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Short communication

Determination of volatile *N*-nitrosamines in irradiated fermented sausage by gas chromatography coupled to a thermal energy analyzer

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Abstract

Volatile *N*-nitrosodimethylamine (NDMA) and *N*-nitrosopyrrolidine (NPYR) in irradiated pepperoni and salami sausages were determined using a gas chromatography coupled to a thermal energy analyzer (GC–TEA). These fermented sausages with aerobic or vacuum packaging were irradiated at 0, 5, 10, and 20 kGy, and then stored for 4 weeks at 4 °C. Both NDMA and NPYR in the fermented sausage were significantly reduced by irradiation. The vacuum packaging showed significantly lower ($P < 0.05$) *N*-nitrosamine levels than that of the aerobic ones. After storage, the contents of NDMA and NPYR in the irradiated sausage were lower than those of the non-irradiated control. Results indicated that a high dose of irradiation (>10 kGy) was needed to reduce the carcinogenic *N*-nitrosamines in the fermented sausage during storage and the GC–TEA analysis was effective in determining the *N*-nitrosamines in irradiated meats even at low trace levels.

Keywords: Irradiation; Sausages; Nitrosamines; Thermal energy analyzer

1. Introduction

Nitrite is one of the very important additives in the cured meat production process in terms of desirable color, texture, lipid oxidation, and especially for preventing a toxin formation by *Clostridium botulinum* [1]. Recent evidence has suggested that the nitrite is a bactericidal for gastrointestinal, oral and skin pathogenic bacteria when ingested and mixed with gastric acid [2]. But significant concerns exist because nitrite may react with amines and amino acids to produce *N*-nitrosamines, which are known to be carcinogenic, mutagenic and teratogenic [3–5]. The formation of *N*-nitrosamines in cured meat products is dependent on the cooking method, residual and/or added nitrite concentration, ascorbate or α-tocopherol concentration, nitrosamine precursors, slice thickness, preprocessing procedures and conditions, moisture content, lean to adipose tissue ratio, presence of nitrosation catalysts and inhibitors, and possibly the smoking process [6–8]. Among the numerous chemical carcinogens that have been detected in foods and drinks, *N*-nitrosamines are distinguished by being very potent. Volatile *N*-nitrosamines induce tumors in a variety of organs, including the liver, lung, kidney, bladder, pancreas, esophagus and tongue depending on the species, but not in the skin, brain, colon or bone. For example, *N*-nitrosodimethylamine at the levels of 20 ppm can induce liver cancer in a human [9]. The formation of *N*-nitrosamines in foods occurs due to an addition of nitrite, smoking, drying with combustion gas, salting, pickling, fungal contamination or food contact materials [10]. Therefore, the nitrite in meat products is a primary problem in the formation of carcinogenic volatile *N*-nitrosamines under high-temperature condition. Lijinsky [9] reported that *N*-nitrosodimethylamine, *N*-nitrosopyrrolidine and *N*-nitrosopiperidine were found in sausage, however the species and contents of the *N*-nitrosamines vary with different countries and societies. Hu and Song [15] reported that the *N*-nitrosodimethylamine in dried shrimp bran and fish meal was drastically reduced by an irradiation with 5 kGy or above. Fiddler et al. [20] reported that irradiation sterilization (30 kGy irradiation at −40 °C)

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reduced the residual nitrite in bacon prior to frying, thereby reducing the volatile nitrosamines after frying. Simie [21] reported that the radiolytic degradation of food components by irradiation occurred due to a reaction with the hydroxyl radical produced by the radiolysis of the water. Therefore, bacon at a frozen state might be less affected by the radical attacks induced from irradiation than that of a non-frozen state.

Food irradiation is allowed in many countries for enhancing the shelf life or technological properties of the food. Recently, it was reported that irradiation was effective in the reduction of the volatile *N*-nitrosamines in an aqueous model system and also the nitrosamine reformation from the breakdown products was not observed under simulated stomach conditions [10]. These results indicated that irradiation could be used in a wide range of food systems encountering the nitrosamine problems. Many studies have shown that the nitrosamine formation can be inhibited by several additives such as nitrite scavengers [11–13], but the preformed nitrosamines in foods are hard to control. Wierbicki and Brynjolfsson [14] reported previously that irradiation sterilization with ^{60}Co and ^{137}Cs reduced the nitrite and preformed volatile nitrosamine levels in cured meat products. Later, Hu and Song [15] reported that the major irradiated products of nitrosodimethylamine in an aqueous solution were dimethylamine and nitrous acid. However, a more specific identification of the breakdown products from *N*-nitrosamines is required.

