Food Control 27 (2012) 194–199

Contents lists available at SciVerse ScienceDirect

Food Control

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

The synergistic effects of combined NaOCl, gamma irradiation and vitamin B_1 on populations of *Aeromonas hydrophila* in squid

Shin Young Park, Bo-Yeon Kim, Hyun-Ha Song, Sang-Do Ha*

School of Food Science and Technology, Chung-Ang University, 72-1 Nae-Ri, Daeduck-Myun, Ansung, Kyunggido 456-756, Republic of Korea

ARTICLE INFO

Article history:
Received 7 February 2012
Accepted 15 March 2012

Keywords:
Aeromonas hydrophila
Gamma irradiation
NaOCl
Vitamin B_1
Synergistic effects
Squid

ABSTRACT

The present study investigated the synergistic disinfection effects of combined NaOCl/gamma irradiation and NaOCl/vitamin B_1 treatment against *Aeromonas hydrophila* ATCC 7966 in tryptic soy broth (TSB) and squid. The synergistic effects were not dependent on the concentration of NaOCl or the dose of gamma irradiation, but were dependent on the vitamin B_1 concentration. Synergistic values for NaOCl/gamma irradiation treatment in TSB and squid were −0.37 to 2.09 $\log_{10}$ CFU/mL and 0.00–2.92 $\log_{10}$ CFU/g, respectively. The largest synergistic values, 2.09 $\log_{10}$ CFU/mL in TSB and 2.92 $\log_{10}$ CFU/g in squid, were as a result of treatment with a combination of 40 ppm NaOCl and 1.0 kGy gamma irradiation and 80 ppm NaOCl and 2.0 kGy gamma irradiation, respectively. Synergistic values for NaOCl/vitamin B_1 treatment in TSB and squid were 0.59–2.98 $\log_{10}$ CFU/mL and 0.06–1.35 $\log_{10}$ CFU/g, respectively. The largest synergistic values, 2.98 $\log_{10}$ CFU/mL in TSB and 1.35 $\log_{10}$ CFU/g in squid, were as a result of treatment with a combination of 300 ppm NaOCl and 1000 ppm vitamin B_1 and 1000 ppm NaOCl and 1000 ppm vitamin B_1, respectively. The results in the broth study suggest that the combination of 40 ppm NaOCl and 1.0 kGy gamma irradiation and 300 ppm NaOCl and 1000 ppm vitamin B_1 could be regarded as a potential optimum hurdle approach for application to real target foods and(or) food surfaces for the control of *A. hydrophila*. Moreover, the results in the food study indicate that the combination treatment of 80 ppm NaOCl and 2.0 kGy gamma irradiation and 100 ppm NaOCl and 1000 ppm vitamin B_1 could possibly be used in seafood production, processing, and distribution processes to enhance seafood safety.

1. Introduction

A member of the family Aeromonadaceae, *Aeromonas hydrophila* is a facultative anaerobic, motile, Gram-negative rod. *A. hydrophila* is common in the environment and is often associated with water and water supplies (Cunliffe & Adcock, 1989). *A. hydrophila* as an emerging human pathogen (Altwegg & Geiss, 1989) has attracted attention due to its ability to grow at refrigeration temperatures like *Listeria monocytogenes* and *Yersinia enterocolitica* (Palumbo, 1986). In general, *A. hydrophila* is neither salt (<5) nor acid (min. pH 6) tolerant (Daskalov, 2006). *A. hydrophila* can be isolated from many kinds of foods including vegetables (Callister & Agger, 1987), meat (Okrend, Rose, & Bennet, 1987), fish (Gobat & Jemmi, 1993), seafood (Palumbo, 1986), raw milk (Palumbo, 1986), and chicken (Hänninen, 1993). *A. hydrophila* survives for long periods in the environment (Araujo, Arrlebas, & Pares, 1991; El-Khashab & El-Yazed, 2001). For these reasons, *A. hydrophila* is of public health significance.

