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Mechanisms of volatile production from amino acid esters by irradiation

Xi Feng [a], Eun Joo Lee [b], Kichang Nam [c], Cheorun Jo [d], Kyungyuk Ko [e], Dong Uk Ahn [a,*]

[a] Department of Animal Science, Iowa State University, Ames, IA 50010, United States
[b] Department of Food and Nutrition, University of Wisconsin-Stout, Menomonie, WI 54751, United States
[c] Department of Animal Science and Technology, Sunchon National University, Suncheon 540-742, Republic of Korea
[d] Department of Agricultural Biotechnology, Center for Food and Bioconvergence and Research Institute for Agriculture and Life Science, Seoul National University, Seoul 151-921, Republic of Korea
[e] Division of Food Additives and Packaging, Department of Food Safety Evaluation, Ministry of Food Drug Safety, Cheongwon-gun, Chungbuk 363-700, Republic of Korea

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ABSTRACT

Proteins and its constituents (amino acids) are known as the major contributors to the off-odor in irradiated meat. However, radiolytic degradation of amino acids occurred not only at side chains but also at amino- and carboxyl-groups of the α-carbon. A model system with amino acid esters was used to elucidate the mechanisms of volatile production at side chains of amino acids by irradiation. The low-molecular weight aldehydes, which contributed to the irradiation off-odor, were mainly from acidic, aliphatic and aliphatic hydroxyl group amino acid esters through the radiolysis of amino acid side chains or Strecker degradation. However, the contribution from non-sulfur amino acids was minor compared with sulfur amino acids. Among the sulfur-containing amino acids, methionine made the greatest contribution to the irradiation off-odor not only through the direct cleavage of the side chain. However, the chemical reactions of sulfur compounds with other compounds produced by irradiation also played significant roles to the off-odor of irradiated meat.

1. Introduction

Irradiation is the most effective method to inactivate foodborne pathogens in meat (Delincée, 1998; Diehl, 2002). In addition to improving the meat safety, irradiation leaves no chemical residues, minimizes nutrient loss, eliminates further cross contamination after processing, and prolongs shelf-life of meat (Farkas, 2006; Roberts, 2014). However, the use of irradiation on meat products is limited because of off-odor production by irradiation (Brewer, 2009; Lee & Ahn, 2004).

All irradiated meat produces characteristic irradiation odor such as "hot fat," "burned oil", "burned feathers", "bloody and sweet", or "barbecued corn-like" odor, regardless of degree of lipid oxidation (Hashim, Resurreccion, & McWatters, 1995; Heath, Owens, Tesch, & Hannah, 1990; O'Bryan, Crandall, Ricke, & Olson, 2008). Several off-odor volatile compounds, including 2-methyl butanal, 3-methyl butanal, 1-heptene, 1-octene, 1-nonene, hydrogen sulfide, sulfur dioxide, mercaptomethane, dimethyl sulfide, methyl thioacetate, dimethyl disulfide and dimethyl trisulfide, were newly generated or increased in meat by irradiation (Arvanitoyannis, 2010; Fan, Sommers, Thayer, & Lehotay, 2002; Lin et al., 2007; Panseri et al., 2015; Patterson & Stevenson, 1995). These researches also indicated that radiolysis of amino acids played an important role in the production of off-odor volatiles in irradiated meats, because more than 70% of meat is water, the second major components in meat are proteins and its constituents (amino acids), which are constituted by a variety of amino acid groups, including acidic, amide, basic, aromatic, aliphatic, aliphatic hydroxyl and sulfur containing groups (Lawrie & Ledward, 2006).

Ahn (2002) used amino acid homopolymers and Ahn et al. (2016a,b) used amino acid monomers to elucidate the production mechanisms of off-odor volatiles in meat by irradiation. However, the production mechanisms of off-odor volatiles in irradiated meats cannot be fully explained by analyzing the volatiles from irradiated amino acid homopolymers or monomers because they found that the radiolytic degradation of amino acids occurred not only at side chains but also at amino and carboxyl groups of the α-carbon. In this study, amino acid esters were used to determine the contribution of the side chains to the production of volatiles from amino acids by irradiation. The amino acid ester samples were randomly divided into 2 groups and irradiated at 0 kGy (control group) or 5 kGy (treatment group) using a linear accelerator. The objective of this study were 1) to determine the volatile compounds newly produced from each amino acid ester by irradiation, 2) to elucidate the production mechanisms of off-odor volatiles from amino acid esters by irradiation, and 3) to characterize the odor and evaluate the

* Corresponding author.
E-mail address: duahn@iastate.edu (D.U. Ahn).

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contribution of volatiles from amino acids to the odor of irradiated model systems.

2. Materials and methods

2.1. Sample preparation

Twenty one amino acid esters, including aspartic acid di-methyl ester, aspartic acid β-methyl ester, glutamic acid γ-methyl ester, alanine methyl ester, isoleucine methyl ester, leucine methyl ester, proline methyl ester, valine ethyl ester, serine ethyl ester, threonine methyl ester, asparagine *t*-butyl ester, glutamine *t*-butyl ester, phenylalanine ethyl ester, tryptophan ethyl ester, tyrosine ethyl ester, arginine ethyl ester, histidine methyl ester, lysine ethyl ester, cysteine ethyl ester, cystine di-ethyl ester, or methionine methyl ester, were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used to make the aqueous model system. Each amino acid ester (5 mg/L) was dissolved in a citrate–phosphate buffer (100 mM, pH 6.0), transferred to a 40 ml vial and irradiated at 0 kGy or 5.0 kGy absorbed dose using an Electron Beam irradiator (Circe IIIR, Thomson CSF Linac, St. Aubin, France). Alanine dosimeters were placed at the top and bottom of a vial and read using a 104 Electron Paramagnetic Resonance Instrument (Bruker Instruments Inc., Billerica, MS) to check the absorbed dose. Four replications were prepared for each amino acid ester. Immediately after irradiation, four 2 mL-portion amino acid ester solutions were taken from each replication, and they were individually transferred to four different sample vials, flushed with helium gas (99.999% purity) for 5 s at 40 psi, and then capped. One portion of each replication was used to analyze volatile profiles, and the other three were used to determine odor characteristics. Volatile profiles and odor characteristics of irradiated amino acid esters were studied. A purge-and-trap dynamic headspace/GC–MS was used to quantify and identify volatile components, and trained sensory panelists evaluated the overall odor characteristics of the samples.

