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Short communication

Evaluation of Non-bacterial factors contributing to histamine accumulation in fish fillets

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ABSTRACT

Histamine is one cause of scombroid foodborne poisoning. The bacterial decarboxylation of amino acids leads to the formation of biogenic amines such as histamine. This study examined histamine accumulation in tuna and yellowtail fillets that had been sterilized by gamma irradiation to confirm whether factors other than bacterial activity are able to induce histamine accumulation. Fish fillets were sterilized by gamma irradiation at 35 kGy and stored for 7 days at 5, 15, or 25 °C, and the resulting histamine concentrations were measured. The histamine concentrations in all tested samples of tuna and yellowtail were below the detection limit of the assay used (<10 mg/kg). In contrast, the tuna and yellowtail meats inoculated with *Morganella morganii*, a known histamine-producing bacterium, showed significant histamine accumulation at 15 and 25 °C. These results indicated that non-bacterial factors do not promote histamine accumulation. Therefore, the proper control of histamine-producing bacteria is both necessary and sufficient to prevent histamine accumulation.

1. Introduction

Foodborne illness may be caused by the consumption of foods that contain high levels of histamine, especially at histamine concentrations higher than 500 mg/kg (Gonzaga, Lescano, Huaman, Salmon-Mulanovich & Blazes, 2009), and histamine poisoning is a major problem in public health and food safety. High levels of histamines have vasoactive effects in humans (Taylor, 1985) and may cause several symptoms, including difficulty breathing, itching, rash, vomiting, fever, and hypertension (Naila, Flint, Fletcher, Bremer & Meerdink, 2010). A total of 187 outbreaks and 752 patients were reported from 2000 to 2006 in the USA (Toda, Yamamoto, Uneyama & Morikawa, 2009). In Japan, 89 outbreaks were occurred from 1998 to 2008, and 1577 patients were reported (Toda et al., 2009). The actual number of cases could be higher; many incidents may be unreported due to the mildness of the disease (Lehane & Olley, 2000). Thus, histamine poisoning is a major problem for public health and food safety. The guidelines for safe levels of histamine concentrations in seafood were established at 50 mg/kg by the Food and Drug Administration (FDA) and at 200 mg/kg in the European Community.

One of the major challenges in preventing histamine poisoning is the difficulty in judging histamine accumulation in fish meats based on their appearance (Lehane & Olley, 2000). Histamine is known as a heat-stable compound (Tapingkae et al., 2010), and once it is produced in fish meats, its removal is difficult, even with cooking and prolonged heating. Accordingly, controlling the production and accumulation of histamine in foods is the key to reducing the risk of histamine poisoning. Histamine-producing bacteria such as *Morganella morganii*, *Raoultella planticola*, and *Photobacterium phosphoreum* play an important role in histamine accumulation in fish meats. Fish contain high levels of histidine in their muscles, and these bacteria express histidine decarboxylases. The bacterial decarboxylation of amino acids leads to the formation of biogenic amines such as histamine (Santos, 1996). Thus, the inhibition of bacterial growth is considered an effective way to reduce the risk of histamine poisoning (Bakar, Yassoralipour, Bakar & Rahman, 2010). However, one previous study reported histamine accumulation in canned anchovy without a contaminant increase in bacteria (Kim et al., 2004). In the study conducted by Kim et al. (2004), although the bacterial populations in canned anchovy with histamine accumulation were below detection limit (2 log CFU/g), some bacteria such as *Bacillus* spp. and *Staphylococcus* spp. were isolated after enrichments. The isolated bacteria produced negligible histamine in culture broth, and Kim et al. (2004) concluded that the accumulation of histamine in canned anchovy is due to poor quality of raw fish with high histamine levels. On the

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other hand, the present study suspected that non-bacterial factor growth such as the activity of enzymes within the fish tissues might be related to histamine accumulation. If histamine can accumulate significantly in sterilized fish meat, then bacterial control would not be sufficient to inhibit histamine accumulation in all cases.

The objective of this study was to investigate the contribution of non-bacterial factors to histamine accumulation. The histamine accumulation in tuna (*Thunnus obesus*) and yellowtail (*Seriola quinqueradiata*) sterilized by gamma irradiation was studied and compared to that in fish meat that was artificially inoculated with histamine-producing bacteria to accomplish this objective.

2. Materials and methods

2.1. Test products

Tuna and yellowtail were purchased from a supermarket in Tsukuba City, Japan. The fish meat was cut into 2-cm fillets with sterile knives and then placed in individual polyethylene bags. Subsequently, the pieces were frozen at −80 °C, and the frozen pieces were sterilized by gamma irradiation (Gamma Cell-220, Nordion International, Inc., Kanata, Ontario, Canada) at 35 kGy with dry ice. Pilot tests showed that a 35 kGy dose was sufficient to sterilize the naturally contaminating bacteria. To evaluate the influence of gamma irradiation on enzyme activities in fish tissues, the changes in *K* value of fish fillets affected by enzyme activities related to autolytic degradation were determined (Aubourg et al., 2007; Howgate, 2005; Ocaño-Higuera et al., 2011; Surette, Gill & LeBlanc, 1988). As a result, no significant change ($p > 0.05$) in the *K* value by gamma irradiation was observed (data not shown). 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). The actual dose was typically within 2% of the target dose. The fish fillets were stored at −80 °C until use in the experiments.

