The effects of X-ray treatments on bioaccumulated murine norovirus-1 (MNV-1) and survivability, inherent microbiota, color, and firmness of Atlantic oysters (*Crassostrea virginica*) during storage at 5 °C for 20 days

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Abstract

In this study, we investigated the inactivation of human norovirus (HuNoV) surrogate Murine norovirus (MNV-1) by X-ray in whole-shell Atlantic oysters (*Crassostrea virginica*). We also investigated the effects of X-ray treatments on the survivability, inherent microbiota, color, and firmness of treated whole-shell Atlantic oysters during storage at 5°C for 20 days. Plaque assays revealed that X-ray significantly reduced the population of internalized MNV-1 in live oysters from $4.3 \pm 0.4 \log \text{PFU g}^{-1}$ to 3.6 ± 0.5 , 3.2 ± 0.2 , 2.8 ± 0.2 , and $2.5 \pm 0.1 \log \text{PFU g}^{-1}$, by 1.0, 2.0, 3.0, and 4.0 kGy X-ray, respectively. The population of MNV-1 was reduced to less than $2.0 \log \text{PFU g}^{-1}$ at 5.0 kGy X-ray. The survivability of live oysters was not significantly affected by treatment with 1.0 and 5.0 kGy X-ray, in comparison with the control, for up to 15 and 10 days, respectively, during storage at 5°C. Treatment of whole-shell oysters by 5.0 kGy X-ray significantly reduced the inherent microbiota and kept them lower than the control until day 20. No significant effect of X-ray treatment on oyster color or firmness was detected.

1. Introduction

Estimates of foodborne illness revealed that human norovirus (HuNoV), an acute gastroenteritis pathogen, causes more than 58% of the U.S. foodborne illnesses, 11% foodborne deaths and 25% hospitalizations, annually (Scallan et al., 2011). HuNoV transmission occurs through the fecal-oral route (person-to-person contact, contaminated food, and contaminated water) or airborne transmission of viral particles. HuNoV is highly contagious with an infectious dose as low as 10 particles (D'Souza et al., 2006). Symptoms of HuNoV infection, including diarrhea, vomiting, nausea, abdominal cramps, headache, and fever, usually last for 24-48 h in healthy individuals, but may be prolonged in the immunocompromised patients, children and the elderly (Iwamoto et al., 2010, Partridge et al., 2012).

Shellfish are known as vectors for human pathogens including norovirus (Le Guyader et al., 2012; Campos, & Lees, 2014, McIntyre, et al., 2012). Several post-harvest techniques have been proposed to reduce HuNoV in live oysters including cold and high temperature, UV treatment, and high-hydrostatic pressure. However, most of these treatments have low log reduction on norovirus or they kill oysters (Araud et al., 2016, Ye et al., 2015; Venugopal, 2005).

Ionizing irradiation is a non-thermal process which has been used to inactivate pathogenic and spoilage microorganisms and extend shelf life of food products (Thakur, & Singh, 1994). Gamma ray, electron beam and X-ray are the three kinds of ionizing irradiation that approved to treat shellfish to up to 5.5 kGy (USFDA, 2015). The biological effects of ionizing irradiation were reported mainly linked to the DNA damage by free radicals during the irradiation process and the ionizing radiation-generated reactive oxygen species (Anh et al., 2013). In the last decade, our group, at Mississippi State University, has extensively studied the use of X-ray to inactivate different pathogens in different food products. Mahmoud, (2012a) reposted that X-ray

significantly ($p < 0.05$) reduce the initial level of artificially inoculated *Listeria monocytogenes* on smoked salmon from 3.7 log CFU g⁻¹ to 2.0 log CFU g⁻¹ after treatment with 0.5 kGy X-ray, while exposure to 1.0 kGy X-ray reduced *L. monocytogenes* to below the detection limit (<1.0 log g⁻¹). Mahmoud (2009b) reported that greater than a 6-log reduction of *Vibrio vulnificus* was achieved with 3.0 kGy in whole-shell oysters. Treatment with 3.0 kGy X-ray treatment did not affect the survivability of live oysters. Wu et al., (2016) stated that X-ray is a potential antiviral agent against HuNoVs surrogate murine norovirus (MNV-1) in food products including salmon sushi and tuna salad. We have to rely on proper surrogates to study the survival of human of HuNoV because HuNoV is not cultivable (Ye et al., 2015). Compared with feline calicivirus (FCV) and Tulane virus, we chosen Murine norovirus (MNV-1) for our research because it is a closer genetic relative of HuNoV in the norovirus genus, has been used in UV irradiation, gamma irradiation and electron beam (Richards, 2012). MNV-1 was propagated and assayed in an efficient microglial cell line murine BV-2 cells (Cox et al., 2009).

