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Effect of UV- C radiation on survival of *Escherichia coli* O157: H7 inoculated fish fillets from fresh water and marine sources

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ABSTRACT

Radiation technique using UV-C was applied in this study on fresh-water Nile tilapia fish (*Oreochromis niloticus*) and marine-water Japanese threadfin bream fish (*Nemipterus japonicas*) fillets injected by *Escherichia coli* strain O157:H7. Tilapia fish (average weight and length are 150 ± 10 g, 15 ± 5 cm, respectively) and threadfin bream (average weight and length are 120 ± 5 g and 15 ± 2 cm, respectively) were collected from fresh fish market at Suez, Egypt. Fish samples were filleted, injected with *Escherichia coli* strain O157:H7, and subjected to UV treatment (15W). Samples exposed to UV irradiation (monochromatic, 253.7 nm) at four different distances: 12, 24, 30 and 45 cm. Each UV-C radiation treatment was performed for 5, 10, 15, and 20 min. Maximum reduction in the total count of *E. coli* strain O157:H7 (1.82 logs CFU/g) was associated with threadfin bream marine fish samples treated at 45 cm for 20 min. While in tilapia samples, count of *E. coli* strain O157:H7 was reduced to 1.1 logs CFU/g when treated at 24 cm for 15 min. Therefore, this study recommends this simple method of radiation using UV-C as a successful technique to expand the safety of fish fillets from fresh water and marine sources.

INTRODUCTION

Marine and Fresh-water systems cover about three quarters of the surface of the Earth, result in a series of critical-ecosystem-services (Younis, 2018). There has been a growing recognition of the interrelationship between human health and the oceans, especially due to the increase in the development of human activities near coastal areas and their diversity (Said *et al*. 2006; Shreadah *et al*., 2006; Younis & Nafea 2012; Younis *et al*., 2014; El Zokm *et al*., 2015; Soliman *et al*., 2018). This could cause risk to human health and therefore seafood contamination (Younis *et al*., 2018; Amin *et al*., 2018).

Fish filleting is the most commercial practice to process fish with the aims of adding value to the product, maintain high quality and preserve the harvested fish from potential contamination by gut, and head contents. Fish fillets are exposed to high-risk of foodborne contamination, if processed under poor hygienic measures. To reduce risks of contamination, several non-thermal physical techniques of sanitation and disinfection to food surfaces have been proposed. Ultraviolet (UV) irradiation has been recognized as one of the most important technology with promising wide applications (Sheen *et al*., 2012; Karlsen *et al*., 2015; and Freitas *et al*., 2015). It was

reported used in food for inactivating foodborne bacterial pathogens (Kim *et al*., 2017).

Escherichia coli were usually investigated as a fecal contamination indicator to assess the cleanliness and safety of seafood (Martínez *et al*., 2009). Although *E. coli* O157:H7strains are not commonly associated with seafood, still some outbreaks were reported (Surendraraj *et al*., 2010 and Matulkova *et al*., 2013). Fish are very sensitive to heat, and non-thermal technologies are preferred to control seafood borne pathogens. Therefore, this study investigated the application of UV-C radiation technology under different treatment times and distances on the survival of *Escherichia coli* O157:H7 inoculated in fish fillets. Compare the effect of these treatments on inoculated fresh water fish (Nile tilapia) fillets versus inoculated marine fish (Japanese threadfin bream) fillets.

MATERIALS AND METHODS

Fish samples

Freshwater tilapia (*Oreochromis niloticus*) fish (10 kg) and marine Japanese threadfin bream (Nemipterus japonicas) fish (10 kg) samples were purchased from local fish market in Suez Governorate, Egypt in 2018. The average weight and length were 150 ± 10 g and 15 ± 5 cm for tilapia, and 120 ± 5 g and 15 ± 2 cm for threadfin bream, respectively. Each fish species were transported to the laboratory packed in a sterilized cooler filled with ice (at < 2℃). The fillets were prepared manually (50 ± 1 g, and 38 ± 1 cm), carefully washed with tap water, and immediately prepared for the experiment.