2.2. Volatile compounds analysis

A purge-and-trap apparatus (Precept II and Purge & Trap Concentrator 3000, 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, 2002). Sample (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 4 h 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/charcoal/silica 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.

An 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 μm nominal; Hewlett-Packard Co.), and an HP-Wax column (7.5 m × 0.25 mm i.d., 0.25 μm 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 final temperature. Constant column pressure at 20.5 psi was maintained. The ionization potential of mass selective detector (Model 5973; Hewlett-Packard Co.) was 70 eV, and the scan range was 19.1–350 m/z. Identification of volatiles was achieved by comparing mass spectral data of samples with those of the Wiley library (Hewlett-Packard Co.). Standards, when available, were used to confirm the identification by the mass selective detector. The area of each peak was integrated using the ChemStation (Hewlett-Packard Co.), and the total peak area was reported as an indicator of volatiles generated from the sample.

2.3. Odor characteristics

Twelve 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 were selected as anchors to identify the overall odor characteristics of samples. Each sample was placed in a glass vial with a randomly selected 3-digit number, and the sample temperature was brought to 25 °C before samples are tested. During the tests, one treatment was presented to each panelist each time, and the order of presentation was randomized. Panelists characterized overall odor characteristics. All the sensory evaluation tests were done at 25 °C in a sensory panel room equipped with white fluorescent lighting.

2.4. Statistical analysis

Newly formed volatiles in irradiated amino acid esters after irradiation (5 kGy) were reported as mean values with standard deviation.

3. Results and discussion

3.1. Acidic, amide, basic and aromatic group amino acid esters

Two different aldehydes, acetaldehyde and propanal, were produced by irradiated acidic group amino acid esters (aspartic acid *di*-methyl ester, aspartic acid β-methyl ester, and glutamic acid γ-methyl ester) (Table 1). Ahn et al. (2016a) indicated three possible pathways to produce aldehydes from aspartic acid and glutamic acid: 1) the side chain and $-NH_2^-$ group cleavage from the α-carbon: generates an acetic acid or propionic acid, which is further converted to acetaldehyde through the oxidation–reduction reactions (McMurry, 2004); 2) the Strecker degradation removes $-NH_2^-$ and –COOH from the α-carbon moiety in amino acid through the actions of ozone (produced by irradiation) and generates aldehyde (Yaylayan, 2003); and 3) the acetic acid formed from aspartic acid and glutamic acid can react with hydroxyl radical (•OH) and produce an ethen-1-ol (CH_2CHOH), which can be further converted to acetaldehyde because keto isomers are more stable than the enol ones (Perez & Toro-Labb, 2000). In this study, the amount of acetaldehyde from irradiated aspartic acid β-methyl ester was 25 times greater than that from irradiated aspartic acid *di*-methyl ester, indicating that the cleavage of a bond between α-carbon and side chain (pathway 1) is the major pathway to produce acetaldehyde in aspartic acid. Meanwhile, the amount of acetaldehyde produced from irradiated glutamic acid γ-methyl ester was greater than that of the propanal, which agreed with the finding made by Ahn et al. (2016a): the cleavage of a bond between $-CH_2-CH_2-$ of glutamic acid by irradiation is very difficult (Berg, Tymoczko, & Stryer, 2012). It also suggested that pathway 1 is the major mechanism to produce acetaldehyde from glutamic acid γ-methyl ester.

A small amount of butane was also produced from glutamic acid γ-methyl by irradiation. Ahn et al. (2016a) suggested that a possible reaction pathway to form cyclohexane from glutamic acid is decarboxylation of side chain and cyclic reaction (Mehta & Mehta, 2005). A similar pathway could be involved here to produce butane: decarboxylation of propionic acid produces $-CH_2-CH_2-$, which undergo condensation reaction to produce butane (Thakur & Singh, 1994).

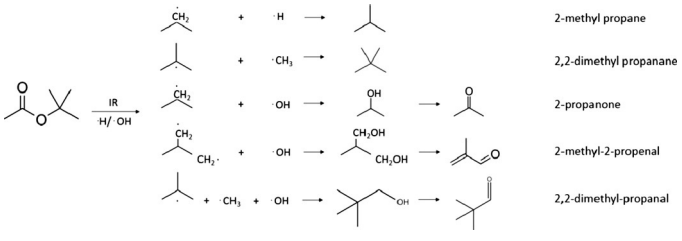
Chen et al. (2012) reported that GC–MS was not capable of analyzing samples that contain high molecular mass nitrogen compounds because of their low volatility. In this study, we also observed that side chains with nitrogen atom (arginine, histidine, lysine, and tryptophane) produced smaller amounts of volatiles than other amino acids (Table 1). However, several volatiles were detected in irradiated amide group amino acid esters, and the majority of these volatiles were C_3, C_4 or C_5 compounds (2-methyl propane, 2-propanone, 2-methyl-2-propenal, 2,2-dimethyl propanane, 2,2-dimethyl-propanal). The side chain structure of asparagine *t*-butyl ester and glutamine *t*-butyl ester suggested