The aim of this study was to investigate the effects of X-ray treatments on bioaccumulated murine norovirus-1 (MNV-1) and survivability, inherent microflora, color, and firmness of whole-shell Atlantic oysters (*Crassostrea virginica*) during storage at 5°C for 20 days

2. Materials and methods

2.1. Murine norovirus (MNV-1)

Because of human norovirus (HuNoV) is not cultivable, we had to rely on proper surrogates to study the survival of HuNoV in oysters. Murine norovirus (MNV-1) has been chosen, for our investigation, due to its genetic similarity and environmental stability, compared with other surrogates such as feline calicivirus and Tulane virus (Richards, 2012; Ye et al., 2015). Murine norovirus (MNV-1) was generously provided by Dr. Herbert W. Virgin IV, Washington University School of Medicine, St. Louis, MO. MNV-1 was propagated in murine BV-2 cells cultured in Dulbecco's modified eagle's medium (DMEM, HyClone Laboratories, Logan, UT) supplemented with 10% fetal bovine serum (FBS, HyClone Laboratories, Logan, UT), 10 mM HEPES (HyClone Laboratories, Logan, UT), 1 mM non-essential amino acid (HyClone Laboratories, Logan, UT), 2mM-glutamine (HyClone Laboratories, Logan, UT), 100 U/ml penicillin (HyClone Laboratories, Logan, UT) and 100 mg/ml streptomycin (HyClone Laboratories, Logan, UT) (Cox et al., 2009; Gonzalez-Hernandez et al., 2012). The MNV-1 stocks were prepared and stored as previously described (Wu, et al., 2016).

2.1 Atlantic oysters (Crassostrea virginica)

Fresh harvested Atlantic oysters were purchased from an oyster processor (Crystal Seas Seafood, Pass Christian, MS) and transported immediately (within 1 h) in a cooler with cubed ice to the MSU Experimental Seafood Processing Laboratory in Pascagoula, MS. After washing with

tap water and removing mud from the shells, oysters with 7-9 cm were chosen for the experiments.

2.2 MNV-1 bioaccumulation in whole-shell oysters

Whole-shell oysters accumulated MNV-1 as previously described (Araud et al., 2016) with a few modifications. For each batch, 18 live oysters were cultivated in polyethylene tank containing recirculating 60 liters of artificial seawater (1.5% sea salt, Oceanic system, Dallas, TX) at room temperature (20–22 °C). Oysters were feed using phyto-plankton (Reef Phytoplankton, Seachem, Madison, GA) according to the instruction of the manufacturer. The seawater was contaminated with MNV-1 at 10^4 PFU/mL where oysters were cultivated for 24 h before X-ray treatments (the initial level of MNV-1, in oysters, was about $4.3 \log \text{PFUg}^{-1}$).

2.3 X-ray treatment

Specific irradiation doses (0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 kGy X-ray; at rate of 31.25 Gy/min) were generated using the RS 2400 industrial cabinet X-ray irradiator (Rad Source Technologies, Inc., Alpharetta, GA). Samples were treated with X-ray at 22 °C and 55–60% relative humidity placed at same distance around the generator X-ray tube as described by Mahmoud (2009a). For inactivation of bio-accumulated MNV-1 study, oysters (3 contaminated oysters) were treated with 0, 1.0, 2.0, 3.0, 4.0 or 5.0 kGy X-ray. For survivability study, oysters (20 uncontaminated oysters for each treatment) were subjected to 0.0, 1.0, 2.0, 3.0, 4.0 or 5.0 kGy X-ray treatments.

For inherent microbiota, texture, and color studies, oysters (3 uncontaminated oysters) were subjected to 0.0, 1.0, 2.0, 3.0, 4.0 or 5.0 kGy X-ray.