2.2. Inoculation of histamine producing bacteria

M. morganii was cultured at 37 °C in 40 ml of TSB medium (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan). The cultures were transferred to the TSB by the loop method at three successive 24 h intervals immediately before they were used as inocula. The cells were collected by centrifugation (3000 × *g*, 5 min, 20 °C) and resuspended in 40 ml of sterile phosphate-buffered saline (PBS, pH 7.2). The suspension was diluted with PBS, and a final suspension containing approximately 5 log CFU/ml was maintained at 22 ± 2 °C. The frozen fish fillets were thawed at 4 °C before inoculation. The 100 μL of prepared suspension was applied to sterilized fish fillets within 30 min after preparation.

2.3. Storage conditions

The fish fillets inoculated with *M. morganii* were packed in sterilized polyethylene bags and stored at 5, 15, or 25 °C for 7 days. Three samples were taken out from the incubator at 3, 5 and 7 days, and the number of *M. morganii* and histamine concentrations were determined. The sterilized, non-inoculated fish fillets were also stored at the same temperature conditions to examine the histamine accumulation in sterilized fish fillets. After storage for 7 days, the histamine concentrations of sterilized fish fillets were quantified. Forty sterilized fish fillet samples were evaluated for each storage temperature and fish type. The histamine concentrations were quantified using an enzymatic histamine test kit (Check Color Histamine, Kikkoman Co., Noda, Japan) according to manufacturer's instruction (Sato, Horiuchi & Nichimura, 2005). Briefly, 24 mL of extraction buffer (0.1 M of EDTA) was added to the 1 g of homogenized samples. The sample extracts were boiled for 20 min. Subsequently, the sample extracts were centrifuged at 10,000 ppm for 5 min and supernatants were collected. Enzyme reagent and colorimetric reagent were added to the extracted samples, and incubated at 37 °C for 15 min. After the incubation, the histamine contents were quantified by measuring absorbance of the extracted samples at 470 nm.

2.4. Microbial analysis

A 10 g sample of each fish fillet was placed in a stomacher bag with 90 ml of peptone-buffered water (pH 7.2). The fish meats were then homogenized for 60 s. Appropriately diluted samples were pour-plated in quadruplicate on tryptic soy agar (TSA) plates to obtain a count of the population of *M. morganii*. The inoculated enumeration media were incubated at 37 °C for 24 h–48 h before the presumptive colonies were counted.

2.5. 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 a 5% level of significance using SPSS (SPSS Inc., Chicago).

3. Results and discussion

3.1. Growth of histamine-producing bacteria during storage

The populations of *M. morganii* found on tuna and yellowtail filets during storage are shown in Fig. 1. The initial values of the population were approximately 3.0 log CFU/g. Rapid growth was observed at 25 °C, and the population exceeded 9.0 log CFU/g after 3 days storage on tuna and yellowtail filets. This result was consistent with the values reported by other researchers (Kim et al., 2002; Lorca et al., 2001; Tao, Sato, Yamaguchi & Nakano, 2009). Similarly, the populations of *M. morganii* increased significantly upon incubation at 15 °C ($p < 0.05$), reaching 8.0 log CFU/g after 7 days storage. However, no significant change in the population was detected after storage at 5 °C for 7 days ($p > 0.05$). These results are similar to those of a study conducted by Yamamoto et al. (1991), who observed no growth for *M. morganii* inoculated on yellowtail at 5 °C over 7 days of storage.

3.2. Histamine accumulation in fish fillets inoculated with *M. morganii*

The changes in the histamine concentration in tuna and yellowtail filets that were inoculated with *M. morganii* are shown in Fig. 2. Before storage, no histamine accumulation (<10 mg/kg) was detected in the fish filets. Increases in the histamine concentrations in tuna filets were observed during storage at 25 °C, reaching 4176 mg/kg and 5307 mg/kg at 3 days and 7 days, respectively. Similarly high levels of histamine accumulation were also reported in another study. Tao et al. (2009) indicated that histamine is accumulated at a level above 4000 mg/kg in tuna meats inoculated with *M. morganii* after storage at 25 °C. Significant accumulations of histamine in tuna fillets were also observed at 15 °C ($p < 0.05$), and the histamine concentration after storage for 7 days was 3273 mg/kg. Similar results were obtained in yellowtail fillets, in which significant increases in histamine concentration were detected at 3 days of storage at 15 °C and 25 °C. After storage for 7 days, the