Effect of gamma-radiation and microwave cooking on *Aeromonas hydrophila* in local fish fillet

Amal A. Meghahed[*], Hanan A. El-Ghaiaty[**], Taghreed A. Hafez[**] and Wafaa S Mohamed[***]

Department of Bacteriology[*] and Food Hygiene[**], Animal Health Research Institute, Port-Said Lab., and National Center for Radiation Research and Technology[***]

Abstract

The objectives of this study were to determine the bactericidal effect of gamma and microwave radiation on *A. hydrophila* inoculated fish fillets. The study revealed that treatment of fish fillets with a dose of 1 KGy of gamma radiation reduced the population of *A.hydrophila* by 10^4 cfu/g, while the dose of 2 and 3 KGy completely eliminated the microorganism. Treatment of fish fillets with microwave cooking for 1 and 2 minutes completely eliminated the microorganism. The physical examination of fish after gamma radiation revealed that the used doses had no significant changes on fish fillets.

Key words: *Gamma-radiation, Microwave, fish fillets, A.hydrophila* .

Introduction

Aeromonas spp. are pathogens that cause food-borne gastroenteritis in human with extraintestinal symptoms such as septicemia, meningitis, endocarditis and osteomyelitis with a high mortality rate in immunocompromised persons **(Gold and Salit 1993)**. The main virulent factors that have effect on pathogenicity are; extracellular toxins (enterotoxin, hemolysin and protease), structural features (pilli, S- layer, lipopolysaccharide), adhesion and invasion **(Cahill 1990** and **Janda and Abbott 1998)**. *Aeromonas spp.* can grow and produce toxins in refrigerated conditions **(Eley *et al.* 1993)** indicating that refrigeration cannot be effective enough to control the pathogen **(Kirov 1993)**.

Because *Aeromonas spp.* are widely distributed in freshwater, estuarine, and marine environments **(Holmes *et al.* 1996)**, seafood have been considered as an important vehicle of *Aeromonas spp*. infections **(Altwegg *et al.* 1991, Kirov 1993, Mattick and Donovan 1998)**, so that elimination of this pathogen is extremely important to maintain human health.

Processing of food by gamma radiation and microwaves can be used to destroy and eliminate the bacteria and reduces the incidence of food-borne illness **(IAEA 1995, and FSIS 2000).**

At low doses of gamma radiation, nutrient losses are either not measurable or of no significance (**Radomyski *et al.* 1994)**. Irradiation is a safe and effective technology that can prevent many food borne diseases and spoilage microorganisms while maintaining wholesomeness and sensory quality **(IFT, 1998, IAEA, 2000,** and **EFSA 2011).**

The mechanism of gamma radiation in killing bacteria was described by **(IAEA 1995)**; when gamma rays strikes bacteria and other microbes, its high energy breaks the chemical bonds in molecules that can lead to DNA and cellular damage while not elevating the product temperature. Therefore, refrigerated and deep- frozen products may be processed by gamma radiation without sensory and quality changes. Thus, the implementation of widespread seafood irradiation would be quite beneficial to both fishermen and consumers **(IAEA 2000).**

Microbial destruction by microwaves has great potential in the pasteurization of foods **(Rosenberg and Bogl 1987)**. Its short heating and exposure time is less destructive to food

than longer conventional heating **(Harlfinger 1992)**.

Microwave heating is known to inactivate many microorganisms in various foods **(Fung and Cunningham 1980, Spite 1984, and Harlfinger 1992)**. Despite many studies on microbial destruction by microwave the mechanism of destruction is not fully understood. Two mechanisms are proposed for inactivation of microorganisms by microwaves. The first states that microwaves inactivate microorganisms entirely by heat **(Heddleson and Doores 1994)**. A second proposed mechanism involves non- thermal inactivation or "cold pasteurization": selective heating, electroporation, cell membrane rupture, and magnetic field coupling **(Kozempel *et al.*1998).**

The aim of this study is to evaluate the effectiveness of both gamma radiation and microwave radiation in eliminating *Aeromonas hydrophila* from fish fillet.

Materials and Methods

1- Preparation of samples:

Fish fillets were obtained from fish market in Port- Said City and transferred directly in ice box to be examined bacteriologically for the presence of *A. hydrophila.*

2-Preparation of the inoculum: according to **Doyle *et al.* (2001**

A.hydrophila strain (obtained from The Animal Health Research Institute- Food Hygiene Department, Agriculture Center Research, Egypt) was used as the inoculum. The bacterial strain was transferred to test tubes containing 10 ml of tryptic soy broth by the inoculation loop. The broth was incubated at 37^{o}C for 24hours. After incubation, the cultures were transferred to sterile centrifuge tubes and centrifuged at 5000xg for 10 minutes. The supernatant was discarded and pellets were resuspended in 10 ml of 0.1% peptone water and serially diluted with peptone water to concentrations of $10^{-1}$to 10^{-8}. Preliminary work was conducted to determine the concentration of inoculum needed to yield 10^{5} cfu/g of fish fillets.

