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PROOF

Effects of TiO_2 Nanocomposite Packaging and Gamma Irradiation on the Shelf-life of Rainbow trout Stored at (+4°C)

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Abstract

Gamma irradiation is used to assure effects on decay prevention by sterilizing the microorganisms and elaborating the safety without arbitrating the nutritional properties and sensory quality of the food. In this research, the effect of Tio_2 nanocomposite (1% concentration), gamma irradiation (3 and 5 kGy), and their combination on the shelf life of filleted "*Oncorhynchus mykiss"* during cold storage (+4°C) were examined periodically every 4 days (during 20 days). For this purpose, fillet samples were packaged by Tio_2 nanocomposites and then they were irradiated at room temperature and they were preserved. Microbial, chemical and sensory effects were considered during storage to control, pack and irradiate treatment. The result indicated that TiO_2 nanocomposite and irradiation at 3 kGy would enable longer retention of the chemical, microbial, and sensory characteristics and extend the shelf life of fish fillets during the cold storage.

Keywords: Nanopackaging, tio_2 nanoparticles, gamma ray, preservation, fish fillet.

Introduction

Polymer nanocomposites refer to those composites which one of its phases is morphologically nano-scale such as nanoparticles, nano-tubes or lamellar nanostructure and the other phase is composed of polymers matrix (Camargo, Satyanarayana & Wypych, 2009). Polymer nanocomposites have attracted considerable attentions and investments in research and development worldwide. Nanocomposites emerged as a good candidate to produce food package because of their good barrier, antimicrobial and mechanical properties (Ratto, Froio, Thellen & Lucciarini, 2009; Massey, 2003; Thellen, Schirmer, Ratto, Finnigan & Schmidt, 2009; Avella, *et al*, 2005). Indeed, preservation of the desired qualities of fish and fishery products in the seafood industries depends on the ability to extend shelf life through the packaging material. Currently, there is considerable interest in the self-disinfecting properties of Tio_2 for qualifying hygienic requirements in the substrates used for food processing and packaging (Emamifar, 2011). It has been reported that Tio_2 nanoparticles have bactericidal and fungicidal effects on *E. coli*, *Salmonella choleraesuis*, *Vibrio parahaemolyticus*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Stayphylococcus aureus*, *Diaporthe actinidiae* and *Penicillium expansum* (Emamifar, 2011). Further, they aren't required to be released in a medium that is considered as a critical advantage over Ag-based and all other biocide agents. Biocidal capacity of Tio_2 nanoparticles is a result of new electronic states that lank them in individual components. In the last few years, in order to increase the gas barrier, property of polymer films focus has been shifted to organic nanocomposites (Ke & Yongping, 2005; Liang, Xu, Bao & Xu, 2004; Zeng, Yu, Lu & Paul, 2005) while in this study, Tio_2 was used instead of organic matters due to its better chemical and thermal stability together and its innocuity as is compared to montmorillonite. Tio_2 nanocomposites as novel antimicrobial agents with the unique chemical and physical barrier properties can protect foods more efficiently. However, food irradiation with ionizing radiation was introduced as a facilitated and credible technological procedure in order to mitigate spoilage losses and improve hygienic quality and extend shelf life through more than 50 year research. Food irradiation with 10 kGy is safe toxicologically and adequate nutritiously when its dose is level or below (Byun, Kim, Byun & Lee, 2008; Lee, Kim & Han, 2009). Bacteriological property and fish spoilage are in direct relation to each other and result in food poison outbreak (Nilla, Khan, Khan, Ahsan & Mustafa, 2012). The treatment of fish and fishery products by ionizing radiations has a potential to contribute to preserve fish. Irradiation has been shown to reduce effectively the spoilage caused by micro-organisms and to slowdown the deterioration process, without denaturing the treated product, and without changing its palatability, as usually occurs with heating (cooking, canning, frying), freezing, drying or smoking, etc. Shelf life of fish can be considerably prolonged without any detectable change in flavor, odor, tissue and form, i.e. sensory quality of fresh fish or fishery product. In addition, irradiation has also been advantageously combined with other food processing methods to the extent which made them less perishable. Another special feature of fish irradiation is that the fresh or processed product can be irradiated in the final packing because of the easy penetration of gamma rays through packaging materials. In this way, not only some bacteria of public health significance can be eliminated without altering the sensory qualities of the product, but also shelf life can be prolonged drastically by improving chemical and microbial qualities. In this study, we compared the effects resulting from gamma irradiation and Tio_2 nanocomposite packaging on shelf life of the rainbow trout. The combined effects have also been reviewed.

