Effect of Nisin and Gamma Irradiation Treatments on *Listeria monocytogenes* on Some High Economic Valued Aquaculture Products

Yüksek Ekonomik Değeri Olan Bazi Su Ürünlerinde *Listeria monocytogenes* Kontaminasyonunun Giderilmesi için Nisin ve Gama Işınlama Kombinasyonlarının Etkisi

Research Article

Ahmet Koluman[1*], Abdullah Dikici[2]

[1]National Food Reference Laboratory, Microbiology Department, Ankara, Turkey.

[2]Tunceli University, Engineering Faculty, Food Engineering Department, Tunceli, Turkey.

ABSTRACT

Fish and other aquaculture products play an important role in global consumption. Sea foods represented a higher risk of *L. monocytogenes* contamination compared with other foods. The risk of *L.monocytogenes* should be decreased using some novel protection methods. Various studies have shown that Nisin alone or combined with another technique is efficient for control of *L. monocytogenes* in different foods and different conditions. Additionally, gamma radiation has a significant potential for the extension of refrigerated shelf life and decontamination of pathogens in fishery products. We applied different doses of gamma irradiation on Pangasius (Pangasius *hypophthalmus*, Ln.) fillets, Herring caviars, Scallops and Pollock (*Pollachius pollachius*, Ln.) fish fillets weather treated with Nisin or not. The results showed that without Nisin application *L. monocytogenes* can be eradicated with 5 kGy of gamma irradiation, but when Nisin application is done the effective dose decreases to 3 kGy. It can be suggested that Nisin combined with gamma irradiation is effective on *L.monocytogenes* attached to different aquacultures.

Key Words

Nisin, Gamma irradiation, *L. monocytogenes*, Aquaculture, Combined treatment

ÖZET

Küresel tüketimde balık ve diğer su ürünleri önemli rol oynamaktadır. Deniz ürünleri diğer ürünlerle kıyasla daha fazla *Listeria monocytogenes* kontaminasyonu riski taşımaktadır. *L. monocytogenes* riski belirli üretim yöntemleri ile düşürülmelidir. Çeşitli çalışmalar Nisin uygulamasının tek veya kombine olarak farklı koşullardaki farklı gıdalarda etkili olduğunu göstermiştir. Bunlara ek olarak, gama ışınlama su ürünlerinde raf ömrünün uzatılması ve patojenlerin dekontaminasyonu için belirgin bir potansiyele sahiptir. Farklı dört adet ekonomik değeri yüksek ürüne gama ışınlaması Nisin içerecek veya içermeyecek şekilde uygulandı. Sonuçlara göre Nisin uygulanmamış gruplarda *L. monocytogenes* 5 kGy ışın dozu ile eradike edilebilirken, Nisin kombinasyonu uygulanması gereken dozu 3 kGy seviyesine çekmiştir. Buna bağlı olarak farklı su ürünlerinde, Nisin ve ışınlama kombinasyonlarının *L. monocytogenes* kontaminasyonunun giderilmesinde beraber uygulanmasının verimli olacağı düşünülmüştür.

Anahtar Kelimeler

Nisin, Gama ışınlama, *L. monocytogenes*, Su ürünleri, Kombine uygulama.

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Correspondence to: A. Koluman, National Food Reference Laboratory, Microbiology Department, Ankara, Turkey.

Tel: +90 312 327 4181 Fax: +90 312 327 4156 E-Mail: ahmetkoluman@hotmail.com

INTRODUCTION

Fish and other aquaculture products play an important role in global consumption. According to Food and Agriculture Organization, food balance sheets 142.901.000 tons of fish and aquaculture is produced globally. Global consumption is hardly the same with the production [1,2].

Listeriosis has become a major public health concern after a major outbreak caused by coleslaw in 1981 [3]. Sea foods represented a higher risk of *L. monocytogenes* contamination compared with other foods [4]. In Canada, ready to eat (RTE) foods with long shelf life has been under inspection since 2000 [5]. Shrimp, smoked mussels, imitation crab meat have been listed as a major source of *L. monocytogenes*. In a study, cold smoked fish products were reported to be contaminated with *L. monocytogenes* [6]. Huss et al. (2000) classified sea foods as high risk potential foods for listeriosis: (I) mollusks, including frozen and fresh mussels, clams, oysters in shells or shucked (ii) raw fish whole or fillets (iii) salted, marinated, fermented or other light preserved fish products (iv) mildly heat processed fish products or other crustaceans [7]. Pangasius and Pollock fillets, caviar and scallops are found in limited areas of the world. The limited source and high levels of demand causes these products to have a high economic value. The products are usually consumed raw. No additives or preservatives are applied due to significant effect on consumer acceptance [8-12].

