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Food Chemistry 89 (2005) 199–205
Food Chemistry
www.elsevier.com/locate/foodchem

Effects of gamma irradiation on the biogenic amines in pepperoni with different packaging conditions

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Received 20 October 2003; received in revised form 11 February 2004; accepted 11 February 2004

Abstract

Combined effects of gamma irradiation and packaging on biogenic amine (BA) formation in pepperoni sausage were investigated during storage. Pepperoni (fermented sausage) was made and packaged with air, vacuum and CO_2/N_2 (25%/75%) gas and then gamma-irradiated at 0, 5, 10 and 20 kGy. The pH was decreased after storage and non-irradiated samples showed a lower pH than irradiated. Lactic acid bacteria (LAB) were not detected after 5 kGy of gamma irradiation, while the LAB in the non-irradiated samples were 4–5 log counts during storage. A total of six different BAs, putrescine, cadaverine, β-phenylethylamine, spermidine, spermine and tyramine, were found in the pepperoni sausage. Detected BAs were statistically low under the irradiation or packaging conditions, except for cadaverine and β-phenylethylamine. Gamma irradiation was effective in reducing putrescine, spermidine, spermine and tyramine. Irradiation effects were not observed on the β-phenylethylamine level, while the CO_2/N_2 packaging caused increasing of the level. Most BAs detected were reduced by gamma irradiation of the pepperoni sausage during storage. However, CO_2/N_2 packaging was an improper condition for BA reduction in the pepperoni sausage.

Keywords: Biogenic amines; Gamma irradiation; Pepperoni

1. Introduction

Biogenic amines (BAs) are organic bases with aliphatic, aromatic or heterocyclic structures and known as toxic substances that could cause food poisoning symptoms, such as stimulating the nerves and blood vessels in man and animals (Joosten, 1988). Several BAs exist in nature, but most BAs are formed by the action of microorganisms through the decarboxylation of amino acids in foods (Shalaby, 1996; Silla Santos, 1996). Major BAs found in foods are putrescine, cadaverine, β-phenylethylamine, spermine, spermidine, histamine, tryptamine tyramine and agmatine. BAs are also known as possible precursors of carcinogens, such as *N*-nitrosamines (Ayala, Fiddler, Gates, & Pensabene, 1994). They are frequently found in high concentrations in food and not reduced by a high-temperature treatment (Shalaby, 1996; Silla Santos, 1996). High amounts of BAs can be found in fermented foods derived from raw materials with high protein content, on fermented sausages such as pepperoni and salami (Suzzi & Gardini, 2003). Some strains of lactic acid bacteria (LAB) can produce BAs and the decarboxylating activities of the LAB isolated from fermented sausages have been widely studied. The microorganism is often responsible for BA formation in fermented sausage (Bauer, Seuss, Paulsen, & Vali, 1994; Maijala & Eerola, 1993; Paulsen & Bauer, 1997).

Several studies, using microorganisms as starters that do not produce BAs (Bover-Cid, Izquierdo-Pulido, &

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doi:10.1016/j.foodchem.2004.02.026

Vidal-Carou, 1998, 2000a; Chin & Koehler, 1983; Dapkevicius, Nout, Rombouts, Houben, & Wymenga, 2000; Fernández-García, 1999), and for controlling the manufacturing conditions, such as raw materials or temperature (Bover-Cid, Izquierdo-Pulido, & Vidal-Carou, 2000b; Chiu & Chen, 2000; Nout, Ruikes, & Bouwmeester, 1993), have been conducted for the reduction of BAs formation. However, more studies on the reduction of BAs are needed.

Food irradiation has been used for the purposes of inhibition of sprouting, destruction of food borne insects and parasites, delay of physiological ripening, extension of shelf life or improvement of food qualities (Loaharanu, 1989; Radomyski, Murano, Olson, & Murano, 1994; Thayer, 1994). Irradiation is effective in reducing microorganisms and viruses, and is known as a good method for inactivating pathogens in food materials (Stewart, 2001). Furthermore, besides the sanitary purpose, irradiation technology is applied to reduce toxic substances such as carcinogenic *N*-nitrosamine and nitrite in meat products, allergenicity in foods or BAs in foods (Ahn et al., 2002a, 2002b, 2002c; Byun et al., 2000; Fiddler, Gates, Pensabene, & Phillips, 1981; Kim et al., 2003a, 2004). Thus, our previous study (Kim et al., 2003a, 2004) showed that irradiation was effective in reducing BA levels in fermented soybean paste during fermentation by controlling the microbial growth. This study was designed to investigate irradiation and packaging, as means for reducing the BAs in fermented sausage. After the manufacturing of the pepperoni, residual microorganisms may play a role in the continuous formation of BAs during storage or distribution. Therefore, effective tools for controlling microorganisms might be needed, after fermentation, for the inhibition of BA formation in pepperoni.

