Food Control 28 (2012) 143–146

Contents lists available at SciVerse ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Short communication

Effectiveness of gamma irradiation in the inactivation of histamine-producing bacteria

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ARTICLE INFO

Article history:
Received 21 November 2011
Received in revised form
25 April 2012
Accepted 1 May 2012

Keywords:
Gamma ray
Histamine
Morganella morganii
Enterobacter aerogenes
Raoultella planticola
Tuna meat

ABSTRACT

Histamine is a cause of scombroid foodborne poisoning. The control of histamine-producing bacteria is a method that can be used to avoid the accumulation of histamine. This study examined the effectiveness of gamma irradiation in inactivating histamine-producing bacteria. The histamine-producing bacteria *Morganella morganii* (JCM 1672), *Enterobacter aerogenes* (ATCC 43175) and *Raoultella planticola* (ATCC 43176) were suspended in tryptic soy broth and were gamma-irradiated at 0.5–4.0 kGy at room temperature. The bacterial populations declined with higher absorbance doses, and the radiation D_{10} (the dose of radiation required to cause a 90% reduction in the number of survivors) values ranged from 0.32 to 0.42 kGy. Histamine-producing bacteria were inoculated on tuna and were gamma-irradiated; the D_{10} values were between 0.31 and 0.34 kGy, depending on the type of bacteria. Complete inactivation of the histamine-producing bacteria that were inoculated on tuna was achieved at 4.0 kGy. Although gamma irradiation was effective in controlling histamine-producing bacteria in order to reduce the risk of histamine poisoning, excessive doses were associated with color changes in the tuna.

1. Introduction

Foodborne illnesses caused by histamine occur after the consumption of foods containing histamine. The symptoms of histamine poisoning include difficulty in breathing, itching, rash, vomiting, fever and hypertension (Naila, Flint, Fletcher, Bremer, & Meerdink, 2010). One of the difficult problems in histamine poisoning is the difficulty in judging histamine accumulation based on the appearance of fish meats (Lehane & Olley, 2000). In total, 187 histamine poisoning incidents and 752 incidents were reported from 2000 to 2006 in the USA (Toda, Yamamoto, Uneyama, & Morikawa, 2009). In Japan, 89 outbreaks and 1577 cases were reported between 1998 and 2008 (Toda et al., 2009). The actual number of cases could be higher because there may be unreported incidents due to the mildness of the disease (Lehane & Olley, 2000). Thus, histamine poisoning is a large problem for public health and food safety. Accordingly, methods controlling the production and accumulation of histamine in foods play an important role in reducing the risk of histamine poisoning.

Bacterial decarboxylation of amino acids leads to the formation of biogenic amines such as histamine (Santos, 1996), and the inhibition of bacterial growth is an effective way to reduce the risk of histamine poisoning. Low temperature storage is a basic strategy for reducing histamine accumulation because histamine formation is temperature dependent (Bakar, Yassoralipour, Bakar, & Rahman, 2010; Emborg & Dalgaard, 2008; Visciano, Campana, Annunziata, Vergara, & Ianieri, 2007). However, some bacteria, such as *Photobacterium* spp., are able to grow and produce histidine decarboxylase at low temperatures (Emborg, Laursen, & Dalgaard, 2005), and temperature control alone is not always sufficient to inhibit the accumulation of histamine. In addition, low temperature control throughout the food chain is difficult to achieve and temperature abuse is usually observed during distribution (Nei, Uchino, Sakai, & Tanaka, 2005). The combination of decontamination treatments and subsequent temperature control would be more effective to avoid histamine poisoning.

Irradiation is an attractive technology that reduces the risk of biogenic amine poisoning (Naila et al., 2010). Although the degree of public acceptance of irradiated foods is not always high in some consumer communities (Gunes & Tekin, 2006), irradiation of several types of foods, such as meat products, fish and seafood, and fresh vegetables, is widely recognized and is approved in more than 50 countries. The mechanism of microbial inactivation by ionizing radiation is primarily damage to nucleic acids. Direct or indirect damage is caused by oxidative radicals originating from the radiolysis of water. The gamma irradiation is a safe, efficient, environmentally

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0956-7135/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodcont.2012.05.006

clean and energy-efficient process and is particularly valuable as a decontamination procedure (Farkas, 1998). Irradiation is reported to control biogenic amines through radiolysis of the amines. Kim et al. (2004) indicated that 5–100% of biogenic amines were destroyed by irradiation in the range of 2.5–25 kGy in a model system. Kim et al. (2003) irradiated materials of fermented soybean paste at 5–15 kGy and reported significant decreases in microbial populations of major bacteria in the paste such as *Bacillus* spp. and lactic-acid bacteria. In addition, they indicated that the accumulation of biogenic amines was reduced during the fermentation of soybean pastes. Thus, some research on the effectiveness of irradiation in controlling biogenic amines and the bacteria that produce them has been conducted. However, data on the resistance to gamma ray irradiation by other biogenic amine-producing bacteria, such as *Morganella morganii*, are not available.