2.4 MNV-1 enumeration (Plaque assay)

Virus was extracted as described by Praveen et al., (2013) with a few modifications. For each plaque assay, three contaminated oysters were shucked and pooled in 200 mL of phosphate buffer (0.15 M Na₃PO₄, pH 9.5) and homogenized by a laboratory blender (Waring Inc., New Hartford, CT) for 3 min. The homogenized tissues were centrifuged with a benchtop centrifuge. The supernatant was neutralized with 2N HCl. Tenfold serial dilutions of neutralized supernatant were made in Earle's balanced salt solution (EBSS) (Gibco-Invitrogen, Grand Island, NY). Plaque assays were performed as described by Wu et al., (2016). A series of 10-fold dilutions of sample were inoculated onto the 6-well plates after the formation of a confluent monolayer of BV-2, aspiration of the medium and two cell washes with DMEM-0. The inoculums were incubated for one hour at room temperature with gentle rock every 15 min for even distribution. The viral inoculum was removed at the end of incubation. To each well, two mL of overlay ((1:1 mixture of 3% w/v Sea-Plaque agarose (Lonza, Allendale, NJ) and 2 X MEM media (Gibco-Invitrogen, Grand Island, NY)) were slowly poured on the edge. The plates were placed into the tissue culture incubator after solidified for 10 min at room temperature. The neutral red staining solution (3%) was prepared with neutral red (Sigma Chemical Co., St. Louis, MO) and culture grade phosphate-buffered saline (Gibco-Invitrogen, Grand Island, NY). Two mL staining solution was added directly onto the overlay. Plaques were count in wells where plaques are separated clearly.

2.5 The survivability of oysters

Live uncontaminated oysters were kept at the refrigerator temperature (5 °C) for up to 20 days and their survival was determined every 5 days. The survivability of treated oysters was determined on the basis of difficulty to open the valves during storage (Mahmoud, 2009a).

2.6 Effect of X-ray treatment on the inherent microbiota on oysters

Uncontaminated oysters were kept at the refrigerator temperature (5 °C) and live oysters were sampled at day 0, 5, 10, 15 and 20. Oysters were shucked, mixed and 25g-samples were placed in sterile Whirl-Pak bags, followed by addition of 225 mL of sterile Alkaline Peptone Water (APW). The samples were homogenized for 2 min using a Stomacher 80 Lab-blender. Serial 10-fold dilutions were prepared in APW. Mesophilic and psychrotrophic counts were performed as previously described (Mahmoud, 2012b). For mesophilic counts, 0.1 mL of each dilution was plated onto TSA and incubated at 37 °C for 24 h. For psychrotrophic counts, 0.1 mL of each dilution was plated onto TSA and incubated at 5 °C for 10 days. Viable counts were expressed as log CFU g⁻¹.

2.7 Effect of X-ray treatment on color and firmness

Uncontaminated oysters were pulled from the refrigerator (5°C) at day 0, 5, 10, 15 and 20. The texture and color were analyzed as previously described by Mahmoud et al., (2016). The

texture was measured by a puncture test using an Instron 4944 (Instron, Norwood, MA). Meat of oysters were punctured with a star-shape probe (5 sharp points, 10 mm diameter). Crosshead speed was set at 50 mm/min. Force-distance curves were obtained from the puncture tests and firmness was taken as the force (N) required puncturing the thickest point of the oyster samples. The color of oyster meat was evaluated using the Hunter colorimeter values (Lab Scan XE Hunter Colorimeter; Hunter Associates Laboratory, Inc., Reston, VA) by L*(lightness), a*(redness-greenness), and b*(yellowness-blueness).

2.8 Statistical Analysis

All experiments were replicated three times. The mean values and standard deviations were determined using XLStat (2015, Addinsoft USA, New York, NY). Significant differences among means were made with Tukey honestly significant difference (HSD) analysis using probability level of 0.05.