Nisin is produced by some strains of *Lactococcus lactis* and it is effective over Gram positive bacteria, spore formers. Nisin effects the membrane of bacteria and the effect of Nisin increases as the aw (activity of water) of the food increases [13]. The antagonistic effect of Nisin on *L. monocytogenes* has been shown and its effect strongly depended on the food matrix. Various studies have shown that Nisin alone or combined with another technique is efficient for control of *L. monocytogenes* in different foods and different conditions [14-18]. Low doses of Nisin have been shown to be a subject of resistance by *L. monocytogenes* [19].

Gamma radiation has a significant potential for the extension of refrigerated shelf life and decontamination of pathogens in fishery products [20-24]. Low doses of gamma irradiation (1-3 kGy) are known to have effect on *L. monocytogenes* populations. In a study, 109 cfu/ml *L. monocytogenes* (ATCC) in buffered broth was irradiated and 3 kGy was determined as LD_{50}. D value of *L. monocytogenes* strains are approximately 0.43 kGy. The dose applied for gamma irradiation decreases as it is combined with other preservation methods. Consumer demands low dose gamma irradiation treatment and minimum levels of chemicals [25]. In a study, Nisin and gamma irradiation combination was compared with Nisin alone and irradiation alone. Authors reported that treatment with combinations of Nisin and gamma radiation resulted in an additive antimicrobial effect when inoculated meat was tested during the first 24 h and in a synergistic effect when tested after 72 h of storage at 4°C [26]. There is limited data about the combination of Nisin and gamma irradiation treatment in high economic value aqua products.

This study was designed to determine the combined effect of Nisin and gamma irradiation on *L. monocytogenes* attached to high economic value aqua products.

MATERIALS AND METHODS

Materials

Fish samples were obtained from markets. The samples were chosen according to some specifications such as; price, no additive or preservative treatment and consumption. Pangasius (*Pangasius hypophthalmus*, Ln.) fillets were obtained frozen in vacuumed packaging. Herring caviars were obtained in jars (approx. 50 g) and stored at +4°C. Scallops were prepared as ready to cook meat-balls and prepared without additives. Pollock (*Pollachius pollachius*, Ln.) fish fillets were frozen and vacuum packed. All samples were prepared under aseptic conditions and 10 g of Caviar and 25 g of other samples were dispatched into sterile plastic containers. All samples were grouped and code numbers of each sample is given. Design of treatment groups is given in Table 1.

Table 1. Groups and treatments.

Group No	Food Matrix	Nisin treatment	Irradiation	Group	Food Matrix	Nisin treatment	Irradiation
1	Pangasius fillets	500 IU	None	No	Scallop	500 IU	None
2	Pangasius fillets	500 IU	1 kGy	17	Scallop	500 IU	1 kGy
3	Pangasius fillets	500 IU	3 kGy	18	Scallop	500 IU	3 kGy
4	Pangasius fillets	500 IU	5 kGy	19	Scallop	500 IU	5 kGy
5	Pangasius fillets	-	None	20	Scallop	-	None
6	Pangasius fillets	-	1 kGy	21	Scallop	-	1 kGy
7	Pangasius fillets	-	3 kGy	22	Scallop	-	3 kGy
8	Pangasius fillets	-	5 kGy	23	Scallop	-	5 kGy
9	Caviar	500 IU	None	24	Pollock fillets	500 IU	None
10	Caviar	500 IU	1 kGy	25	Pollock fillets	500 IU	1 kGy
11	Caviar	500 IU	3 kGy	26	Pollock fillets	500 IU	3 kGy
12	Caviar	500 IU	5 kGy	27	Pollock fillets	500 IU	5 kGy
13	Caviar	-	None	28	Pollock fillets	-	None
14	Caviar	-	1 kGy	29	Pollock fillets	-	1 kGy
15	Caviar	-	3 kGy	30	Pollock fillets	-	3 kGy
16	Caviar	-	5 kGy	31	Pollock fillets	-	5 kGy
				32			

Preparation of *L. monocytogenes* Strains

L. monocytogenes 4e (ATCC 19118), *L. monocytogenes* 4a (ATCC 19114) and *L. monocytogenes* 4b (ATCC 13932) were enriched in Brain Heart Infusion broth (BHI, Oxoid) for 18 hours at 37°C. The strains were streaked on Ottoviani Agosti agar (ALOA, Biomerieux) plates and incubated for 24-48 hours at 37°C. One typical colony for each strain were chosen and transferred into BHI broth and incubated at 37°C for 18 hours. Both of the enriched strains were transferred into one sterile centrifuge tube and centrifuged at 3000 rpm for 5 minutes. Supernatant was discarded and pellet was washed with sterile saline solution (0.85% NaCl) and centrifuged again at 3000 rpm for 5 minutes. The supernatant was discarded and pellet was suspended in 100 mL of sterile BHI broth.