Bacterial strain

E. coli strain O157:H7 was acquired from Ain Shams University, Microbiological Resources Center, at Cairo, Egypt. Stock cultures were sub-cultured twice into 10 mL of Brain Heart Infusion broth (BHI, Lab M, Neogen Company, UK), incubated at 37°C for 24 hr, and streaked onto Tryptic Soy Agar slants (TSA, Lab M, UK). Culture from TSA plates were streaked on Sorbitol MacConkey agar (SMAC, Lab M, Neogen Company, UK) and examined for typical colony morphology. Typical colonies confirmed by biochemical tests (indole, citrate, Methyle Red, Voges–Proskauer), API 20 E biochemical test strips (Biomerieux, France), and serological method using slide agglutination test with O157, and H7 antisera (pro-lab, USA). Then, the bacterial culture was used immediately for the preparation of the inoculum.

Inoculation of fish fillets

Population of *E. coli* strain O157: H7 at 7–8 logs CFU/mL was prepared from the confirmed bacteria. The inoculum was cultured in Tryptic soy broth (TSB, Lab M, Neogen Company, UK) incubated at 35°C for 24 h. Cell suspensions were centrifuged (Eppendorf, 14,000 rpm) for 10 min and pellet was suspended in buffered peptone water (BPW, Oxoid, UK). Bacterial cell counts were performed on TSA plated and incubated for 24 hr. at 35°C. Fish fillets samples were cut into 5 g pieces and used immediately after inoculation of bacterial suspension (1ml) on each sample. The inoculated samples in a sterilized petri-dish were set in laboratory for 2 hours and then exposed different UV-C treatments (Chun *et al*., 2010).

Exposure to UV-C surface irradiation

The UV-C irradiation experiments were performed with a bench top UV reactor contained two UV lamps (each one 15 W, monochromatic, 253.7 nm). The exposure to UV light was set to 0, 5, 10, 15, and 20 min on the inoculated fish sample surface, with different exposure heights of 12, 24, 30, and 45 cm from UV lamp to

the exposed fish surface. The UV lamp was turned on for at least 30 min before each experiment to ensure a constant UV intensity (Lee *et al*., 2016).

Bacteriological analysis

Inoculated samples (5 g) were homogenized in sterile bags for 2 min. sterile 0.1% peptone water (45 ml), and then serial dilution in 0.1% PW was conducted. Each dilution (1 ml) were plated in triplicate on SMAC, and incubated for 24 h. at 37°C. Clear colonies were considered positive for *E coli* O157:H7 and counted at a range of 25–250 CFU per plate.

Statistical analysis

All treatments were conducted in triplicates and bacterial counts were reported as means (log CFU/ g). Analysis of variance (ANOVA) was applied on this experiment using SPSS version 12. The average log reduction was pairwise compared between treatment times and distances from the UV lamps using Tukey test with a 95% confidence level.

RESULTS AND DISCUSSION

UV-C irradiation reduced the total *E. coli* strain O157: H7 count in tilapia fish fillets as shown in Figure (1). The highest reduction ($P<0.05$) of bacterial count was found of tilapia fillets located at 24 and 30 cm from UV lamps, respectively. The results show that the overall average counts exposed to UV-C for 5, 10, 15, and 20 min were 6.5 ± 0.32, 6.35 ± 0.13, 6.2 ± 0.82, and 6.4 ± 0.57 log CFU/g (Fig. 3), respectively compared to 7.3 ± 0.1 log CFU/g of non-treated control as shown in Table 1. The log reductions of *E. coli* strain O157: H7 were 0.8, 0.95, 1.1, and 0.9 logs CFU/g, respectively.

