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Irradiation and organic acid treatment for microbial control and the production of biogenic amines in beef and pork

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Abstract

The effect of irradiation and organic acid treatment in controlling the growth of microorganisms and the formation of biogenic amines (BAs) in ground beef and pork was studied. *Bacillus cereus*, *Enterobacter cloacae*, and *Alcaligenes faecalis* were inoculated into the ground beef and pork with approximately 10^7 CFU/g. A gamma irradiation was used with absorbed doses of 0, 0.5, 1, and 2 kGy, as irradiation treatment and 2 M solutions of acetic, citric, and lactic acid were used as organic acid treatment. Irradiation was effective in reducing the inoculated bacteria and achieved about 3 decimal reductions by 2 kGy. The levels of putrescine, tyramine, spermine, and total amount of biogenic amines were significantly reduced by irradiation of ground beef and pork inoculated with different microorganisms tested. On the other hand, organic acid treatment showed only 2 decimal reductions or less. The reduction of the BAs content was also limited and variable by organic acid treatment. Therefore, the results indicate that, for the controlling of microorganisms and production of biogenic amines in ground beef and pork, irradiation was more effective than organic acid treatment.

Keywords: Biogenic amines; Irradiation; Organic acids; Beef; Pork

1. Introduction

BAs are formed in foods by bacterial decarboxylation of amino acids (Halasz, Barath, Simon-Sarkadi, & Holzapfel, 1994). The ingestion of biogenic amines (BAs) can cause headache, hypertension, pyrexia, or heart disease. It has been reported that the ingestion of a large amount of histamine (HIM) can cause symptoms, such as nausea, dyspnea, flush, perspiration, palpitation and hypertension or hypotension (Bartholomew, Berry, Rodhouse, & Grilbert, 1987; Franzen & Eysell, 1969; Min, Lee, Jang, Lee, & Kim, 2004; Taylor, 1986). The ingestion of significant amounts of tyramine (TYM) can also cause hypertension and an associated headache (Min et al., 2004; Stratton, Hutkins, & Taylor, 1991).

Fresh foods have low BAs contents while BAs are found at a high level in spoiled or fermented foods. Both spoiled and fermented foods contain a large number of microorganisms, either by contamination or addition for fermentation. Under ambient conditions, such microorganisms may produce many BAs. During meat fermentation, microbial growth, acidification and proteolysis can generate the conditions that are favourable for BAs production. In a fermented sausage common in Spain, TYM, putrescine (PUT), and cadaverine (CAD) were detected during sausage production (Hernandez-Jover, Izquierdo-Pulido, Veciana-Nogues, Marine-Font, & Vidal-Carou, 1997a). The authors indicated that the high background microflora naturally present on the raw meat and lard seemed to have a strong influence on BAs formation during ripening. TYM and CAD were the main BAs formed during ripening (Bover-Cid, Izquierdo-Pulido, & Vidal-Carou, 2001). Bruna, Fernandez, Hierro, Ordonez, and de la Hoz (2000)

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reported that the addition of Pronase E and *Penicillium aurantiogriseum* extracts enhanced BAs production in salchichon, a salami-like dry fermented sausage. BAs are known to accumulate over time during meat storage (Smith, Kenney, Kastner, & Taylor, 1993).

The freezing, thawing, and processing of meat and the presence of bacteria are major factors in BA formation. Maijala, Eerola, Aho, and Hirn (1993) observed an increase of pH values during meat storage/spoilage and noted that the accumulation of BAs in meat could contribute to an increase in pH. Kaniou, Samouris, Mouratidow, Eleftheriadou, and Zantopoulos (2001) determined BA levels in fresh unpacked and vacuum-packed beef during storage at 4 °C and reported that PUT and CAD levels in the unpacked beef were 10.4 and 5.2 μg/g, respectively, after 12 days of storage. After 35 days of storage, PUT, CAD and HIM levels in the vacuum-packed beef were 36.3, 13.3, and 19 μg/g, respectively.

Irradiation is a well known method for controlling microorganisms (Lee, Sebranek, Olson, & Dickson, 1996; Min, Kim, & Lee, 1999; Min, Lee, Kim, & Jung, 1997; Min, Shin, Lee, Kim, & Lee, 1998; Zhao, Sebranek, Dickson, & Lee, 1996). International organizations, such as the WHO, IAEA and FAO, have officially pronounced the safety and wholesomeness of food irradiation (Murano, Murano, & Olson, 1995). Organic acids also have an antimicrobial activity, due to their ability to lower the pH, resulting in instability of bacterial cell membranes (Ingram, Ottoway, & Coppock, 1956; Luck & Jager, 1997; Macris, 1975). Treatment with acetic acid effectively reduced *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on carcass surfaces (Conner, Kotrola, Mikel, & Tamblyn, 1997; Cutter, Dorsa, & Siragusa, 1997). Prasai et al. (1997) reported that the pronounced antibacterial effects of lactic acid in limiting the growth of bacteria on vacuum-packaged strip loins were augmented at colder storage temperatures. Fang and Tsai (2003) observed that, in ground beef, 1.0, 1.5 and 2% acetic acid could effectively inhibit the growth of *E. coli* O157:H7 at 10 and 30 °C.