Inoculation and Attachment of *L. monocytogenes*

Bacterial suspension was homogenized using a vortex and 1 mL of suspension was dispensed on samples and all samples were incubated at 37°C for 45 minutes. Initial level of *L. monocytogenes*

was counted from the suspension and untreated samples after attachment.

Preparation of Nisin Suspension

Pure Nisin powder of *Lactococcus lactis* was obtained from Sigma. Nisin stock solutions were prepared by solubilizing the appropriate amounts of powder in a Nisin diluent (0.02 N HCl–0.75% NaCl, pH 1.8) to obtain 500 IU of solution. The solution was then autoclaved at 121°C for 15 min and stored at 4°C until treatment.

Following the attachment 1 mL of Nisin solution was sprayed on the samples. After Nisin treatment, all samples were stored at +4°C until gamma irradiation.

Irradiation of Samples

Irradiation of samples was done in Gamma-Cell which has ^{60}Co as radiation source and was built in 1994 with an activity of 10568 Ci by ISSLEDOVATELJ. All samples were stored at +4°C following irradiation. Three external dosimeters were applied to samples and were analyzed to determine effective dose applied to the samples. All results were calculated to determine mean effective treatment dose.

Methods

Sample preparation

All matrices were analyzed on 0, 1st, 3rd, 6th, 9th, 12th days of treatment. All samples were weighted in sterile bags and diluted 1:9 with buffered peptone water (BPW, Oxoid) and homogenized for 30 sec. Another part of the sample was diluted 1:9 in Half Fraser broth (HF, Biomerieux) and homogenized for 30 sec.

Determination of *L. monocytogenes*

Plate Count Method

The homogenate prepared in BPW was transferred to sterile beakers and replaced in automatic spiral plater (IUL, Spain) and 100 µL was plated at 7 log level to two ALOA petri dishes. Petri dishes were allowed to dry and incubated for 24-48 hours at 37°C. The colonies were counted using a grid and calculated using the standard formula given in user's manual.

Enrichment Method

All HF bags were incubated at 30°C for 48 hours. During incubation at 24th hour one loopful of enrichment was streaked on ALOA agar and incubated at 37°C for 48 hours. At the end of incubation period (48th hour) 100 µL of HF was transferred to Fraser broth (FB, Biomerieux) and incubated at 37°C for 24 hours. One loopful of enrichment was streaked on two ALOA agar plates and incubated at 37°C for 48 hours. All blue, lescitinase positive colonies were confirmed using API Listeria.

pH Metrics

Three gram of each sample was diluted in 6 mL of deionized distilled water and homogenized. Sample was read 3 times with a pH-meter (Mettler Toledo, Spain).

Statistical Analysis

Statistical analysis was performed using Statistica version 8.0 software for Windows 7 (StatSoft, Inc., Tulsa, OK, USA). A multivariate analysis of variance (MANOVA) and Tukey's HSD *post hoc* tests were used to analyze significant differences between all groups. The level of significance was set up at $p<0.05$. Results were presented as mean ± standard error.

RESULTS and DISCUSSION

Initial levels of *L. monocytogenes* in suspension were approximately 7.845098 $\log_{10}$cfu/mL. Likewise, the initial contamination levels of Pangasius fillets, Pollock fillets, scallops and caviars were 7.042706739, 7.075546961, 7.102662342, and 7.092252655 $\log_{10}$cfu/mL.

The dosimeters of each group were evaluated and mean effective dose of each sample are given in Figure 1. Optimal distribution of gamma irradiation doses in three trials were 1.03 kGy, 3.05 kGy and 5.05 kGy.

According to the multivariate test MANOVA test results, there is a statistically significant correlation between days and Nisin content in between the groups ($p<0.05$). The statistical difference between Nisin treated and non Nisin treated groups were given in Figure 2. Figure 2a

