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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

The effect of UV-C radiation technique on survival of *Escherichia coli* O157:H7 inoculated the freshwater Nile tilapia (*Oreochromis niloticus*) and marine Japanese threadfin bream (*Nemipterus japonicas*) fillets were investigated. Tilapia samples (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 purchased from local fish market in 2018 and filleted. The fish fillet samples were inoculated with *Escherichia coli* O157:H7 strain, and subjected to UV treatment (15 W) at four different distances (12, 24, 30 and 45 cm) from the UV lamps that emitted monochromatic UV irradiation at 253.7 nm. Each UV-C radiation treatment was performed for 5, 10, 15, and 20 min. Results showed that the maximum reduction of *E. coli* O157:H7 of 1.82 logs CFU/g in threadfin bream samples treated for 20 min treatment at 45 cm. whereas in tilapia samples maximum reduction of *E. coli* O157:H7 was 1.1 logs CFU/g treated for 15 min at 24 cm. In conclusion, this study recommends that UV-C radiation could be used as a successful technique to improve the safety of fish fillets from fresh water and marine sources.

INTRODUCTION

Fresh-water and marine ecosystems cover more than three-quarters of the Earth's surface, providing a series of critical ecosystem services (Younis, 2018). There has been a growing recognition of the interrelationship between human health and the oceans. Due to the increase in the development of human activities near coastal areas there is concern that some areas are struggling to maintain their habitat (Shreadah *et al*., 2006; Said *et al*. 2006; Younis *et al.*, 2014; El Zokm *et al*., 2015; Soliman *et al.*, 2018) and their diversity (Younis & Nafea 2012;). 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 identified as an important technology with promising wide applications (Sheen

et al., 2012; Lázaro *et al.*, 2014; Freitas *et al.*, 2015 and Karlsen *et al.*, 2015). Kim *et al.*, 2017) reported that UV-C has been used on several microorganisms, including gram positive and gram negative bacteria, in food for the purpose of inactivating and disinfecting food-borne pathogens.

Escherichia coli have been used as a standard fecal contamination indicator to assess the cleanliness and safety of seafood and various commercial food products (Martínez *et al.*, 2009 and Kagambèga *et al.*, 2011). Although only some *E. coli* strains are pathogenic to humans, certain pathogenic serotypes, such as *E. coli* O157:H7, have caused numerous outbreaks associated with seafood (Surendraraj *et al.*, 2010 and Matulkova *et al.*, 2013). Therefore, this study was designed to investigate the effects of UV-C radiation technology under different treatment times and distances on the survival of *Escherichia coli* O157:H7 inoculated freshwater tilapia (*Oreochromis niloticus*) and marine Japanese threadfin bream (*Nemipterus japonicas*) 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 packed in a sterilized cooler filled with ice (at < 2 ± 1°C) and transported to the laboratory within 1 hour. 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 O157:H7 ATCC strain was obtained from the Microbiological Resources Center (MIRCEN), Faculty of Agriculture, Ain Shams University. Stock cultures (10^8 CFU/mL) stored at -70°C in 15% (w/v) glycerol was inoculated into 10 mL of Brain Heart Infusion broth (BHI, Lab M, Neogen Company, UK). *E. coli* O157:H7 was cultured twice at 37°C for 24 hr in BHI, and then was streaked onto Tryptic Soy Agar (TSA, Lab M, Neogen Company, UK) plates. The TSA plates were incubated at 37°C for 24 hr and were examined for typical and colony morphology on Sorbitol MacCkoncy agar. Typical colonies confirmed by O157, and H7 antisera (pro-lab, USA) using slide agglutination tests. Then, the bacterial culture was used immediately for the preparation of the inoculum.

Inoculation of fish fillets

Population of *E. coli* O157: H7 at 7–8 logs CFU/mL was prepared from the confirmed bacteria. The inoculum was prepared using Tryptic soy broth (TSB, Lab M, Neogen Company, UK) by incubation at 35°C for 24 h. Cell suspensions were centrifuged (Eppendorf, Model 5418, Maximum speed/RCF: 14,000 rpm) for 10 min and pelleted material was suspended in 10 mL of buffered peptone water (BPW), and bacterial cell counts were determined by plating on TSA and incubating for 24 hr at 35°C. Fish fillets samples were cut into 5 g pieces and used immediately after preparation by the addition of 1 mL of bacterial suspension on each sample. The inoculated samples were placed in a sterilized petri-dish and exposed immediately for UV-C.

Exposure to UV-C surface irradiation

The UV-C irradiation experiments were conducted with a bench-scale collimated beam UV reactor equipped with two UV lamps (each one 15 W) that emitted monochromatic UV irradiation at 253.7 nm. The irradiance of exposed 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 surface. To ensure a constant UV intensity output, the UV lamp was turned on for at least 30 min before each experiment (Lee *et al.*, 2016).