The objective of this study was to investigate and to compare the effects of irradiation and organic acid treatment on BAs formation in raw ground beef and pork.

2. Materials and methods

2.1. Chemicals

Amine standards, including β-phenylethylamine hydrochloride, putrescine (PUT) dihydrochloride, cadaverine (CAD) dihydrochloride, histamine (HIM) dihydrochloride, serotonin (SER) creatinine sulfate, tyramine (TYM) hydrochloride, spermidine (SPD) trihydrochloride, spermine (SPM) tetrahydrochloride and 1,7-diaminoheptane, sodium bicarbonate, sodium hydroxide, ammonium acetate and dansyl chloride were purchased from Sigma Chemical Co. (St. Louis, USA). Ammonia and perchloric acid (70%) were purchased from Showa Chemical Co. (Tokyo, Japan) and acetonitrile and acetone (HPLC grade) were purchased from TEDIA (Cincinnati, USA).

2.2. Meat samples

Beef and pork loin were purchased from a slaughterhouse at 24 h postmortem (Suwon, Korea). The samples were transferred into an ice box and transported to the laboratory. Lean flesh was taken from the samples and used for this study.

2.3. Inoculation of bacteria

To eliminate the contaminated microorganisms present in the sample, an ultraviolet radiation was administered for 15 min on a clean bench. Samples were then ground aseptically through a 9 mm plate by a grinder. Ground sample (10 g) was transferred into a sterilized 50 ml polypropylene conical tube (Becton Dickinson & Co., Franklin Lakes, USA) for bacterial inoculation.

According to a previous study, *Alcaligenes faecalis*, *Bacillus cereus*, and *Enterobacter cloacae* can produce large amounts of BAs (Min et al., 2004). Therefore, these bacteria were selected for an inoculation test and obtained from the Korean Culture Center of Microorganisms (Seoul, Korea). Each bacterium was inoculated into a test tube (15 × 250 mm) containing 30 ml of trypticase soy broth, nutrient broth, and nutrient broth, respectively and incubated in a shaking incubator (220 rpm) for 20 h at its optimal temperature (30 °C, each of them). The enriched broth (1 ml) was taken and inoculated into the secondary medium and incubated under the same conditions as above. These procedures were repeated 3 times to activate each bacterium. The 1 ml of enriched bacterial broth (approximately 10^8 CFU/ml) was inoculated into the meat sample in a 50 ml polyethylene conical tube. The inoculated sample was then incubated at the optimal temperature for 24 h and the amounts of BAs were determined. Nutrient broth and trypticase soy broth were purchased from Becton Dickinson and Co. (Sparks, USA).

2.4. Irradiation treatment

After 24 h of incubation of samples, irradiation was performed at the Korea Atomic Energy Research Institute (Daejeon, Korea) using a Co^{60} gamma irradiator (point source, AECL, IR-79, MDS Nordion International Co., Ltd., Ottawa, Canada) with a source strength of 100 kCi. The dose rate used was 83.3 Gy/min at 12 ± 0.5 °C and the applied absorbed doses were 0, 0.5, 1, and 2 kGy. Dosimetry was performed using a 5 mm diameter alanine dosimeter (Bruker Instruments, Rheinstetten, Germany), and the free radical signal was measured using a Bruker EMS 104 EPR Analyzer. The actual dose was within ±2% of the target dose. After irradiation, samples were transported to the laboratory in an ice box and stored at 4 °C for 20 h.

2.5. Organic acid treatment

Acetic acid (glacial, Showa Chemical Co. Ltd., Tokyo, Japan), citric acid (monohydrate, Sigma Chemical Co., St Louis, USA) and lactic acid (Kanto Chemical Co. Inc., Tokyo Japan) were each prepared as 0.2 M solutions using distilled water. The pHs were 2.6, 1.7, and 1.9 for acetic acid, citric acid, and lactic acid, respectively. Each organic acid (1 ml) was added to the ground meat sample (10 g) and mixed with a sterile glass stick. The treated samples were stored at 4 °C for 20 h.

2.6. Microbiological study

Sample was aseptically removed from the 50 ml tube and transferred into a test tube containing 9 ml of 0.1% peptone water. The sample mixture was vortexed for 2 min and additional decimal dilutions were carried out for the Aerobic Plate Count Petrifilm (Microbiology Products 3 M Health Care, USA; AOAC, 1995). The Petrifilms were incubated at 30 °C for 48 h. Colony forming units (CFU) per gramme were counted at a dilution giving 30–300 CFU per plate.