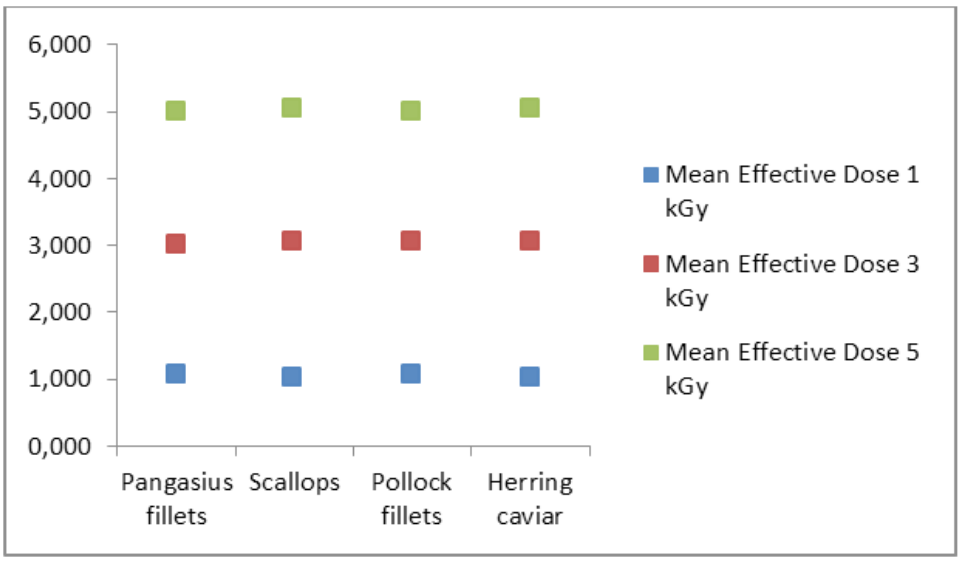

Figure 1. Mean effective dose distribution of samples.

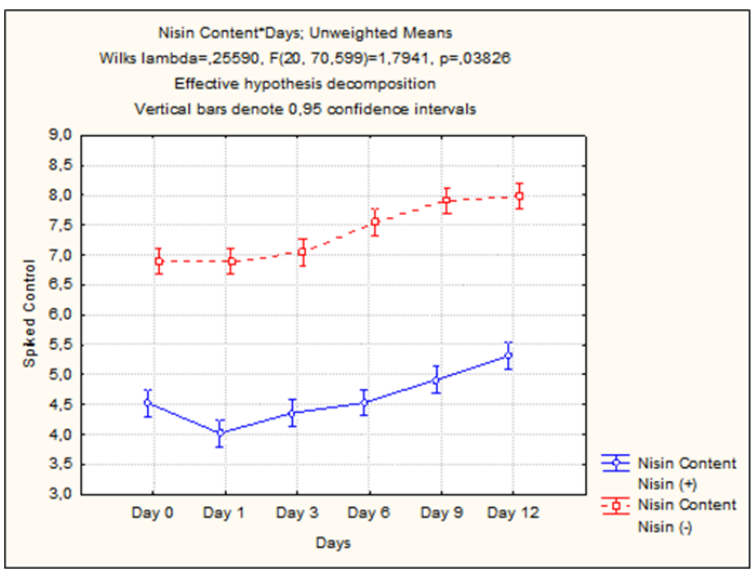

Figure 2a. The changes in results with Nisin treatment by days of spiked control (0 kGy) Pangasius fillets.

is the results of spiked control (0 kGy), Figure 2b, 2c and 2d are the results of 1 kGy, 3 kGy and 5 kGy irradiated Pangasius fillets, respectively.

According to MANOVA test results there is a statistically significant correlation between days and Nisin content in between the groups ($p<0.05$). The statistical difference between Nisin treated and non Nisin treated groups were given in figure 3. Figure 3 is the results of spiked control (0 kGy), Figure 3b, 3c and 3d are the results of 1 kGy, 3 kGy, 5 kGy irradiated scallops, respectively.

According to MANOVA test results, there is a statistically significant correlation between days and Nisin content in between the groups ($p<0.001$). The statistical difference between Nisin treated and non Nisin treated groups were given in Figure 4. Figure 4a is the results of spiked control (0 kGy), Figure 4b and 4c are the results of 1 kGy and 3 kGy irradiated Pollock fillets, respectively. *L. monocytogenes* spiked on 5 kGy treatment group was not cultivable.

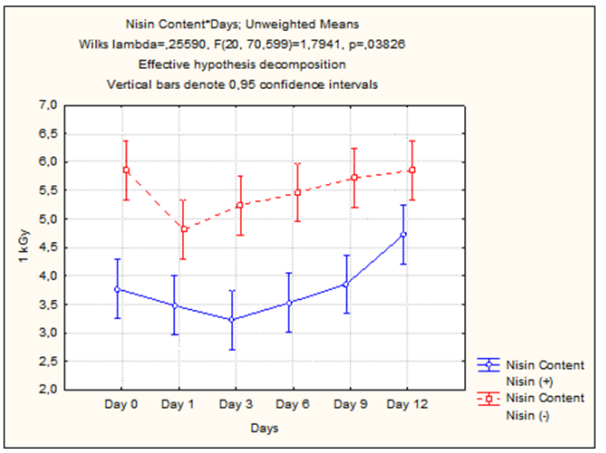

Figure 2b. The changes in results with Nisin treatment by days of 1 kGy irradiated Pangasius fillet samples.

