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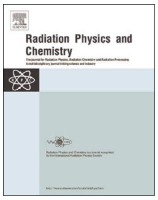

Mechanisms of volatile production from sulfur-containing amino acids by irradiation

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HIGHLIGHTS

- Radiolysis of amino acid side chains were the major cause of volatile production.
- The Strecker degradation were involved in the production from sulfur amino acids.
- Odor of the irradiated sulfur amino acids were similar to that of irradiated meat.
- Methionine was the major amino acid in the production of irradiation off-odor.

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ABSTRACT

Sulfur-containing amino acids were used to study the mechanisms of off-odor production in meat by irradiation. Irradiation not only increased the amounts of volatiles but also produced many new volatiles from sulfur-containing amino acid monomers. We speculate that the majority of the volatiles were the direct radiolytic products of the side chains, but Strecker degradation as well as deamination and decarboxylation of radiolytic products were also involved in the production of volatile compounds from sulfur amino acids. The volatile compounds produced in amino acids were not only the primary products of irradiation, but also the products of secondary chemical reactions after the primary compounds were produced. Cysteine and methionine produced odor characteristics similar to that of the irradiated meat, but the amounts of sulfur volatiles from methionine were far greater than that of cysteine. Although the present study was carried out using an amino acid model system, the information can be applied to the quality indexes of irradiated meats as well as other food products.

1. Introduction

Previous studies indicated that many volatile compounds such as 2-methyl butanal, 3-methyl butanal, 1-hexene, 1-heptene, 1-octene, 1-nonene, hydrogen sulfide, sulfur dioxide, mercaptomethane, dimethyl sulfide, methyl thioacetate, dimethyl disulfide, cis-3- and trans-6-nonenals, oct-1-en-3-one and bis(methylthio-)methane and trimethyl sulfide were greatly increased or newly produced from meat by irradiation (Patterson and Stevenson, 1995; Ahn, 2002; Fan et al., 2002). Among the volatiles, sulfur compounds played the most important roles in irradiation off-odor of meat, and the production of sulfur compounds and the intensity of sulfury odor was irradiation dose-dependent (Ahn et al., 2000; Ahn and Lee, 2002). However, the amounts of aldehydes, the indicators of lipid oxidation, were not influenced by irradiation (Jo and Ahn, 2000), and the volatiles produced from lipids accounted for only a small part of the off-odor in irradiated meat (Ahn et al., 1998, 1999). These studies also indicated that irradiation off-odor was different from warmed-over flavor in oxidized meat, and the mechanisms and the volatiles involved in irradiation odor were different those of oxidation odor (Ahn et al., 2000; Jo and Ahn, 2000).

We hypothesize that sulfur amino acids will be the major components involved in the production of off-odor in irradiated

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meat. This is the second part of the model system study that uses amino acid monomers to determine the mechanisms of volatiles production from irradiated meat. Only the volatile generation mechanisms from sulfur amino acids by irradiation were discussed here. The objectives of this study were to (1) determine the volatile compounds produced from sulfur amino acids by irradiation, (2) elucidate the production mechanisms of volatiles from sulfur amino acids by irradiation, and (3) characterize the odor and evaluate the contribution of volatiles from sulfur amino acids to the overall odor of irradiated meat systems.

2. Materials and methods

2.1. Sample preparation

Three sulfur-containing amino acid monomers that include methionine, cysteine, and cystine (Sigma-Aldrich, St. MO, USA) were tested. Each amino acid monomer (50 mg/10 mL) was dissolved in a citrate–phosphate buffer (100 mM, pH 6.0) and irradiated at 0 or 5.0 kGy absorbed dose using an Electron Beam irradiator (Circe IIIR Thomson CSF Linac, St. Aubin, France). Four replications were prepared for each amino acid. Immediately after irradiation, 2-mL portions of the amino acid solution (4 portions) were transferred to sample vials, flushed with helium gas (99.999% purity) for 5 s at 40 psi, and then capped. One of them was used to analyze volatile profiles, and the other three were used to determine odor characteristics. Volatile profiles and odor characteristics of irradiated and nonirradiated amino acid monomers were compared. A purge-and-trap dynamic headspace/GC–MS was used to quantify and identify volatile components, and trained sensory panel evaluated the overall odor characteristics of the samples.