Materials and Methods

Fish Samples and Preparation

Fresh farmed rainbow trout (average weight 300-450g and length 30-32 cm) was purchased from local market (Karaj, Iran). Samples were transported to the laboratory within 30 minutes in iced condition with a fish/ice ratio of 1:3 (w/w).

Samples were scaled, gutted, and washed with water. Then, scaled and gutted fish were filleted, packaged with Tio_2 nanocomposite for test group and without Tio_2 nanocomposite for control group. Samples in iced condition were transported for irradiation.

Gamma Irradiation

The fish samples irradiations was performed in 60 cobalt irradiator (gamma cell Model: PX30, dose rate 2.28 Gry/Sec) to 3 and 5 kGy at ambient temperature. Three samples were used for each irradiation dose. All samples were analyzed chemically, microbiologically and sensory every 4 days during 20 days.Trial groups were included: Control, packaged by Tio_2 nanocomposites (P), irradiated by 3 and 5 kGy dose without tio_2 nanocomposites and irradiated by 3 and 5 kGy dose with tio_2 nanocomposites.

Chemical Analysis

Total volatile base-nitrogen (TVB-N), pH, peroxide value (PV), Thiobarbituric acid (TBARS) and oxidation of protein through carbonyl index were determined according to the methods suggested by (Pearson, 1997; Suvanich, Jahncke, & Marshal, 2000; Pearson, 1997; Natseba, Lwalinda, Kakura, Muyanja, & Muyonga, 2005; Levine, Williams, Stadtman, & Shacter, 1994) respectively.

Microbial Assay

10 g sample was placed in 90 ml of 0.85% NaCl solution and homogenized with a stomacher after microbiological analyses. Different decimal dilutions were prepared from this dilution and appropriate media were used to plate them. The pour plate method was used to

determine psychrotrophic counts and total viable aerobic bacterial using plate count agar (PCA, Merk, Darmstadt, Germany) at 7°C for 10 days and 37°C for 2 days. Incubation of *Enterobacteriaceae* on double-layered plates of Violet Red Bile Glucose agar (VRBG, Oxide) was done at 30°C for 48 h [before incubation 5 ml of Tryptone Soy Agar was added (Merck, Darmstadt, Germany) and incubated for 1 h at room temperature]; lactic acid bacteria was incubated on double-layered plates of MRS Agar (Oxide) at 30°C for 48 h. The inoculated plates were re-incubated at 37°C for 2 days in order to determine total viable counts, and the plates were re-incubated at 10 °C for 7 days to determine psychrotrophic counts. All counts were converted as log10 CFU/g in triplicate. (Arashisar, Hisar, Kaya, & Yanik, 2004; Sallam, 2007).

Sensory Assessment

Selection criteria were availability for the assessments, interest to participate in the study, the absence of aversions, allergies or intolerance against fish or bread, and normal perception abilities. Same lots of fish fillet of trial samples were used for analysis. 6-member trained panel (one woman and five men ranging 27 - 45 years age) was used to assess the samples based on a five point scale to determine: texture; discoloration; odor; and overall acceptability (5meaning extremely desirable and 1 extremely unacceptable) of the samples. As the sensory attributes diminished below 4, the sample would be rejected based on the shelf life criteria. (Fan *et al*, 2009; Nollet, & Toldra, 2010; Rehbein, & Oehlenschlager, 2009).

Statistical Analysis

The data obtained from the analyses were subjected to statistical analysis using the Standard Statistical Package (SPSS, version 19.00). Analysis of variance (ANOVA) based Duncan's Multiple Range Test, Kruskal-Wallis and Mann–Whitney U tests was used for statistical analysis. The significance of differences was calculated at 5% level (P<0.05).

