EFFECT OF THE GAMMA RADIATION AND TEMPERATURE ON HISTAMINE PRODUCTION, LIPID PEROXIDATION AND ANTIOXIDANT PARAMETERS IN SARDINE (*Sardina pilchardus*)

Nadica Maltar-Strmečki[1], *Blanka Ljubić-Beer*[2], *Renata Laškaj*[3], *Jasna Aladrović*[2] *and Petar Džaja*[2]
[1]Ruđer Bošković Institute, Zagreb, Croatia
[2]Faculty of Veterinary Medicine, Zagreb, Croatia
[3]University Hospital for Infectious Diseases "Dr. Fran Mihaljević", Croatia
nstrm@irb.hr

INTRODUCTION

Sardine (*Sardina pilchardus* Walbaum, 1792) is pelagric fish widely distributed in the Adriatic Sea and one of the most commercially important fish species in the fisheries of all countries located along the coast of the Adriatic Sea. Safety and hygienic quality, as well as economic efficiency are directly related to the duration between when the sardine is caught and when it reaches the end consumer as well as upon the harvest methods, the on-board handling and temperature exposures throughout the processing, transit and storage. Radiation processing of fish is recognised as a safe and effective method among the existing technologies for preservation[1].

Histamine is only amine with established legal limits for the human consumption [2] because it is extremely variable and is a function of species and individual fish, the part of fish sampled, time and temperature throughout the processing, transit and storage [3]. Besides that, oxidative changes are the main factor responsible for spoilage[4]. Lipid peroxidation is probably the main cause of the decrease of nutritional value and meat quality during storage at different temperatures leading to the formation of odour and the loss of taste, texture and consistency[4]. EPR (electron paramagnetic resonance) spectroscopy has been used as the only method that determines the antioxidant potential by direct detection of free radicals [5]. Therefore this study was undertaken to investigate the effects of irradiation dose on histamine production and the antioxidant defences in the muscles of sardine during the storage at two different temperatures 4°C and 30°C.

MATERIAL AND METHODS

Fresh samples of Sardine (*Sardina pilchardus* Walb., 1792) were purchased from fisherman after harvesting, delivered to the laboratory on ice and under hygienic conditions (N = 480) and divided in three groups for irradiation at different dose. Within 3 hours, two groups were irradiated with panoramic ^{60}Co source at the Ruđer Bošković Institute with a dose rate capacity of 3 Gy/s until a mean level of respectively 1 and 3 kGy had been reached. After irradiation, each group was stored at two different temperatures (4°C and 30°C) throughout the experimental period. Initial determinations were made within 8 hours of purchase. The concentration of histamine was determined by using the commercial competitive enzyme immunoassay Histamin Food ELISA (DRG Instruments GmbH) according to the kit instructions. Lipid peroxide concentration measured as thiobarbituric acid reactive substances (TBARS) was performed according to the method of Ohkawa *et al.* (1979) [6]. EPR spectroscopy was applied on the galvinoxyl free radical as proposed by Quiles *et al.* [5]. Measurements were performed at room temperature using a Varian E-109 spectrometer equipped with a Bruker ER 041 XG microwave bridge. Statistical analysis was performed by Mathematica 8 (Wolfram Research, Inc.). Data were expressed as mean ± standard deviation (SD). Normality of distribution was tested by the Kolmogorov-Smirnov test. Analysis of variance (ANOVA) was used for a comparison of parameters between the subgroups and differences among the mean values were processed by *post hoc* Duncan multiple range. $P < 0.05$ was accepted to indicate a significant difference.

RESULTS

Changes of histamine in gamma irradiated fish samples stored at two different temperatures are shown in Tables 1 and 2. Significant differences were found in histamin level within non-irradiated and irradiated samples stored at 4°C after 24 hours. The histamine concentrations of irradiated samples for both doses (1 and 3 kGy) were lower. Indeed, as expected histamine concentrations remain safe for consumption, <50 ppm = 5 mg/100g [2] during the 24 hours. The storage at 30 °C increases the histamine production rapidly. The histamine concentration when stored at 30 °C for 12 hours became toxic (>500 ppm). In this study, irradiation indicated that this safe consumption can be prolonged by irradiation with dose of 3 kGy which is in agreement with previously reported results [3, 7].