2.7. Determination of biogenic amines (BAs)

The modified method of Eerola, Hinkkanen, Lindfors, and Hirvi (1993) was used to determine BAs. The sample (2 g) was homogenized (Ultra-Turrax 25, IKA-Labortechnik, Staufen, Germany) with 10 ml of 0.4 M perchloric acid. The homogenized sample was centrifuged for 10 min at 3000 rpm (Union 5KR, Hanil Co., Incheon, Korea) and the supernatant was filtered through a filter paper (Whatman No. 1, Whatman International Ltd., Maidstone, England). Perchloric acid (0.4 M, 10 ml) was added to the remnant and the whole mixed thoroughly in a vortex mixer (Vortex-Genie2, Scientific Industries, Inc., Bohemia, USA). This mixture was centrifuged for 10 min at 3000 rpm and the supernatant was filtered. Finally, the volume of the filtrate was adjusted to 25 ml with 0.4 M perchloric acid.

The sample extract (1 ml) was placed in a 15 ml polypropylene conical tube (Becton Dickinson & Co., Franklin Lakes, USA) and 50 μl of internal standard (1000 ppm of 1,7-diaminoheptane) were added. Then, sodium hydroxide (2 N, 200 μl), saturated sodium bicarbonate (300 μl) and dansyl chloride solution (2 ml, 10 mg dansyl chloride dissolved in 1 ml of acetone) were added to the sample extract. After incubation at 40 °C for 45 min, 100 μl of ammonia were added to the reaction mixture for the removal of residual dansyl chloride. After 30 min at ambient temperature, the volume of the reaction mixture was adjusted to 5 ml with acetonitrile. This reaction mixture was centrifuged for 5 min at 2500 rpm. The supernatant was filtered with a 0.45 μm syringe filter with a PVDF Membrane (Acrodisc® LC13 PVDF minispike, Pall Co., Ann Arbor, USA).

The filtered sample (10 μl) was injected into an HPLC apparatus with a diode array detector (Agilent 1100, Agilent Technologies Inc., Wilmington, USA), equipped with a Spherisorb ODS_2 column (4.6 × 150 mm i.d., 5 μm, Waters, Milford, USA). A gradient elution programme was used with a mixture of 0.1 M ammonium acetate as solvent A and with acetonitrile as solvent B. Both solvents were vacuum-filtered using a membrane filter (47 mm PTFE 0.45 μm, Pall Co., Ann Arbor, USA) and degassed with an ultrasonicator (5210, Branson Ultrasonic Co., Danbury, USA). The flow rate used was 1 ml/min. The gradient began at 50% and ended at 90% after 19 min. Subsequent analysis was delayed for 10 min to achieve equilibrium. The column temperature was 40 °C. The column effluent was analyzed at 254 nm with 550 nm as a reference. All experiments were done in triplicate.

2.8. Statistical analysis

Statistical analysis was performed with SAS 8.01 for Windows (2000). Descriptive statistics were used to calculate the mean and standard error. One-way analysis of variance was performed and, when significance ($P < 0.05$) was found, the differences of mean values were identified with Duncan's multiple range test.

3. Results and discussion

3.1. Effects of irradiation on microbial reduction and biogenic amines (BAs) production

Gamma irradiation treatment significantly reduced the number of all bacteria tested but the reduction rate was slower at 1 or 2 kGy of dose (Table 1). The initial number of inoculated *B. cereus*, *E. cloacae*, *A. faecalis* were 6.3, 5.9, and 7.5 Log CFU/g in beef and 6.1, 6.0, and 7.5 Log CFU/g in pork, respectively. All bacterial counts showed about a 2 decimal points decrease by a 0.5 kGy and about a 3 decimal reduction by a 2 kGy irradiation dose. Jo, Lee, Kang, Shin, and Byun (2004) observed the D_{10}-vlaues of *B. cereus*, *Staphyloccoccus aureus*, *Salmonella* Typhimurium, *E. coli* in marinated beef rib and reported these as 0.663 ± 0.01, 0.594 ± 0.05, 0.636 ± 0.02 and 0.538 ± 0.01 kGy, respectively, after gamma irradiation. Grant, Mixon, and Patterson (1993) have reported D_{10}-values in the range 0.252–0.316 kGy and 0.126–0.288 kGy for *S. aureus* and *B. cereus*, respectively, in roast beef meal components. Bhide, Paturkar, Sherikar, and Waskar (2001) reported that gamma irradiation was not very effective against Gram positive spore-forming bacteria such as *B. cereus* and *Clostridium* spp. Meanwhile, Shay, Egan, and Wills (1988) required a 2–5 kGy dose of irradiation to destroy the vegetative bacteria of *B. cereus*.