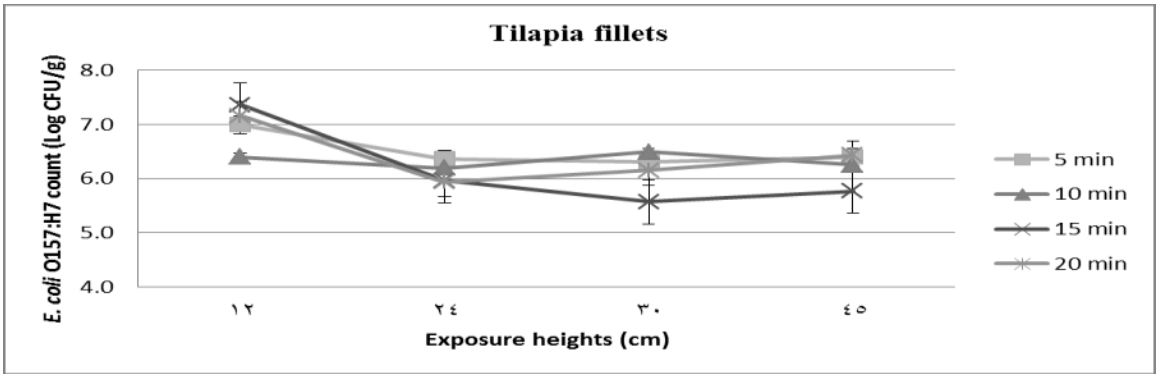

Fig. 1: Reduction of UV-C radiation on *E. coli* strain O157:H7 count (Log CFU/g) in Tilapia fillets at different heights (cm).

On the other side, UV-C irradiation reduced the total count of *E. coli* strain O157: H7 in threadfin bream fish fillets as shown in Figure (2). The highest reduction ($P<0.05$) was found at height 45 cm followed by 24 cm from UV lamps (Fig. 2). The overall average E. coli counts of marine fillets exposed to UV-C were 6.43 ± 1.06, 5.85 ± 0.37, 5.8 ± 0.41, and 5.38 ± 0.30 log CFU/g for 5, 10, 15, and 20 min (Fig.4), respectively compared with control (7.2 ± 0.1 log CFU/g) (Table 1). The reductions of bacterial count were 0.77, 1.35, 1.4, and 1.82 logs CFU/g, respectively.

Table 1: Counts of *E. coli* strain O157:H7 in raw tilapia and Japanese threadfin bream fish fillets (control).

Fish species	*E. coli* O157:H7 count (log CFU/g)
Tilapia fillets	7.3 ± 0.1
Japanese threadfin bream fillets	7.2 ± 0.1

These results (n3) were expressed as Mean ± SD.

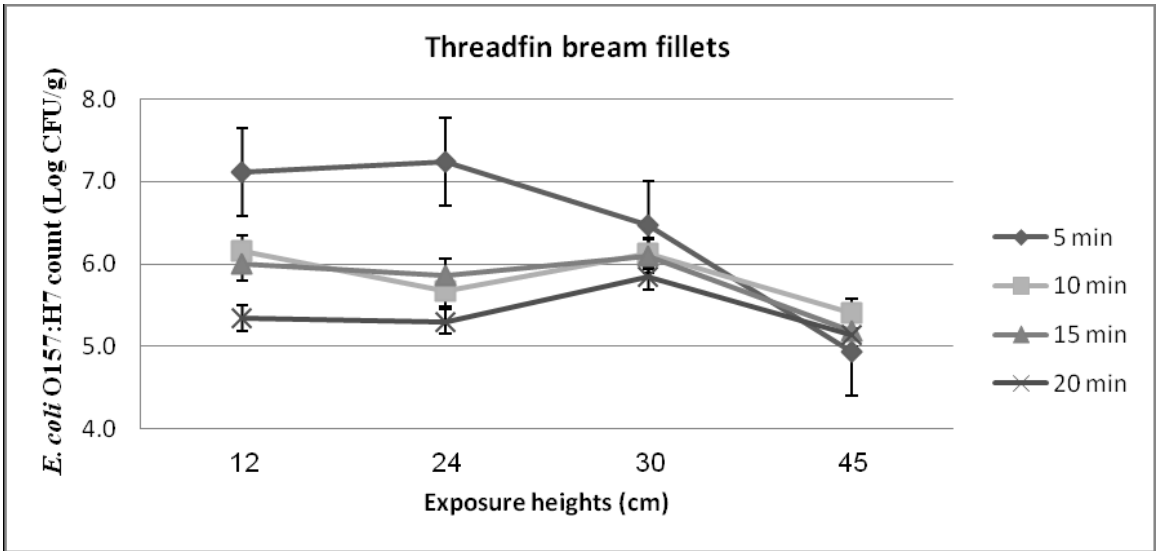

Fig. 2: Reduction of UV-C radiation on *E. coli* strain O157:H7 count (Log CFU/g) in threadfin bream fillets at different heights (cm).