In the last decade, several single-agent disinfection and sanitization techniques have been proposed to reduce pathogenic microorganisms in foods. Physical sanitization and disinfection methods, such as heating (Browne & Dowds, 2001), electron beam irradiation (Sarrias, Valero, & Salmeron, 2003), chemical sanitization, and disinfectant methods using chlorine, hydrogen peroxide, ethanol, and ozone (Beuchat, 1997; Chang, Han, Song, Chung, & Shin, 2004; Mermelstein, 1998; Piernas & Guiraud, 1998; Wisniewsky, Glatz, Gleasin, & Reitmeier, 2000) have been used to reduce the amounts of pathogenic bacterial contamination in foods.

Sodium hypochlorite (NaOCl) is the best chlorine compound used as a disinfectant. The bactericidal efficacy of NaOCl is based on the penetration of the chemical and its oxidative action on essential enzymes in the cell (Kumar & Anand, 1998; Lomander, Schreuders, Russek-Cohen, & Ali, 2004). Gamma irradiation is the most widely accepted form of radiation sterilization and thus it is commonly used when materials cannot be heated to a high temperature for sterilization (WHO, 1999, pp. 9–37). Recently, sanitizer-treated rice had a greater reduction than water-treated rice, while sanitizer-

* Corresponding author. Dept. of Food Science and Technology, Chung-Ang University, 72-1 Nae-ri, Daeduck-myun, Ansung, Gyunggido 456-756, Republic of Korea. Tel.: +82 31 670 4831; fax: +82 31 675 4853.
E-mail address: sangdoha@cau.ac.kr (S.-D. Ha).

0956-7135/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodcont.2012.03.015

treated rice with vitamin B_1 produced an even greater reduction (Lee & Ha, 2008; Lee, Ha, & Ha, 2010). Kim, Cho, and Lee (2005) also showed that vitamin B_1 possessed antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*.

Many studies have been performed to evaluate the inactivation efficiency or synergy of combined chemical treatments and physical sanitization techniques, such as ethanol, hydrogen peroxide, sodium hypochlorite, ozone, ultraviolet processes, and gamma irradiation compared with the individual treatments. Studies of combined disinfection treatments are common in the literature (Chawla, Chander, & Sharma, 2006; Kanatt, Chawla, Chander, & Sharma, 2006). Some researchers have reported that the combination of chemical methods and gamma irradiation is highly effective against microorganisms. Sommers, Fan, Handel, and Sokorai (2003) noted a reduction in *L. monocytogenes* in frankfurters containing 10% citric acid at all gamma radiation doses. In addition, Bhide, Paturkar, Sherikar, and Waskar (2001) reported that acetic acid in combination with 3 kGy gamma irradiation was effective against *Bacillus cereus* in sheep/goat meat without any adverse effects on sensory attributes. Kim et al. (2011) recently reported that combined disinfection with NaOCl and vitamin B1 followed by gamma irradiation had synergistic effects in reducing the microbial population in oysters and short-necked clams. The purpose of the current study was to investigate the synergistic disinfection effects of combined treatment with NaOCl and gamma irradiation and NaOCl and vitamin B_1 on populations of *A. hydrophila* in tryptic soy broth and fresh squid.

2. Materials and methods

2.1. A. hydrophila

In this study, *A. hydrophila* ATCC 7966, which was isolated from a tin of milk with a fishy odor in the United States, was purchased from the ATCC culture collection. A stock culture was maintained at −70 °C in tryptic soy broth (TSB, Difco Laboratories, Detroit, MI, USA) containing 30% glycerol. To obtain a working culture, each strain was cultured twice at 37 °C for 18–24 h in TSB, streaked onto a tryptic soy agar (TSA; Difco, Becton Dickinson) plate, incubated at 37 °C for 18–24 h, examined for typical and homogeneous colony morphology, and then used immediately at room temperature.

2.2. Inoculation of TSB and squid

Populations of *A. hydrophila* ATCC 7966 used for the inoculum comprised 7–8 $\log_{10}$ CFU/mL. The inoculum was prepared in TSB by incubation at 35 °C for 24 h. Cell suspensions were centrifuged and suspended in 10 mL of buffered peptone water, and populations were determined by plating on TSA and incubating for 24 h at 35 °C. Stainless steel chips (20 mm by 20 mm) were inoculated with 0.25 mL of the cell suspension, which was spread onto the top surface of each chip and allowed to attach for 30 min at ambient temperature (approximately 25 °C) under a laminar flow hood.