3-Treatment of the samples:

Tilapia fish fillets were aseptically weighed and divided into six groups each of 100 grams then inoculated by *A.hydrophila* to obtain 10^{5}cfu/g concentration for each group. The first group was not treated and used as control group. The 2nd, 3rd, and 4th groups were treated by 1, 2, and 3 KGy of gamma rays respectively. The irradiation was carried out with the CO^{60} gamma rays source using Russian Facility (CM-20) Gamma cell located at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The source was giving a dose rate of irradiation of about 6 kGy / hour at the time of experiments; this means that the fish were exposed to 1 KGy every 10 minutes. The 5th and 6th groups were subjected to microwave cooking for 1 and 2 minutes respectively. Microwave radiation was performed in household microwave oven (Clatronic, type MWG- 748, with a rotating glass plate, frequency of 2,450 MHz, and power of 850W).

4-Bacterial count: according to (**Umadatt 1997).**

Twenty five grams were transferred aseptically from each sample to a sterile stomacher bag containing 225 ml of sterile 0.1% peptone water and agitated for 60 seconds. 0.1 ml aliquot was aseptically transferred from each mixture and spread over the surface of duplicate plates of Aeromonas agar to which Aeromonas selective supplement was added. The plates were incubated at 37^{o}C for 24 hr. After incubation, colony forming units from each plate were counted, recorded, averaged, and reported as colony- forming units per gram.

5-Physical examination of the irradiated samples:

Fish fillets which were treated by gamma radiation were examined by panelists for any changes in; color, texture, and smell. Non-irradiated fillet was used as standard. This standard was presented to panelists together with the treated and compared. Panelists scored each sample using a 5-point grade: 1, very poor; 2, poor; 3 common; 4, good; 5, very good **(Szczesniak 1987)**. Data were submitted to ANOVA and the least significant difference test, in order to compare the mean values of the

investigated parameters.

6- Statistical analysis:

Statistical analysis was carried out on the averages of the triplicate results. Data were analyzed by the analysis of variance (one- way ANOVA) method with significant level of $p \leq 0.05$ **(Draper and Smith 1998)**. All statistical procedures were computed using the Microsoft Excel 2007.

Results and Discussion

The results obtained from (Table 1) indicated that there were 10^4 cfu/g reductions in the population of *A. hydrophila* of fish fillets in group 2 after the treatment with 1 KGy gamma radiation when compared to the control untreated group which was 10^5 cfu/g of *A. hydrophila*. Treatment of fish fillets in group 3 and 4 with 2 and 3 KGy dose levels of gamma radiation respectively resulted in complete elimination of *A. hydrophila* population in these groups.

The obtained results agreed with **IAEA (2000)** which stated that the bacterial level of fish fillets was markedly reduced by 1.0 KGy gamma radiation and **Nagar and Bandekar (2011)** who observed that gamma radiation with 1.5 KGy dose resulted in complete elimination of 10^5 cfu/g of *A. hydrophila* from mixed sprouts, chicken and fish samples. A study done by **Ozbas *et al.* (1996)** stated that the count of *A. hydrophila* in meat ball samples decreased when irradiation level increased, They also stated that a dose of 0.75 KGy was sufficient to destroy approximately 10^4 cfu/g of *A. hydrophila* in meat ball. **Mahin *et al.* (2011)** examined the effect of 2.5, 5.0, 7.5, and 10 KGy gamma radiation on Mola fish and observed that 2.5 KGy completely eliminated the *Aeromonas spp.*.The results obtained by gamma radiation treatment indicated that *A. hydrophila* was very sensitive to gamma radiation as all Gram- negative microorganisms **Thayer *et al.* (1993), and Monk *et al.* (1995)**

The effect of microwave cooking on *A. hydrophilla* in fish fillets was shown in (Table 2). Treatment of fish fillets with microwaves at 1 and 2 minutes eliminated all the *A. hydrophila* populations and these results are consistent with that obtained by **Huang *et al.* (2006)** who observed that catfish fillets which were inoculated by 10^8 cell/ cm of *A. hydrophila* and cooked by microwave to internal temperature 70^oc became free from the microorganism. Also **Quesada *et al.* (2009)** who inoculated minced meat with 10^7 and 10^9 cfu/ml of *A. hydrophila* and subjected it to microwaves for 30, 60, 90, and 120 seconds, observed that the rate of destruction of bacteria was significant ($P< 0.005$) and the number of bacteria diminished dramatically as the exposure time increased, even though, for the complete elimination of the microorganism at 90 and 120 seconds. **Aziz *et al* (2002)** investigated the effect of microwave treatments on the survival of bacteria in different beef products and found that bacterial counts were reduced by 1 log cycle in 20 s and by 2 log cycles in 30 s exposure and this indicated that the use of longer exposure time can result in complete elimination of the bacteria. A study done by **Arzina *et al.* (2012)** observed that the application of microwave for 1 min to pizza resulted in decrease in total bacterial count but after 2 min the bacterial concentration became below the detection limit.