2.2. Volatile compounds analysis

A purge-and-trap apparatus (Precept II and Purge & Trap Concentrator 3100, Tekmar-Dohrmann, Cincinnati, OH, USA) connected to a gas chromatography/mass spectrometry (GC/MS, Hewlett-Packard Co., Wilmington, DE, USA) was used to analyze volatiles produced (Ahn et al., 2000). Sample solution (2 mL) was placed in a 40-mL sample vial, and the vials were flushed with helium gas (40 psi) for 5 s. The maximum waiting time of a sample in a refrigerated (4 °C) holding tray was less than four hours to minimize oxidative changes before analysis. The sample was purged with helium gas (40 mL/min) for 12 min at 40 °C. Volatiles were trapped using a Tenax column (Tekmar-Dohrmann) and desorbed for 2 min at 225 °C, focused in a cryofocusing module (−90 °C), and then thermally desorbed into a column for 30 s at 225 °C.

A HP-624 column (7.5 m × 0.25 mm i.d., 1.4 mm nominal), an HP-1 column (52.5 m × 0.25 mm i.d., 0.25 mm nominal; Hewlett-Packard Co.), and an HP-Wax column (7.5 m × 0.25 mm i.d., 0.25 mm nominal) were connected using zero dead-volume column connectors (J & W Scientific, Folsom, CA). Ramped oven temperature was used to improve volatile separation. The initial oven temperature of 0 °C was held for 2.50 min. After that, the oven temperature was increased to 15 °C at 2.5 °C/min, increased to 45 °C at 5 °C/min, increased to 110 °C at 20 °C/min, increased to 210 °C at 10 °C/min, and then was held for 2.5 min at the temperature. Constant column pressure at 20.5 psi was maintained. The ionization potential of the mass selective detector (Model 5973; Hewlett-Packard Co.) was 70 eV, and the scan range was 18.1–250 m/z. Identification of volatiles was achieved by comparing mass spectral data of samples with those of the Wiley library (Hewlett-Packard Co.). The area of each peak was integrated using the ChemStation (Hewlett-Packard Co.), and the total peak area (pA*sx10^4) was reported as an indicator of volatiles generated from the sample.

2.3. Odor characteristics

Ten trained sensory panelists characterized the odor of samples. Panelists were selected based on interest, availability, and performance in screening tests conducted with samples similar to those to be tested. During training, a lexicon of aroma terms to be used on the ballot was developed, and references that can be used to anchor the rating scale were identified. Samples were placed in glass vials, and the sample temperature was brought to 25 °C before samples were tested. All the treatments were presented to each panelist, and the order of presentation was randomized. Panelists characterized overall odor characteristics.

Table 1
Production of volatile compounds from sulfur-containing amino acid monomers solution by irradiation.

Amino acid	Volatiles	0 kGy	5 kGy	SEM
		——————————Total ion counts × 10^4————————————		
Cysteine	Mercaptomethane	0^b	4684^a	661
	Carbon disulfide	0^b	17841^a	1108
	Dimethyl disulfide	0^b	3555^a	656
Cystine	Acetaldehyde	0^b	62647^a	7742
	2-propanone	0^b	4723^a	284
	Carbon disulfide	102^b	472^a	43
	3-methyl pentane	796^a	89^b	125
	Hexane	35484^a	4087^b	4795
	Methyl cyclopentane	6671^a	2110^b	879
Methionine	Mercaptomethane	0^b	5198^a	1335
	2-Propenal	0^b	3757^a	846
	2-Propanone	0^b	655^a	38
	Dimethyl sulfide	0^b	120056^a	899
	Hexane	222^b	689^a	39
	Butanal	0^b	164^a	13
	(methylthio)-ethane	0^b	1510^a	67
	Thiophene	0^b	74^a	3
	3-(methylthio)-1-propene	0^b	230^a	8
	Dimethyl disulfide	131^b	208770^a	990
	Methyl ethyl disulfide	0^b	417^a	22

[a,b] Means with no common superscript differ significantly ($P < 0.05$), $n = 4$.

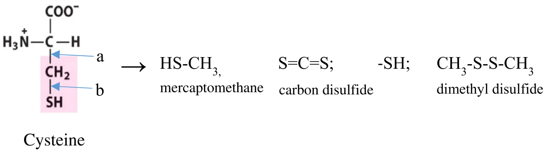
2.4. Statistical analysis

Data were analyzed using the generalized linear model procedure of SAS software (version 9.1, NC, USA); the Student's *t*-test was used to compare differences between irradiated and non-irradiated means. Mean values and standard error of the means (SEM) were reported. Significance was defined at $p < 0.05$.