Squid (*Sepioteuthis sepioidea*) was purchased from a local market in Anseong, Korea. To remove microorganisms already present on the squid, 10 g of each sample was washed with sterile, distilled water for 2 min, and then dried for 2 min. The samples were transferred into sterilized oxygen-impermeable nylon bags (2 mL O_2/m^2/24 h at 0 °C, 0.09 mm thickness; Sunkyung Co. Ltd., Seoul, Korea) on a clean bench. The packs were sealed and transferred to a refrigerator (4 °C) before irradiation. Packed samples were irradiated in a cobalt-60 gamma irradiator (Point source, ACEL, IR-79, MDS Nordion, Ontario, Canada) at the Korea Atomic Energy Research Institute, Jeongeup, Korea. The applied dose in this study was 1.0 kGy.

2.3. NaOCl and vitamin B_1 treatment

The efficacy of NaOCl or vitamin B_1 disinfectant was estimated using the European EN 1276 method based on quantitative suspension testing (AOAC, 1995; British Standards Institution, 1997). The Korea Food and Drug Administration use the EN method as the official method. Eight milliliters of NaOCl or vitamin B_1 was added to a mixture containing 1 mL TSB (7–8 $\log_{10}$ CFU/mL) or one piece of sliced squid (7–8 $\log_{10}$ CFU/g) and 1 mL of an interfering substance. This mixture was allowed to react at 20 °C (mean standard deviation [SD]) for 5 min and then agitated, and 1 mL was then added to a mixture containing 8 mL of neutralizing agent and 1 mL of distilled disinfecting product. This mixture was maintained for 5 min at 20 °C to ensure complete neutralization, after which 1 mL of the mixture was immediately applied to a sterilized TSA plate to count the number of surviving bacteria.

The sterile interfering substance was prepared by melting 0.3 g of bovine serum albumen (Sigma, St. Louis, MO, USA) in 100 mL of water and then filtering this mixture through a membrane filtration system (0.45-mm pore size; Sartorius AG 3770770, Gottingen, Germany) before use. The neutralizing agent was created by combining 3 g of lecithin (Fluka, Buchs, Switzerland), 30 g of polysorbate 80 (Fluka), 5 g of sodium thiosulfate (Sigma), 1 g of L-histidine (Sigma), and 30 g of saponin (Fluka) in a 1-L flask. The mixture was then diluted with a diluting agent to increase its mass, melted, and sterilized before use.

2.4. Gamma irradiation

TSB and packed squid were irradiated in a cobalt-60 gamma irradiator (Point Source, ACEL, IR-79, MDS Nordion, Ontario, Canada) at the Korea Atomic Energy Research Institute, Jeongeup, Korea. The source strength was approximately 11.1 PBq at a dose rate of 10 kGy/h. Dosimetry was performed using 5 min diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany), and the free radical signal was measured using a Bruker EMS 104 EPR Analyzer. The dosimeters were calibrated against an international standard developed by the International Atomic Energy Agency (Vienna, Austria).

2.5. Combined sodium hypochlorite and gamma irradiation treatment

NaOCl at a concentration of 20, 40, 60 or 80 ppm (NaOCl 12%, Shimadzu Co., Kyoto, Japan) was used as the chemical disinfectant. The solutions were diluted with tap water to their respective normal target concentrations. The applied dose of gamma irradiation was 0.1, 0.3, 0.5, 1.0 or 2.0 kGy. After irradiation, the samples were stored in a refrigerator at 4 °C prior to further analysis.

2.6. Combined NaOCl and vitamin B_1 treatment

NaOCl at a concentration of 50, 100, 200 or 300 ppm (NaOCl 12%, Shimadzu Co., Kyoto, Japan) was used as the chemical disinfectant. The sanitizer solutions were diluted with tap water to their respective normal target concentrations. Vitamin B_1 (thiamine dilauryl sulfate, Shinko Sci. Osaka, Japan) at a concentration of 100, 500 or 1000 ppm was used and the solution was immediately added to the sanitizer after being dissolved. Vitamin B_1 (thiamine dilauryl sulfate) was dissolved in 30% ethanol and the solution was immediately added to the sanitizer solutions.

2.7. Synergistic effects

The inactivation efficacies of the combined treatments were compared with those for the individual treatments to assess any