The physical examination of fish fillets which were treated by gamma radiation at 1, 2, and 3 KGy revealed that there were no significant differences between them and the untreated control group as shown in (Table 3). These results can be attributed to the fact that gamma radiation is a non- heat sanitary method called (cold pasteurization) and can be used without significant changes in the sensory characteristics of food **WHO (1981) and IAEA (2000).** The obtained results in line with **Mohamed and El- Mossalami (2009)** who observed that doses of 1,2,and 3 KGy had no changes in the sensory attributes of tilapia fish fillets, and **Ozden *et al.* (2007)** who found that 2.5 and 5 KGy of gamma radiation did not affect the sensory quality of sea bass. **Aworh *et al* (1999)** who concluded that low dose gamma radiation up to 6KGy, inhibited the microbial growth and extended the shelf life of Nigerian fish products with no significant differences in ap-

pearance, flavor, texture, and over all acceptability, and **Chen *et al.* (2010)** who observed that low dose gamma radiation up to 2 KGy significantly reduced all microorganisms in crab meat without change in its physical properties.

Conclusion

According to the data of this study we recommend that locally produced fish fillets should be exposed to 2 kGy dose of irradiation that will be sufficient to eliminate *A. hydrophila* microorganism that contaminate it without affecting the sensory attributes of the product. Also we recommended cooking of fish fillets by microwave for 1 to 2 min. if the product was not treated by any method of bacterial decontamination to be sure that the product is safe for consumption.

Table 1: Mean values of the effect of treatment with gamma radiation on the count of *A. hydrophila* in fish fillets.

Dose level	Cfu/g
0 KGy (control)	6.4×10^5
1 KGy	4.8×10^1
2KGy	< 10
3 KGy	< 10

Table 2: Mean values of the effect of treatment with microwave cooking on the count of *A. hydrophila* in fish fillets.

Time of cooking	Cfu/g
0 min (control)	6.4×10^5
1 min	< 10
2 min	< 10

Table 3: Effect of treatment with gamma radiation on the physical characters of fish fillets.

Dose level	Color	Odor	Texture
0 KGy(control)	4.62 ± 0.02^a	4.71 ± 0.10^b	4.91 ± 0.03^c
1 KGy	4.58 ± 0.12^a	4.70 ± 0.13^b	4.93 ± 0.02^c
2 KGy	4.56 ± 0.152^a	4.67 ± 0.152^b	4.88 ± 0.12^c
3 KGy	4.60 ± 0.10^a	4.64 ± 0.13^b	4.90 ± 0.04^c

All regarding are mean of triplicates± SD.

Means with the same letter in any given column are not significantly different ($P \leq 0.05$).

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تأثير كل من أشعه جاما والطهى بالميكروويف على ميكروب الايروموناس هيدروفيلا فى اسماك الفيليه المحلية

امل احمد مجاهد*، حنان عباس الغياتى ، تغريد احمد حافظ**، وفاء سيد محمد*****

قسم الميكروبيولوجى* ، صحة الاغذية** معهد بحوث صحة الحيوان- معمل فرعى بورسعيد

و مركز تكنولوجيا الاشعاع- هيئة الطاقة الذرية ***

الملخص العربى

تم اجراء هذة الدراسة للتعرف على فاعلية المعالجة باشعة جاما و كذلك الطهى بالميكروويف فى القضاء على ميكروب الايروموناس هيدروفيلا المحقون فى فيلية السمك البلطى. و قد اثبتت الدراسة انه عند تعريض فيلية السمك للمعاجة باشعة جاما عند ١كيلو جراى حدث انخفاض كبير فى اعداد ميكروب الايروموناس هيدروفيلا بينما تم القضاء نهائيا على الميكروب عند الجرعات ٣,٢ كيلو جراى على التوالى. كذلك اثبتت الدراسة ان طهى الفيلية بالميكروويف لمدة دقيقة و دقيقتين قد قضى نهائيا على ميكروب الايروموناس هيدروفيلا. هذا و قد تم اجراء اختبار الخواص الطبيعية لفيلية السمك المعاج باشعة جاما و اثبت انة لا يوجد اختلافات معنوية من حيث اللون أو الرائحة أو التماسك بينة و بين الفيلية الغير معالج بالاشعاع.