The objective of the present study was to investigate the effects of gamma irradiation and packaging on BA formation in pepperoni during storage.

2. Materials and methods

2.1. Sample preparation

Pepperoni sausage was made by the formula in Table 1. 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 −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 seven days. After that, the made 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 (−7 to −5 °C). After manufacture, the sample was sliced to a thickness of about 10 cm, and then packaged with air, vacuum, or CO_2/N_2 (25%/75%) gas in oxygen-impermeable nylon bags (2 ml of $O_2/m^2/24$ h at 0 °C, 20 × 30 cm; Sunkyung Co. Ltd., Seoul, Korea) with a packaging machine (Leepack, Hanguk Electronic, Kyungi, Korea). Air was flushed into the packaging bag without sealing for the aerobic packaging. The gases used were ultra pure grade (99.999%). All the samples were stored at 4 °C before gamma irradiation.

Table 1
Formula of pepperoni sausage manufacture using this study

Ingredients	Amount (kg)
Pork lean meat	57.109
Pork back fat	19.036
Beef lean meat	19.036
Iced water	0.952
Salt	2.094
Phosphate	0.190
Sodium nitrite ($NaNO_2$)	0.0156
Ascorbate	0.020
Spice mix[a]	0.286
Mustard	0.761
Dextrin	0.2864
Starter (LAB)	0.024
Sorbate-k	0.190
Total	100

[a] Spice mix: coriander, glucose, red pepper and onion powder (Sewon Co. Ltd., Seoul Korea).

2.2. Gamma irradiation

The packaged samples were irradiated in a cobalt-60 irradiator (point source, AECL, IR-79, MDS Nordion International Co. Ltd., Ottawa, ON, Canada). The source strength was about 100 kCi with a dose rate of 5 kGy/h at 12 ± 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 irradiation doses in this study were 0, 5, 10 and 20 kGy and the actual doses were within ±0.2 kGy of the target dose. After irradiation, the samples were stored at 4 °C for 4 weeks. The analyses were carried out at 0 and 4 weeks, respectively.

2.3. pH and lactic acid bacterial counts

50 ml of deionized distilled water (DDW) were added to 10 g of a sample and homogenized with a homogenizer (DIAX 900, Heidolph, Schwabach, Germany) for 1 min. The samples were filtered with filter paper (No. 4, Whatman International Ltd., Kent, UK) and the pH was measured using a pH meter (Orion 520A, Orion Research Inc., Boston, MA, USA). The samples were aseptically homogenized with a stomacher lab blender

(Model 400, Tekmar Co., Cincinnati, Ohio, USA) for 2 min. Series of decimal dilutions were prepared with a sterile saline solution (0.85%). Each diluent (100 μl) was poured on to de Man, Rogosa and Sharpe agar (MRS, Merck, Darmstadt, Germany) and the lactic acid bacteria were incubated at 30 °C for 72 h. All the procedures were replicated 3 times.

2.4. Biogenic amine analysis

The analysis procedure of BAs was based on that described by Hwang, Chang, Shiua, and Chai (1997) and García-García (2000) with slight modifications. Standard amines, putrescine (PUT), cadaverine (CAD), β-phenylethylamine (PHE), spermidine (SPD), spermine (SPM), tryptamine (TRP), histamine (HIS), tyramine (TYR) and agmatine (AGM), were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The standard amines were dissolved in DDW with a concentration of 1000 mg% and used as the working solution.

To each 10 g of pepperoni sausage, 30 ml of trichloroacetic acid were added and mixed in a homogenizer (Heidolph) for 3 min, and the samples were made up to 50 g with trichloroacetic acid, and then filtered with a filter paper (No. 4, Whatman International Ltd.). Sodium hydroxide (2 M, 1 ml) was added to each 2 ml of the standard amine solution and the prepared sample diluents (the homogenate), followed by 10 μL of benzoyl chloride. The solution was mixed using a vortex mixer (G-560, Scientific Industries Inc., Bohemia, NY, USA) and placed in a water bath at 30 °C for 40 min for benzoylation. By adding 2 ml of saturated sodium chloride, the benzoylation was stopped, and then the sample was extracted with 3 ml diethyl ether by a vortex mixer (Scientific Industries Inc.) at maximum speed for 3 min. After the extraction, the extracted samples were centrifuged at 2500 rpm for 20 min and 1.5 ml of the upper organic layer were transferred into a glass tube (10 ml) and evaporated by nitrogen gas. The residue was dissolved in 1 ml of methyl alcohol (HPLC grade) and filtered with a membrane filter (Teflon Syringe Filters, Acrodisc 25 CR PTFE, 25 mm × 0.45 μm, Young-Wha Scientific Co., Ltd, Seoul, Korea), and the filtrate was used for HPLC analysis.