3. Results and discussion

3.1. Volatile production from cysteine

Three new volatiles, including mercaptomethane, carbon disulfide, and dimethyl disulfide, were produced from cysteine by irradiation (Table 1). It is assumed that mercaptomethane (CH_3SH) is produced through the direct cleave of cysteine side chain (arrow 'a' in Reaction 1), and carbon disulfide (CS_2) can be produced by the reaction of H_2S with CO_2 as shown below (Reactions 2 and 3) (Good et al., 1961). Dimethyl disulfide ($CH_3–S–S–CH_3$) is formed from the reaction of two mercaptomethane (CH_3SH).



(Reaction 1)

$H_2S + CO_2$ COS (carbonyl sulfide)$+ H_2O$ (Reaction 2).

$COS + H_2S$ $CS_2 + H_2O$ (Reaction 3).

Irradiation of water (at 25 °C) produces various products including e^-_{aq}, H_3O^+,.OH, H^+, H_2, and H_2O_2 (Simic, 1983). Among the irradiation products of water, aqueous electron (e^-_{aq}), hydroxyl radical (.OH), hydrogen atom (H^+) and hydrogen peroxide (H_2O_2) can be actively involved in various reactions with amino acids, proteins, lipids, vitamins, and carbohydrates. Packer and Winchester (1968) reported that radiolysis of neutral aqueous solutions of cysteine $HO_2CCH(NH_2)CH_2SH$ in the presence of oxygen produced hydrogen sulfide (H_2S), cysteine $[SCH_2CH(NH_2)CO_2H]_2$, and hydrogen peroxide (H_2O_2). The bond cleavage at $–CH_2–SH$ (arrow 'b' in Reaction 1), which produces H_2S, can easily occur by aqueous electron (e^-_{aq}), hydrogen radical (.H), or hydrogen atom (H^+) (Armstrong and Wilkening, 1964; Barton and Packer, 1970; Al-Thannon et al., 1974). Fan et al. (2011) identified SO_2 and H_2S in irradiated cooked turkey breasts meat but those compounds were not detected in this study probably because of the following reasons: (1) all the H_2S formed were reacted with other irradiation products to form new products such as carbon disulfide (Reactions 2 and 3) and sulfuric acid (Reactions 4–6); and (2) the H_2S formed are converted to sulfuric acid (H_2SO_4), which is non-volatile (Reactions 4–6) (Mortimer, 1967; Tso and Lee, 1984; Sharma et al., 1997). H_2SO_4 can also be formed from SO_2 when H_2O_2 is available (as in irradiated water, Reactions 7 and 8) (Martin and Damschen, 1981).

$2H_2S + 3O_2$ $2H_2O + 2SO_2$ (Reaction 4)

$2SO_2 + O_2$ $2SO_3$ (Reaction 5)

$SO_3 + H_2O$ H_2SO_4 (Reaction 6)

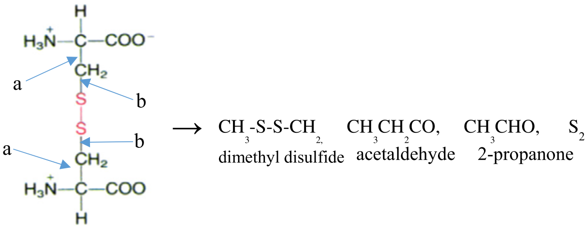
$2H_2O_2 + S_2^-$ $SO_2^{2-} + 2H_2O$ (Reaction 7)

$SO_2 + H_2O_2$ H_2SO_4 (Reaction 8)

3.2. Volatile production from cystine

Two new volatiles, including acetaldehyde and 2-propanone, were newly produced, and one volatile, carbon disulfide, was significantly increased from cystine by irradiation (Table 1). Shu et al. (1985) studied the thermal degradation of cystine in water at pH 5.5 and pH 2.3, respectively, and identified 42 compounds, which were mainly thiazoles, sulfides, thiolanes, and thians. They reported that sulfur compounds were generated more readily at low pH, and suggested that the thermal stability of the S–S bond of cystine can vary depending upon pH values. Therefore, it is assumed that acetaldehyde, 2-propanone and carbon disulfide were the predominant compounds produced from cystine in this study (pH 6.0).