Thermal energy analyzer detection, the only detection method that is recognized as specific for nitrosamines, is based on the chemiluminescence generated by the decay of the NO_2 group when it is electronically excited [16,17]. As mentioned above, irradiation reduced the *N*-nitrosamine level in foods, and after irradiation the detected levels of *N*-nitrosamines were very low. Accordingly, GC–TEA is an effective detecting method for *N*-nitrosamines in irradiated meat even at a low trace level. The presence of *N*-nitrosamines in fermented sausage has been reported [9]. Therefore, this study was designed to offer information on the irradiation effects to the *N*-nitrosamine levels in fermented sausage.

The objective of the present study is to investigate the possible reduction of the volatile *N*-nitrosamines in irradiated pepperoni and salami sausages with aerobic and vacuum packaging during refrigerated storage using GC–TEA detection.

2. Experimental

2.1. Preparation of fermented sausage

Pepperoni and salami sausages were prepared by the formula in Table 1, respectively. Pepperoni was made by the commercially used procedure in a domestic plant, and chilled (−6 to −5 °C) pork lean meat, beef and pork back fat were cut, weighed, and minced in a bawl chopper (C-20-2, Fatosa s.a., Barcelona, Spain) with the spices and ingredients, and then stopped at a −2 °C internal temperature of the mixture. Approximately 1 kg of the mixture was stuffed in a fibrous casing (40 mm diameter) and then dried and aged in a dry chamber (ES-3, NU-VU Food Service, Menominee, MI, USA; 18 °C) for 7 days. After that, the sausage was thermally treated as follows; dried at 55 °C for 25 min, smoked at 58 °C for 25 min, cooked at 62 °C for 70 min, and chilled from −7 to −5 °C. Salami sausage was prepared by the commercially used procedure in a domestic plant (Table 1). In brief, superchilled (−6 to −5 °C) pork lean meat, beef and pork back fat were cut, weighed, and minced in a bawl chopper (C-20-2, Fatosa s.a., Barcelona, Spain) with the spices and ingredients, and then stopped at a −2 °C internal temperature of the mixture. Approximately 1 kg of the mixture was stuffed in a fibrous casing (40 mm diameter) and then dried and aged in a dry chamber (ES-3, NU-VU Food Service, Menominee, MI, USA; 18 °C) for 18–20 days. After drying and aging, it was subjected to thermal treatment as earlier described.

Table 1
Formulas (kg) of the pepperoni and salami sausages prepared in this study

Ingredients	Pepperoni	Salami
Pork lean meat	57.109	52.295
Pork back fat	19.036	19.016
Beef lean meat	19.036	23.771
Iced water	0.952	0.951
Salt	2.094	2.100
Phosphate	0.190	0.380
Sodium nitrite ($NaNO_2$)	0.016	0.016
Ascorbate	0.020	0.020
Spice mix (liquid)	0.286	–
Mustard	0.761	0.152
Dextrin	0.286	0.513
Starter (lactic acid bacteria)	0.024	0.024
Sorbate-*k*	0.190	–
Pepper	–	0.246
Anise	–	0.230
Fennel	–	0.143
Garlic (powdered)	–	0.048
Total	100	100

After preparation, both the pepperoni and salami sausages were sliced with a thickness of about 10 cm, and then packaged with air or vacuum condition in oxygen-impermeable nylon bags (2 ml of $O_2/m^2/24$ h at 0 °C, 20 cm × 30 cm; Sunkyung Co. Ltd., Seoul, Korea) with a packaging machine (Leepack, Hanguk Electronic, Kyungi, Korea). All the samples were stored at 4 °C before gamma irradiation.

2.2. Gamma irradiation

The fermented sausages with aerobic and vacuum packaging were irradiated in a cobalt-60 irradiator (Nordion International, Ottawa, Ontario, Canada). The source strength was ca. 100 kCi with a dose rate of 5 kGy h^{-1} at 11 ± 0.5 °C. Dosimetry was performed using 5 mm diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany), and the free-radical signal was measured using a Bruker EMS 104 EPR

Analyzer. The absorbed doses in this study were 0, 5, 10 and 20 kGy, and the actual doses were within ±2% of the target dose.