3. Results and discussion

No MNV-1 was found, in the live oysters, before the bio-accumulation experiments. Our results indicated that the viral (MNV-1) titers was $4.3 \pm 0.4 \log \text{PFUg}^{-1}$ in oyster meat when oyster was harvested after accumulation of MNV-1. This result is in agreement with that of Araud et al. (2016) who reported that a $3\text{--}4 \log \text{PFUg}^{-1}$ of MNV-1 uptake in oyster when harvested at 24 h. The treatment of whole-shell oysters with 1, 2, 3, and 4 kGy X-ray significantly reduced the $\log \text{PFUg}^{-1}$ from 4.3 ± 0.4 to 3.6 ± 0.5 , 3.2 ± 0.2 , 2.8 ± 0.2 , and 2.5 ± 0.1 , respectively, however, exposure to 5.0 kGy X-ray reduced the population of MNV-1 to less than $2.0 \log \text{PFU g}^{-1}$ (Fig. 1). This study demonstrated that X-ray had a strong antiviral activity against norovirus (MNV-1) compared with gamma and e-beam irradiation.

The ionizing irradiation was not considered as a feasible process for inactivating viruses in contaminated shellstock because some of the previous studies found that the radiation (gamma rays and electron beam) exposures needed to significantly reduce PFU of viruses in hard-shelled shellfish was higher than the maximum FDA-allowable dose of 5.5 kGy which substantially increase the shellfish mortality. Harewood et al., (1994) stated the viral indicator F-coliphage was not significantly affected by gamma ray with a high D10 value (13.50 kGy) in shellfish. Praveen et al., (2013) reported that 4 kGy electron beam only reduced MNV-1 by $1 \log \text{PFUg}^{-1}$ on whole-shell oysters. Further understanding of the mechanism of viral inactivation in whole-shell oyster is needed to guide the food industry to properly use the X-ray irradiation.

Irradiation as the only known method to pasteurize live molluscs without significantly affecting mortality (Mbarki et al., 2009). In our study, the survivability of control group was 19.7 ± 0.6 of 20 oysters (98.5%) after storage of oysters at 5°C for 20 days (Table 1). Our study

also found that the treatment of whole-shell oysters with up to 5 kGy did not kill oysters (day 0). These findings are in agreement with those reported by Farcy et al., (2011) who stated that mollusks are irradiation-resistant and can survive after exposure to doses up to 10^3 kGy. Jakabi et al. (2003) reported that the gamma ray irradiation did not kill the oysters nor affect their sensory attributes at 3.0 kGy. The average survivability decreased, during storage, with higher X-ray doses, however, there was no significant differences among all treatment doses at day 5 or 10. Furthermore, the average survivability significantly decreased with higher X-ray doses at day 15 and 20 (Table 1). The survivability of oysters treated with 5.0 kGy X-ray (9.3 ± 2.0) was significantly lower than the survivability of oysters treated with 1.0 kGy X-ray (14.3 ± 1.2) on day 20. These results are in agreement with those obtained by Mahmoud (2009b) who reported that 3.0 kGy of X-ray treatment did not affect the survivability of live oysters up to 7 days.

The mesophilic counts for the control samples of whole-shell oyster were 5.09 ± 0.05 , 5.69 ± 0.21 , 5.83 ± 0.48 , 6.84 ± 1.08 and 6.98 ± 1.27 log CFU g⁻¹ on day 0, 5, 10, 15 and 20, respectively, during storage at 5°C (Table 2). On day 0, treatment with 1 kGy X-ray significantly reduced the mesophilic count log CFU g⁻¹ from 5.09 ± 0.05 to 3.11 ± 0.05 . Furthermore, treatment with 2-5 kGy X-ray significantly reduced the mesophilic count to below the detection level (2 log CFU g⁻¹). Treatment with X-ray kept the mesophilic counts significantly lower than the control until day 20.