The HPLC systems used were the following: separations module (Waters 2690, Waters Co., Milford, MA, USA), photodiode array detector (PDA, Waters 996; Waters Co.), millennium 32 chromatography manager (System Software, Workstation version 3.0, Waters Co.), and a symmetry® C18 column (3.9 × 150 mm, 5 μm, Waters Co.). The gradient elution programme was set with a flow rate of 0.9 ml/min and compositions of methyl alcohol in DW (50%, 70%, 85%, 100%). The injection volume, column temperature and PDA detection wavelength were 20 μl, 25 ± 2.0 °C and 225 nm, respectively. All the procedures were replicated three times.

2.5. Statistical analysis

Experiments were designed as a 4 (irradiation dose) × 3 (packaging condition) factorial for each storage period. The data were analyzed by ANOVA using SAS (version 8.2, SAS Institute Inc., Cary, NC, USA) and the differences among the mean values were processed by the Student–Newman–Keuls multiple range test. Significance was defined at $P < 0.05$.

3. Results and discussion

3.1. pH and lactic acid bacteria

The changes in pH of the pepperoni during storage are shown in Table 2. Immediately after gamma irradiation, the pH of the sample irradiated at 20 kGy showed statistical differences between the different packaging conditions, but no significant difference was observed

Table 2
Changes in pH of pepperoni sausage by gamma irradiation with different packaging conditions

Storage periods (weeks)	Packaging[a]	Gamma irradiation[b]				SEM[c]
		0	5	10	20	
0	Air	6.28	6.30	6.30	6.31x	0.005
	Vacuum	6.28	6.25	6.31	6.28y	0.011
	CO_2N_2	6.24	6.21	6.23	6.24z	0.006
	SEM[d]	0.012	0.018	0.016	0.014	
4	Air	5.81d	5.90c	6.00b	6.11a	0.042
	Vacuum	5.81c	5.94b	6.09a	6.19a	0.055
	CO_2/N_2	5.90c	5.96c	6.05b	6.19a	0.042
	SEM[d]	0.020	0.012	0.019	0.020	

Different letters (a–c) within same row, and different letter (x–z) within same column and storage periods differ significantly ($p < 0.05$).
[a] Gas purity was 99.9999% and CO_2/N_2 ratio was 25%/75%.
[b] Gamma irradiation dose (kGy).
[c] SEM: Standard error of the means ($n = 12$).
[d] SEM: Standard error of the means ($n = 9$).

among the samples by the packaging conditions at 4 weeks. The pH appeared lower in the control than in the irradiated samples and significant differences were observed by irradiation dose within the same packaging condition after 4 weeks of storage. The pH is an important factor for fermentation and the formation of BAs because the amino acid decarboxylase activity is stronger in an acidic environment, pH 3–6 (Silla Santos, 1996). Therefore, BA formation depends on the amount of growth of the decarboxylating bacteria (Yoshinaga & Frank, 1982).

LAB were not detected after gamma irradiation at 5 kGy or above in any samples during storage (data not shown). Statistically significant differences were observed in packaging conditions and cell counts of LAB in the CO_2/N_2 packaging (5.15 and 4.58 log CFU/g at 0 and 4 weeks, respectively) were higher than those under the air or vacuum conditions during storage.

These results indicated that irradiation is effective in inhibiting the growth of LAB and the decline of the pH in pepperoni, resulting in inhibition of the BA formation.