Results and Discussions

Chemical Analysis

pH Measurement

Figure1 depicts pH changes in the fish fillet. Different treatments had presumably different effects on the pH value of samples during storage. The pH value that in control sample was more than other samples at the end of storage (P<0.05) indicating the quality loss (Can, 2011). The pH has not affected by irradiation considerably (P>0.05). However, Tio_2 nanocomposites caused decrease in pH value during refrigerator storage (P<0.05). A similar trend in irradiation effect on fish, was observed for farmed sea bass, turbot on ice and rainbow trout in refrigerator storage (Papadopoulos, Chouliara, Badeka, Savvaidid & Kontominas, 2004; Moini *et al*, 2009). There was no report for Tio_2 nanocomposites effect in this basis. Basic amines generation such as alkaline volatile amines by spoilage bacteria is likely reason for increase in pH, although the increased psychrotrophic bacterial counts could be also contributed in this regard (Debevere & Bosku, 1996; Manju, Jose, Srinivasa Gopal, Ravishankar, & Lalitha, 2007; Masniyom, Soottawat, & Visessanguan, 2005; Reddy, Villanueva & Kautter, 1995) Furthermore, the increased values of pH are in conformity with the increased TVB-N mostly composed of alkaline volatile amines (Hyytia, Hielm, Mokkila, Kinnunen, & Korkeala, 1999). It was reported in Tio_2 nanocomposites treatment that psychrotrophic bacterial counts were reduced in conformity with the pH values. Reduced pH of fish samples may be ascribed to the solvability of CO_2 in the samples (Fan, Chi & Zhang, 2008; Hultmann & Rustad, 2004; Manju *et al*., 2007) or it is likely due to the fact that Lactic Acid Bacteria increases organic acids level. LAB dies under irradiation particularly at higher doses and a smaller level of lactic acid was produced (P>0.05). Also several factors like storage conditions and buffering potential of meat can be associated to the different pH of treatments (Pacheco-Aguilar, Lugo-Sanchez & Robles-Burgueno, 2000).

Value of TVB-N

TVB-N level in all samples increased gradually over storage period (P<0.05) (Figure2). The difference between treatment was significant (P<0.05). TVB-N values for 3kGy, 5kGy and 3kGy+ Tio_2 nanocomposite, were 23.73, 20.43 and 19.73 mg N/100g respectively. Tio_2 nanocomposite with gamma ray at 5 kGy showd the lowest level of TVB-N value during 20 days at 4°C. Results of irradiated samples were supported by (Khan, Banu, Hossain, & Hossain, 1997; Moini *et al*, 2009; Ahmed *et al*, 2009). There was no research report about TVB-N value in packaged food by Tio_2 nanocomposite. Ammonia and primary, secondary and tertiary amines are main components of fish TVB-N (Beatty, 1938; Manju *et al*., 2007). TVB-N is a product of bacterial spoilage and endogenous enzymes and is related to protein breakdown, the content is considered as a quality index for seafood products (Erkan, Ozden, Alakavuk, Yildirim, & Lnugur, 2006; Masniyom *et al*., 2005; Mendes & Goncalvez, 2008). The highest acceptable level is proposed to be less than 25 mg N/100 g in flesh (Kilincceker, Dogan, & Kucukoner, 2009). At the end of storage, Tio_2 nanocomposite and control samples were in below acceptable level, 46.13 and 25.61 mg N/100g respectively. TVB-N values in irradiated samples, was below the limit of acceptable quality limit (AQL) in our study during entire storage period. Production of TVB-N in samples was delayed significantly, comparing to the control samples. Tio_2 nanocomposite with gamma ray at 3 and 5 kGy led to rate of TVB-N formation during storage to be