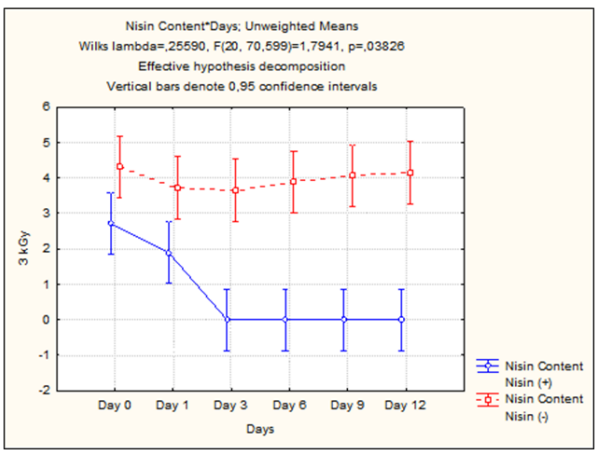

Figure 2c. The changes in results with Nisin treatment by days of 3 kGy irradiated Pangasius fillet samples.

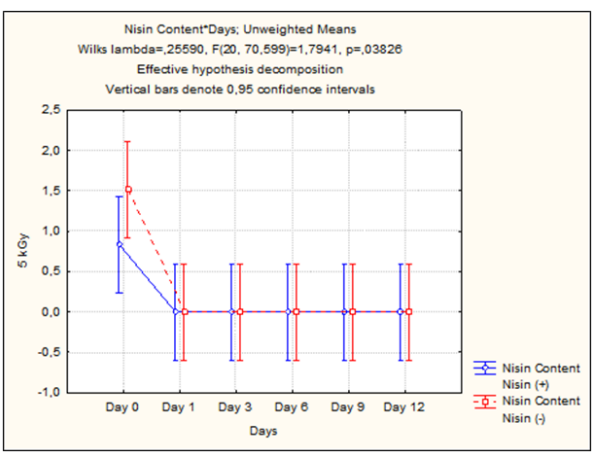

Figure 2d. The changes in results with Nisin treatment by days of 5 kGy irradiated Pangasius fillet samples

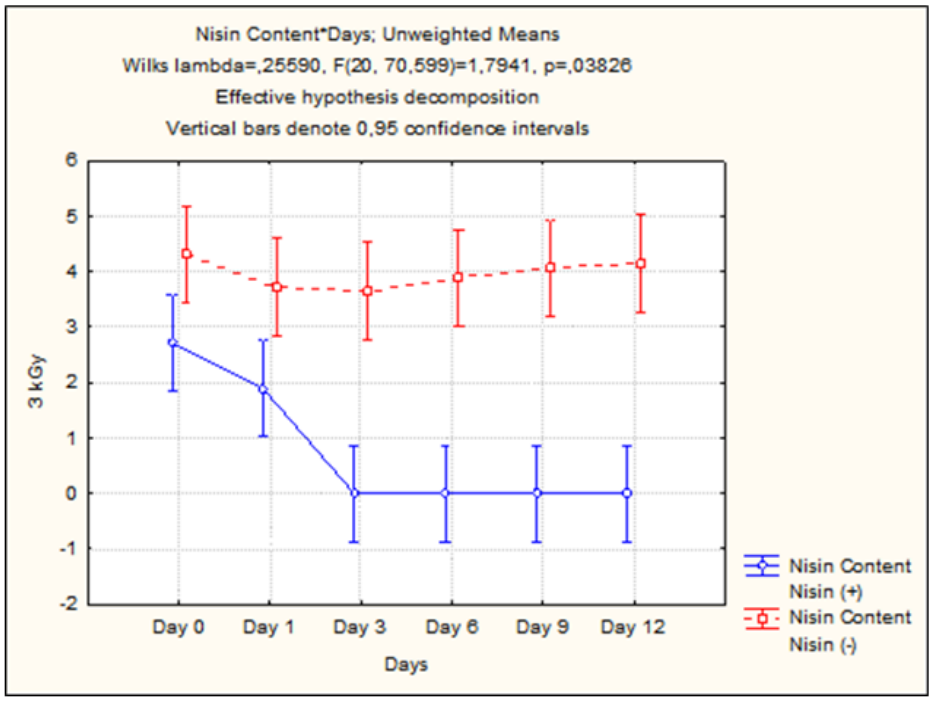

Figure 3a. The changes in results with Nisin treatment by days of spiked control (0 kGy).