Table 1
Production of volatile compounds from acidic, basic, aromatic, and amide group amino acid ester solution by irradiation.[a]

Volatiles	5 kGy
	Total ion counts $\times 10^4$
Acidic group amino acid esters	
Aspartic acid di-methyl ester	
Acetaldehyde	2833 ± 292
Aspartic acid b-methyl ester	
Acetaldehyde	73,411 ± 8346
2-Methoxy-methyl propane	1292 ± 80
Glutamic acid g-methyl ester	
Butane	3295 ± 312
Acetaldehyde	74,626 ± 4414
Propanal	11,615 ± 950
Amide group amino acid esters	
Asparagine t-butyl ester	
2-Methyl propane	25,895 ± 582
2-Propanone	27,665 ± 4592
2-Methyl-2-propenal	2070 ± 386
Acetic acid ethenyl ester	951 ± 58
2,2-Dimethyl propanane	1364 ± 104
Formic acid, 1,1-dimethylethyl ester	1704 ± 220
Glutamine t-butyl ester	
2-Methyl propane	65,873 ± 2108
2-Methyl-2-propenal	1899 ± 262
2-Methoxy butane	6723 ± 378
2,2-Dimethyl-propanal	3490 ± 86
2,2-Dimethyl-3-pentanol	4591 ± 292
2,3,Dihydro-1,4-dioxine	3915 ± 182
Basic group amino acid esters	
Arginine ethyl ester	
Acetaldehyde	67,663 ± 4254
Histidine methyl ester	
Hexane	3340 ± 132
2-Propenoic acid methyl ester	870 ± 176
Lysine ethyl ester	
Acetaldehyde	65,252 ± 1764
Ethanol	6939 ± 2294
2-Propanone	6322 ± 508
2-Methoxy-2-methyl propane	668 ± 20
Acetic acid ethyl ester	847 ± 76
Acetic acid, 1-methyl ethyl ester	627 ± 88
Aromatic group amino acid esters	
Phenylalanine ethyl ester	
Toluene	30,053 ± 2100
Tryptophane ethyl ester	
None	–
Tyrosine ethyl ester	
Hexane	859 ± 46

[a] Only the newly formed volatiles in irradiated amino acid esters (5 kGy) were listed (n = 4).

that these volatiles could be from the *t*-butyl ester, not the side chains (Reaction 1) (Guillard, Charton, & Pichat, 2003).



(1)

The production of acetic acid ethenyl ester from asparagine *t*-butyl ester is consistent with the results of Ahn et al. (2016a) who indicated that the bond between α-carbon and side chain can be easily broken. However, no acetaldehyde was produced from irradiated asparagine *t*-butyl ester, which is unexpected. In our previous study (Ahn et al., 2016a), acetaldehyde was formed from irradiated asparagine through the oxidation–reduction reaction of the acetic acid (McMurry, 2004). However, only a small amount of acetic acid ethenyl ester was formed after irradiation, and thus only very small amount of acetamide residues could be left to produce acetic acid as a reactant (Cordell, Pandya, Hubbard, Turner, & Monks, 2013).

In irradiated glutamine *t*-butyl ester, two other volatiles, 2-methoxy butane and 2,3-dihydro-1,4-dioxine, were produced. Glutamine and glutamine *t*-butyl ester have relatively high boiling points, 445.6 °C and 359.5 °C, respectively (SCB, Santa Cruz Biotechnology, 2015). In our previous study, only hexane was formed after irradiation (Ahn et al., 2016a). We deduced that these two volatiles were from the condensation reactions with the fragment of $–CH_2–CH_2–$ from the side chain. At the same time, some oxidation–reduction reaction and isomerization reaction should be also involved (McMurry, 2004).

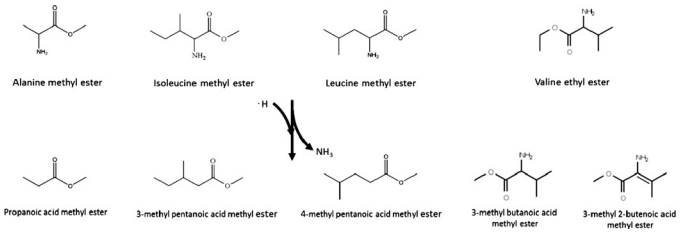
This observation confirmed that the pathway of producing acetaldehyde from asparagine: the bond between α-carbon and side chain can be broken easily and the $–NH_2$ group from side chain can also be removed easily. It also indicated that the reactions of the side chain from glutamine were different from those from aspartic acid, because no acetaldehyde nor propanal was formed in irradiated glutamine *t*-butyl ester.

Because no acetaldehyde could be produced from arginine by irradiation (Ahn et al., 2016a), the production of acetaldehyde in irradiated arginine ethyl ester should be from the ethyl ester group ($CH_3–CH_2–O–$) through an oxidation–reduction reaction (McMurry, 2004). Volatiles produced in histidine methyl ester by irradiation include hexane and 2-propenoic acid methyl ester. It is assumed that 2-propenoic acid methyl ester was formed through the following reactions: irradiation cleaves an $–NH_2^-$ and imidazole group, which generated a 1-hydroxy-propanoic acid methyl ester or 2-hydroxy-propanoic acid methyl ester by reacting with hydroxyl radical (•OH). 1-Hydroxy-propanoic acid methyl ester or 2-hydroxy-propanoic acid methyl ester was further converted to 2-propenoic acid methyl ester by the dehydration reaction. There is no six-carbon chain in histidine methyl ester. Thus, it is assumed that hexane was produced by a polymerization reaction among the irradiated free radicals of methyl, ethyl and propyl groups (Thakur & Singh, 1994). Irradiation of lysine ethyl ester produced 6 volatile compounds: acetaldehyde, ethanol, 2-propanone, 2-methoxy-2-methyl propane, acetic acid ethyl ester, and 1-methyl acetic acid ethyl ester. The assumption is that acetaldehyde was produced in the same way as explained in irradiated arginine ethyl ester (McMurry, 2004). 2-Propanone in irradiated lysine ethyl ester was possibly formed through the ketonic decarboxylation (Renz, 2005). Other volatile compounds produced from lysine ethyl ester by irradiation include 2-methoxy-2-methyl propane, acetic acid ethyl ester, and 1-methyl-acetic acid ethyl ester, but their amounts were less than 1/10 of the key volatile compounds from lysine ethyl ester (Table 1).