Cystine is the amino acid formed by the oxidation of two cysteine molecules that covalently link via a disulfide bond. Because of the shift in electron clouds towards –S–S– bond, the bond strength between $S–CH_2$ (arrows 'b' in Reaction 9), the side chain and the α-carbon (arrows 'a' in Reaction 9), becomes weaker, and thus this part can be broken easily by e^-_{aq} (Packer and Winchester, 1970).



Cystine (Reaction 9)

The expected primary radiolytic volatile products of cystine include methane (CH_4), dimethyl disulfide ($CH_3–S–S–CH_3$), S2, acetaldehyde (CH_3CHO) and 2-propanone (CH_3CH_2CO) (Reaction 9). However, irradiation of cystine produced only two new volatile compounds (acetaldehyde and 2-propanone) and increased carbon disulfide, which is different from what was predicted (Table 1). It is assumed that the production of acetaldehyde and 2-propanone is through the following reaction mechanisms: acetaldehyde was produced (1) via the radiolysis of bonds between the two $–CH_2–S–$ positions (arrow 'b' in Reaction 9), which produced two alanines $[CH_3CH_2(NH_2)COOH]$ and S_2, and then (2) the Strecker degradation removed $–NH_2^-$ and –COOH from the α-carbon moiety through the actions of ozone produced by irradiation as shown in the Reaction 10 (Schonberg and Moubacher, 1952).

$RCH(NH_2)COOH + O_3 + O_2 + H_2O \rightarrow RCHO + CO_2 + NH_3 + H_2O_2 + O_2$ (Reaction 10)

To produce 2-propanone from cystine, the radiolysis between $–S–CH_2$ of side chain (arrow 'b' in Reaction 9) and the removal of $NH_2–$ from α-carbon first, and then the ketonic decarboxylation are necessary (Reactions 11 and 12) (Swendseid et al., 1942). Irradiation of cysteine in air-containing aqueous solution produced ammonia, sulfur and pyruvic acid ($CH_3COCOOH$) as the final products, and the pyruvic acid can be decarboxylated to produce acetaldehyde or 2-propanone as shown in Reaction 12 (Soji et al., 1966).

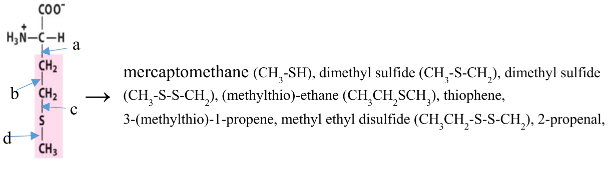
$NH_2(CH_3COO) \rightarrow NH_3 + CH_3COO^-$ (Reaction 11)

$CH_3COO \rightarrow CO_2 + (CH_3)2CO$ (Reaction 12)

The amount of acetaldehyde produced from cysteine was much higher than 2-propanone, indicating that the Strecker degradation is more favorable pathway than the ketonic decarboxylation. However, both pathways require cleavage at $–CH_2–S–$ positions of cysteine (arrow 'b' in Reaction 9).

3.3. *Volatile production from methionine*

Irradiation of methionine produced many new volatile compounds, including mercaptomethane (CH_3–SH), 2-propenal (C_2H_3CHO), 2-propanone (CH_3CH_2CO), dimethyl sulfide (CH_3–S–S–CH_3), butanal ($CH_3CH_2CH_2CHO$), (methylthio)-ethane ($CH_3CH_2SCH_3$), thiophene (C_4H_4S), 3-(methylthio)-1-propene and methyl ethyl disulfide (CH_3CH_2–S–S–CH_2), majority of which were sulfur compounds. Also, the amount of total newly generated sulfur volatiles from methionine was more than 15-fold of cysteine (Table 1).



(Reaction 13)

The production of mercaptomethane (CH_3–SH) from methionine by irradiation is through the direct cleavage between –CH_2–S– (arrow ‘c’ in Reaction 13) or –CH_2–S– and CH_3–S–(arrows ‘b’ and ‘d’ in Reaction 13). One of the main effects of ionizing radiations on methionine is the scission of –CH_2–S– group (arrows ‘c’ in Reaction 13), but cleavage of –CH_3 (arrow ‘d’ in Reaction 13) can also occur easily. Shimazu et al. (1964) and Tajima et al. (1969) reported that a direct bond cleavage between –CH_2–S– (arrow ‘c’ in Reaction 13) from the deaminated methionine by .OH or e^-_{aq} produced α-aminobutryic acid and mercaptomethane.

