Food Control 34 (2013) 132–137

Contents lists available at SciVerse ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Effect of the gamma radiation on histamine production, lipid peroxidation and antioxidant parameters during storage at two different temperatures in sardine (*Sardina pilchardus*)

Nadica Maltar-Strmečki [a,*], Blanka Ljubić-Beer [b], Renata Laškaj [c], Jasna Aladrović [b], Petar Džaja [d]

[a] Division of Physical Chemistry, Ruđer Bošković Institute, Bijenička c. 54, 10000 Zagreb, Croatia
[b] Department of Physiology and Radiobiology, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia
[c] University Hospital for Infectious Diseases "Dr. Fran Mihaljević", Mirogojska 8, 10000 Zagreb, Croatia
[d] Department of Forensic and Judicial Veterinary Medicine, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia

ARTICLE INFO

Article history:
Received 26 November 2012
Received in revised form
24 March 2013
Accepted 30 March 2013

Keywords:
Radiation
Histamine
Lipid peroxidation
Antioxidant
Quality assurance

ABSTRACT

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. In the present study, the effect of gamma irradiation and temperature of storage on the histamine production, lipid peroxidation and antioxidant parameters in sardine (*Sardina pilchardus*, Walbaum, 1792) were investigated. Fish samples were irradiated with different doses (0 kGy, 1 kGy and 3 kGy) and monitored during the storage at two different temperatures (4 °C and 30 °C). The results indicate that histamine concentration was reduced by gamma irradiation and that the safe consumption can be prolongated for both temperatures of storage. However, irradiation treatment induced oxidative damage, as evidenced by changes in levels of lipid peroxidation, activity of the antioxidant enzymes glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) as well as in the radical kinetic rate detected by EPR (electron paramagnetic resonance) spectroscopy. These results suggest that gamma radiation undoubtedly induces antioxidant defense 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.

1. Introduction

Sardine (*Sardina pilchardus* Walbaum, 1792) is pelagic 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 (Pesic et al., 2010; Sinovcic, 2000; Sinovcic, Kec, & Zorica, 2008).

Sardine belongs to the Clupeidae family and lives between 15°–66° N and 23°–42°E (Sinovcic, 2003). These migratory species live in larger or smaller schools. The spawning takes place mid-autumn to early spring. They feed mainly by planktonic crustaceans and other larger planktonic animals. They can reach up to 21 cm in length and 60 g in weight (approximately 30 g). As contain high level of fat (approx. 13%), sardines are considered rich-oil fish (Bandarra, Batista, Nunes, Empis, & Christie, 1997; Caponio, Lestingi, Summo, Bilancia, & Laudadio, 2004). Higher water content that in warm-blooded animals makes it more digestive. Furthermore, the important fact is that sardines do not accumulate hazardous substances such as heavy metals and pesticides in their tissues because of short life cycle (Sinovcic & Alegria-Hernandez, 1997) and therefore are highly recommendable as a food source for humans.

Safety and hygienic quality of sardine is directly related to the duration between when the sardine is caught and when it reaches the end consumer and depends upon conditions how the sardine is handled and upon which conditions. The "safe shelf life" is dependent upon the harvest methods, the onboard handling and time as well as temperature exposures throughout the processing, transit and storage.

Regarding to that, economic efficiency of a harvest of fish depends on the method of sterilization. Radiation processing of fish is recognised as a safe and effective method among the existing technologies for preservation. It is a physical method of food processing consisting of exposing food to radiation during a limited

* Corresponding author. Tel.: +385 1 4561 127.
E-mail address: nstrm@irb.hr (N. Maltar-Strmečki).

0956-7135/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodcont.2013.03.046

period of time (Olszyna-Marzys, 1992). It is effective of reducing microorganisms and bacterial pathogens as well as for reducing the risk of biogenic amine poisoning (Naila, Flint, Fletcher, Bremer, & Meerdink, 2010; Nei, Kawasaki, Inatsu, Yamamoto, & Satomi, 2012). Histamine is most widely amine investigated in fishery products, and it is only amine with established legal limits for the human consumption (Lehane & Olley, 2000; Prester, 2011). At any time, exposure of certain fish to elevated temperatures after the catch and before consumption can cause formation of histamine from histidine by bacterial histidine decarboxylases, which are inevitably present. The growth of histamine-forming bacteria is more rapid at higher temperatures (Lehane & Olley, 2000; Visciano, Campana, Annunziata, Vergara, & Ianieri, 2007). Histamine production in fresh fish 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 (Benner, Staruszkiewicz, & Otwell, 2004; Staruszkiewicz et al., 2004; Vinagre, Madeira, Narciso, Cabral, & Diniz, 2012).

Besides microbiological factors, oxidative changes are the main factor responsible for spoilage of meat (Martinaud et al., 1997; Min & Ahn, 2005; Xiong et al., 2007).