As in aromatic group amino acid monomers (Ahn et al., 2016a), irradiation did not increase the amounts of total volatile much from the aromatic amino acid esters although a few volatile compounds were produced after irradiation. Irradiation of phenylalanine produced 1 volatile compound (toluene). Benzene and toluene have the same ring structure as the side chain of phenylalanine. So, it is assumed that toluene was formed directly from the side chain of phenylalanine. The production of hexane in tyrosine ethyl ester could be through the formation of benzene and the rearrangement of benzene: under high temperature, benzene can be rearranged to generate methyl cyclopentane (Hu, Shima, & Hou, 2014), and a ring-opening reaction could occur later to form hexane (Zhao, Moskaleva, & Rösch, 2013).

Irradiation changed the volatile profiles of alanine methyl ester, isoleucine methyl ester, leucine methyl ester, and valine ethyl ester most among the aliphatic group amino acids esters: many volatile compounds were produced from the four amino acid esters by irradiation, but the most predominant volatiles in aliphatic group amino acids esters were 2-propenonic acid methyl ester and propanoic acid methyl ester form alanine methyl ester; 3-methyl pentanoic acid methyl ester

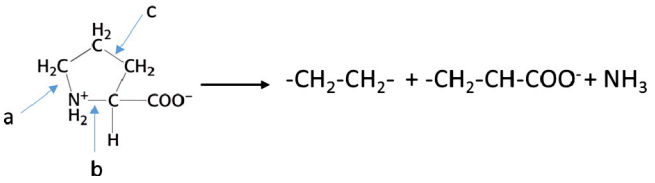
from isoleucine methyl ester; 4-methyl pentanoic acid methyl ester from leucine methyl ester; and 3-methyl butanoic acid methyl ester and 3-methyl 2-butenoic acid methyl ester from valine ethyl ester. These compounds were formed by the following reactions: 1) irradiation cleaved an $-NH_2^-$ from α-carbon and the remaining part reacted with hydrogen radical (•H), a main product of irradiation (Thakur & Singh, 1994), to produce the respective compound from each of the amino acid ester (Reaction 2).



(2)

In addition to those ester compounds, the production of 2-methyl butanal from isoleucine methyl ester; 3-methyl butanal from leucine methyl ester; and 2-methyl propanal from valine ethyl ester by irradiation was also prominent. Mottram, Wedzicha, and Dodson (2002) found that the Strecker degradation can generate aldehydes from the branched-chain of amino acids. Similar reaction pathway was reported by Weenen and van der Ven (2001) and Yaylayan (2003). Ahn et al. (2016a) also found that deamination and decarboxylation from α-carbon of the amino acids by ozone can produce 2-methyl butanal, 3-methyl butanal and 2-methyl propanal from the three amino acids. This indicated that the Strecker degradation could be the main pathway for producing volatile compounds from aliphatic group amino acids by irradiation.

The production of 2-propenoic acid methyl ester from proline methyl ester by irradiation suggested a deamination (arrow 'a' and 'b' in Reaction 3) and a bond breakage at the side chain (arrow 'c' in Reaction 3) were occurred. These reactions also indicated that a $-CH_2-CH_2-$ group might be formed at the imidazole group. The other produced volatile, acetaldehyde, further supported our assumption, because acetaldehyde (CH_3CHO) can be formed from $-CH_2-CH_2-$ reacting with hydroxyl radical (•OH) and further oxidation–reduction reaction (McMurry, 2004).



(3)

The significant increase of cyclohexane in irradiated proline methyl ester also confirmed the production of ($-CH_2-CH_2-$) from side chain because a cyclic reaction will be involved in the formation of cyclohexane. Through the similar mechanisms, methyl cyclopentane, cyclohexane and 2-methyl-1,4-pentadiene were also produced (Mehta & Mehta, 2005; Thakur & Singh, 1994).

Acetaldehyde as well as several other volatile compounds including 2-butanone, 2-propenoic acid methyl ester, and propanoic acid methyl ester were produced from serine ethyl ester, while acetaldehyde and 2-butenoic acid methyl ester were produced from threonine methyl ester by irradiation. The formation of acetaldehyde from serine ethyl ester by irradiation is through a two-step reaction: first, amino and carboxyl residues are cleaved from the α-carbon to generate ethen-1-ol, and then forms acetaldehyde. Sato, Quitain, Kang, Daimon, and Fujie (2004) found that deamination and isomerization can form pyruvic acid from serine in high temperature and high pressure water. It is assumed that 2-butanone should be formed through a ketonic decarboxylation of an acetic acid and a pyruvic acid (Reaction 4). Acetaldehydes were also formed from threonine methyl ester. However, it was not formed through the same reaction mechanisms as in serine but through the production of CH_3CHOH^- residue from the side chain by irradiation because no propanal was detected.

$$CH_3COOH + CH_3COCOOH \longrightarrow CH_3C(O)CH_2CH_3 + CO_2 \quad (4)$$

The formation of 2-propenoic acid methyl ester, propanoic acid methyl ester, and 2-butenoic acid methyl ester by irradiation followed similar reaction pathways as described in aliphatic group amino acids esters: irradiation cleaved an $-NH_2^-$ from α-carbon and reacted with hydroxyl radical (•OH), and then dehydration reaction was involved to produce double bonds (Table 2).

