetic acid, the increase was rapid at low doses (1−2 kGy) and lessened at doses above 2 kGy. The only sulfur

Table 2. Changes in Volatile Sulfur Compounds of Turkey Breast During Storage at 5 °C

compound	storage time (days)	dose (kGy)			
		0	24	4	LSD
hydrogen sulfide	0	34.1	68.0	69.5	10.4
	7	40.0	16.6	14.7	25.8
	14	0	7.4	17.8	9.9
	LSD	13.5	21.2	13.7	
sulfur dioxide	0	---	45.6	63.3	5.1
	7	---	---	13.7	14.9
	14	---	---	---	
	LSD		1.2	12.8	
methanethiol	0	32.6	905.6	1130.0	57.5
	7	30.6	435.6	524.5	113.5
	14	3.8	144.8	245.8	62.8
	LSD	10.5	109.8	81.1	
carbon disulfide	0	675.8	392.9	189.4	83.6
	7	494.9	295.3	188.7	119.4
	14	355.0	246.5	170.0	61.6
	LSD	81.5	99.8	81.1	
dimethyl disulfide	0	---	149.0	188.2	15.6
	7	---	72.8	111.9	20.3
	14	---	59.9	32.1	36.8
	LSD		50.9	15.4	
dimethyl trisulfide/(methylthio) acetic acid	0	---	72.2	96.5	5.1
	7	---	19.7	35.1	12.6
	14	---	22.4	11.8	98.3
	LSD		14.2	9.8	
total	0	742.5	1633.4	1737.0	124.0
	7	565.5	840.0	894.9	254.2
	14	358.8	481.0	477.3	94.9
	LSD	83.8	189.1	163.7	

compound that was reduced by irradiation was carbon disulfide. The decrease was almost linear as the function of dose based on square root of peak area.

During storage at 5 °C, all sulfur compounds decreased, even in the nonirradiated controls (**Table 2**). Sulfur dioxide was undetectable after 14 days storage. For most of the sulfur compounds, the decrease (in terms of square root of peak area) during the 14-day storage was more than 5-fold. But for carbon disulfide, the reduction was less than 2-fold based on square root of peak area.

Previous studies showed that sulfur compounds always decreased in aerobically packaged pork, presumably due to volatility of sulfur compounds; but in a vacuum-packaged pork, concentrations of sulfur compounds either decreased or increased (*9*, *10*). Our results show that all sulfur compounds decreased during storage. Our results seems to be in agreement with earlier observations that storage of products after irradiation generally improves the odor of irradiated meats (*24*, *25*). It is not clear whether the decrease in sulfur compounds observed in the present study was due to leakage of volatile sulfur compounds from the vials or to their reaction with other components of cooked turkey breast. The samples were tightly sealed in 40-mL vials during storage. Different sets of vials were used for each sampling day (0, 7, and 14 days).

Most of the sulfur compounds have very low odor thresholds, and have a pungent, unpleasant odor (*26*). The odor thresholds of all sulfur compounds detected in the turkey breast study are in the ppb or sub-ppb range. Methanethiol probably has one of the lowest odor thresholds in water (0.02 ppb) (*26*). Our results show that methanethiol was the most sensitive compound to irradiation. Using the standard curve established in water, the concentration of methanethiol in the turkey breast was estimated to be 185 ppb at a dose of 2 kGy. Even after storage for 14 days, the concentration was 25 ppb in the irradiated samples. These concentrations are several 1000-folds higher than the odor threshold of methanethiol in water. Of course, the odor threshold of sulfur compounds in turkey breast is likely to be higher than that in water due to the presence of other volatile compounds. It should also be pointed out that the methanethiol concentration calculated using the standard curve prepared from water will likely be different from the actual amount. Nevertheless, our results suggest that methanethiol and many other sulfur compounds probably have an impact on the irradiation odor observed in irradiated meats.

Carbon disulfide was the major compound detected in nonirradiated cooked turkey breast in terms of peak area. It has been shown that low amounts of volatile sulfur compounds contribute to the characteristics of cooked meats (*26*−*28*). It is unclear whether carbon disulfide itself contributes to savory, meaty, roasted, or boiled flavor of cooked meats. Most of the sulfur compounds were promoted by irradiation, but carbon disulfide decreased. It is unknown why carbon disulfide was reduced by irradiation while all other sulfur compounds were increased by irradiation. The sulfur compounds are believed to be generated from the side-chains of sulfur-containing amino acids in protein and/or subsequent reaction with other compounds (*6*, *26*, *27*), although the exact mechanism is unclear. Formation of these sulfur compounds may vary in response to irradiation and cooking. Carbon disulfide may be formed in turkey breast upon cooking but converted to other sulfur compounds by irradiation. The development of the off-odor may be due not only to the increase in sulfur compound concentration, but also to the difference in concentration among the sulfur compounds. Carbon disulfide is reduced by irradiation in pork muscle strip (*9*) but increased in pork loin (*10*).

In an attempt to identify compounds responsible for the irradiation odor, Batzer and Dotty (*10*) tentatively identified hydrogen sulfite and methanethiol in irradiated beef. Merritt et al. (*29*) identified 10 compounds in irradiated meats. Five of the compounds were sulfur compounds. Wick et al. (*30*) suggested that methional, *n*-nonanal, and phenylacetaldehyde were responsible for the off-odor. In our present study, we did not detect any methional. All of these earlier studies were conducted using sterilization doses (above 15 kGy) of irradiation. More recently, Patterson and Stevenson (*31*) found that dimethyl trisulfide is the most potent compound, followed by *cis*-2 and *trans*-6-nonenals, oct-1-en-3-one, and bis(methylthio-)methane in irradiated chicken breast. Ahn et al. (*9*, *10*), using a dynamic headspace extraction technique coupled with GC−MS, identified several sulfur compounds, including methyl sulfide, dimethyl disulfide, methanethiol, (methylthio) acetic acid, and carbon disulfide. We used cooked turkey breast, whereas most of the earlier studies used raw meats.

To achieve a 5-log reduction of common pathogens, doses of 2.45−3.6 kGy are probably required (*1*−*3*, *32*). At those doses, an increase in most of the sulfur compounds would be anticipated, while carbon disulfide would be reduced. Whether this change in the profile of sulfur compounds will impact the odor of cooked turkey breast is unclear and requires further study. The irradiation-induced sulfur compounds may be reduced using low dose radiation in conjunction with other techniques.

In summary, irradiation at doses of pathogen inactivation significantly increased the concentrations of most of the sulfur compounds. The increase was more rapid at low doses. Our results provide evidence that sulfur compounds may be involved in the development of irradiation odor. The concentrations of these sulfur compounds may well exceed their odor thresholds although exact concentrations of these sulfur compounds were not able to be determined because of the limitations of SPME and complexity of the sample matrix used in this study.

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