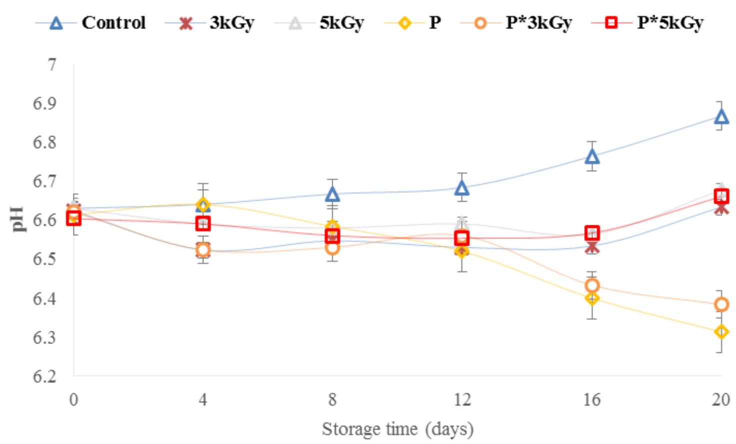

Figure 1. Changes in pH values of fish samples during 20 days at +4°C

diminished. This was done, reducing the initial levels of the common spoilage bacteria ($P<0.05$). The results obtained from Figure2 showed that as the irradiation applied dose is increased, the rate of TVB-N formation is reduced. It can be concluded that Tio_2 nanocomposite and gamma irradiation when are combined can inhibit the decomposition of fish fillets effectively regarding TVB-N.

Measuring Peroxide Value (PV)

One of the main factors in sensory deterioration of fish after death is lipid oxidation due to high levels of unsaturated fatty acid (Guillen & Ruiz, 2004). Polyunsaturated and monounsaturated fatty acids are abundant in rainbow trout (26 and 50%, respectively) and this fish is very sensitive to lipid oxidation (Kotakowska, Domiszewski, Kozlowski, & Gajowniczek, 2006). Figure3 depicts extent of lipid oxidation in the samples during storage. Treated groups were significantly different in PV compared to control samples ($P<0.05$). Irradiated samples had higher PV compared to control, irradiated samples plus Tio_2 nanocomposite and Tio_2 nanocomposite alone. Our results in irradiation effect on PV consistent with the results of Mohamed, EL-Mossalam, and Nosier (2009); Ahmed *et al* (2009); Snauwaert, Tobback, Maes, and Thyssen (1977); Al-Kahtani *et al* (1996). The best performance in primary lipid oxidation retardation was observed for samples treated with Tio_2 nanocomposite may be as a result of reducing the oxygen level in Tio_2 nanocomposites. Nasiri, Shariaty-Niasar, and Akbari (2012) concluded that almond packages reduce the oxygen permeability of Tio_2 nano-composites. Similar results for deceasing oxidation using hurdle technology in combination with irradiation have been reported by several authors (Fan, 2007, Trindade, Mancini, & Villavicencio ,2009).

Gamma irradiation results in oxidative stress and affects biomolecules. This is done through conformational changes, oxidation, covalent bonds break and free radicals formation (Cheftel, Cuq & Lorient, 1985). Molecular properties of the proteins and lipids could be modified by radicals like hydroxyl ($HO^{\bullet}$) and superoxide anion ($O^{2\bullet-}$) generated by radiation. Upper limit for the foodstuffs is considered PV value of 10-20 meq O_2 kg^{-1} oil (Huss, 1995). In our study, the PV of the control reached the upper limit of 20 meq O_2 kg^{-1} oil on day 8 while it took 12

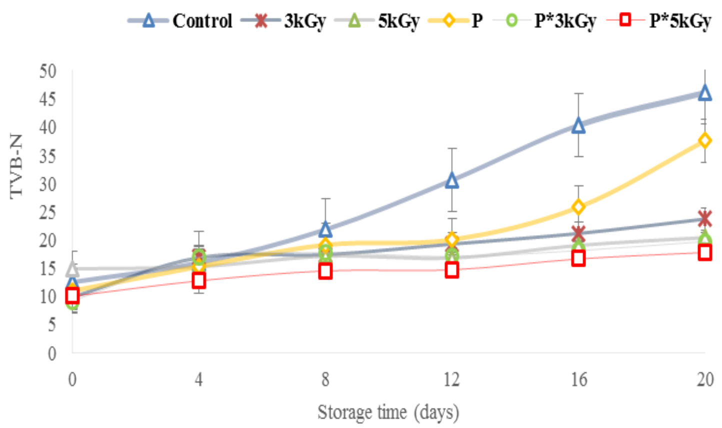

Figure 2. Changes in TVB-N values of fish samples during 20 days at +4°C.