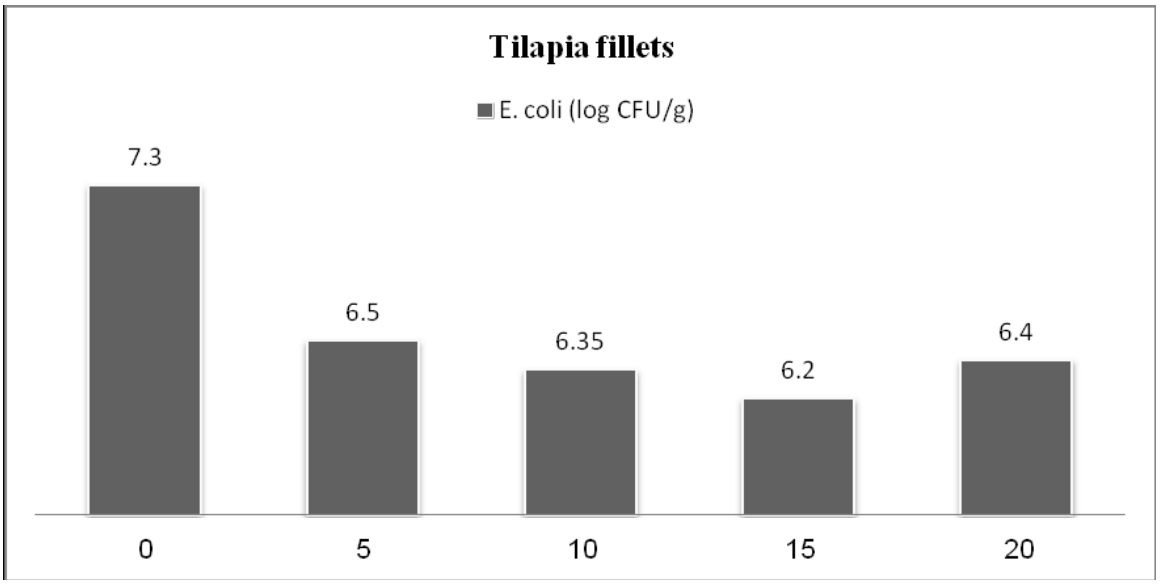

Fig. 3: Reduction rate of UV-C radiation on *E. coli* O157:H7 count (Log CFU/g) in Tilapia fish for different times (min).

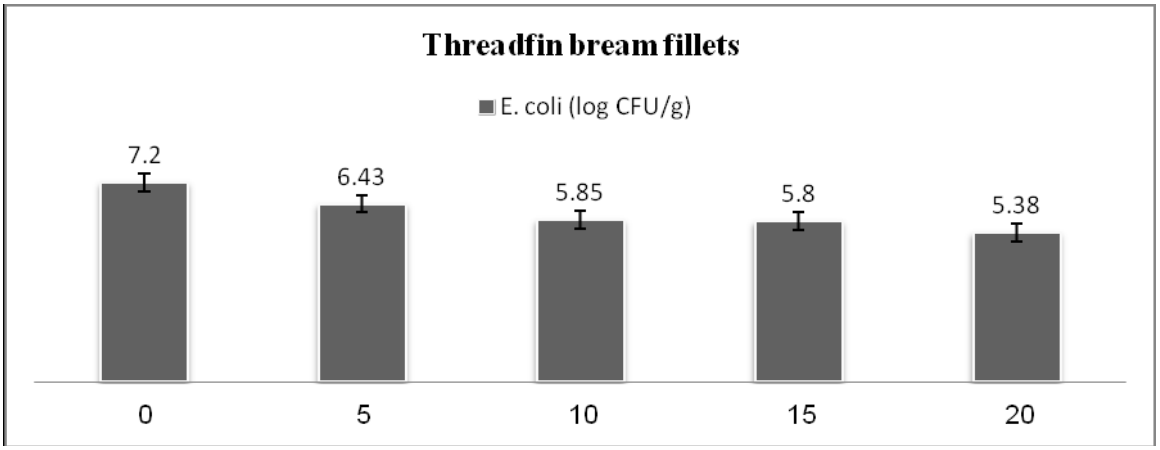

Fig. 4: Reduction rate of UV-C radiation on *E. coli* strain O157:H7 count (Log CFU/g) in Threadfin bream fillets for different times (min).

