



Irradiated Chinese Rugao ham: Changes in volatile *N*-nitrosamine, biogenic amine and residual nitrite during ripening and post-ripening

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ABSTRACT

N-nitrosamines, biogenic amines and residual nitrite are harmful substances and often present in cured meat. The effects of gamma-irradiation (γ -irradiation) on these chemicals in dry-cured Chinese Rugao ham during ripening and post-ripening were investigated. Rugao hams were irradiated at a dose of 5 kGy before ripening and were then ripened in an aging loft. Although γ -irradiation degraded tyramine, putrescine and spermine, on the other hand, it promoted the formation of spermidine, phenylethylamine, cadaverine and tryptamine. Residual nitrite was significantly reduced by γ -irradiation. *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA) and *N*-nitrosopyrrolidine (NPYR) were found in Chinese Rugao ham during ripening and post-ripening but could be degraded with γ -irradiation. The results suggest that γ -irradiation may be a potential decontamination measure for certain chemical compounds found in dry-cured meat.

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1. Introduction

Investigation of the safety of meat products includes the assessment of biogenic amines, residual nitrite and volatile *N*-nitrosamines, as these compounds can present a health hazard through direct toxic effects or by forming reaction products. For example, nitrite and its derivatives may react with some biogenic amines to produce volatile *N*-nitrosamines. Also, biogenic amines can affect blood pressure and excessive quantities of these amines in foods can trigger migraines in sensitive individuals, in addition to gastric and intestinal problems and allergic responses (Stratton, Hutkins, & Taylor, 1991). Nitrite can also oxidize the reduced form of iron, Fe²⁺, in haemoglobin which is responsible for the transport of oxygen in mammalian blood, to its oxidized form, Fe³⁺ which is then converted to methaemoglobin (MeHb). The resulting MeHb is unable to release oxygen to body tissues because of its high dissociation constant, which produces a situation that is lethal to humans and can be caused by the presence of approximately 1 g nitrite (Cassens, 1997). Moreover nitrite can be converted into nitrosating agents that may easily react with secondary amines to produce carcinogenic *N*-nitrosamines (Shahidi, Pegg, & Sen, 1994). In addition, some biogenic amines may react with nitrosat-

ing agents to produce *N*-nitrosamines. It has also been suggested that *N*-nitrosopyrrolidine (NPYR) may be formed from spermidine and spermine; and *N*-nitrosopiperidine (NPIP) from cadaverine or piperidine (Ansorena et al., 2002; Smith, 1980; Warthesen, Scanlan, Bills, & Libbey, 1975). *N*-Nitrosamines have been shown to induce tumors in liver, lung, esophagus, bladder and pancreas in various experimental animal species (Lijinsky, 1999).

Therefore, great efforts have been made to investigate and reduce the presence of these compounds in meat products. It has been shown that the formation of biogenic amines is influenced by certain microorganisms, the nature of the meat components used in the product, and the procedures used during the processing of meat products (Capillas & Colmenero, 2004). The concentration of residual nitrite can be reduced by adding ascorbic acid, sorbate, EDTA and natural colorants during meat processing (Bloukas, Arvanitoyannis, & Siopi, 1999; Radcliffe, Lamb, Blinkhorn, & Drucker, 2003; Shuib & Abdullah, 2002), or by giving a dietary supplement of α -tocopheryl acetate to animals used for meat production (Dineen et al., 2000). The reduction of nitrite concentration and the virtual elimination of nitrate have been undertaken by manufacturers on both a voluntary basis and, in many countries, by government regulations (Lijinsky, 1999). It has also been reported that the formation of *N*-nitrosamines can be inhibited by the addition of vitamin C and E (Tricker & Preussmann, 1991). However γ -irradiation has also emerged as an effective measure to reduce the content of biogenic amines, residual nitrite and

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volatile *N*-nitrosamines in meat products (Ahn, Kim, Jo, Yook, & Byun, 2003; Ahn et al., 2004a; Kim et al., 2005).