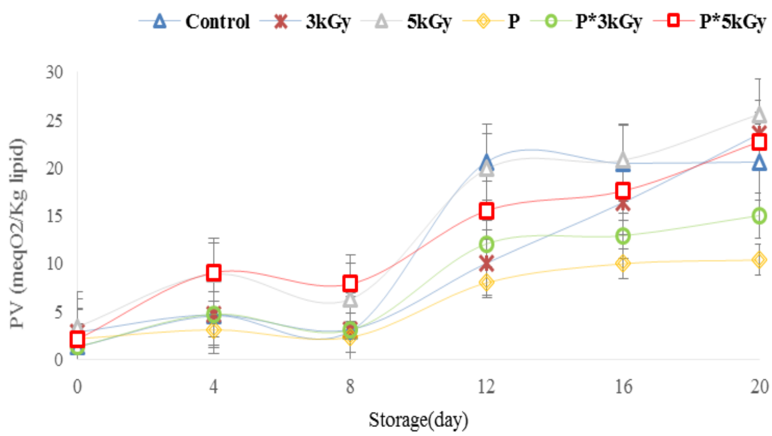

Figure 3. Changes in PV of fish samples during 20 days at +4°C.

days for the 5kGy irradiated sample and 16 days for the samples treated with Tio_2 nanocomposites and 5kGy to reach the same level. Therefore, packaging type has considerable effects during storage. It is seen the PV was increased only slightly in Tio_2 nanocomposites, likely as a result of the proper antioxidant response in Tio_2 nanoparticles. Further, nanocomposites have gas barrier properties attributed to modifications of the polymer chain flexibility and arrangement of the polymeric matrix itself, which ultimately reduces the penetration level in the nanocomposite and modifies the solubility parameters and the impermeable inorganic phase represents a physical barrier to diffusing molecules, which are forced to a more tortuous path through the polymer (Nasiri *et al*., 2012). The oxygen penetrates depending on oxygen concentrations in the packaging atmosphere temperature, pH, species and muscle type. However, the oxidative damage of irradiated fish must be investigated in further studies. Therefore, oxidation level can be increased in fish meat store in high-oxygen atmosphere but in samples treated by Tio_2 nano-composites, the oxidation is increased slightly. This is because of Tio_2 nanoparticles have antioxidant properties and the fact that nanocomposites have barrier quality.

TBARS changes

Thiobarbituric acid reactive substances (TBARS) is a product of peroxides degradation into aldehyde and ketones in second stage and is mostly used as a lipid oxidation indicator (Lindsay, 1991). TBARS value is an index of Malondialdehyde (MDA) concentration which is considered as the product of lipid oxidation. Figure 4 depicts TBARS Changes in samples. Generally, TBARS was increased in all samples in line with storage time augmentation ($P>0.05$) although did not show a consistent trend during refrigerated storage for both the control and irradiated groups. Our results support Yang *et al* (2014) who reported TBARS values increase for samples irradiated by electron beam and vacuum pack fish fillet. Fan *et al* (2008) concluded that the TBARS value of silver carp increased during storage. Al-kahtani *et al* (1996) and Mohamed, Mira and Abou-Zied (2008) reported similar results. The variations can be explained as a result of the different phases of decomposition of the peroxides and formation of carbonyls (Manjanaik, Kavya, Shetty, Somashekarappa, & Rajashekar, 2017). Significant differences ($P<0.05$) in the TBA values were found between the control, irradiated samples and Tio_2 nanocomposite samples during the storage period. Irradiated samples outperform Tio_2 nanocomposite sample producing higher TBARS positive correlations with the applied dose was resulted from TBARS levels. TBARS values were increased after irradiation was attributed to the lipid oxidation derived from free radicals. Polyunsaturated fatty acids are abundant in fish muscle lipid that makes it prone to oxidative reaction (Stammen, Gerdes & Caporaso, 1990). Therefore, Tio_2 nanocomposite delayed the oxidation of fillets by chelation of pro-oxidant metal ions. This supports O_2 exerting a great positive influence on lipid oxidation that extends shelf life.

Protein Oxidation of (Carbonyl Index)

Irradiation leads to chemical reactions of proteins that depend on several parameters including the amino acid composition, protein structure, and physical status, other substances in the food, and irradiation treatment and other hurdle technology. Proteins can be modified by compounds product of lipid oxidation that induces cross linking and undesirable changes in food properties can be resulted (EFSA[1], 2011). We assessed the protein oxidation based on the formation of protein carbonyl groups. Results showed a lower level of protein oxidation in case of Tio_2 nanocomposite treatments. Protein oxidation in meat during chill storage in high-oxygen atmospheres (70-80% O_2 and 20-30% CO_2) compared to storage of meat without oxygen was investigated by Marianne (2016). Protein oxidation was found to increase significantly in high-oxygen atmospheres compared to storage without presence of oxygen.