2.3. N-Nitrosamine extraction

The extraction of the volatile *N*-nitrosamine in the fermented sausages was performed using the Hotchkiss method [18] with some modification. The sausage (20 g) was homogenized with a homogenizer (DIAX 900, Heidolph, Schwabach, Germany), and the homogenized sample was steam-distilled once. The sample was acidified to pH 1 with 0.1 N sulfuric acid containing sulfamate to prevent artificial nitrosamine formation and 1 ml of *N*-nitrosodipropylamine (1 mg/kg) was added as an internal standard for extraction efficiency. The sample was steam-distilled on a steam generator and 150 ml of the distillate was collected and then was transferred to a 250 ml flask to which 60 ml of dichloromethane (DCM) and 500 mg of sodium chloride were added. The distillate was extracted three times with 180 ml DCM. The pooled DCM extracts were dried over anhydrous sodium sulfate, concentrated to 3–5 ml in a Kuderna–Danish apparatus, and then blown down under nitrogen to a final volume of 1 ml. The mean recovery values for the internal standard was 90.2 ± 3.28%.

2.4. GC–TEA analysis

Volatile *N*-nitrosamines were analyzed according to the method of Sen et al. [19] with some modification. Volatile *N*-nitrosodimethylamine (NDMA) and *N*-nitrosopyrrolidine (NPYR) in DCM concentrates were determined quantitatively by a gas chromatography (GC, Model 5890II, Hewlett-Packard Co., Wilmington, DE, USA) coupled to a thermal energy analyzer (TEA, Thermo Electron Model 502B, Waltham, MA, USA). The concentrated sample (2 μl) was injected into a GC–TEA. Analyses were carried out with a non-polar SPB-5 fused silica capillary column (0.53 mm i.d. × 30 m, Supelco Co., Bellefonte, PA, USA), which was introduced into the ceramic pyrolysis tube at the end of the TEA. Helium was used as the carrier gas at a flow rate of 3.5 ml/min. The injection port was set at 220 °C and the temperature of the column port was ramped; 50 °C for 5 min, increased to 200 °C at 5 °C/min. The volatile *N*-nitrosamines, especially NDMA and NPYR, which were identified by pure standards, were detected to the levels of 0.1 μg/kg. For identification, the NDMA and NPYR standards were prepared to the concentration at 10, 50 and 100 ppb, and the retention time was 2.34 min for NDMA and 9.97 min for NPYR under this chromatographic condition.

2.5. Statistical analysis

The experiment was designed as 4 (irradiation dose) × 2 (packaging) × 2 (storage periods) factorials, and the experiment from sample preparation to analysis was repeated in triplicate. The data was then analyzed by SAS software (SAS Institute, Cary, NC). The general linear model procedure was processed and Duncan's multiple range test was used to compare the mean values at $P < 0.05$. Mean values and the pooled standard error of the mean (S.E.M.) were recorded.

3. Results and discussion

The contents of NDMA and NPYR in pepperoni sausage during refrigerated storage for 4 weeks were detected by GC–TEA (Table 2).

A statistically significant difference was observed in the samples with irradiation treatment, and irradiation significantly reduced the contents of NDMA and NPYR ($P < 0.05$). After 4 weeks of storage, a reduction of the NDMA and NPYR levels by irradiation was also observed. Exceptionally, the NDMA contents in irradiated pepperoni sausage under vacuum packaging were not significantly different when compared to the non-irradiated control.

Some packaging effects were observed, and NDMA and NPYR with the vacuum state showed lower contents compared to the aerobic condition. Thicker [10] reported that an anaerobic condition or the use of a nitrite scavenger is helpful for the inhibition of the carcinogenic *N*-nitrosamine formation, because nitrite is an important precursor for forming *N*-nitrosamines in foods. Ahn et al. [22] reported that the reduction state combined with the direct physical effect produced by irradiation in an anaerobic environment may change NO_2^- to NO, which is not a nitrosating agent. NO would stay in the gaseous state or be changed to other compounds by a further reaction. Thicker [10] reported that neither nitrite nor nitrous acid (HONO) are nitrosating agents, but are intermediates in the formation of the nitrosating agents such as dinitrogen trioxide (N_2O_3), dinitrogen tetraoxide (N_2O_4) and the nitrous acidium ion (H_2O^+NO).

During irradiation, foods suffer radiolysis, and accordingly radiolytic degradation of the *N*-nitrosamines in the foods might occur. The low molecules of radiolytic products produced during irradiation may interrupt the chromatographic analysis. However, in this study, we accurately detected the NDMA and NPYR using a TEA detector even in the presence of the large amounts of radiolytic byproducts caused by radiolysis.

The contents of NDMA and NPYR in the salami sausage during storage at 4 °C are shown in Table 3. Irradiation significantly reduced the NDMA in the salami sausage at 0 week, while the NPYR was not detected in the sausage irradiated over 5 or 10 kGy ($P < 0.05$). As for the results of the pepperoni sausage, the vacuum packaging effects, which are the lower nitrosamine contents than the aerobic condition, were somewhat observed. After storage for 4 weeks, the irradiated salmi showed low NDMA and NPYR contents compared to non-irradiated ones.