Gamma irradiation is an effective method of eliminating pathogens and controlling the growth of pathogenic and spoilage bacteria in meat and meat products. Recently, significant progress has been made in using irradiation to reduce the concentration of harmful chemicals such as biogenic amines in pepperoni (Kim et al., 2005), and volatile *N*-nitrosamines and nitrite in sausage (Ahn et al., 2003; Ahn et al., 2004a). However, little information is available about volatile *N*-nitrosamines in dry-cured meat products, especially the simultaneous investigation of *N*-nitrosamine, biogenic amines and residual nitrite in irradiated dry-cured ham during ripening and post-ripening.

Chinese Rugao ham is one of the most famous dry-cured meat products in China. The objective of this work was to investigate the effect of γ -irradiation on the volatile *N*-nitrosamines, biogenic amines and residual nitrite in dry-cured ham during ripening and post ripening.

2. Materials and methods

2.1. Chinese Rugao ham preparation

Procedures for the preparation of Chinese Rugao ham consist of six stages: preparation of green ham, salting, soaking and washing, sun-drying and shaping, ripening, and post-ripening. The details are as follows, green hams were salted (9–10 kg salt per 100 kg of green ham, salting-room temperature 0–8 °C and relative humidity 60–80%) and stored in piles (10–12 layers placed on platforms) in salting-room for 40 days during which time salt was added on six occasions and the piles of ham were turned eight times. A total of 150 mg of sodium nitrite per 1 kg of green ham was added to the salt during salting. After soaking and washing for 24 h and sun-drying (under natural conditions outdoors) for 15 days, the hams were transferred to an aging loft (the room or space under a roof and above the ceiling of the uppermost story, often used for storage) for ripening over a period of seven months, after ripening the hams were post-ripened at room temperature for two months.

Sixty trimmed green hams, of 8–9 kg produced from the native Jiangquhai swine, were purchased from the Chinese Rugao ham Company of the Jiangsu Longlife Group, PR China, and randomly divided into two groups. Thirty green hams were treated with γ -irradiation at a dose of 5 kGy at the end of the sun-drying period (55 d), and were then transferred to an aging loft for ripening. The other green hams were used as controls. All the green hams were processed following the procedures described above for Chinese Rugao ham.

2.2. γ -Irradiation

After the sun-drying period (55 d) 30 hams were irradiated in a cobalt-60 irradiator (Nantong Michael Irradiation CO., LTD.), and were then transferred to an aging loft to ripen. The source strength was ca. 150 kCi. The irradiation dose used in this study was 5 kGy and the dose rate was 10–15 kGy/h. To confirm the target dose, 2 alanine dosimeters per cart were attached to the top and bottom surfaces of the sample. The alanine dosimeter was read using a 104 Electron Paramagnetic Resonance instrument (Bruker Instruments, Billerica, MA). The final doses were 5.16 ± 0.46 kGy for 5 kGy treatments.

2.3. Sampling

Five hams were randomly taken from each group at the following stages: after γ -irradiation (55 d), mid-ripening (135 d), end-

ripening (265 d) and post ripening (325 d). Approximately 600 g of muscle from the *Biceps femoris*, *Semimembranosus* and *Semitendinosus* was obtained from each ham and then minced. The minced samples were vacuum-packaged and stored at –20 °C for further analysis.

2.4. Determination of volatile *N*-nitrosamines

2.4.1. Extraction and purification of volatile *N*-nitrosamines

A triple steam-distillation method (Rongmei & Lishan, 1980) was adopted to extract and purify the volatile *N*-nitrosamines. Two hundred grams of minced sample were homogenized in 100 ml of ultrapure water with 120 g of sodium chloride using a Warring Blender (Cole-Parmer, Vernon Hills, Illinois, USA) at high speed for 2 min. The homogenate was then steam-distilled and approximate 400 ml of the distillate was collected. The distillate was mixed with 80 g of sodium chloride and 4 g of sodium hydroxide and then steam-distilled again. During the second steam-distillation, 300 ml of distillate was collected and 40 g sodium chloride and 6 g tartaric acid were then stirred into the distillate and the mixture was then steam-distilled again for a third time. Finally, about 250 ml of distillate was collected and transferred to a 500 ml separating funnel. Eighty grams of sodium chloride and 40 ml of dichloromethane (DCM) were added to the distillate and evenly mixed. The mixture was extracted four times with 160 ml of DCM. The DCM extracts were pooled and dried with anhydrous sodium sulfate, and then concentrated to about 10 ml in a Kuderna-Danish apparatus (Wenzhou T-zone Machinery Manufacturing Co., Ltd., PR China). Finally, the concentrate was evaporated under nitrogen to a final volume of 1 ml in water bath at 40 °C.