Table 1. Changes in histamine of irradiated sardine during storage at 4°C

Storage time	**Histamine concentration** *(ppm)*		
T (h)	0 kGy	1 kGy	3 kGy
0	3.27 ± 0.92^{a}	3.72 ± 1.52^{a}	2.51 ± 1.85^{a}
1	1.93 ± 0.75^{a}	3.35 ± 0.52^{b}	4.21 ± 1.24^{b}
3	3.90 ± 1.11^{a}	4.65 ± 1.12^{a}	4.98 ± 0.86^{a}
6	3.16 ± 1.24^{a}	2.16 ± 0.48^{a}	2.61 ± 1.48^{a}
12	3.83 ± 2.22^{a}	2.52 ± 1.04^{a}	4.71 ± 3.99^{a}
24	13.04 ± 4.02^{a}	6.17 ± 0.77^{b}	6.13 ± 1.49^{b}
30	11.02 ± 2.07^{a}	6.87 ± 2.79^{b}	4.69 ± 2.40^{b}
48	17.18 ± 10.01^{a}	4.60 ± 2.13^{b}	3.39 ± 2.11^{b}

a, b: different letters within a same row indicate significantly different values ($p < 0.05$).

Table 2. Changes in histamine of irradiated sardine during storage at 30°C

Storage time	**Histamine concentration** *(ppm)*		
T (h)	0 kGy	1 kGy	3 kGy
0	3.52 ± 0.73^{ab}	2.71 ± 1.63^{a}	4.73 ± 0.73^{b}
1	3.98 ± 1.89^{a}	4.34 ± 0.72^{a}	3.32 ± 0.92^{a}
3	12.24 ± 8.01^{a}	4.39 ± 2.64^{b}	3.74 ± 0.88^{b}
6	102.18 ± 44.2^{a}	11.88 ± 14.35^{b}	2.65 ± 1.56^{b}
12	568.45 ± 206.21^{a}	363.81 ± 244.53^{b}	63.55 ± 59.92^{b}

a, b: different letters within a same row indicate significantly different values ($p < 0.05$).

Lipid peroxidation (TBARS) levels (Figure 1) in fish meat stored at 4 °C in the first three hours was the highest in fish irradiated with 1 kGy, and the third hour reveals significantly higher level than the levels obtained in the other two groups. During further storage at 4 °C the TBARS level was significantly higher in the irradiated fish for both doses compared to non-irradiated samples. In the study reported previously for sardine fish no differences in TBARS values between the irradiated samples were detected

[8], while the study of the Nile tilapia lipid-oxidation showed a tendency to increase when increasing the irradiation dose [9]. Reduced oxidative stability was previously found in irradiated chicken meat [10] where during storage at 30 °C, as the initial value and the value after 3 hours of storage were lower in the fish meat irradiated with a dose of 3 kGy compared to non-irradiated samples. Storage of meat leads to increase of lipolytic activity and thereby, free fatty acid in meat. Beside that irradiation of meat slows down the process of lipolysis and thus may lead to lower levels of lipid peroxides. Therefore, it can be concluded that there are differences because the complex effects of both processes, as well as the influence of the storage conditions, the radiation dose and of the type of fish meat.