The changes of BAs that occurred after irradiation in the ground beef and pork samples inoculated with *B. cereus* are shown in Table 2. Putrescine (PUT), tyramine (TYM), spermine (SPM) and the total amount of biogenic amines (TABA) in ground beef were significantly lower in the irradiated sample than were those of the non-irradiated one,

Table 1
Irradiation effect on the total bacterial counts (Log CFU/g) of raw beef and pork loin artificially inoculated with *Bacillus cereus*, *Enterobacter cloacae* and *Alcaligens*

Samples	Inoculum	Irradiation dose (kGy)			
		0	0.5	1	2
Beef	*Bacillus cereus*	6.3 ± 0.07^{a}	4.7 ± 0.10^{b}	3.0 ± 0.00^{c}	3.0 ± 0.00^{c}
	Enterobacter cloacae	5.9 ± 0.10^{a}	4.4 ± 0.12^{b}	3.3 ± 0.00^{c}	3.3 ± 0.00^{c}
	Alcaligenes faecalis	7.5 ± 0.06^{a}	5.3 ± 0.15^{b}	4.1 ± 0.00^{c}	4.0 ± 0.80^{c}
Pork	*Bacillus cereus*	6.1 ± 0.07^{a}	4.2 ± 0.15^{b}	3.5 ± 0.00^{c}	ND[d,1]
	Enterobacter cloacae	6.0 ± 0.04^{a}	4.3 ± 0.73^{b}	3.8 ± 0.00^{b}	3.0 ± 0.00^{c}
	Alcaligenes faecalis	7.5 ± 0.01^{a}	5.0 ± 0.11^{b}	4.3 ± 0.05^{c}	3.3 ± 0.00^{d}

[a–d] Means ± SE within the same row with the same superscript were not significantly different.
[1] Viable cell was not detected with detection limit $<10^{2}$ CFU/g.

Table 2
Contents of biogenic amines (μg/g) produced in irradiated beef and pork loin artificially inoculated with *Bacillus cereus* after irradiation

Samples	BAs	Irradiation dose (kGy)			
		0	0.5	1	2
Beef	PUT	4.7 ± 0.25^{a}	2.1 ± 0.13^{c}	2.8 ± 0.13^{b}	2.0 ± 0.10^{c}
	CAD	0.1 ± 0.14	ND[1]	ND	ND
	HIM	1.9 ± 0.09	2.4 ± 0.16	2.3 ± 0.13	2.2 ± 0.11
	SER	3.5 ± 0.17	3.5 ± 0.90	2.9 ± 0.09	2.4 ± 0.08
	TYM	24.7 ± 0.08^{a}	15.7 ± 0.59^{b}	12.5 ± 0.38^{c}	9.3 ± 0.41^{d}
	SPD	1.6 ± 0.01	1.7 ± 0.11	1.9 ± 0.14	1.6 ± 0.06
	SPM	28.4 ± 0.77^{a}	25.1 ± 0.77^{bc}	25.3 ± 1.26^{b}	22.4 ± 0.22^{c}
	TABA	64.9 ± 0.55^{a}	50.5 ± 1.84^{b}	47.7 ± 1.79^{b}	39.9 ± 0.57^{c}
Pork	PUT	2.3 ± 0.07^{a}	0.5 ± 0.51^{b}	0.3 ± 0.01^{b}	0.3 ± 0.15^{b}
	CAD	0.2 ± 0.11	0.3 ± 0.33	ND	ND
	HIM	0.2 ± 0.10^{b}	0.7 ± 0.04^{a}	0.9 ± 0.24^{a}	0.6 ± 0.07^{ab}
	SER	2.3 ± 0.32	1.9 ± 0.11	2.3 ± 0.21	2.0 ± 0.12
	TYM	1.3 ± 0.53	0.9 ± 0.10	1.2 ± 0.02	0.8 ± 0.03
	SPD	1.7 ± 0.06	2.2 ± 0.44	1.9 ± 0.04	1.7 ± 0.03
	SPM	31.3 ± 0.34^{a}	26.8 ± 1.65^{b}	28.2 ± 0.23^{b}	25.9 ± 0.85^{b}
	TABA	39.3 ± 0.97^{a}	33.4 ± 2.50^{b}	34.7 ± 0.63^{ab}	31.3 ± 1.08^{b}

[a–c] Means ± SE within the same row with the same superscript were not significantly different ($P < 0.05$).
Abbreviation: PUT, putrescine; CAD, cadaverine; HIM, histamine; SER, serotonin; TYM, tyramine; SPD, spermidine; SPM, spermine; TABA, total amount of biogenic amines.
[1] Not detected.

indicating that the microbial control by irradiation treatment may have an impact on the production of BAs, even at the initial stage of storage. Particularly, among the BAs tested, the reduction of TYM was the greatest. On the other hand, Kim et al. (2003) reported that a significant difference was not observed in BAs content between control and irradiated Korean fermented soybean pastes immediately after gamma irradiation; however, 4 BAs, namely PUT, TYM, spermidine (SPD) and histamine (HIM), showed significant reduction by irradiation during fermentation. Similarly, Kim et al. (2005) studied the combined effects of gamma irradiation and modified atmosphere packaging on the BAs formation in pepperoni sausage. A total of 6 different BAs were found in the study, including PUT, CAD, β-phenylethylamine (PHE), SPD, SPM and TYM, in the pepperoni sausage. Irradiation decreased the major BAs but there was no significant reduction for CAD and PHE. When proteins are irradiated, their tertiary structure can be denatured (Stevenson, 1992). Since meat and poultry contain substantial amounts of water, the radicals induced by water molecules activated by irradiation can change the structure of proteins by reforming their physicochemical properties through fragmentation, cross-linking, coagulation, and/or oxidation.