The psychotropic counts for the control samples of whole-shell oyster were slightly increased during storage at 5°C (Table 3). On day 0, treatment with 1 and 2 kGy reduced the psychotropic count from 5.70 ± 0.18 to 3.74 ± 0.03 and 2.67 ± 0.48 log CFU g⁻¹, respectively. Furthermore, treatment with 3-5 kGy X-ray significantly reduced the psychotropic count to below the detection level (2 log CFU g⁻¹). Treatment with X-ray kept the psychotropic counts

significantly lower than the control until day 20. These results show same trend as those reported by (Mahmoud & Burrage, 2009) where treatment with 1.0 kGy significantly reduced the inherent microbiota in whole-shell oysters from 4.6 log CFU to undetectable limit (1.0 log CFU g⁻¹). The microbial community structure and sensitivity can be changed, on oyster, throughout seasons (Pierce et al., 2016). Lopez-Caballero et al., (2000) stated bacteria would be the reason of high total volatile base (TVB) and small amounts TMAO (Trimethylamine N-oxide) during oyster spoilage because it produces amines, ammonia and reduced the pH by proteolytic and saccharolytic process. Through improving the microbial quality might alleviate biochemical changes during refrigerated storage (Mbarki et al., 2009). Buzin et al. (2011) reported cold storage of Pacific oyster (*Crassostrea gigas*) remained a low mortality rate and the sensory attributes, odor, appearance, texture and flavor, did not show significant variations. A further investigation on chemical changes on live oyster by X-ray preservation is in progress in our laboratory.

It is critical to retain the color of seafood products when considering development of intervention methods of pathogens. For example, high pressure treatment was found to changes the color of oysters at pressures above 300 MPa due to the protein denaturation and the extent of color change increased gradually with the increase of treatment pressure (Wang et al., 2015). The firmness and the color of oyster meat during storage at 5°C for 20 days were shown in Tables 4 and 5. The results indicated that X-ray treatment did not significantly affect firmness nor a*, b* and saturation values of oyster meat on day 0 or throughout the storage (20 days). The hue value of oyster meat with 5 kGy X-ray treatment was significantly higher than day 0, 5, 10, and 15 on day 20 but no significant difference compared with other X-ray doses on day 20.

4. Conclusion

To our knowledge, this is the first report about the X-ray inactivation of internalized MNV-1 on whole-shell oysters. The results of this study indicate that the efficacy of X-ray irradiation against internalized MNV-1 increased with increasing X-ray doses. For application of X-ray technology in oyster industries, the shelf-life studies were performed on survivability, inherent microbiota, color, and firmness on whole-shell oyster. The results indicated that X-ray is effective in reducing spoilage microflora. The main advantage of using X-ray for reducing MNV-1 in oysters is that the X-ray treatment did not kill the oysters (at 0 day) even at the highest dose tested (5 kGy) and the survivability was not significantly affected by treatment with X-ray within 10 days of storage at 5 °C. Our results suggest that X-ray irradiation is a feasible process for inactivating norovirus and inherent microbiota for the oyster industry.

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Fig. 1.

Inactivation of internalized MNV-1 (log PFU g⁻¹) on whole shell oyster by X-ray.

Error bars represent standard error. Different lower case letters are significantly different ($p < 0.05$). ND = not detectable ($<2.0 \log \text{CFU g}^{-1}$).

Table 1.

The number of surviving of treated whole-shell oysters (*Crassostrea virginica*) with 0, 1, 2, 3, 4 and 5 kGy X-ray during storage at 5°C for 20 days.

Days	X-ray dose (kGy)					
	0	1	2	3	4	5
0	20.0 $\pm$ 0.0 aA	20.0 $\pm$ 0.0 aA	20.0 $\pm$ 0.0 aA	20.0 $\pm$ 0.0 aA	20.0 $\pm$ 0.0 aA	20.0 $\pm$ 0.0 aA
5	20.0 $\pm$ 0.0 aA	20.0 $\pm$ 0.0 aA	19.0 $\pm$ 1.7 aAB	19.0 $\pm$ 1.0 aA	19.0 $\pm$ 1.0 aA	17.0 $\pm$ 1.7 aAB
10	20.0 $\pm$ 0.0 aA	19.7 $\pm$ 0.6 aA	18.0 $\pm$ 1.7 aAB	17.0 $\pm$ 3.0 aA	17.3 $\pm$ 0.6 aAB	16.3 $\pm$ 1.5 aAB
15	19.7 $\pm$ 0.6 aA	17.3 $\pm$ 2.5 abAB	15.7 $\pm$ 1.5 abcBC	11.7 $\pm$ 2.1 cB	13.0 $\pm$ 1.0 cB	13.7 $\pm$ 0.6 bcB
20	19.7 $\pm$ 0.6 aA	14.3 $\pm$ 1.2 bB	13.3 $\pm$ 0.6 bC	9.3 $\pm$ 1.2 bcB	8.0 $\pm$ 3.6 bcC	9.3 $\pm$ 2.0 cC

Mean values with different lower case letters in same row or capital letters in same column are significantly different ($p < 0.05$).