Antioxidant enzymes glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase as well as numerous non-enzymatic molecules (glutathione, albumin, uric acid vitamins E, A and C) participate in protecting muscle cells of live animals and meat (Descalzo et al., 2005). The activity of an antioxidant can be estimated by quantitatively determining primary or secondary products from the autoxidation of lipids (Shahidi & Wanasundara, 1992). It was shown that the autoxidative process induced by irradiation in fat is much the same as that which occurs without irradiation, but it is quite accelerated (Sant'Ana & Mancini-Filho, 2000). Fats are present in the muscles as structural components of muscle membrane, but muscle cells have the ability to store fatty substances (Goodpaster & Kelley, 1998). Fatty acid composition of meat affects the profile of compounds produced in the process of lipid oxidation which depends on endogenous factors such as total fat, reducing compounds (ascorbic acid) and antioxidants and the exogenous factors, oxygen, heat, salt addition, temperature during handling, distribution and prolonged storage of meat (Skibsted, Mikkelsen, & Bertelsen, 1998). Lipid peroxidation is probably the main cause of the decrease in meat quality during storage at different temperatures. As well as leading to the formation of odor, lipid peroxides cause the loss of taste, texture and consistency, all this leads to reduced nutritional value of meat (Gray, Gomaa, & Buckley, 1996).

Anyway, all food containing water are likely to undergo both oxidation and reduction reaction during irradiation, because of radiolitic products of water, notably the hydroxyl radical, is a powerful oxidizing agent and aqueous electron or hydrogen atom is reducing agent (Kim et al., 2004).

The present study was undertaken to investigate the effects of irradiation dose on histamine production and the antioxidant defenses in the muscles of sardine during the storage at two different temperatures 4 °C and 30 °C.

2. Materials and methods

2.1. Experimental setup

Sardine (*S. pilchardus* Walbaum, 1792) specimens, caught in coastal and open sea waters of the Zadar area (Croatia) in the Adriatic see by local fishermen, were used. In this study, 480 samples were analysed. Fresh samples were purchased from fisherman after harvesting and delivered to the laboratory on ice and under hygienic conditions and divided in three groups for irradiation at different dose (0 kGy, 1 kGy and 3 kGy). Within 3 h, fish samples (without control) were irradiated with a ^{60}Co Gamma Ray source 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 and were taken during 48 h at 4 °C and during 12 h at 30 °C, for the further analyses. Initial determinations were made within 8 h of purchase.

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

Histamine concentration (ppm)			
Storage period (h)	Control (0 kGy)	1 kGy	3 kGy
0	3.3 ± 0.9	3.7 ± 1.5	2.5 ± 1.9
1	1.9 ± 0.8[a]	3.4 ± 0.5[b]	4.2 ± 1.2[b]
3	3.9 ± 1.1	4.7 ± 1.1	5.0 ± 0.9
6	3.2 ± 1.2	2.2 ± 0.5	2.6 ± 1.5
12	3.8 ± 2.2	2.5 ± 1.0	4.7 ± 4.0
24	13.0 ± 4.0[a]	6.2 ± 0.8[b]	6.1 ± 1.5[b]
30	11.0 ± 2.1[a]	6.9 ± 2.8[b]	4.7 ± 2.4[b]
48	17.2 ± 10.0[a]	4.6 ± 2.1[b]	3.4 ± 2.1[b]

The data are represented by the mean of independent trials and standard deviations ($N = 20$ for control and $N = 25$ for irradiated samples). Different letter within a same row differ significantly ($p < 0.05$).

2.2. Irradiation and dosimetry

Samples were irradiated from panoramic ^{60}Co source of the Ruđer Bošković Institute with a dose rate capacity of 3 Gy/s. For maintaining temperature during irradiation, samples were placed in the container box filled with ice and dry ice for refrigeration and freezing conditions, respectively. Routine dosimetry was performed with 5-mm-diameter alanine dosimeters (Bruker Instruments, Germany) using a Varian E-109 spectrometer equipped with a Bruker ER 041 XG microwave bridge for readout. The actual doses were within ±2% of the target dose.

2.3. Sample preparation

10 g of the muscle tissue samples were homogenised in MQ water in the ratio 1:10 (w/v) and cooled, with a Schüthomogen^{plus} Teflon glass homogeniser (Schütt Labortechnik GmbH, Germany) at 2800 rotations per minute during 30 s on ice. The tissue homogenates were centrifuged at 18,000 *g* over 30 min at 4 °C. The obtained supernatants were stored at −80 °C.

2.4. Biochemistry analysis

The concentration of histamine determined by using the commercial competitive enzyme immunoassay Histamine Food ELISA (DRG Instruments GmbH, Germany) according to the kit instructions.

Lipid peroxide concentration measured as thiobarbituric acid reactive substances (TBARS) was performed according to the method of Ohkawa, Ohishi, and Yagi (1979).

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

Histamine concentration (ppm)			
Storage period (h)	Control (0 kGy)	1 kGy	3 kGy
0	3.5 ± 0.7[ab]	2.7 ± 1.6[a]	4.7 ± 0.7[b]
1	4.0 ± 1.9	4.3 ± 0.7	3.3 ± 0.9
3	12.2 ± 8.0[a]	4.4 ± 2.6[b]	3.7 ± 0.9[b]
6	102.2 ± 44.2[a]	11.9 ± 14.4[b]	2.7 ± 1.6[b]
12	568.5 ± 206.2[a]	363.8 ± 244.5[b]	63.6 ± 59.9[b]

The data are represented by the mean of independent trials and standard deviations ($N = 20$ for control and $N = 25$ for irradiated samples). Different letter within a same row differ significantly ($p < 0.05$).