The risk of volatile *N*-nitrosamines in foods has been assessed, especially, in cured meats. It is reported that the

Table 2
N-Nitrosodimethylamine (NDMA) and *N*-nitrosopyrrolidine (NPYR) levels (μg/kg) in the pepperoni sausage irradiated at 0, 5, 10 and 20 kGy during a refrigerated storage for 4 weeks[a,b]

Storage (week)[a]	Packaging[b]	Irradiation dose (kGy)				S.E.M.[c]
		0	5	10	20	
0	NDMA					
	Air	5.0a	5.2ax	3.6ab	2.8b	0.64
	Vacuum	4.3	3.1y	2.1	2.2	0.78
	S.E.M.[d]	0.26	0.39	0.45	0.16	
	NPYR					
	Air	3.8a	3.0a	−b	−b	0.51
	Vacuum	6.2a	4.6a	3.3a	−b	0.37
	S.E.M.[d]	0.52	0.19	0.39	–	
4	NDMA					
	Air	6.2ax	3.9b	3.2b	3.0b	0.47
	Vacuum	3.3y	2.8	3.0	2.3	0.53
	S.E.M.[d]	0.23	0.30	0.61	0.48	
	NPYR					
	Air	4.2a	3.1ax	2.7a	−b	0.56
	Vacuum	3.1a	−by	−b	−b	0.41
	S.E.M.[d]	0.39	0.26	0.32	–	

[a] Values with different letters (a, b) within a row differ significantly ($P < 0.05$).
[b] Values with different letters (x, y) within a column differ significantly ($P < 0.05$).
[c] Standard error of the mean ($n = 12$).
[d] Standard error of the mean ($n = 6$).

Table 3
N-Nitrosodimethylamine (NDMA) and *N*-nitrosopyrrolidine (NPYR) levels (μg/kg) in the salami sausage irradiated at 0, 5, 10 and 20 kGy during a refrigerated storage for 4 weeks[a,b]

Storage (week)[a]	Packaging[b]	Irradiation dose (kGy)				S.E.M.[c]
		0	5	10	20	
0	NDMA					
	Air	7.3a	8.2ax	5.3ax	3.2b	0.93
	Vacuum	5.2a	3.6ay	−by	−b	0.51
	S.E.M.[d]	0.33	0.73	0.12	0.68	
	NPYR					
	Air	3.4a	2.7a	−b	−b	0.46
	Vacuum	3.2	–	–	–	0.72
	S.E.M.[d]	0.28	0.31	–	–	
4	NDMA					
	Air	5.8	4.3	5.0x	3.2	0.62
	Vacuum	4.2	3.5	3.1y	2.9	0.75
	S.E.M.[d]	0.86	0.92	0.09	0.77	
	NPYR					
	Air	4.3a	3.7a	−b	−b	0.48
	Vacuum	2.9	3.1	2.5	–	0.34
	S.E.M.[d]	0.52	0.63	1.01	–	

[a] Values with different letters (a, b) within a row differ significantly ($P < 0.05$).
[b] Values with different letters (x, y) within a column differ significantly ($P < 0.05$).
[c] Standard error of the mean ($n = 12$).
[d] Standard error of the mean ($n = 6$).

N-nitrosamine level is 10 ppb or none in the U.S. commercial meat products [23]. Meanwhile, Park and others [24] have reported that the NDMA levels in Korean commercial pork sausages ranged from 0.5 to 36.8 ppb. However, if the increasing trend of meat products in Korea is considered, it is important to secure the chemical and microbiological safety associated with meat products. It is suggested that a gamma irradiation at below 5 kGy was effective in destroying the volatile *N*-nitrosamines in an aqueous model system without the reformation from breakdown products of the *N*-nitrosamines, however a high dose of irradiation should be applied in real food systems, including sausage [25,26]. Recently, the Joint FAO/IAEA/WHO study group [27] concluded that food irradiated to any dose appropriate to achieve

the intended technological objective is both safe to consume and nutritionally adequate. Accordingly, irradiated foods are deemed wholesome throughout the technologically useful dose range from below 10 kGy to the envisioned doses above 10 kGy. Thereby, food irradiation can be used to secure the chemical safety of food as well as the microbiological safety.

4. Conclusions

The present study showed that irradiation reduced the carcinogenic *N*-nitrosodimethylamine and *N*-nitrosopyrrolidine in fermented sausage during storage, and these volatile *N*-nitrosamines were adequately determined by GC–TEA even at low trace levels. This study indicated that food irradiation can be used not only to improve food quality by extending shelf life, but to reduce the toxic compounds in foods.

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