Table 2.

Changes in the mesophilic bacterial counts (log CFU g⁻¹) of treated whole-shell oysters with 0, 1, 2, 3, 4 and 5 kGy X-ray during storage at 5°C for 20 days

Days	X-ray dose (kGy)					
	0	1	2	3	4	5
0	5.09 $\pm$ 0.05aA	3.11 $\pm$ 0.05bA	ND	ND	ND	ND
5	5.69 $\pm$ 0.21aA	3.49 $\pm$ 0.57bAB	ND	ND	ND	ND
10	5.83 $\pm$ 0.48aA	4.65 $\pm$ 0.38bBC	4.59 $\pm$ 0.30bcA	3.89 $\pm$ 0.33bcA	3.70 $\pm$ 0.32cA	ND
15	6.84 $\pm$ 1.08aA	5.99 $\pm$ 0.35aCD	5.58 $\pm$ 0.92aAB	5.84 $\pm$ 0.09aB	5.77 $\pm$ 0.18aB	5.02 $\pm$ 0.79aA
20	6.98 $\pm$ 1.27aA	7.04 $\pm$ 0.98aD	7.23 $\pm$ 0.97aB	7.23 $\pm$ 0.89aC	7.48 $\pm$ 0.91aC	6.82 $\pm$ 0.38aB

Mean values with different lower case letters in same row or Capital letters in same column are significantly different ($p < 0.05$). ND = not detectable (<2.0 log CFU g⁻¹).

Table 3

Changes in the psychrotrophic bacterial counts (log CFU g⁻¹) of treated whole-shell oysters with 0, 1, 2, 3, 4 and 5 kGy X-ray during storage at 5°C for 20 days

Days	X-ray dose (kGy)					
	0	1	2	3	4	5
0	5.70±0.18 aA	3.74±0.03 bA	2.67±0.48 cA	ND	ND	ND
5	5.79±0.40 aA	4.96±0.13 abB	4.22±0.47 bAB	4.00±0.95 bA	3.97±0.49 bA	ND
10	6.33±0.48 aA	5.89±0.31 aAB	5.82±0.41 aBC	5.60±0.49 aB	5.89±0.43 aB	5.40±0.65 aA
15	6.76±0.41 aA	6.33±5.55 aBC	6.58±0.90 aC	5.72±0.38 aB	6.51±0.62 aBC	5.89±0.21 aAB
20	6.90±1.39 aA	7.11±0.61 aC	6.83±1.19 aC	6.67±0.58 aB	7.53±0.91 aC	6.29±0.20 aB

Mean values with different lower case letters in same row or Capital letters in same column are significantly different ($p < 0.05$). ND = not detectable (<2.0 log CFU g⁻¹).

Table 4.

Changes in the firmness [by a firmness test using an Instron 4944 (Maximum load (N)) of the meat of treated whole-shell oysters with 0, 1, 2, 3, 4 and 5 kGy X-ray during storage at 5°C for 20 days

Days	X-ray dose (kGy)					
	0	1	2	3	4	5
0	0.24 $\pm$ 0.22 aA	0.69 $\pm$ 0.28 aA	0.49 $\pm$ 0.19 aA	1.02 $\pm$ 0.21 aA	0.97 $\pm$ 0.41 aA	0.78 $\pm$ 0.35 aA
5	0.65 $\pm$ 0.11 aAB	0.55 $\pm$ 0.33 aA	0.64 $\pm$ 0.37 aAB	0.80 $\pm$ 0.47 aA	0.93 $\pm$ 0.34 aA	0.92 $\pm$ 0.84 aA
10	0.59 $\pm$ 0.66 aAB	1.37 $\pm$ 0.11 aA	0.98 $\pm$ 0.19 aAB	0.72 $\pm$ 0.09 aA	0.89 $\pm$ 0.08 aA	1.18 $\pm$ 0.56 aA
15	1.39 $\pm$ 0.29 aB	1.36 $\pm$ 0.92 aA	1.24 $\pm$ 0.16 aAB	1.55 $\pm$ 0.79 aA	1.46 $\pm$ 0.38 aA	1.60 $\pm$ 0.33 aA
20	0.86 $\pm$ 0.31 aAB	1.07 $\pm$ 0.47 aA	1.35 $\pm$ 0.49 aB	1.27 $\pm$ 0.19 aA	1.80 $\pm$ 0.47 aA	1.21 $\pm$ 0.22 aA