Table 3 shows the effects of irradiation on the production of BAs for beef and pork loin inoculated with *E. cloacae*. In the samples of irradiated ground beef, PUT, TYM, spermidine (SPD) and TABA showed significantly lower values than those of the non-irradiated control. However, in pork, a lower TYM value but higher SPM and TABA values were observed in irradiated samples. Kim et al. (2005) also found that, immediately after irradiation, the contents of β-phenylethylamine in the irradiated samples were higher than those of the control but, after 4 weeks of storage at 4 °C, the samples irradiated at 20 kGy showed significantly lower values than those of the others. This suggests that the direct breakdown effect of the BAs in meat products is less possible than the indirect reduction of the BAs, caused by the initial microbial control. There was no significant BAs reduction effect, by gamma irradiation, on ground pork loin

Table 3
Contents of biogenic amines (μg/g) produced in irradiated beef and pork loin artificially inoculated with *Enterobacter cloacae*

Samples	BAs	Irradiation dose (kGy)			
		0	0.5	1	2
Beef	PUT	5.5 ± 0.21^{a}	2.4 ± 0.17^{c}	3.5 ± 0.03^{b}	3.3 ± 0.25^{b}
	CAD	0.1 ± 0.10	ND[1]	ND	ND
	HIM	2.0 ± 0.06^{c}	2.4 ± 0.05^{b}	2.7 ± 0.05^{a}	2.3 ± 0.15^{b}
	SER	2.0 ± 1.01	2.4 ± 0.35	3.4 ± 0.19	3.1 ± 0.14
	TYM	19.8 ± 0.20^{a}	11.0 ± 0.48^{b}	12.3 ± 0.36^{b}	12.3 ± 0.84^{b}
	SPD	3.0 ± 0.07^{a}	1.9 ± 0.06^{c}	2.2 ± 0.04^{b}	1.8 ± 0.13^{c}
	SPM	31.1 ± 0.76	25.9 ± 0.84	29.2 ± 0.53	27.5 ± 2.17
	TABA	63.5 ± 1.93^{a}	46.0 ± 0.36^{c}	53.2 ± 0.80^{b}	50.3 ± 3.65^{bc}
Pork	PUT	2.2 ± 0.18	2.6 ± 0.36	2.3 ± 0.32	2.5 ± 0.22
	CAD	0.1 ± 0.10	ND	ND	ND
	HIM	0.2 ± 0.08	0.4 ± 0.02	0.2 ± 0.17	0.2 ± 0.11
	SER	1.9 ± 0.20	1.6 ± 0.21	2.3 ± 0.11	2.1 ± 0.18
	TYM	1.7 ± 0.14^{a}	1.0 ± 0.03^{b}	1.0 ± 0.05^{b}	1.1 ± 0.29^{b}
	SPD	1.7 ± 0.04	2.4 ± 0.32	1.9 ± 0.04	1.9 ± 0.07
	SPM	28.0 ± 0.94^{b}	35.9 ± 0.96^{a}	36.5 ± 1.06^{a}	35.8 ± 0.83^{a}
	TABA	35.8 ± 1.50^{b}	43.8 ± 0.99^{a}	44.2 ± 0.74^{a}	43.6 ± 0.65^{a}

[a–c] Means ± SE within the same row with the same superscript were not significantly different ($P < 0.05$).
Abbreviation: PUT, putrescine; CAD, cadaverine; HIM, histamine; SER, serotonin; TYM, tyramine; SPD, spermidine; SPM, spermine; TABA, total amount of biogenic amines.
[1] Not detected.

patty inoculated with *A. faecalis* (Table 4). On the other hand, PUT, TYM and TABA were decreased significantly in ground beef by gamma irradiation ($P < 0.05$).

Tabor and Tabor (1984) and Pegg (1986) reported that PUT, SPD and SPM were ubiquitous components of meat. In this study, all the bacteria tested produced a relatively large quantity of PUT, TYM and SPD in beef and pork samples. Hernandez-Jover, Izquierdo-Pulido, Veciana-Nogues, Marine-Font, and Vidal-Carou (1997b) reported that fresh pork and beef had only SPD and SPM. However, the beef and pork, inoculated with three different microorganisms, produced a substantial amount of other BAs. From the results, it can be recognized that irradiation at the initial stage of storage can be an effective tool for reducing microbial counts and the formation of BAs in fresh beef and pork.