Dimethyl sulfide (CH_3–S–CH_2) is the primary product of the side chain breakage at bond position between –CH_2–CH_2– (arrow ‘b’ in Reaction 13). Dimethyl disulfide (CH_3–S–S–CH_2) can be formed when two mercaptomethane (CH_3SH) are combined. Dimethyl disulfide can also be produced by the Strecker degradation: in this pathway, methionine appears to be degraded first to its Strecker aldehyde, methional (3-methylthiopropanal), which is unstable and decomposes to mercaptomethane, and finally to dimethyl disulfide (Schutte and Korenders, 1972). (Methylthio)-ethane ($CH_3CH_2SCH_3$) is the direct product of radiolytic breakage of the side chain at the α-carbon (arrow ‘a’ in Reaction 13). Rest of the sulfur compounds (thiophene, 3-(methylthio)-1-propene, methyl ethyl disulfide) should have been formed through the multiple-step reactions of irradiation products after the primary irradiation products are formed. Accordingly, the amounts of them were much smaller than the primary sulfur compounds.

The results indicated that the two predominant sulfur volatiles produced from methionine by irradiation were dimethyl sulfide and dimethyl disulfide: the amount of dimethyl sulfide (CH_3–S–CH_2) was 23 times more, while that of the dimethyl disulfide (CH_3–S–S–CH_2) was 40 times more than that of the mercaptomethane (CH_3–SH). This indicated that the most susceptible bonds in the side chain of methionine are between (1) the α-carbon and –CH_2 (arrows ‘a’ in Reaction 13), (2) the –CH_2–S– (arrows ‘c’, and ‘b’ and ‘d’ in Reaction 13), and (3) the –CH_2–CH_2– and –S–CH_3 (arrows ‘b’ and ‘d’ in Reaction 13).

2-Propenal (C_2H_3CHO) should have been produced by the removal of –SCH_3 moiety from methionine, followed by deamination and decarboxylation. Since the dimethylthio group (–H_2C–S–CH_2–) has a low reactivity with aqueous electrons (e_{aq}), methionine is more likely to react with hydroxyl radicals (Simic, 1983). The formation of several compounds with double bonds [2-propenal, thiophene, and 3-(methylthio)-1-propene] indicated that hydrogenation reactions are also involved in the production of volatiles from methionine by irradiation.

Ohara (1965) reported that sulfur amino acids are the most radiation sensitive among amino acids. Allen and Parks (1975) also reported that all amino acids could undergo degradations, but sulfur-containing amino acids were the most susceptible to radiolytic degradation among the amino acid groups. Among the three sulfur amino acid monomers, methionine produced the largest amounts of volatiles, indicating that it is the most susceptible to radiolytic attack (Table 1). This is consistent with the study of Ahn (2002) who reported that methionine and cysteine were the major amino acids to produce the most important volatiles in irradiated meat. Ahn also reported that the amounts of sulfur compounds produced from cysteine were only 0.25% to 0.35% of methionine. Lee and Ahn (2002) observed that methionine was one of the major sources of gas production (CO and CH_4) when irradiated at 0, 2.5, 5, or 10 kGy, indicating that methional can also be produced by irradiation. Dimick (1978) identified methional as a light-induced flavor in the sunlight-exposed skim milk. Methional can be further converted to mercaptans, sulfides and disulfides to produce “cabbage-like” or “burnt feather” odor (Day et al., 1958).

Table 2
The major volatiles and the odor characteristics of irradiated sulfur amino acids.

Amino acid	Major volatiles	Odor characteristics
Cysteine	Carbon disulfide, mercaptomethane, dimethyl disulfide	Boiled egg, sulfury
Cystine	Acetaldehyde, 2-propanone	Ethanol
Methionine	Dimethyl disulfide, dimethyl sulfide, methanethiol	Boiled cabbage, boiled vegetables