Bacteriological analysis

The treated fish samples (5 g) were homogenized for 60s in sterile bags containing 45 mL of sterile 0.1% pepton water (PW), and then diluted in 0.1% PW of test tube. Aliquots were taken from the homogenate samples, diluted with 0.1% PW and plated in triplicate on Sorbitol Mackoncy plates (SMAC, Lab M, Neogen Company, UK). The plates were incubated at 37°C for 24h. Clear colonies were considered positive for *E coli* O157:H7 and counted at a range of 25–250 CFU per plate.

Statistical analysis

Count of *E. coli* O157: H7 in raw tilapia and threadfin bream were transformed into log CFU/ g for statistical analysis. All treatments were carried out in triplicates and bacterial populations were reported as means (log CFU/ g). An analysis of variance (ANOVA) was performed using SPSS version 12. Tukey test was used to compare the average log reduction between pairs of treatment times and distances from the UV lamps. Pairwise comparison among means with a 95% confidence level was used.

RESULTS AND DISCUSSIONS

The effect of UV-C irradiation on the reduction of *E. coli* O157: H7 count in tilapia fish fillets is 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 (Table 1). The log reductions of *E. coli* O157: H7 were 0.8, 0.95, 1.1, and 0.9 logs CFU/g, respectively.

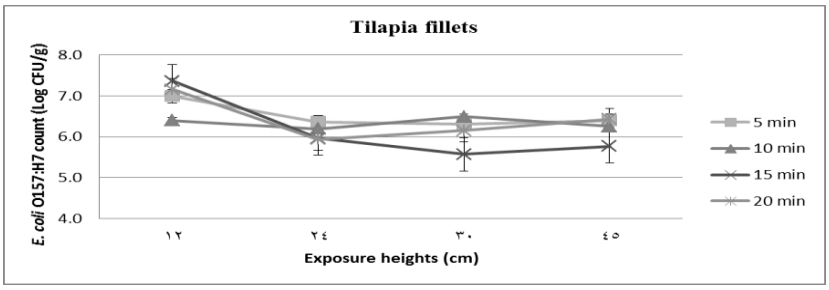

Fig. 1: Reduction effect of UV-C radiation on *E. coli* O157:H7 count (Log CFU/g) in Tilapia fillets at different heights (cm).

On the other side, the effect of UV-C irradiation on the reduction of *E. coli* O157: H7 in threadfin bream fish fillets is 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: The counts of *E. coli* 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.

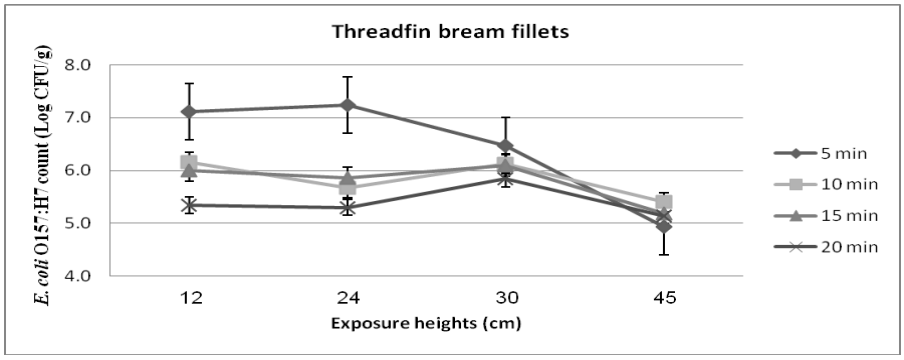

Fig. 2: Reduction effect of UV-C radiation on *E. coli* O157:H7 count (Log CFU/g) in threadfin bream fillets at different heights (cm).

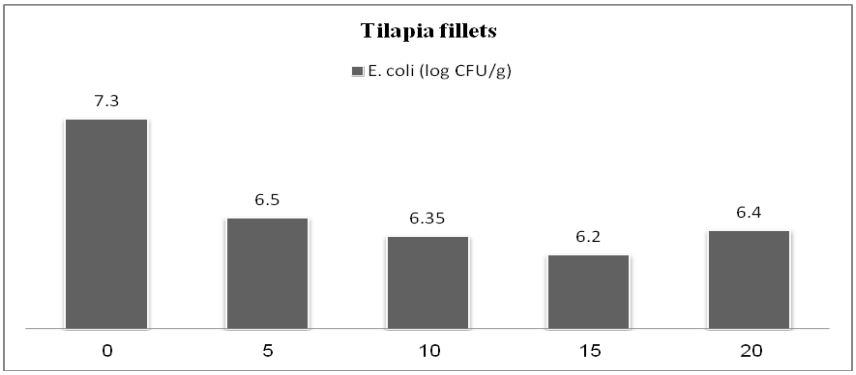

Fig. 3: Reduction rate of UV-C radiation on *E. coli* O157:H7 count (Log CFU/g) in Tilapia fish for different times (min).