3.2. Effects of organic acid treatment on microbial reduction and BAs production

The organic acid treatment achieved microbial reduction of beef and pork but the reduction range was quite

Table 4
Contents of biogenic amines (μg/g) produced in irradiated beef and pork loin artificially inoculated with *Alcaligenes faecalis*

Samples	BAs	Irradiation dose (kGy)			
		0	0.5	1	2
Beef	PUT	2.5 ± 0.08^{a}	1.9 ± 0.19^{b}	1.9 ± 0.06^{b}	1.4 ± 0.07^{b}
	CAD	0.5 ± 0.04	ND[1]	0.2 ± 0.19	ND
	HIM	2.3 ± 0.12	2.6 ± 0.06	2.4 ± 0.06	2.7 ± 0.18
	SER	3.4 ± 0.14	4.8 ± 0.49	3.9 ± 0.31	3.8 ± 0.23
	TYM	17.7 ± 0.61^{a}	17.7 ± 0.32^{a}	15.4 ± 0.30^{b}	11.9 ± 0.47^{c}
	SPD	2.0 ± 0.10	2.2 ± 0.11	2.0 ± 0.05	2.0 ± 0.08
	SPM	32.6 ± 1.89	30.5 ± 1.10	29.1 ± 0.58	30.4 ± 1.41
	TABA	61.0 ± 1.85^{a}	59.5 ± 1.17^{ab}	54.9 ± 0.58^{bc}	52.3 ± 2.07^{c}
Pork	PUT	1.1 ± 0.71	ND	ND	ND
	CAD	0.6 ± 0.06	ND	ND	ND
	HIM	0.7 ± 0.14	0.7 ± 0.34	0.6 ± 0.08	0.8 ± 0.13
	SER	2.4 ± 0.33	3.0 ± 0.52	2.1 ± 0.19	2.7 ± 0.65
	TYM	0.9 ± 0.16	1.2 ± 0.01	1.1 ± 0.26	1.1 ± 0.22
	SPD	1.7 ± 0.07	2.2 ± 0.37	2.0 ± 0.20	1.9 ± 0.28
	SPM	26.4 ± 0.65	27.6 ± 1.16	30.4 ± 2.67	25.8 ± 0.90
	TABA	33.8 ± 1.22	34.6 ± 0.66	36.1 ± 3.21	32.3 ± 0.99

[a–c] Means ± SE within the same row with the same superscript were not significantly different ($P < 0.05$).
Abbreviation: PUT, putrescine; CAD, cadaverine; HIM, histamine; SER, serotonin; TYM, tyramine; SPD, spermidine; SPM, spermine; TABA, total amount of biogenic amines.
[1] Not detected.

Table 5
Effect of organic acid treatment on the reduction of *Alcaligenes faecalis*, *Bacillus cereus*, and *Enterobacter cloacae* incubated at 4 °C for 24 h

Samples	Inoculum	No. of microorganisms (Log CFU/g)			
		Control	Acetic acid	Citric acid	Lactic acid
Beef	*Bacillus cereus*	6.3 ± 0.07^{a}	6.2 ± 0.09^{a}	5.1 ± 0.03^{c}	5.8 ± 0.12^{b}
	Enterobacter cloacae	5.9 ± 0.10^{a}	4.7 ± 0.02^{b}	4.7 ± 0.18^{b}	5.6 ± 0.04^{c}
	Alcaligenes faecalis	7.5 ± 0.06^{a}	7.3 ± 0.06^{b}	6.7 ± 0.02^{c}	7.2 ± 0.03^{b}
Pork	*Bacillus cereus*	6.1 ± 0.07^{a}	5.8 ± 0.08^{b}	3.8 ± 0.02^{c}	5.8 ± 0.01^{b}
	Enterobacter cloacae	6.0 ± 0.04^{a}	3.3 ± 0.31^{c}	3.7 ± 0.04^{bc}	4.2 ± 0.10^{b}
	Alcaligenes faecalis	7.5 ± 0.01^{a}	7.2 ± 0.08^{b}	7.0 ± 0.03^{c}	7.3 ± 0.06^{b}

[a–c] Means ± SE within the same sample row with the same superscript were not significantly different ($P < 0.05$).

limited (Table 5). Among the organic acids tested, the treatment of citric acid (2 M) was the best to control *A. faecalis*, *B. cereus* and *E. cloacae*, resulting in approximately 1 decimal reduction in the ground beef loin and 2 decimal reductions in the ground pork loin (Table 5). Acetic acid was also effective on the reduction of *E. cloacae* while *A. faecalis* was the most resistant microorganism against the organic acid treatment. Overall, the 2 M organic acid treatment was less effective in microbial control than was irradiation treatment at 2 kGy.

Organic acids are generally recognized as safe (GRAS) but may produce adverse sensory changes. However, the dilute solutions of organic acids (1–3%) are generally without effect on the desirable sensory properties of meat when used as a carcass decontaminant (Smulders & Greer, 1998). Generally, vinegar is composed of 4% acetic acid and the concentration of the organic acids for this study was less than 1% in meat.

Lactic acid treatment of beef inoculated with *B. cereus* showed the highest TYM and TABA contents, demonstrating even higher levels than those of the non-treated control (Table 6). However, the lactic acid treatment of pork gave the lowest contents of SPM and TABA in ground pork. The contents of BAs in ground beef inoculated with *E. cloacae* and *A. faecalis* resulted in fluctuating data by different acid treatments (Tables 7 and 8). The contents of PUT were lower in ground pork inoculated with both *E. cloacae* and *A. faecalis* by different acid treatments. However, no other difference was found in the contents of BAs in pork samples.