3.2. Biogenic amines

The contents of BAs in the pepperoni sausage with various packaging conditions are shown in Tables 3–7. Six different BAs, putrescine (PUT), cadaverine (CAD), β-phenylethylamine (PHE), spermidine (SPD), spermine (SPM) and tyramine (TYR), were found in the pepperoni sausage. Most BAs detected showed statistically significant differences with respect to the irradiation or packaging conditions, except for CAD. PUT in pepperoni under an air and vacuum condition, was reduced by irradiation during storage. Particularly, in the samples irradiated at 10 or 20 kGy, PUT was not detected. The results indicated that irradiation might directly affect the PUT. Kim et al. (2004) reported that irradiation induced radiolysis of PUT in an aqueous model system at 2.5 kGy, and PUT was completely destroyed at 5 kGy. In the CAD contents, no difference was observed in any samples during storage, and CAD showed the lowest contents (0.02–0.14 mg%) compared to the other detected BAs (data not shown). Immediately after irradiation, the contents of PHE in the irradiated samples with air and vacuum packaging were higher than those of the control. However, after 4 weeks, samples irradiated at 20 kGy showed significantly lower levels than those of the others. Packaging effects were also observed, and the non-irradiated sample with CO_2/N_2 packaging showed the highest levels of PHE. SPD decreased in the air-packaged and 20 kGy-irradiated samples, and no significant difference was observed within the other samples by the packaging environments at 0 week. Pepperoni samples with CO_2/N_2-packaging showed higher levels than those of the other packaging conditions at 0 week. After 4 weeks of storage, SPD reduction was observed in irradiated samples under air and vacuum conditions. SPM in the 5 kG- and 10 kGy-irradiated samples with CO_2/N_2 gas showed significantly higher levels than the air packaged samples at 4 weeks. TYR in the vacuum- and CO_2/N_2-packaged samples was significantly reduced by gamma irradiation at 4 weeks.

Meat and meat products may contain PUT, CAD, PHE, TYR, SPD and SPM (Koehler & Eitenmiller, 1978; Nakamura, Wada, Saway, & Kawabata, 1993; Santos-Buelga, Pena-Egido, & Rivas-Gonzalo, 1986; Shalaby, 1993, 1996). Hernandez-Jover, Izquierdo-Pulido, Veciana-Nogués, Mariné-Font, and Vidal-Carou (1997) reported high amounts of CAD in Danish and pepperoni sausages, while SPD and SPM were found at low levels, which is not consistent with our results. In general, quite variable quantities of BAs are reported for

Table 3
Putrescine levels in pepperoni sausage under various packaging and gamma irradiation conditions during storage (mg%)

Storage periods (week)	Packaging[a]	Gamma irradiation[b]				SEM[c]
		0	5	10	20	
0	Air	0.61a	0.43b	–[d]c	–c	0.032
	Vacuum	0.45a	0.42a	0.21b	0.23b	0.027
	CO_2N_2	0.46	0.53	0.43	0.30	0.020
	SEM[e]	0.034	0.029	0.027	0.029	
4	Air	0.26ax	–b	–b	–b	0.023
	Vacuum	0.19ay	0.12a	–b	–b	0.018
	CO_2/N_2	0.16y	0.15	0.18	0.17	0.013
	SEM[e]	0.021	0.014	0.014	0.025	

Different letters (a,b) within same row, and storage periods differ significantly ($p < 0.05$).
[a] Gas purity was 99.9999% and CO_2/N_2 ratio was 25%/75%.
[b] Gamma irradiation dose (kGy).
[c] SEM: Standard error of the means ($n = 12$).
[d] –, Not detected.
[e] SEM: Standard error of the means ($n = 9$).

Table 4
β-Phenylethylamine levels in pepperoni sausage under various packaging and gamma irradiation conditions during storage (mg%)

Storage periods (weeks)	Packaging[a]	Gamma irradiation[b]				SEM[c]
		0	5	10	20	
0	Air	0.19cy	0.20by	0.23abz	0.29ay	0.017
	Vacuum	0.30y	0.33y	0.30y	0.29y	0.009
	CO_2N_2	0.82bx	0.91abx	1.02ax	1.00ax	0.047
	SEM[d]	0.132	0.139	0.159	0.150	
4	Air	0.28by	0.51az	0.44ay	0.17b	0.052
	Vacuum	0.84xy	1.01y	1.25x	0.91	0.108
	CO_2/N_2	1.87ax	1.56abx	1.00bxy	0.83b	0.172
	SEM[d]	0.317	0.193	0.162	0.167	

Different letters (a,b) within a same row, and different letters (x–z) within same column and storage periods differ significantly ($p < 0.05$).
[a] Gas purity was 99.9999% and CO_2/N_2 ratio was 25%/75%.
[b] Gamma irradiation dose (kGy).
[c] SEM: Standard error of the means ($n = 12$).
[d] SEM: Standard error of the means ($n = 9$).