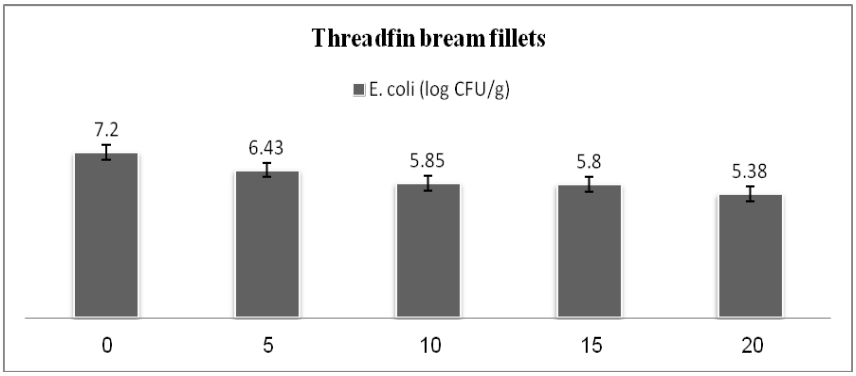

Fig. 4: Reduction rate of UV-C radiation on *E. coli* O157:H7 count (Log CFU/g) in Threadfin bream fillets for different times (min).

The effect of UV-C irradiation on the reduction of *E. coli* O157: H7 count was more effective (*P< 0.05*) (1.82 logs CFU/g) in threadfin bream fish fillets than tilapia fish fillets (1.1 logs CFU/g). This may be as a result of marine fish has smoother flesh than tilapia fish and UV surface treatment is strongly influenced by surface topography. Effect of pulsed UV-light to inactivate of *E. coli* O157:H7 and *Listeria monocytogenes* Scott A on salmon fillets at different treatment times and distance from the UV strobe was reported by Ozer and Demirci (2006). Log reduction of *E. coli* O157:H7 (1.09 $\log_{10}$ CFU/ g) was achieved on muscle side at 8 cm for 60 sec treatment, whereas 0.86 log CFU/ g reduction on skin at 5 cm for 30 sec treatment.

Direct application of UV-C radiation on food products was found to be an effective method for inactivation of spoilage and pathogenic microorganisms in many food products such as poultry (Chun *et al.*, 2010; Haughton *et al.*, 2011 and Lázaro *et al.*, 2013), and rainbow trout (Rodrigues *et al.*, 2016). The UV-C wavelengths between 200 and 280 nm were showed germicidal activity. At 253.7 nm there was a maximum lethal effect (Bintsis *et al.*, 2000) as it causes damage in DNA of microorganisms and causes the cell growth interruption (Koutchma *et al.*, 2009). This UV-C radiation technology has several benefits as it is easy to implement, low cost, and do not produce reactivity, chemical waste or undesirable by-products that might change the sensory properties of products, such as odor, flavor and color (Chang *et al.*, 1985 and Chun *et al.*, 2009). On the other hand, UV light exposure initiates free radical oxidation and catalyzes other stages of the oxidation process. Lipid radicals, superoxide radicals and H_2O_2 are formed due to UV light (Kolakowska 2003). Superoxide radicals can further induce carbohydrate cross linking, protein cross linking, protein fragmentation, peroxidation of unsaturated fatty acid, and loss of membrane fluidity function. Furthermore, it is well known that UV-C radiation promotes biochemical changes such as protein degradation (Koutchma *et al.*, 2009). Kolakowska (2003) reported that there are obvious changes in the chemical composition of food components and product quality deterioration when the UV light treatment is applied in high doses. Using UV light treatment for food has been found not to cause any adverse effects, especially if UV light is applied in moderate amounts (Krishnamurthy 2006). Therefore, it is mandatory to properly optimize the disinfection process so that the quality of the food products is maintained and its safety is ensured.

UV-C radiation technology efficacy for disinfecting food products varied according to many factors, such as the characteristics of bacterial strain and species, growth rate (Wright *et al.*, 2000), initial bacterial population density as well as composition and food type (Guerrero-Beltran and Barbosa-Canovas, 2004). Moreover, the mode of action of UV-C technique concentrated only on the food's surface (Morgan, 1989), and surface irregularities may act as physical protection against UV-C rays, contributing to bacterial survival (Korhonen *et al.*, 1981).

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* 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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