Several BAs exist in nature, but most BAs are formed by the action of microorganisms through the decarboxylation of amino acids in foods. Therefore, an inhibition of the decarboxylase activity and also prevention of bacterial growth would be very important for controlling the amine content of food. Nout, Ruikes, Bouwmeester, and Beljaars (1993) reported that TYM, PUT and CAD levels in samples were low or high, depending on the applied manufacturing process: soaking soybeans, type of fermentative microorganisms, boiling, home-cooking by stewing or frying in oil, and storage temperature.

The pH level is an important factor influencing amino acid decarboxylase activity, which is stronger in an acidic environment, the optimum pH being between 4.0 and 5.5

Table 6
Contents of biogenic amines (μg/g) in beef and pork inoculated with *Bacillus cereus* after treatment with organic acids

Samples	BAs	Control	Acetic acid	Citric acid	Lactic acid
Beef	PUT	4.7 ± 0.25	4.3 ± 0.19	4.5 ± 0.11	4.1 ± 0.20
	CAD	0.1 ± 0.14	0.3 ± 0.16	0.2 ± 0.10	0.3 ± 0.14
	HIM	1.9 ± 0.09	1.8 ± 0.10	1.8 ± 0.04	1.8 ± 0.08
	SER	3.5 ± 0.17	3.1 ± 0.07	3.2 ± 0.10	2.9 ± 0.43
	TYM	24.7 ± 0.08^{b}	18.1 ± 0.52^{c}	13.5 ± 0.24^{d}	38.0 ± 0.57^{a}
	SPD	1.6 ± 0.01^{b}	2.3 ± 0.10^{a}	1.7 ± 0.20^{b}	1.9 ± 0.04^{b}
	SPM	28.4 ± 0.77	26.9 ± 1.57	28.2 ± 0.98	30.5 ± 0.92
	TABA	64.9 ± 0.55^{b}	56.9 ± 2.09^{c}	53.0 ± 1.11^{c}	79.5 ± 1.51^{a}
Pork	PUT	2.3 ± 0.07	1.1 ± 0.54	1.5 ± 0.05	1.5 ± 0.02
	CAD	0.2 ± 0.11	0.2 ± 0.10	0.2 ± 0.12	0.2 ± 0.11
	HIM	0.2 ± 0.10	0.2 ± 0.11	0.2 ± 0.01	0.3 ± 0.02
	SER	2.3 ± 0.32	2.1 ± 0.37	1.8 ± 0.08	1.9 ± 0.08
	TYM	1.3 ± 0.53	1.1 ± 0.39	1.2 ± 0.47	0.9 ± 0.40
	SPD	1.7 ± 0.06	1.6 ± 0.04	1.8 ± 0.02	1.6 ± 0.03
	SPM	31.3 ± 0.34^{a}	26.6 ± 0.96^{bc}	28.2 ± 1.54^{b}	24.4 ± 0.20^{c}
	TABA	39.3 ± 0.97^{a}	32.8 ± 1.02^{bc}	34.8 ± 1.14^{b}	30.9 ± 0.32^{c}

[a–d] Means ± SE within the same row with the same superscript were not significantly different ($P < 0.05$).
Abbreviation: PUT, putrescine; CAD, cadaverine; HIM, histamine; SER, serotonin; TYM, tyramine; SPD, spermidine; SPM, spermine; TABA, total amount of biogenic amines.

Table 7
Contents of biogenic amines (μg/g) in beef and pork inoculated with *Enterobacter cloacae* after treatment with organic acids

Samples	BAs	Control	Acetic acid	Citric acid	Lactic acid
Beef	PUT	5.5 ± 0.21^{a}	5.4 ± 0.14^{a}	4.4 ± 0.09^{b}	4.0 ± 0.10^{b}
	CAD	0.1 ± 0.10	0.1 ± 0.10	ND[1]	ND
	HIM	2.0 ± 0.06	1.7 ± 0.06	1.7 ± 0.08	1.7 ± 0.03
	SER	2.0 ± 1.01	3.2 ± 0.22	3.0 ± 0.35	2.9 ± 0.30
	TYM	19.8 ± 0.20^{b}	15.4 ± 0.16^{c}	14.9 ± 0.28^{c}	34.6 ± 0.13^{a}
	SPD	3.0 ± 0.07^{a}	1.7 ± 0.06^{b}	3.3 ± 0.71^{a}	1.7 ± 0.02^{b}
	SPM	31.1 ± 0.76^{a}	30.8 ± 1.31^{a}	27.1 ± 0.21^{b}	26.3 ± 0.14^{b}
	TABA	63.5 ± 1.93^{b}	58.3 ± 1.85^{c}	54.5 ± 1.39^{c}	71.2 ± 0.12^{a}
Pork	PUT	2.2 ± 0.18^{a}	1.8 ± 0.06^{b}	1.5 ± 0.02^{c}	1.7 ± 0.08^{bc}
	CAD	0.1 ± 0.10	0.1 ± 0.09	0.1 ± 0.07	ND
	HIM	0.2 ± 0.08	0.2 ± 0.09	0.1 ± 0.09	0.1 ± 0.14
	SER	1.9 ± 0.20	2.1 ± 0.13	1.7 ± 0.18	1.9 ± 0.17
	TYM	1.7 ± 0.14	1.5 ± 0.06	1.5 ± 0.08	1.6 ± 0.00
	SPD	1.7 ± 0.04	1.8 ± 0.05	2.2 ± 0.40	1.7 ± 0.03
	SPM	28.0 ± 0.94	25.3 ± 0.31	27.1 ± 0.57	26.4 ± 0.59
	TABA	35.8 ± 1.50	32.8 ± 0.63	34.1 ± 1.17	33.5 ± 0.68