The objectives of this study are (1) to evaluate the resistance of histamine-producing bacteria (*M. morganii*, *Enterobacter aerogenes* and *Raoultella planticola*) to gamma irradiation in pure cultures and on tuna fillets and (2) to evaluate the visible quality changes in tuna after irradiation.

2. Materials and methods

2.1. Test strain

The histamine-producing bacteria *M. morganii* (JCM 1672), *E. aerogenes* (ATCC 43175) and *R. planticola* (ATCC 43176) were used in this study. To minimize the growth of microorganisms naturally present on tuna, the bacteria were adapted to growth in tryptic soy broth (TSB, Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) supplemented with 50 μg/ml of rifampicin (TSBR). Plating on media containing rifampicin greatly minimized interference by naturally occurring microorganisms and facilitated the detection of test bacteria on the recovery media (Inatsu, Bari, Kawasaki, Isshiki, & Kawamoto, 2005).

2.2. Preparation of inocula

Each bacterium was cultured at 37 °C in 20 ml of TSB medium (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) supplemented with 50 μg/ml rifampicin. The cultures were transferred to the TSBR by loop at three successive 24 h intervals immediately before they were used as inocula. The cells of each strain were collected by centrifugation (3000× *g*, 5 min, 20 °C) and resuspended in 5 ml of sterile phosphate-buffered saline (PBS, pH 7.2). The final suspension, containing approximately 9 log CFU/ml, was maintained at 22 ± 2 °C, and the suspension was applied to tuna within 30 min after preparation.

2.3. Procedure for inoculation

The cell suspension (200 μl) in PBS was applied with a micropipette to the surface of 25 g of tuna (approximately 1.3 cm × 1.3 cm × 1.3 cm), and the inoculated tuna were dried at room temperature (22 ± 2 °C) for 30 min. After drying, the tuna were immediately treated with irradiation.

2.4. Treatments of histamine-producing bacteria in pure culture and on tuna

The centrifuge tubes (diameter of 30 mm) containing 20 ml of TSBR suspensions of *M. morganii*, *E. aerogenes* and *R. planticola* were prepared as described above and were irradiated at room temperature. The targeted doses were 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 or 4.0 kGy, at 5.6 kGy/h, using a cobalt-60 gamma source (Gamma Cell-220, Nordion International, Inc., Kanata, Ontario, Canada). The inoculated tuna were also irradiated after the samples were placed into a polyethylene bag. Dosimetry was performed using a 5-mm-diameter alanine dosimeter (Bruker Instruments, Rheinstetten, Germany), and the free radical signal was measured using an ESR analyzer (EMX-Plus, Bruker Instruments, Rheinstetten, Germany) to evaluate the actual doses. The actual doses were within 3% of the targeted doses.

2.5. Microbial analysis

After irradiation, serial decimal dilutions were prepared with PBS to enumerate the microbial population in the TSBR. When the microbial population on the tuna was counted, 25 g of tuna was placed in a stomacher bag; 225 ml of PBS (pH 7.2) was added and the mixture was pummeled for 60 s. Serial decimal dilutions were prepared with PBS, and the appropriately diluted samples were pour-plated in quadruplicate on tryptic soy agar plates (TSA; Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) supplemented with 50 μg/ml rifampicin (TSAR). All of the ingredients except rifampicin were combined and sterilized by heating at 121 °C for 15 min. The rifampicin solution was added to the molten agar before pouring the medium into petri dishes. Inoculated enumeration media were incubated at 37 °C for 24 h before the presumptive colonies of pathogen were counted.

2.6. Detection of surviving histamine-producing bacteria

The survival of histamine-producing bacteria was confirmed when the population was below detection limits (<1.0 log CFU/g for tuna meats and <1.0 log CFU/mL for TSBR experiments). In total, 20 ml of the irradiated suspension culture was transferred to 180 ml of TSBR and incubated at 37 °C for 24 h. For the irradiation experiments in tuna, the homogenized mixture containing 25 g tuna meat with 225 ml of TSB in a stomacher bag was kept in an incubator at 37 °C for 24 h for enrichment. Subsequently, 0.1 ml of suspensions or homogenates was plated onto TSAR. All of the plates were incubated at 37 °C for 24 h, and the surviving pathogen colonies were counted.

2.7. Color measurements

The color of the tuna was evaluated before and after gamma irradiation. A portable color meter (model SP-62, X-Rite, Grandville, Mich.) was calibrated with a white standard tile before use. The samples were placed on the tiles, which were then placed on a white sheet. The Commission Internationale de l'Eclairage (CIE) parameters (L^*, a^* and b^* values) were determined in six blocks of the tuna for each experimental condition.

2.8. Statistical analysis

The experiments were repeated three times, and the data are shown as the means and standard deviations. Significant differences in average values were established by the Tukey–Kramer multiple-comparison method at the 5% level of significance using SPSS (SPSS Inc., Chicago).

3. Results and discussion

3.1. Effect of gamma irradiation on the population of histamine-producing bacteria in pure culture

The populations of histamine-producing bacteria in TSBR after gamma irradiation are shown in Table 1. Approximately