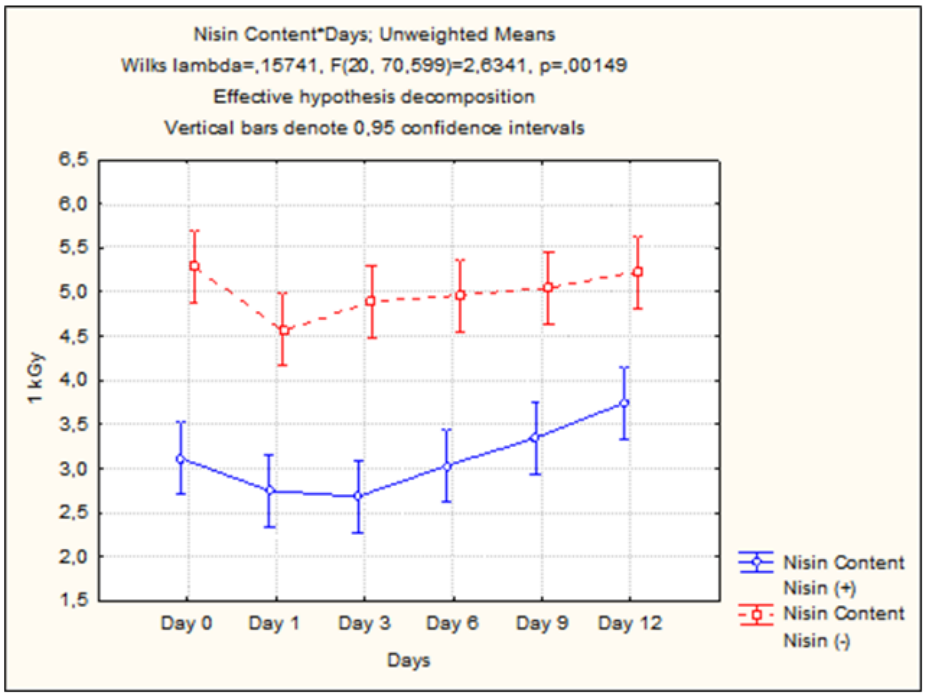

Figure 3b. The changes in results with Nisin treatment by days of 1 kGy irradiated samples.

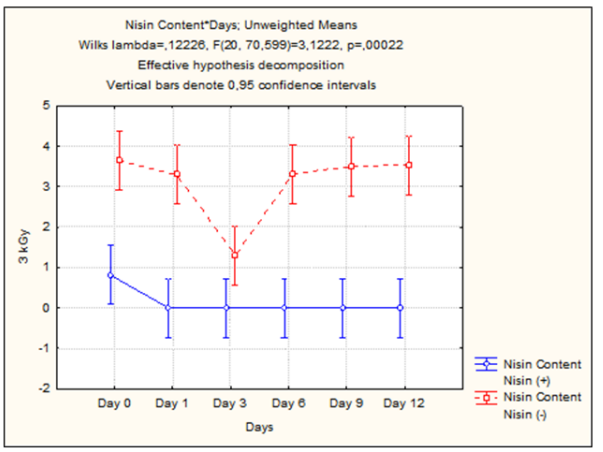

Figure 3c. The changes in results with Nisin treatment by days of 3 kGy irradiated samples.

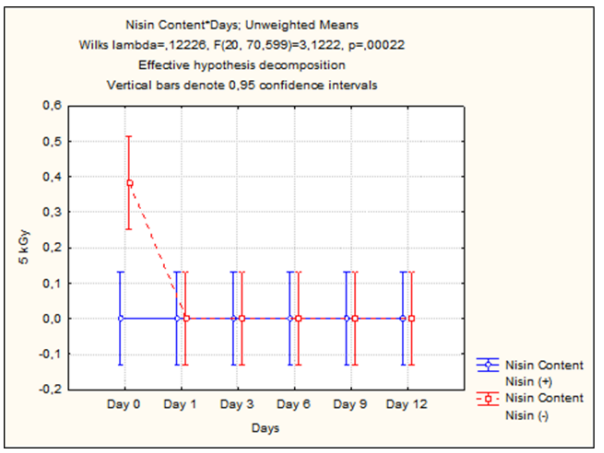

Figure 3d. The changes in results with Nisin treatment by days of 5 kGy irradiated samples.

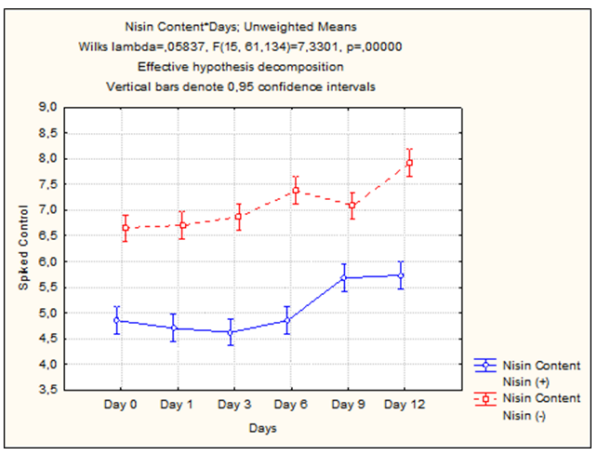

Figure 4a. The changes in results with Nisin treatment by days of spiked control (0 kGy) Pollock Fillets.