Mean values with different lower case letters in same row or Capital letters in same column are significantly different ($p < 0.05$).

Table 5.

Changes in the color (Hunter parameters, *L*, *a*, *b*, Saturation value, and Hue value) of the meat of treated whole-shell oysters with 0, 1, 2, 3, 4 and 5 kGy X-ray during storage at 5°C for 20 days.

(*L*)

Days	X-ray dose (kGy)					
	0	1	2	3	4	5
0	63.7 $\pm$ 7.6 aA	63.4 $\pm$ 5.5 aA	63.0 $\pm$ 4.5 aA	50.5 $\pm$ 18.1 aA	60.2 $\pm$ 9.2 aA	58.9 $\pm$ 2.9 aA
5	49.5 $\pm$ 16.1 aA	48.5 $\pm$ 14.7 aA	53.3 $\pm$ 18.9 aA	61.1 $\pm$ 3.8 aA	56.9 $\pm$ 3.1 aA	49.2 $\pm$ 10.0aA
10	63.6 $\pm$ 5.3 aA	59.3 $\pm$ 3.8 abA	66.4 $\pm$ 0.4 aA	52.7 $\pm$ 8.1 abA	57.9 $\pm$ 4.6 abA	36.7 $\pm$ 21.1 bA
15	47.5 $\pm$ 16.2 aA	54.3 $\pm$ 2.6 aA	45.8 $\pm$ 13.0 aA	50.4 $\pm$ 12.7 aA	49.6 $\pm$ 8.9 aAB	45.9 $\pm$ 12.5 aA
20	57.4 $\pm$ 2.2 aA	59.9 $\pm$ 4.9 aA	63.2 $\pm$ 5.9 aA	54.9 $\pm$ 1.4 aA	37.7 $\pm$ 6.9 bB	57.9 $\pm$ 4.5 aA

(a)

Days	X-ray dose (kGy)					
	0	1	2	3	4	5
0	2.4 $\pm$ 0.5 aA	1.6 $\pm$ 1.4 aA	1.6 $\pm$ 0.2 aA	3.2 $\pm$ 1.4 aA	1.8 $\pm$ 0.8 aA	2.1 $\pm$ 0.8 aA
5	2.2 $\pm$ 0.5 aA	3.6 $\pm$ 1.6 aA	2.0 $\pm$ 0.3 aA	2.2 $\pm$ 0.3 aA	2.7 $\pm$ 0.5 aA	2.9 $\pm$ 0.5 aA
10	2.4 $\pm$ 0.9 aA	3.2 $\pm$ 0.5 aA	3.1 $\pm$ 0.6 aA	2.2 $\pm$ 0.5 aA	2.6 $\pm$ 0.4 aA	4.2 $\pm$ 2.8 aA
15	1.7 $\pm$ 0.9 aA	2.4 $\pm$ 0.4 aA	2.9 $\pm$ 0.4 aA	2.7 $\pm$ 0.7 aA	2.2 $\pm$ 0.9 aA	1.9 $\pm$ 0.4 aA
20	1.8 $\pm$ 0.5 aA	2.3 $\pm$ 0.6 aA	2.4 $\pm$ 1.2 aA	3.4 $\pm$ 0.7 aA	4.2 $\pm$ 2.1 aA	3.2 $\pm$ 0.4 aA

(b)