2.4.2. Gas chromatography–mass spectrometry analysis

Analyses were performed using a Varian CP3800 series gas chromatograph (Walnut Creek, CA, USA) coupled to a CTC CombiPAL autosampler (Zwigen, Switzerland) and a Varian 1200 L MS/MS triple quadrupole mass spectrometer (Walnut Creek, CA, USA). Analytes were separated using a fused-silica capillary column (DB-5 MS, 30 m \times 0.25 i.d, film thickness 0.25 μ m) and high-purity helium (99.999%) was used as the carrier gas with a constant flow of 0.8 ml per minute. The injector temperature was set at 250 °C and splitless injection was employed. The oven temperature was programmed as follows: an initial holding temperature of 40 °C for 2 min, then an increase to 55 °C at a rate of 5 °C per minute followed by a further increase to 250 °C at a rate of 15 °C per minute which was maintained for 5 min. Detection of compounds was carried out in the selected ion monitoring (SIM) mode (Table 1).

2.5. Determination of biogenic amines

The extraction, dansylation, identification and quantification of biogenic amines by RP-HPLC were performed according to the method described by Vinci and Antonelli (2002).

Table 1

Volatile NA included in the studied standard, together with the selected ion for detection in MS SIM mode, the retention time for each analyte and their molecular weight^a

<i>N</i> -nitrosamines	<i>m/z</i>	Retention time (min)	MW
<i>N</i> -nitrosodimethylamine (NDMA)	74	5.173	74
<i>N</i> -nitrosodiethylamine (NDEA)	88	7.793	88
<i>N</i> -nitrosopyrrolidine (NPYR)	100	10.159	100
<i>N</i> -nitrosopiperidine (NPIP)	114	10.679	114
<i>N</i> -nitrosodibutylamine (NDBA)	84	12.310	158

^a *m/z*: mass/charge; MW: molecular weight.

2.6. Determination of residual sodium nitrite

The residual concentrations of sodium nitrite in Chinese Rugao ham were determined according to ISO methods (ISO 2918-1975).

2.7. Statistical analysis

The experiment was replicated twice and the data was analyzed using SAS software (SAS 8.2, Institute Inc., 2001). The effects of γ -irradiation treatment on the content of residual nitrite, biogenic amines and *N*-nitrosamines were evaluated using a hypothesis test and two-sample *t*-test. The changes in concentration of these compounds during processing were analyzed using Duncan's multiple-range test. Significance was defined at $P < 0.05$.

3. Results

3.1. Residual nitrite

Gamma irradiation immediately reduced the residual nitrite content in Chinese Rugao ham. The residual nitrite concentration in irradiated samples was significantly decreased compared to the control on the day (55 d) after irradiation (shown in Table 2) ($P < 0.05$). The degrading effect of irradiation on residual nitrite continued to be observed at mid-ripening (135 d) ($P < 0.05$). The nitrite on the day (55 d) after irradiation was 25.92% less than the control; however, the greatest decrease in percentage occurred at mid-ripening (135 d) and was 69.67%. Gamma irradiation also

Table 2
Changes in residual nitrite (mg/kg) in irradiated Chinese Rugao hams and controls during ripening and post ripening (mean \pm s.d., $n = 5$)^a

Time (days)	Control	Irradiation	Decreasing percentage (%)
55	8.99 \pm 0.83 ^{Aa}	6.66 \pm 0.37 ^{Ab}	25.92
135	9.43 \pm 0.66 ^{Aa}	2.86 \pm 0.12 ^{Cb}	69.67
265	6.40 \pm 0.19 ^{Za}	4.36 \pm 0.35 ^{Bb}	31.88
325	6.03 \pm 0.20 ^{Za}	3.04 \pm 0.95 ^{Cb}	49.58

^a Means indicated by different superscript (capital letters) in the same column differ significantly ($P < 0.05$); means indicated by different lowercase letters in the same row differ significantly ($P < 0.05$); the results are expressed in terms of dry matter.