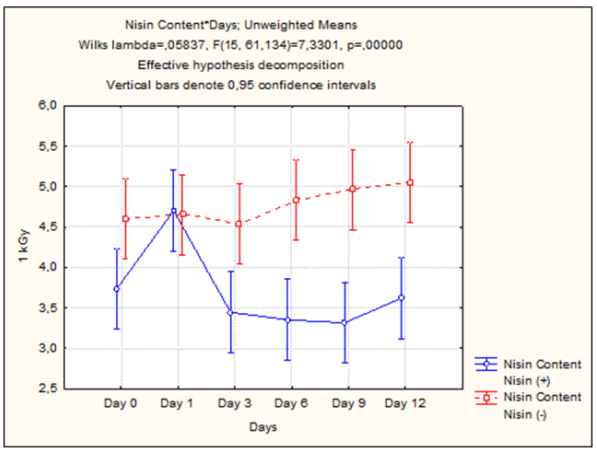

Figure 4b. The changes in results with Nisin treatment by days of 1 kGy irradiated Pollock Fillets.

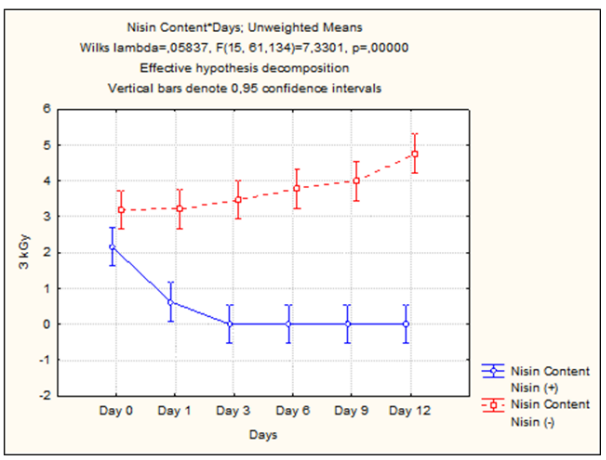

Figure 4c. The changes in results with Nisin treatment by days of 3 kGy irradiated Pollock Fillets.

According to MANOVA test results there is a statistically significant correlation between days and Nisin content in between the groups ($p<0.05$), except for the 1 kGy treated group. The statistical difference between Nisin treated and non Nisin treated groups were given in Figure 5. Figure 5a is the results of spiked control (0 kGy), Figure 5b and 5c are the results of 1 kGy and 3 kGy irradiated caviars, respectively. *L. monocytogenes* spiked on 5kGy treatment group was not cultivable.

DISCUSSION

L. monocytogenes is an emerging food borne pathogen which is widely found in many food matrices. Effective treatments are needed to eliminate the pathogen of concern. Nisin is accepted as one of the most effective lantibiotic of *L. monocytogenes*. Additionally, food irradiation is also effective on the pathogen. The proposed approach to reach *L. monocytogenes* free food is to apply Nisin and Gamma irradiation combinations on high economic value aquaculture products.

Treatment of Pangasius fillets, Pollock fillets, scallops and caviars alone with Nisin approximately 3 $\log_{10}$cfu/g decrease by the first day of treatment but an increase of approximately 1 $\log_{10}$cfu/g by the end of storage period were recorded in all samples. This increase was due to psychrophilic nature of the microorganism. Other researchers reported 1.1-1.5 $\log_{10}$cfu reduction [26-30].

Nisin is a lantibiotic and it is stable during irradiation [31]. Treatment of Nisin (500

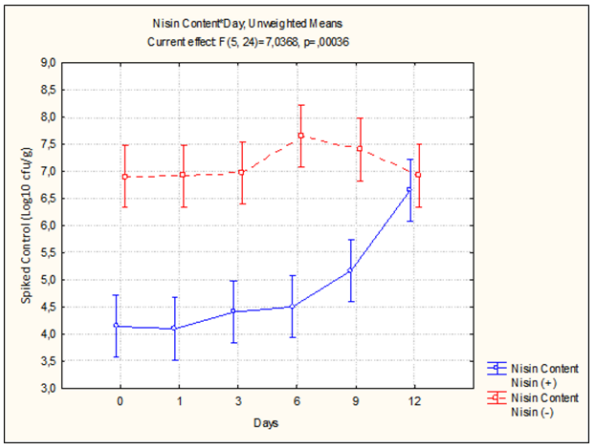

Figure 5a. The changes in results with Nisin treatment by days of spiked control (0 kGy) caviars.