More carbonyl content during storage was observed for samples treated by 5 kGy and 3 kGy irradiation. Carbonyl groups can be formed by the side chains of amino acids, which may result in an increased susceptibility to protein aggregation, leading to a loss of quality (Stammen *et al*, 1990). Such carbonyl compounds formation is the result of oxidation catalyzed by metal ion (Levine, 1984).

In our study, the value of carbonyl index samples dramatically increased during storage period. The rate of increase was more pronounced in the samples treated at the higher dose irradiations ($P<0.05$). Proteins could have exposed to oxidative reactions time because of high concentration proteins and their proximity to hydroperoxides. Free radicals are products of lipid irradiation leading to form the carbonyls in which changes in food nutritional and sensorial specifications can be attributed.

Tio_2 nanocomposite exerts no effect on the formation of protein carbonyl groups during storage because the protective effect is dependent on the antioxidant activity of Tio_2 nanoparticles. The results obtained on lipid and protein oxidation suggested formation of protein carbonyl faster rate than secondary lipid oxidation product group formation in various samples during storage.

Sensory Analysis

Sensory variables of samples like; overall acceptability, texture, color and odor were shown in Table 1. All samples were initially fresh with sensory scores ranging 5- 4 suggesting excellent quality. The

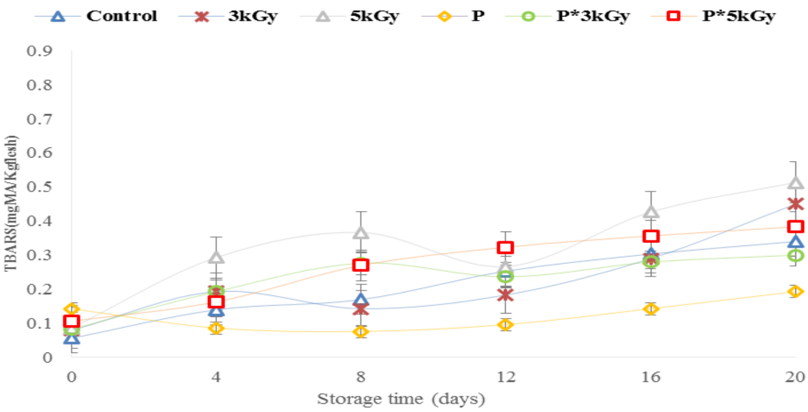

Figure 4. Changes in TBARS values of fish samples during 20 days at +4°C.

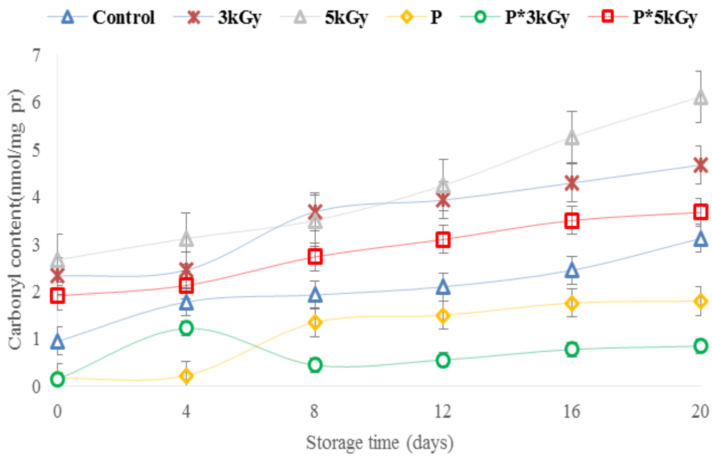

Figure 5. Changes in oxidation of protein-carbonyl content (nmol mg-1 protein) of refrigerated fish samples 20 days at +4°C. nmol mg^{-1} protein.

Table 1. Changes in sensory attributes scores of fish samples stored at +4°C.