Table 3

Changes in biogenic amines (mg/100 g) in irradiated Chinese Rugao ham and controls during ripening and post ripening (mean \pm s.d., $n = 5$)^a

Name/time (days)		55	135	265	325
Total bas	Control	14.97 \pm 1.04 ^{Ab}	11.42 \pm 1.64 ^{Ba}	2.33 \pm 0.33 ^{Cb}	2.07 \pm 0.19 ^{Ca}
	Irradiation	11.93 \pm 0.39 ^{Aa}	14.45 \pm 1.48 ^{Ba}	7.23 \pm 0.88 ^{Ca}	1.64 \pm 0.12 ^{Ca}
Tyramine	Control	3.39 \pm 0.50 ^{Ba}	1.32 \pm 0.85 ^{Aa}	0.26 \pm 0.12 ^{Aa}	0.19 \pm 0.10 ^{Aa}
	Irradiation	0.71 \pm 0.18 ^{Wb}	2.46 \pm 0.38 ^{Xb}	1.18 \pm 0.23 ^{Yb}	0.09 \pm 0.02 ^{Zb}
Phenylethylamine	Control	0.06 \pm 0.01 ^{Ba}	0.06 \pm 0.02 ^{Ba}	0.01 \pm 0.00 ^{Ca}	0.01 \pm 0.00 ^{Ca}
	Irradiation	0.16 \pm 0.01 ^{Xb}	0.06 \pm 0.02 ^{Ya}	0.02 \pm 0.00 ^{Za}	0.04 \pm 0.00 ^{YZb}
Putrescine	Control	2.19 \pm 0.50 ^{Ba}	0.79 \pm 0.14 ^{Ca}	0.34 \pm 0.17 ^{Aa}	0.22 \pm 0.03 ^{Aa}
	Irradiation	0.85 \pm 0.44 ^{Xb}	2.15 \pm 0.91 ^{Yb}	0.99 \pm 0.35 ^{Xb}	0.26 \pm 0.07 ^{Xa}
Spermine	Control	5.87 \pm 0.94 ^{Aa}	5.39 \pm 0.49 ^{Aa}	0.91 \pm 0.11 ^{Ba}	0.82 \pm 0.11 ^{Ba}
	Irradiation	6.30 \pm 0.32 ^{Xa}	3.36 \pm 0.33 ^{Yb}	0.94 \pm 0.04 ^{Za}	0.89 \pm 0.05 ^{Za}
Tryptamine	Control	1.08 \pm 0.20 ^{Aa}	1.20 \pm 0.09 ^{Aa}	nd	0.02 \pm 0.01 ^{Ba}
	Irradiation	1.31 \pm 0.05 ^{Xb}	1.43 \pm 0.12 ^{Yb}	0.03 \pm 0.00 ^Z	0.04 \pm 0.00 ^{Za}
Histamine	Control	nd	nd	nd	nd
	Irradiation	nd	nd	nd	nd
Cadaverine	Control	1.44 \pm 0.11 ^{Ba}	1.61 \pm 0.11 ^{Ca}	0.62 \pm 0.05 ^{Aa}	0.64 \pm 0.09 ^{Aa}
	Irradiation	1.40 \pm 0.11 ^{Xa}	4.05 \pm 0.84 ^{Yb}	3.90 \pm 0.36 ^{Yb}	0.15 \pm 0.00 ^{Zb}
Spermidine	Control	0.95 \pm 0.17 ^{Aa}	1.05 \pm 0.07 ^{Aa}	0.18 \pm 0.02 ^{Ba}	0.16 \pm 0.02 ^{Ba}
	Irradiation	1.21 \pm 0.10 ^{Xb}	0.93 \pm 0.11 ^{Ba}	0.18 \pm 0.02 ^{Ya}	0.17 \pm 0.03 ^{Ya}

nd: not detected; Bas: biogenic amines; the results are expressed in terms of dry matter.