Many volatiles were generated and the amounts of volatiles produced from amino acid esters by irradiation were very high. The results (Tables 1–2) indicated that the side chains are highly susceptible to radiolysis and some side chain groups are more susceptible to radiolytic attack than others. In addition to ester compounds, aldehydes and hydrocarbons were the major volatile compounds in irradiated acidic, aliphatic and aliphatic hydroxyl amino acid ester groups. However, the side chain groups containing hydroxyl group (serine ethyl ester and threonine methyl ester) and nitrogen atom (asparagine *t*-butyl ester, glutamine *t*-butyl ester, arginine ethyl ester, lysine ethyl ester, histidine methyl ester, and tryptophane ethyl ester) produced smaller amounts of volatile compounds because of the high hydrophilicity of the hydroxyl group and the low volatilities of N-containing compounds. Low-molecular weight aldehydes are characterized by their unpleasant and pungent odors, and produce irritating effect to the nose (Turin & Yoshii, 2003). Therefore, it is assumed that these low-molecular weight aldehydes (acetaldehyde, propanal, 2-methyl propanal, 2-methyl butanal, and 3-methyl butanal) may have contributed significantly to the irradiation off-odor.

3.2. Sulfur-containing amino acid group

Volatiles such as mercaptomethane, ethanethiol, ethyl formate, 2-propenoic acid methyl ester were produced from cysteine ethyl ester by irradiation. Mercaptomethane can be produced through the direct cleave of cysteine ethyl ester side chain. Ethanethiol can be formed by the reaction of mercaptomethane with CH_3^-. The free methyl group could be provided by ethyl ester group. Propionic acid methyl ester was detected in irradiated cysteine ethyl ester, which indicated that a methyl group was lost at ethyl ester terminal during irradiation. Meanwhile, ethanethiol also can be formed in a different pathway: after deamination and decarboxylation of cysteine by irradiation, the formation of ethanethiol residue group ($-CHCH_2SH$) further reacted with hydrogen radical (•H) to produce ethanethiol (Thakur & Singh, 1994). The relatively high amount of ethyl formate in irradiated cysteine ethyl ester as an evidence supported the decarboxylation reaction can occur easily in cysteine.

Irradiation of cystine *di*-ethyl ester produced 6 volatile compounds: acetaldehyde, 2-propanone, carbon disulfide, acetic acid methyl ester, 2-propeonic acid methyl ester, and ethyl propanate (Table 3). Cystine is the amino acid formed via a covalent bond derived from two thiol groups of cysteines (Berg et al., 2012). Because of the redistribution of electron clouds in the disulfide bond, it is highly reactive towards radicals and can result in disulfide bond cleavage (Stinson & Xia, 2013).

The formation of 2-propenoic acid methyl ester and ethyl propanate from the primary radiolytic products indicated that a bond between $SH-CH_2$ can be easily broken. After the cleavage of a bond between $SH-CH_2$, two alanines [$CH_3CH_2(NH_2)COOH$] and H_2S can be formed. Acetaldehyde can be produced by removing $-NH_2^-$ and –COOH through the Strecker degradation of alanine. Good, Lacina, and McCullough (1961) found that the reaction of H_2S with CO_2 can produce carbon disulfide (CS_2). Considering that a ketonic decarboxylation reaction was

Table 2
Production of volatile compounds from aliphatic and aliphatic hydroxyl group amino acid esters solution by irradiation.[a]

Volatiles	5 kGy
	Total ion counts $\times 10^4$
Aliphatic group amino acid esters	
Alanine methyl ester	
2-Propenonic acid methyl ester	119,025 ± 5430
Propanoic acid methyl ester	199,886 ± 10,340
2-Methyl propanoic acid methyl ester	841 ± 62
Isoleucine methyl ester	
2-Methyl-1-propene	985 ± 94
Butane	1297 ± 88
1-Butene	2359 ± 420
2-Butene	680 ± 106
2-Methyl propanal	506 ± 94
2-Butanone	3239 ± 1060
2-Methyl butanal	16,057 ± 488
Butanoic acid methyl ester	588 ± 44
2-Butenoic acid methyl ester	531 ± 40
3-Methyl butanoic acid methyl ester	5402 ± 208
Pentanoic acid methyl ester	680 ± 8
3-Hexenoic acid methyl ester	7280 ± 458
3-Methyl pentanoic acid methyl ester	289,010 ± 1698
Hexanoic acid methyl ester	2707 ± 292
2-(Dimethylhydrazono) butanal	27,247 ± 494
Leucine methyl ester	
2-Methyl 1-propene	1795 ± 234
2-Methyl propanal	11,689 ± 826
2-Methyl 2-propenal	423 ± 52
2,2-Oxybis propane	977 ± 40
2-Propenoic acid methyl ester	2011 ± 294
Propanoic acid methyl ester	719 ± 108
3-Methyl butanal	14,414 ± 576
3-Methyl butanoic acid methyl ester	3368 ± 276
4-Methyl 2-pentenoic acid methyl ester	64,936 ± 2248
3-Methyl butanoic acid methyl ester	6211 ± 326
4-Methyl pentanoic acid methyl ester	252,829 ± 3822
4-Methyl 4-pentenoic acid methyl ester	3283 ± 156
2,4-Dimethyl hexanoic acid methyl ester	2268 ± 106
Proline methyl ester	
Acetaldehyde	3573 ± 1090
Hexane	702 ± 8
Methyl cyclopentane	524 ± 10
2-Propenoic acid methyl ester	542 ± 398
Cyclohexane	22,710 ± 2522
2-Methy-1,4-pentadiene	732 ± 214
Valine ethyl ester	
2-Methyl propanal	13,080 ± 2190
Acetic acid ethyl ester	1627 ± 206
Propanoic acid methyl ester	2593 ± 894
3-Methyl butanal	1625 ± 92
3-Methyl butanoic acid methyl ester	381,988 ± 30,348
3-Pentenoic acid methyl ester	33,890 ± 2394
Pentanoic acid methyl ester	1968 ± 378
2-Butenoic acid methyl ester	17,759 ± 2220
3-Methyl 2-butenoic acid methyl ester	176,890 ± 4152
4-Methyl pentanoic acid methyl ester	1516 ± 186
Aliphatic hydroxyl group amino acid esters	
Serine ethyl ester	
Acetaldehyde	1716 ± 896
2-Butanone	1721 ± 688
2-Propenoic acid methyl ester	56,624 ± 7960
Propanoic acid methyl ester	3777 ± 706
Threonine methyl ester	
Acetaldehyde	19,094 ± 3584
2-Butenoic acid methyl ester	626 ± 52