Days	X-ray dose (kGy)					
	0	1	2	3	4	5
0	14.3 $\pm$ 0.7 aA	11.1 $\pm$ 5.4 aA	13.3 $\pm$ 2.4 aA	14.4 $\pm$ 1.9 aA	13.7 $\pm$ 0.2 aA	16.0 $\pm$ 1.3 aA
5	11.8 $\pm$ 3.1 aA	14.0 $\pm$ 4.2 aA	12.2 $\pm$ 3.9 aA	14.2 $\pm$ 0.9 aA	14.0 $\pm$ 0.2 aA	13.9 $\pm$ 2.5 aA
10	13.0 $\pm$ 2.3 aA	14.3 $\pm$ 1.3 aA	14.8 $\pm$ 1.9 aA	12.4 $\pm$ 2.2 aA	13.2 $\pm$ 1.9 aA	19.1 $\pm$ 2.6 aA
15	12.3 $\pm$ 1.3 aA	15.0 $\pm$ 2.4 aA	15.6 $\pm$ 0.9 aA	15.9 $\pm$ 1.7 aA	14.4 $\pm$ 0.9 aA	14.2 $\pm$ 1.1 aA
20	12.9 $\pm$ 0.9 aA	13.4 $\pm$ 0.6 aA	13.0 $\pm$ 1.2 aA	15.1 $\pm$ 0.8 aA	14.2 $\pm$ 2.8 aA	14.7 $\pm$ 0.9 aA

(Saturation value)

Days	X-ray dose (kGy)					
	0	1	2	3	4	5
0	14.9 $\pm$ 0.7 aA	11.2 $\pm$ 5.6 aA	13.4 $\pm$ 2.4 aA	14.7 $\pm$ 2.0 aA	13.9 $\pm$ 0.1 aA	16.2 $\pm$ 1.4 aA
5	12.0 $\pm$ 3.1 aA	14.6 $\pm$ 3.7 aA	12.4 $\pm$ 3.8 aA	14.4 $\pm$ 0.9 aA	14.3 $\pm$ 0.2 aA	14.2 $\pm$ 2.5 aA
10	13.2 $\pm$ 2.5 aA	14.6 $\pm$ 1.4 aA	15.1 $\pm$ 2.0 aA	12.6 $\pm$ 2.3 aA	13.4 $\pm$ 1.9 aA	19.6 $\pm$ 8.1 aA
15	12.4 $\pm$ 1.2 aA	15.2 $\pm$ 2.4 aA	15.8 $\pm$ 0.9 aA	15.3 $\pm$ 1.7 aA	14.6 $\pm$ 0.9 aA	14.3 $\pm$ 1.1 aA
20	12.9 $\pm$ 0.9 aA	13.6 $\pm$ 0.8 aA	13.2 $\pm$ 1.4 aA	15.5 $\pm$ 0.7 aA	14.9 $\pm$ 3.1 aA	15.0 $\pm$ 1.0 aA

(Hue value)

Days	X-ray dose (kGy)					
	0	1	2	3	4	5
0	80.5 $\pm$ 1.8 aA	83.6 $\pm$ 5.2 aA	82.9 $\pm$ 1.8 aA	77.8 $\pm$ 4.9 aA	82.3 $\pm$ 3.5 aA	82.7 $\pm$ 2.1 aA
5	79.4 $\pm$ 2.2 aA	73.7 $\pm$ 11.6 aA	79.7 $\pm$ 4.0 aA	81.1 $\pm$ 0.8 aA	79.0 $\pm$ 2.1 aA	77.9 $\pm$ 0.6 aAB
10	79.8 $\pm$ 2.0 aA	77.4 $\pm$ 0.9 aA	78.2 $\pm$ 0.8 aA	79.9 $\pm$ 0.6 aA	78.8 $\pm$ 0.5 aA	78.3 $\pm$ 3.2 aAB
15	81.8 $\pm$ 5.4 aA	80.7 $\pm$ 0.9 aA	79.5 $\pm$ 1.2 aA	80.0 $\pm$ 2.6 aA	81.6 $\pm$ 3.4 aA	82.3 $\pm$ 1.2 aAB
20	82.1 $\pm$ 1.9 aA	80.1 $\pm$ 2.2 aA	79.9 $\pm$ 4.6 aA	77.2 $\pm$ 2.8 aA	73.9 $\pm$ 6.6 aA	77.7 $\pm$ 1.1 aB

Mean values with different lower case letters in same row or capital letters in same column are significantly different ($p < 0.05$).