^a Means indicated by different capital letters in the same row differ significantly ($P < 0.05$). Means for a particular biogenic amine indicated by different lowercase letters in the same column differ significantly ($P < 0.05$).

changed the fluctuations in residual nitrite in Chinese Rugao ham. The general trend of residual nitrite in control samples was an initial increase followed by a decrease with the highest concentration observed at 135 days. Conversely, the trend in residual nitrite concentration following irradiation was an initial decrease followed by an increase and a then final decrease.

3.2. Biogenic amines

Table 3 shows the biogenic amines concentrations in control and irradiated Chinese Rugao hams during ripening and post ripening. Gamma irradiation resulted in the degradation of some of the biogenic amines but in contrast, it also promoted the formation of other biogenic amines. After irradiation, the concentrations of total biogenic amines, tyramine and putrescine showed a marked and immediate decline ($P < 0.05$), after which, a sharp increase occurred such that concentrations after irradiation treatment were significantly higher than those in the control at mid-ripening (135 d) ($P < 0.05$). Irradiation also lowered the content of spermine at mid-ripening (135 d). However, irradiation increased the concentrations of some biogenic amines such as spermidine, cadaverine, tryptamine and phenylethylamine although there was no difference between irradiation treatment and control at post-ripening (325 d). In the present study, histamine was not detected.

3.3. Volatile *N*-nitrosamines

The changes in concentration of volatile *N*-nitrosamines in hams during ripening and post ripening are shown in Table 4. Gamma irradiation immediately decomposed the volatile *N*-nitrosamines in dry-cured ham. After γ -irradiation, the concentrations of all volatile *N*-nitrosamines found in Chinese Rugao hams decreased immediately ($P < 0.05$). Furthermore, NDEA was still not detected at 55 and 135 days. These facts show that γ -irradiation can degrade volatile *N*-nitrosamines. However, the concentrations of volatile *N*-nitrosamines increased significantly during ripening ($P < 0.05$). No significant differences in NDMA were found between the irradiated and control samples during the entire period of ripening and post-ripening ($P > 0.05$). But the concentrations of total volatile *N*-nitrosamines, NDEA and NPYR were lower in the irradiated than in the control samples during ripening and post-ripening.

Table 4Changes in *N*-nitrosamines ($\mu\text{g}/\text{kg}$) in irradiated Chinese Rugao ham and controls during ripening and post ripening (mean \pm s.d., $n = 5$)^a

Name/time (days)		55	135	265	325
Total VNNA ^b	Control	6.33 \pm 0.44 ^{Aa}	6.57 \pm 0.86 ^{Aa}	42.51 \pm 2.09 ^{Ba}	26.00 \pm 4.62 ^{Ca}
	Irradiation	2.56 \pm 0.22 ^{Bb}	7.82 \pm 0.41 ^{Cb}	21.52 \pm 3.35 ^{Bb}	14.52 \pm 0.17 ^{Ab}
NDMA	Control	0.31 \pm 0.09 ^{Aa}	0.39 \pm 0.04 ^{Aa}	0.67 \pm 0.05 ^{Ba}	0.11 \pm 0.04 ^{Ca}
	Irradiation	0.07 \pm 0.02 ^{Cb}	0.34 \pm 0.05 ^{Aa}	0.77 \pm 0.04 ^{Bb}	0.10 \pm 0.02 ^{Ca}
NDEA	Control	0.07 \pm 0.01 ^B	0.14 \pm 0.08 ^B	0.93 \pm 0.10 ^{Ca}	0.38 \pm 0.18 ^{Aa}
	Irradiation	nd	nd	0.30 \pm 0.05 ^{Yb}	0.26 \pm 0.13 ^{Yb}
NPYR	Control	5.95 \pm 0.47 ^{Aa}	6.03 \pm 0.82 ^{Aa}	40.90 \pm 2.19 ^{Ba}	25.51 \pm 5.49 ^{Ca}
	Irradiation	2.49 \pm 0.28 ^{Xb}	7.48 \pm 0.46 ^{Yb}	20.44 \pm 4.05 ^{Zb}	14.17 \pm 0.04 ^{Wb}

NDMA:*N*-nitrosodimethylamine; NDEA:*N*-nitrosodiethylamine; NPYR:*N*-nitrosopyrrolidine; the results are expressed in terms of dry matter.^a Means indicated by different capital letters in the same row differ significantly ($P < 0.05$); means for a particular *N*-nitrosamine indicated by different lowercase letters in the same column differ significantly ($P < 0.05$).^b VNNA:volatile *N*-nitrosamine.