[a] Only the newly formed volatiles in irradiated amino acid esters (5 kGy) were listed (n = 4).

necessary to form a 2-propanone from two acetic acids, a large amount of CO_2 was produced as the final product in the decarboxylation reaction (Renz, 2005). We can deduce that carbon disulfide (CS_2) was formed by the reaction of H_2S with CO_2 during irradiation in cystine *di*-ethyl ester.

Table 3
Production of volatile compounds from sulfur-containing amino acid ester solution by irradiation.[a]

Volatiles	5 kGy
	Total ion counts $\times 10^4$
Cysteine ethyl ester	
Mercaptomethane	1743 ± 420
Ethanethiol	366 ± 70
Ethyl formate	9091 ± 572
Propionic acid methyl ester	20,012 ± 1454
Cystine di-ethyl ester	
Acetaldehyde	3680 ± 158
2-Propanone	1812 ± 228
Carbon disulfide	537 ± 42
Acetic acid methyl ester	1841 ± 64
2-Propenoic acid methyl ester	177,002 ± 2128
Ethyl propanate	7124 ± 840
Methionine methyl ester	
2-Propanone	747 ± 14
Thiobis methane	2383 ± 98
Methyl thiirane	621 ± 20
2-Propenoic acid methyl ester	2850 ± 44
Butanoic acid methyl ester	7054 ± 350
Dimethyl disulfide	147,591 ± 1892
Cyclopropanecarboxylic acid methyl ester	8135 ± 244

[a] Only the newly formed volatiles in irradiated amino acid esters (5 kGy) were listed (n = 4).

Irradiation of methionine methyl ester produced 2-propanone, thiobis methane, methyl thiirane, 2-propenoic acid methyl ester, butanoic acid methyl ester, dimethyl disulfide, and cyclopropanecarboxylic acid methyl ester. The production of 2-propenoic acid methyl ester, butanoic acid methyl ester, and cyclopropane carboxylic acid methyl ester indicated that the bond between S–CH_2 and the bond between CH_2–CH_2 in the side chain can be easily broken. The formation of thiobismethane and dimethyl disulfide by irradiation indicated that these compounds were produced not only through the radiolytic degradation of side chains but also the chemical reactions of the primary sulfur compounds with other volatile compounds after they were produced (Fan, Lee, & Ahn, 2011). Considering the primary radiolysis products of water are OH•, H•, and e^-_{aq} (Thakur & Singh, 1994), we propose possible radiolytic pathways for methionine methyl ester in Fig. 1. The production of methyl thiirane further indicated that there was a cyclic reaction in the side chain after it was cleaved from methionine methyl ester. The other produced volatiles in methionine methyl ester include 2-propanone, 2-propenonic acid methyl ester, and butanoic acid methyl ester. 2-Propanone should have been formed through the same reaction as in aspartic acid β-methyl ester: the ketonic decarboxylation of two CH_3COO^-. 2-Propenoic acid methyl ester could have been produced through the same reaction pathways as in histidine methyl ester. Butanoic acid methyl ester and cyclopropane carboxylic acid methyl ester should have been produced through the similar mechanisms as have been described in aliphatic group amino acids ester: irradiation cleaved an –NH_2 from α-carbon, –SCH_3 from side chain, and then a reaction with hydrogen radical (•H). To form cyclopropane carboxylic acid methyl ester, a cyclic reaction on the side chain is necessary.

Fan (2012) assumed that methionine is the principal source of volatile sulfur compounds. In this study, we found that the amount of sulfur compounds produced from methionine methyl ester was higher than those of other sulfur-containing amino acid esters (cysteine ethyl ester, cystine di-ethyl ester), confirming that the contribution of methionine to the irradiation odor is far greater than that of the cysteine and cystine.