[a–c] Means ± SE within the same row with the same superscript were not significantly different ($P < 0.05$).
Abbreviation: PUT, putrescine; CAD, cadaverine; HIM, histamine; SER, serotonin; TYM, tyramine; SPD, spermidine; SPM, spermine; TABA, total amount of biogenic amines.
[1] Not detected.

Table 8
Contents of biogenic amines (μg/g) in beef and pork inoculated with *Alcaligenes faecalis* after treatment with organic acids

Samples	BAs	Control	Acetic acid	Citric acid	Lactic acid
Beef	PHM	6.1 ± 1.06	4.3 ± 0.12	2.6 ± 0.24	5.7 ± 2.56
	PUT	2.5 ± 0.08	2.4 ± 0.03	2.5 ± 0.19	2.4 ± 0.19
	CAD	0.5 ± 0.04	0.5 ± 0.06	0.3 ± 0.15	0.6 ± 0.05
	HIM	2.3 ± 0.12	2.3 ± 0.05	1.9 ± 0.14	1.8 ± 0.22
	SER	3.4 ± 0.14	3.3 ± 0.32	3.7 ± 0.43	3.4 ± 0.37
	TYM	17.7 ± 0.61^{b}	19.8 ± 0.58^{b}	13.0 ± 0.69^{c}	27.0 ± 2.25^{a}
	SPD	2.0 ± 0.10^{b}	2.4 ± 0.05^{a}	1.5 ± 0.09^{c}	2.2 ± 0.06^{b}
	SPM	32.6 ± 1.89^{a}	26.2 ± 0.38^{b}	24.9 ± 0.62^{b}	25.5 ± 0.98^{b}
	TABA	67.1 ± 2.91^{a}	61.0 ± 0.47^{b}	50.4 ± 1.26^{c}	68.5 ± 1.24^{a}
Pork	PHM	5.6 ± 0.46^{a}	2.5 ± 0.44^{b}	1.6 ± 0.79^{b}	0.9 ± 0.24^{b}
	PUT	1.1 ± 0.71	1.3 ± 0.63	1.3 ± 0.81	0.6 ± 0.13
	CAD	0.6 ± 0.06	0.5 ± 0.26	0.5 ± 0.03	0.6 ± 0.07
	HIM	0.7 ± 0.14	0.6 ± 0.36	0.4 ± 0.01	0.5 ± 0.28
	SER	2.4 ± 0.33	2.8 ± 0.11	2.7 ± 0.14	2.7 ± 0.14
	TYM	0.9 ± 0.16	1.0 ± 0.19	0.9 ± 0.13	0.9 ± 0.10
	SPD	1.7 ± 0.07	1.5 ± 0.03	1.7 ± 0.06	1.6 ± 0.05
	SPM	26.4 ± 0.65	25.0 ± 0.41	26.3 ± 1.21	26.6 ± 1.13
	TABA	39.4 ± 1.64	35.3 ± 1.20	35.6 ± 1.35	34.6 ± 1.35

[a–c] Means ± SE within the same row with the same superscript were not significantly different ($P < 0.05$).
Abbreviation: PUT, putrescine; CAD, cadaverine; HIM, histamine; SER, serotonin; TYM, tyramine; SPD, spermidine; SPM, spermine; TABA, total amount of biogenic amines.

(Teodorovic, Buncic, & Smiljanic, 1994). Several factors influence the pH of dry sausage, and the addition of glucono-δ-lactone to dry sausages resulted in a significant decrease in pH and in the levels of HIM and PUT, as well as the levels of faecal streptococci, aerobic mesophilic bacteria and coliforms, but did not affect the growth of lactic acid bacteria (Maijala et al., 1993). Furthermore, in such an environment with lower pH, bacteria are more strongly encouraged to produce the amino acid decarboxylase, as a part of their defence mechanisms against the acidity (Teodorovic et al., 1994). Therefore, the acidic conditions caused by addition of organic acids to ground meat may not only reduce organisms but also encourage production of the BAs.

4. Conclusion

Irradiation was effective in reducing the inoculated bacteria and reduction of the major biogenic amines (BAs) in ground beef and pork inoculated with three different microorganisms. On the other hand, organic acid treatment showed only 2 decimal reductions or less from the original inoculation level. The reduction of the BAs

content was also limited and variable by organic acid treatment. Therefore, the results suggest that irradiation was a more effective method than organic acid treatment for controlling microorganisms and the production of biogenic amines in ground beef and pork.

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