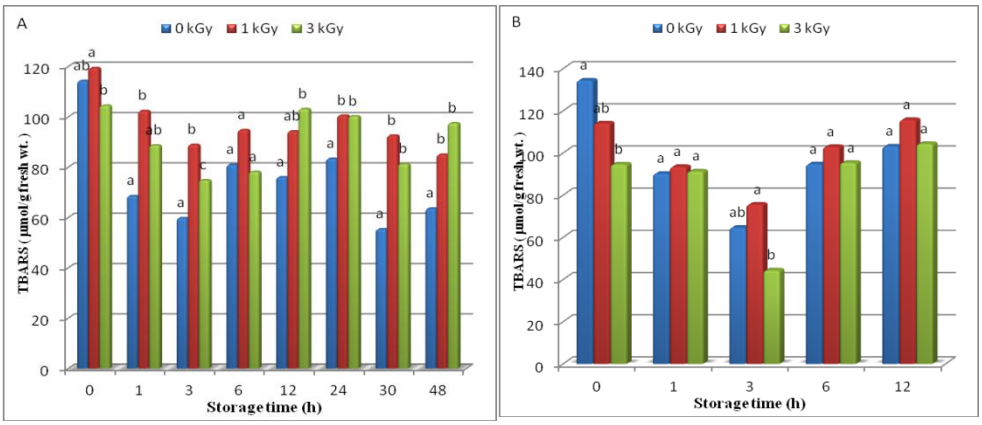

Figure 1. TBARS level for storage temperature at 4°C (A) and 30°C (B). Different letters within the same storage time indicate significant differences ($p < 0.05$) among TBARS values.

The change of radical concentration detected by EPR is the result of the antioxidant activity during reaction time, *t*. Typical decay curves are presented in Figure 2 for samples stored at 30 °C for 12 h. EPR analysis shows that decrease of the signal intensity is a function of reaction time *t* and dose. These results suggest that gamma radiation undoubtedly induces antioxidant defence system in sardine meat. However, further research is necessary to elucidate the precise role that the antioxidant system plays under influence of gamma radiation.

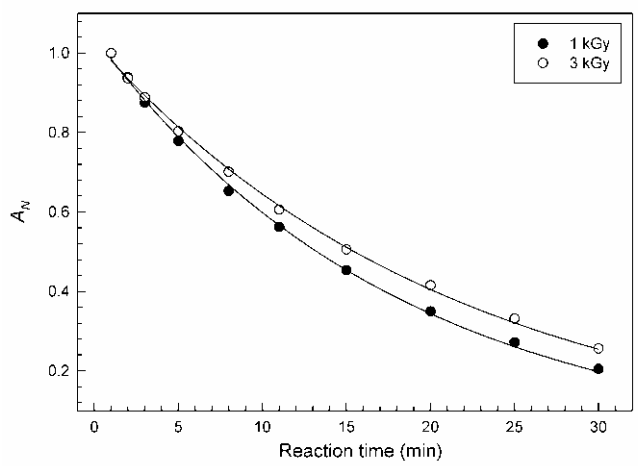

Figure 2. The normalized EPR signal intensity (A_N) of the galvinoxyl radical measured as a function of the reaction time *t*.

CONCLUSION

The results obtained in this study on the histamine production, lipid peroxidation and antioxidant parameters for the application of different dose rates of ionizing radiation to sardine fish during storage at different temperatures could provide the food industry with information concerning the definition of the best processing conditions to maximize the food quality.

Acknowledgment

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nstrm@irb.hr

Radiation processing of fish is recognized as a safe and effective method for reducing microorganisms and viruses as well for inactivating pathogens among the existing technologies for preservation. Safety and hygienic quality is directly related to the duration between when the fish is caught and when it reaches the end consumer and depends upon conditions how the sardine is handled and upon which conditions. As sardine (*Sardina pilchardus* Walbaum, 1792) is pelagric fish widely distributed in the Adriatic Sea and one of the most commercially important fish species in the fisheries of all countries located along the coast of the Adriatic Sea in the present study, the effects of gamma irradiation on the histamine production, lipid peroxidation and antioxidant parameters in sardine during the storage at two different temperatures (4 °C and 30 °C) were investigated. The results indicate that histamine concentration was reduced by gamma irradiation and that the safe consumption can be prolonged for both temperatures of storage. However, irradiation treatment induced oxidative damage, as evidenced by changes in levels of lipid peroxidation and radical kinetic rate detected by EPR (electron paramagnetic resonance) spectroscopy. These results suggest that gamma radiation undoubtedly induces antioxidant defence system in sardine fish. However, further research is necessary to elucidate the precise role that the antioxidant system plays under the influence of gamma radiation and temperature.