3.3. Major volatiles and odor characteristics

Table 4 showed the major volatiles from amino acids and their odor characteristics after irradiation. Off-odors in protein-containing

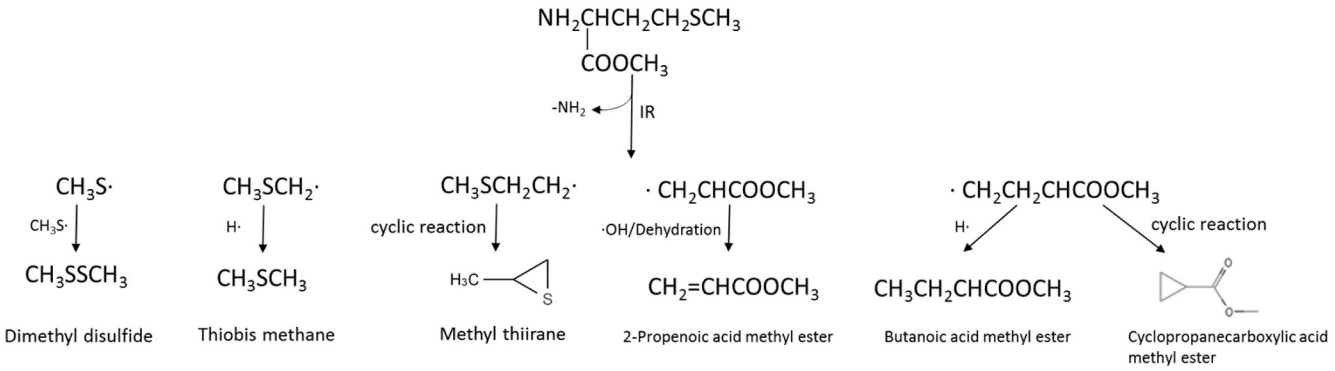


Fig. 1. Proposed formation of voltiles from methionine methyl ester by irradiation.

products are actually due to protein and/or amino acid degradation (Shipe et al., 1978). Various volatile sulfur compounds are produced by irradiation such as hydrogen sulfide, sulfur dioxide, carbon disulfide, mercaptomethane, dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, bis-methylthio-methane, and methyl thioacetate (Fan et al., 2002; Lin et al., 2007; Patterson & Stevenson, 1995). Brewer (2009) reported that sulfur-containing volatiles formed from sulfur amino acids contributed to the irradiation odor. Also, sulfur compounds have extremely low sensory threshold, which bring much stronger effects on the odor of food products (Buttery & Ling, 1998; Frank, Owen, & Patterson, 2004; Hill & Smith, 2000; Landaud, Helinck, & Bonnarme, 2008). Sensory panelists described the odor of irradiated sulfur-containing amino acids as "hard-boiled eggs and sulfury" and "boiled cabbage or vegetables". Typical odor characteristics of sulfur-containing amino acids indicated that sulfur volatiles played the major role in the odor of the irradiated samples (Table 4).

The sources and mechanisms of generating off-odor volatiles are much more complex in the real food system than in the model systems. Two kinds of reactions are discussed most as the cause of odor/flavor generation in foods: Maillard reaction and the Strecker degradation. The Maillard reaction is an important reaction in the formation of aroma compounds in meat (Bailey, 1994). This reaction starts with a condensation reaction between the carbonyl group in a reducing sugar and a free amino group and a series of secondary reactions after that (Martins, Jongen, & van Boekel, 2000). The Strecker degradation is often considered as a sub-reaction within the Maillard reaction (Yaylayan, 2003). The typical Strecker degradation involves the oxidative deamination and decarboxylation of α-amino acid in the presence of α-dicarbonyl compounds. The products of the Strecker degradation are α-aminoketones and Strecker aldehydes containing one carbon less than the corresponding amino acid (Resconi, Escudero, & Campo, 2013). However, the Maillard reaction was not involved in producing

Table 4
The major volatiles and odor characteristics of irradiated amino acid esters solutions.

Amino acid[a]	Major volatiles	Odor characteristics[b]
Acidic group amino acid esters		
Aspartic acid di-ME	Acetaldehyde	No odor
Aspartic acid β-ME	Acetaldehyde	No odor
Glutamic acid γ-ME	Acetaldehyde, propanal	Honey, sweet
Amide group amino acid esters		
Asparagine *t*-BE	2-Methyl propane, 2-propanone, 2-methyl-2-propenal, 2,2-dimethyl propanane	No odor
Glutamine *t*-BE	2-Methyl propane, 2-methyl-2-propenal, 2,2-dimethyl-propanal	Hospital odor
Basic group amino acid esters		
Arginine EE	Acetaldehyde	Bean sprouts, sperm, detergent
Histidine ME	Acetaldehyde, 2-propenoic acid ME	No odor
Lysine EE	Acetaldehyde	Sour
Proline ME	Acetaldehyde, cyclohexane	Sweet and nutty
Valine EE	2-Methyl propanal, 3-methyl butanoic acid ME	Roast nuts
Aromatic group amino acid esters		
Phenylalanine EE	Toluene	Strong solvent odor
Tyrosine EE	Hexane	Alcohol, mild solvent
Aliphatic group amino acid esters		
Alanine ME	2-Propenoic acid ME, propanoic acid ME	Sour, yoghurt, cheese, aftershave, alcohol
Isoleucine ME	2-Methyl butanal, 3-methyl-pentanoic acid ME	Sweet (licorice), roast nuts
Leucine ME	3-Methyl butanal, 4-methyl-pentanoic acid ME	Weak roast nuts, grease, wax, gasoline
Proline ME	Acetaldehyde, cyclohexane	Sweet and nutty
Valine EE	2-Methyl propanal, 3-methyl butanoic acid ME	Roast nuts
Aliphatic hydroxyl group amino acid esters		
Serine EE	Acetaldehyde, 2-butanone 2-propenoic acid ME, propanoic acid ME	Coleslaw, sweet
Threonine ME	Acetaldehyde	Hospital odor
Sulfur-containing amino acid esters		
Cysteine EE	Mercaptomethane, ethyl formate, Propionic acid ME	Boiled egg, sulfury
Cystine *di*-EE	Acetaldehyde, 2-propanone, acetic acid ME, 2-propeonic acid ME, ethyl propanate	Alcohol
Methionine ME	Thiobis methane, 2-propenoic acid ME, butanoic acid ME, dimethyl disulfide, cyclopropanecarboxylic acid ME	Boiled cabbage, boiled vegetables

[a] Abbreviations: ME: methyl ester, EE: ethyl ester.
[b] Odor characteristics of each irradiated amino acid esters.