UV-C irradiation reduced *E. coli* strain O157: H7 total count (1.82 logs CFU/g) effectively ($P< 0.05$) in threadfin bream fish compared to tilapia fish fillets (1.1 logs CFU/g). This might be due to the different texture of marine fish compared to fresh-water tilapia fish, and UV application is strongly influenced by surface topography. Results were different according to the surface of application as reported by Ozer and Demirci (2006). Pulsed UV-light inactivated *E. coli* O157:H7 on salmon fillets (1.09 log CFU/ g) on muscle surface for 60 sec at 8 cm treatment, while, 0.86 log CFU/ g reduction was achieved on skin surface at 5 cm treatment after 30 sec.

Application of UV-C radiation on different food products such as poultry and rainbow trout was reported effective for inactivating spoilage and pathogenic microorganisms (Chun *et al*., 2010; Haughton *et al*., 2011; Lázaro *et al*., 2013; and Rodrigues *et al*., 2016). UV-C wavelengths between 200 and 280 nm were showed germicidal activity. Whereas, wave length at 253.7 nm had the maximum lethal effect on microorganisms as it damaged the DNA and interrupt cell growth (Koutchma *et al*., 2009). UV-C radiation technology has been reported with several benefits when applied in food products (Chang *et al*., 1985). It is easy to implement, low cost, and do not produce any byproducts or chemical waste that might change the odor, flavor or the color of food products (Chun *et al*., 2009). On the other hand, Kolakowska (2003) reported the side effects as it could enhance the oxidation process by initiation of free radical oxidation and catalyzes other stages of the oxidation process. Lipid radicals, superoxide radicals, and H_2O_2 could also be formed due to UV light. Furthermore, superoxide radicals could lead to carbohydrate and protein cross linking, peroxidation of unsaturated fatty acid and protein fragmentation and degradation, and resulted in loss of membrane fluidity and functions (Koutchma *et al*., 2009). When UV light treatment applied in high doses, chemical changes in food composition and total product quality deterioration were obvious (Kolakowska, 2003). While using UV light treatment in moderate doses has not been associated with any adverse effects in food (Krishnamurthy 2006). Therefore, to maintain the quality and the safety of food products, the disinfection process should be properly optimized.

In this study, Maximum reduction in the total count of *E. coli* strain O157:H7 (1.82 logs CFU/g) in threadfin bream marine fish samples treated at 45 cm for 20 min. While in tilapia samples, count of *E. coli* strain O157:H7 was reduced to 1.1 logs CFU/g when treated at 24 cm for 15 min. The treatment had different results according to the type of fish and could not achieve more than 2 logs reduction in total samples. This might be explained as the efficacy of the UV treatment for disinfecting

fish samples influenced by other factors such as bacterial growth rate, initial bacterial population, food composition, and fish type as reported by Wright *et al*. (2000) and Guerrero-Beltran and Barbosa-Canovas (2004). Moreover, UV-C technique applied and concentrated on food surface, and surface irregularities could act as physical barrier against UV rays resulting in bacterial protection and survival (Morgan, 1989).

CONCLUSION

In general, by using non-thermal UV-C radiation technique, this study demonstrated that maximum reduction (1.82 logs CFU/g) of *E. coli* strain O157:H7 could be achieved for marine fish fillets for 20 min treatment at 45 cm distance, whereas 1.1 logs CFU/g reduction for fresh water fish fillets could be achieved for 15 min treatment at 24 and 30 cm distances. Therefore, this study recommends that application of UV-C radiation improved the safety of marine and fresh water fish fillets.

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