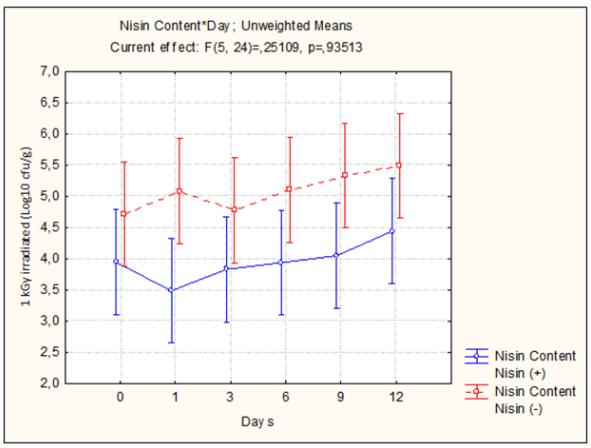

Figure 5b. The changes in results with Nisin treatment by days of 1 kGy irradiated caviars.

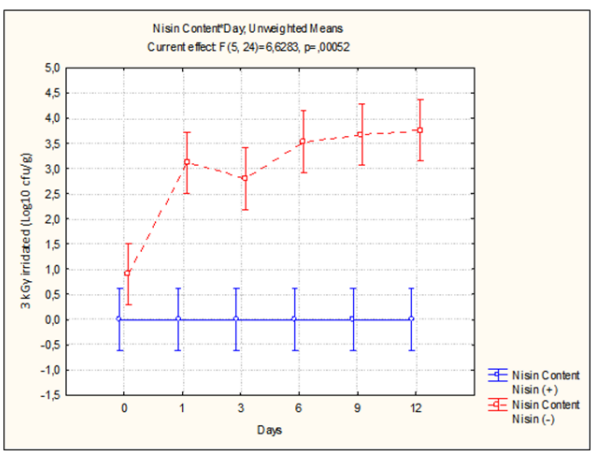

Figure 5c. The changes in results with Nisin treatment by days of 3 kGy irradiated caviars.

IU) followed by gamma irradiation showed synergistic effects in all matrices. Gamma irradiation and Nisin combinations were applied to *L. monocytogenes* on sausage meat, red meat cubes. In the same study held by Turgis et al. [32] sausage meat inoculated with *L. monocytogenes* at levels of 7 log cfu/g and Nisin combined with gamma irradiation treatment made on these samples. The results showed an increase of 3 log cfu decrease in irradiated and Nisin combined groups. The effect of 3 kGy combined with Nisin showed similar effects with 5kGy irradiation alone. In a study by Mohamed et al. [26] synergistic effect of gamma irradiation combined with Nisin on meat samples was observed. Differences between four different aqua products may be described by the size of the matrices. It is known that different food matrices and different sizes of the same matrix may affect the survival of the bacteria (Anonymous 2006). Parallel results were obtained by the studies [29,33] which do underline the importance of meat environment as a protective shield for pathogens. Nisin treatment to fresh salmon showed that the effect of Nisin combined with Microguard© showed 1 logcfu/g reductions and this level maintained for three days [34]

L. monocytogenes has a psychrophilic character which underlines the growth under of cold storage. It can be seen that no *L. monocytogenes* could be cultured in 5 kGy irradiated samples. On the contrary, the color and odor was not acceptable in any products (data not given). It is also significant that Nisin combined with 3 kGy gamma irradiation also gives the same result with 5 kGy alone. Also the sensory properties were more acceptable. In a study held by Mohamed et al. [26] reported that "meat cubes treated with Nisin and gamma radiation were stored at 4°C for 72 h, and the behavior of the pathogen was assessed post treatment. Based on the data of this study, there is no indication of recovery of any injured cells during storage. Moreover, *Listeria* population decreased during storage of meat treated with some combinations of Nisin and gamma radiation. This observation is consistent with the reported data in our study. This event supports the synergy between Nisin and Gamma irradiation and strengthens the hypothesis of our study.

L. monocytogenes numbers were tragically decreased in higher levels of radiation (3 kGy and 5 kGy) and Nisin combinations. This idea also presents in a study conducted by Mohamed et al. [26]. In another study Nisin combined with gamma irradiation improved the overall quality of ready to eat (RTE) foods [35]. Nisin_pectin film coated RTE turkey meals were irradiated to inactivate *L. monocytogenes* and a significant difference between irradiated samples was recorded [36].

The idea of combined irradiation was suggested by other authors [32,37-40] who suggested radio resistant microorganism to be more susceptible to chemical treatments combined. It can be suggested that gamma irradiation causes sub lethal damage with free radicals and DNA damage by strand breaking and as the intact formation of cell disappears the sensitivity to Nisin increases [27,41-43]. These data supports the idea of Nisin and Gamma irradiation combinations for *L. monocytogenes* has a high value on aqua culture products. This combination not only increases the self-life of the products but also will be an additional barrier for food borne pathogens like *L. monocytogenes*. It can be suggested that this combination can be applied by aqua culture producers.

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