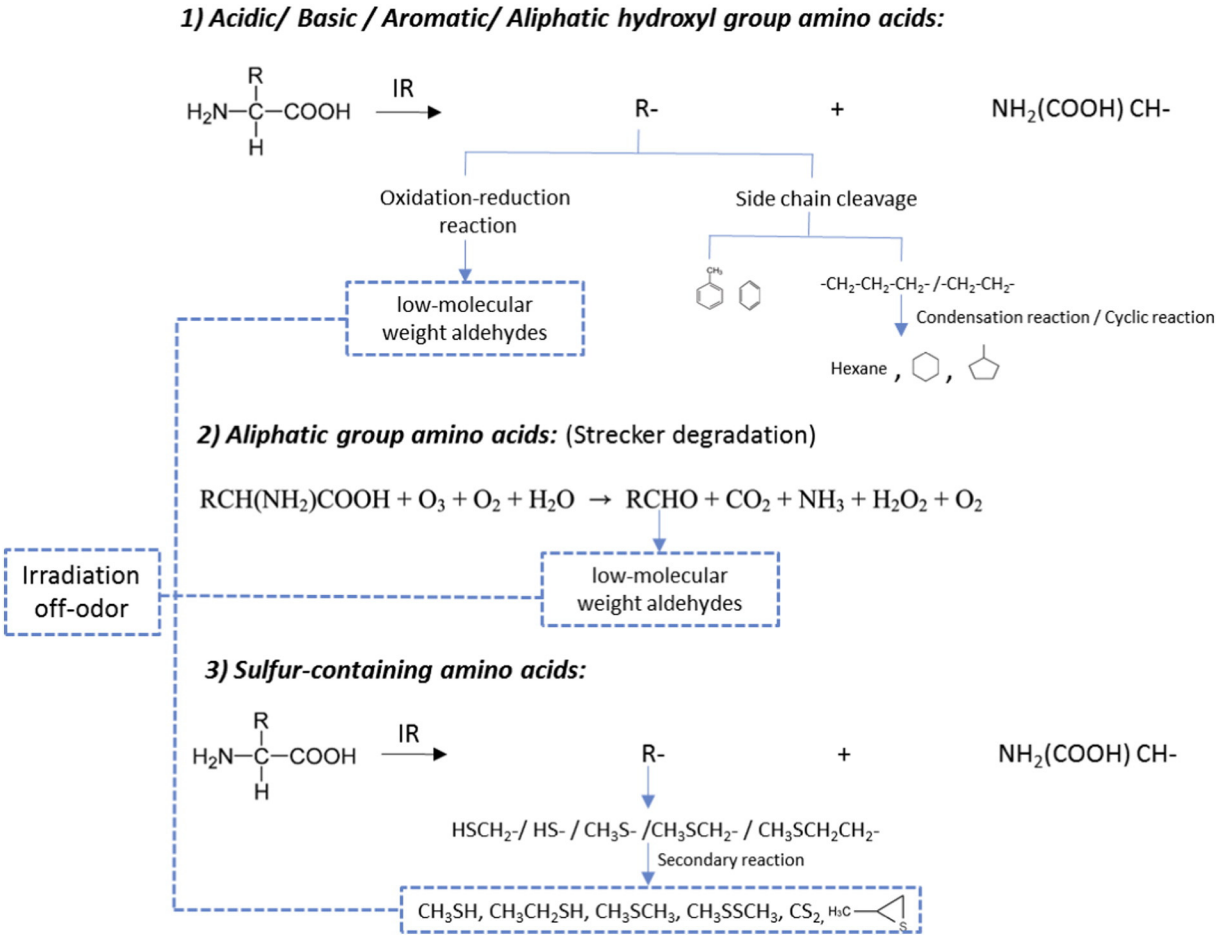


Fig. 2. Proposed formation of off-odor from amino acids by irradiation.

volatiles from irradiated amino acids ester while the Strecker degradation played important roles in volatile production in the irradiated amino acids model systems probably because our study system did not contain any carbohydrates.

4. Conclusions

The majority of volatiles produced from the amino acid esters in model system by irradiation were mainly from the side chains of amino acid esters. However, the volatile compounds produced from amino acid esters by irradiation were not only the primary products of radiolytic degradation, but also the products of extensive chemical reactions, which include deamination, hydrogenation, oxidation–reduction, decarboxylation, dehydration, condensation, isomerization, cyclic reaction and rearrangement of the primary radiolytic products (Fig. 2). The low-molecular weight aldehydes (acetaldehyde, propanal, 2-methyl propanal, 2-methyl butanal, and 3-methyl butanal), which contributed significantly to the irradiation off-odor, were mainly produced from acidic, aliphatic and aliphatic hydroxyl group amino acid esters through the radiolysis of amino acid side chains or the Strecker degradation. However, the contributions from non-sulfur amino acid esters are very small compared with that of the sulfur amino acid esters. Among the sulfur-containing amino acid esters, methionine ester made greater contribution to the irradiation odor than other sulfur amino acid esters. The sulfur amino acid esters produced volatiles not only through the direct cleavage of the side chains, but also the chemical reactions of primary sulfur compounds with other compounds produced by irradiation. This study further confirmed that the main sources and production mechanism of off-odor volatiles in meat by irradiation are meat proteins through the radiolytic and the Strecker degradation of amino acid side chains. Based on this conclusion, several possible solutions could be used to minimize off odor produced from irradiated meat, including masking agents, off-odor absorbers and double packaging.

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