4. Discussion

4.1. Residual nitrite

In the present study, γ -irradiation reduced the residual nitrite, an effect that remained over time. This was consistent with reported data on irradiated cooked pork sausage (Jo, Ahn, Son, Lee, & Byun, 2003). In addition, it has been reported that the effect of irradiation on residual nitrite could still be observed in vacuum-packaged sausages after four weeks of storage, but not in air-packaged samples (Ahn et al., 2004b). Moreover, it has been suggested that γ -irradiation may be effective in the breakdown of nitrite, and that the radiolysis products do not act as a precursor for *N*-nitrosodimethylamine formation (Ahn et al., 2003). It is well known that nitrite in food should be controlled not only because it is inherently toxic but also because it can be converted to nitrosating agents which are the precursors of the carcinogenic *N*-nitrosamines. Therefore, if γ -irradiation were to be adopted as a method of reducing residual nitrite in dry-cured ham, the radiolysis products are not likely to be converted to carcinogenic *N*-nitrosamines.

4.2. Biogenic amines

In this study, γ -irradiation was not only decreased the content of tyramine, putrescine and spermine in dry-cured ham, but also facilitated the formation of spermidine, cadaverine, tryptamine and phenylethylamine. However, in a previous paper the γ -irradiation of pepperoni was shown to reduce the concentration of spermidine, and increase the concentration of phenylethylamine (Kim et al., 2005). Thus γ -irradiation may have a more positive effect in reducing the concentration of biogenic amines in pepperoni than in dry-cured ham. After γ -irradiation treatment, the concentrations of tyramine, putrescine and spermine may recover. This phenomenon may be related to the processing conditions used in the production of Chinese Rugao ham. After irradiation, the hams continue to ripen under natural conditions and this may lead to the presence of large numbers of microorganisms in the hams. These microorganisms may then participate in the production of biogenic amines during the ripening stage.

4.3. Volatile *N*-nitrosamines

Gamma irradiation proved to be effective in reducing the volatile *N*-nitrosamine content in Chinese Rugao ham during ripening. Likewise, γ -irradiation reduced the content of NDMA and NPYR in cooked pork sausage packaged under vacuum, air or a modified atmosphere (Jo et al., 2003; Ahn et al., 2004a; Ahn et al., 2004b) and in fermented anchovy sauce (Ahn et al., 2003). However, in the present study NDEA was also degraded by γ -irradiation. Thus

γ -irradiation is able to degrade at least three volatile *N*-nitrosamines i.e. NDMA, NDEA and NPYR in dry-cured ham.

After γ -irradiation, the concentrations of volatile *N*-nitrosamines may also recover in dry-cured Chinese Rugao ham during ripening. This may be explained by the presence of residual nitrite and some secondary amines. Although gamma irradiation may degrade the nitrite to innocuous products which cannot be converted into nitrosating agents, there will still be some residual nitrite in the samples (shown in Table 2). In addition, it was suggested that γ -irradiation might degrade volatile *N*-nitrosamines to the corresponding amines and other non-nitrosating agents (Ahn, Kim, Yook, & Byun, 2002). Thus the corresponding amines may persist in the hams after γ -irradiation. Furthermore, the hams would once again be exposed to contamination by microorganisms during the period of ripening under natural conditions following irradiation. So some secondary amines would be produced by the microorganisms. The existence of residual nitrite and the increasing concentrations of the corresponding amines would lead to an increase in the concentrations of some volatile *N*-nitrosamines in Chinese Rugao ham during the ripening period.

5. Conclusions

Gamma irradiation before ripening is an effective method of reducing the content of volatile *N*-nitrosamines, biogenic amines and residual nitrite in dry-cured ham, although concentrations of volatile *N*-nitrosamines and biogenic amines recover to some extent during the ripening phase. It may be that treatment of the final product with γ -irradiation would prove to be more effective, considering that the low moisture content and A_w of the finished hams provides an unfavorable environment for the growth of microbes and therefore their production of biogenic amines.

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