3.4. *Major volatiles and odor characteristics*

Table 2 showed the major volatiles from sulfur-containing amino acids and their odor characteristics after irradiation. Sensory panelists described the odor of irradiated sulfur-containing amino acids as “hard-boiled eggs and sulfury” and “boiled cabbage or vegetables” (Table 2). Earlier researchers characterized the sensory of irradiated meat as “bloody and sweet”, “barbecued corn-like”, “hot fat”, “burned oil” or “burned feathers”, and was different from the description of nonirradiated meat (Patterson and Stevenson, 1995; Ahn et al., 2000). Irradiation is very well known to produce various volatile sulfur compounds such as hydrogen sulfide, carbon disulfide, mercaptomethane, dimethyl sulfide, methylthioethane, dimethyl disulfide, bis-methylthio-methane, 3-methylthio-1-propene, ethanoic acid S-methyl ester, methyl thioacetate and dimethyl trisulfide from meat (Patterson and Stevenson, 1995; Fan et al., 2002), and these sulfur-containing compounds were suggested as the main off-odor generators because they have extremely low sensory threshold values (Wick et al., 1967;

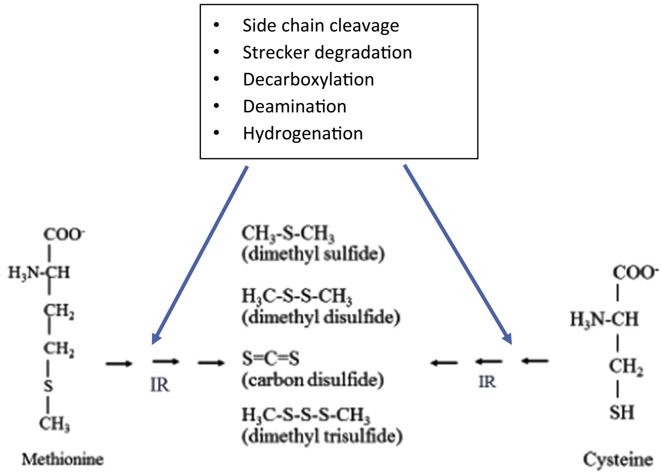


Fig. 1. Production of volatiles from sulfur amino acids by irradiation.

Leonardos et al., 1969).

The production of off-flavors in protein-containing products is actually due to two concurrent chemical reactions: lipid oxidation and protein and/or amino acid degradation (Brewer and Vega, 1995). However, previous studies indicated that the major volatile components responsible for the characteristic off-odor in irradiated meat were sulfur compounds, while volatiles from lipids accounted for only a small part of the off-odor in irradiated meat (Ahn et al., 1998, 1999).

Methionine and cysteine were the major sulfur-containing amino acids among meat components, but the amount of sulfur compounds produced from methionine was more than 99% of the total sulfur compounds produced by irradiation (Table 1). Fig. 1 summarizes the major volatiles and the reaction involved in the production volatiles from sulfur-containing amino acids in meat.

The primary sulfur compounds produced by irradiation interacted with sulfur compounds as well as other volatile compounds to produce secondary sulfur compounds. Fujimaki and Morita (1968) pointed out that not only the primary irradiation species from water molecules (e^-_{aq}, .OH, and H^+), but also the radicals secondarily produced from food constituents played an important role to the off-flavor in irradiated foods. Ahn and Lee (2002) reported that the perception of odor from samples containing sulfur volatiles can be changed depending upon the composition of other volatiles in the sample because the volatiles produced from non-sulfur amino acids can interact with sulfur compounds. However, the release of volatile compounds can be affected by the physicochemical conditions of a product and the interactions with the components in the product (Godshall, 1997; Lubbers et al., 1998). Therefore, the volatile compounds released from real meat systems can be significantly different from those generated through the use of the aqueous model (Jo and Ahn, 1999).

4. Conclusions

Although speculative, we concluded that the majority of the volatiles were produced from the side chains of sulfur-containing amino acids. The volatile compounds produced in amino acids were not only the primary products of radiolytic degradation, but also the products of extensive chemical reactions including deamination, Strecker degradation, hydrogenation, oxidation–reduction, and decarboxylation of the radiolytic products. The side chains of sulfur-containing amino acids were highly susceptible to radiolytic attack and produced various sulfur compounds; the overall odor intensity of irradiated sulfur amino acids was very high and the odor characteristics of sulfur amino acids were similar to the irradiation odor of meat. The contribution of methionine to the irradiation odor was far greater than that of the cysteine and cystine. Although the present study was carried out in a given reaction medium using amino acid monomers, the information can be applied to the quality indexes of irradiated meats as well as other food products.

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