Table 5
Spermidine levels in pepperoni sausage under various packaging and gamma irradiation conditions during storage (mg%)

Storage periods (week)	Packaging[a]	Gamma irradiation[b]				SEM[c]
		0	5	10	20	
0	Air	1.18axy	0.98aby	0.92aby	0.84by	0.025
	Vacuum	1.02y	1.00y	0.88y	0.90y	0.015
	CO_2N_2	1.34x	1.66x	1.78x	1.86x	0.042
	SEM[d]	0.031	0.073	0.092	0.105	
4	Air	0.96a	0.82aby	0.68bz	0.42c	0.026
	Vacuum	1.82a	1.16bx	1.10by	1.06b	0.064
	CO_2/N_2	1.38	1.42x	1.74x	1.84	0.041
	SEM[d]	0.066	0.058	0.099	0.128	

Different letters (a,b) within same row, and different letters (x–z) within same column and storage periods differ significantly ($p < 0.05$).
[a] Gas purity was 99.9999% and CO_2/N_2 ratio was 25%/75%.
[b] Gamma irradiation dose (kGy).
[c] SEM: Standard error of the means ($n = 12$).
[d] SEM: Standard error of the means ($n = 9$).

Table 6
Spermine levels in pepperoni sausage under various packaging and gamma irradiation conditions during storage (mg%)

Storage periods (week)	Packaging[a]	Gamma irradiation[b]				SEM[c]
		0	5	10	20	
0	Air	2.69a	2.43b	2.11c	2.012d	0.102
	Vacuum	2.49a	2.16b	2.06b	2.05b	0.072
	CO_2N_2	2.41	2.39	2.14	2.55	0.090
	SEM[d]	0.059	0.094	0.040	0.121	
4	Air	1.20a	0.72by	0.65bcz	0.31c	0.043
	Vacuum	1.72a	1.29bx	1.78ay	0.88c	0.111
	CO_2/N_2	1.36	1.17xy	1.29x	1.25	0.039
	SEM[d]	0.113	0.061	0.062	0.132	

Different letters (a,b) within same row, and different letters (x, y) within same column and storage periods differ significantly ($p < 0.05$).
[a] Gas purity was 99.9999% and CO_2/N_2 ratio was 25%/75%.
[b] Gamma irradiation dose (kGy).
[c] SEM: Standard error of the means ($n = 12$).
[d] SEM: Standard error of the means ($n = 9$).

sausage. The variable concentration could be due to the variation of the ripening process time, the variation and difference of decarboxylase activity of the natural microflora responsible for the formation, and the biosynthesis and metabolism of the BAs, in addition to the variation in the type and quality of the meat used, the

Table 7
Tyramine levels in pepperoni sausage under various packaging and gamma irradiation conditions during storage (mg%)

Storage periods (week)	Packaging[a]	Gamma irradiation[b]				SEM[c]
		0	5	10	20	
0	Air	0.37a	0.29abx	0.14b	0.25ab	0.033
	Vacuum	0.19	0.22xy	0.17	0.16	0.010
	CO_2N_2	0.26	0.16y	0.11	0.26	0.029
	SEM[d]	0.036	0.025	0.015	0.029	
4	Air	0.09	0.14	0.09	0.02	0.022
	Vacuum	0.20a	0.19a	0.11b	0.05c	0.031
	CO_2/N_2	0.11a	0.08b	0.09b	0.07b	0.007
	SEM[d]	0.032	0.032	0.006	0.009	

Different letters (a,b) within same row, and different letters (x–z) within same column and storage periods differ significantly ($p < 0.05$).
[a] Gas purity was 99.9999% and CO_2/N_2 ratio was 25%/75%.
[b] Gamma irradiation dose (kGy).
[c] SEM: Standard error of the means ($n = 12$).
[d] SEM: Standard error of the means ($n = 9$).

proportion of meat content included, and the length of maturation (Shalaby, 1995, 1996). Our previous study demonstrated that irradiation is effective in reducing BAs in fermented soybean paste, by inducing inhibition of microbial growth during fermentation (Kim et al., 2003a, 2004). Oxygen supply appears to have a significant effect on the biosynthesis of PUT and CAD. On the other hand, the presence of oxygen could inactivate histamine production and a modified atmospheric packaging gives a slight inhibition effect for histamine (Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994; Silla Santos, 1996).

Present results indicate that the BAs detected in the pepperoni sausage were decreased by irradiation, except for CAD and PHE. The packaging effects were shown in each detected BA, differently. Modified atmospheric packaging, such as CO_2/N_2 packaging was not effective in reducing the BA levels of the pepperoni after irradiation. Therefore, more research is needed on various packaging conditions. Gamma-irradiation might be considered to control the microorganisms relevant to the formation of BAs, resulting in a reduction of the BAs in the pepperoni sausage.

Acknowledgements

This research was supported under the Nuclear R & D Programme by the Ministry of Science and Technology in Korea.

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