	Days of storage(day)	0	4	8	12	16	20
Colour	Control	5.000±.00^{a}	±34.4 0.48^{c}	3.34±0.48^{c}	1.65±0.48^{c}	1.00±0.00^{c}	1.00 0.00^{b}
	P	0±00.5.00^{a}	4.9±0.55^{ab}	4.66±0.55^{a}	4.40±0.55^{a}	3.25 ±0.55^{c}	2.33 ± 0.55^{a}
	3kGy	0±00.5.00^{a}	±82.40.38^{ab}	4.82± 0.71^{a}	4.82± 0.71^{a}	4.00±0.38^{a}	3.82±0.38^{b}
	5kGy	0±00.5.00^{a}	4.80±0.55^{ab}	4.40± 0.55^{a}	4.40± 0.55^{a}	3.60±0.55^{b}	2.40± 0.55^{b}
	P*3kGy	0±00.5.00^{a}	0±00.5.00^{a}	4.40±0.55^{a}	4.40±0.55^{a}	4.00±0.00^{a}	3.75±0.00^{a}
	P*5kGy	0±00.5.00^{a}	4.80±0.55^{ab}	3.40±0.55^{b}	3.40±0.55^{b}	±34.2 0.48^{c}	1.18±0.38^{b}
Odour	Control	±00.50.00^{a}	4.750±.48^{a}	3.20±0.84^{c}	2.80±0.84^{c}	2.40±0.55^{c}	1.31 ± 0.47^{c}
	P	±00.50.00^{a}	4.400±.55^{b}	4.75±0.45^{a}	4.66±0.45^{a}	3.40±0.45^{b}	3.31±0.96^{a}
	3kGy	4.860±.00^{b}	±82.4 0.38^{a}	4.48±0.50^{a}	4.48±0.50^{a}	3.31±0.96^{b}	2.28±0.76^{bc}
	5kGy	4.86±0.00^{b}	4.75 ± 0.35^{b}	4.66±0.48^{b}	3.82±0.38^{b}	3.17±0.48^{b}	2.40±0.50^{bc}
	P*3kGy	5.00±0.00^{a}	4.91±0.00^{a}	4.82±0.48^{a}	4.66±0.48^{a}	4.48±0.50^{a}	3.40±0.45^{a}
	P*5kGy	±00.50.00^{a}	4.80±0.45^{ab}	4.00±0.45^{b}	3.80±0.45^{b}	3.60±0.55^{b}	2.00±0.00^{bc}
Texture	Control	±00.50.00^{a}	±00.50.00^{a}	3.20±0.55^{c}	3.20±0.55^{c}	1.60±0.55^{c}	1.17±0.38^{c}
	P	±00.50.00^{a}	4.00±0.00^{b}	4.80±0.45^{ab}	3.80±0.45^{b}	3.60±0.45^{b}	3.34±0.51^{b}
	3kGy	±00.50.00^{a}	4.80±0.45^{ab}	3.40±0.45^{b}	3.40±0.45^{b}	3.40±0.55^{b}	3.34±0.48^{b}
	5kGy	±00.50.00^{a}	4.80±0.45^{ab}	3.60±0.45^{b}	3.60±0.45^{b}	3.40±0.45^{b}	2.40±0.55^{c}
	P*3kGy	±00.50.00^{a}	±00.50.00^{a}	4.48±0.50^{a}	4.48±0.50^{a}	4.48±0.50^{a}	4.00±0.00^{a}
	P*5kGy	±00.50.00^{a}	4.80±0.45^{ab}	3.80±0.45^{b}	3.80±0.45^{b}	3.60±0.55^{b}	2.00±0.00^{c}
Overall acceptability	Control	±00.50.00^{a}	4.80±0.45^{ab}	3.00±0.00^{b}	3.00±0.00^{b}	2.40±0.55^{c}	1.16 ± 0.48^{c}
	P	±00.50.00^{a}	4.40±0.45^{ab}	4.60±0.55^{ab}	4.33±0.55^{ab}	4.00±0.55^{b}	3.31±0.69^{b}
	3kGy	00.0±00.5^{a}	±00.50.00^{a}	4.40±0.45^{ab}	3.4±0.45^{ab}	3.4±0.00^{ab}	2.34±0.48^{b}
	5kGy	0±00.5.00^{a}	4.40±0.45^{ab}	4.00±0.71^{a}	4.00±0.71^{a}	00.0±00.3^{b}	2.31± 0.47^{b}
	P*3kGy	±00.50.00^{a}	5.00±0.00^{a}	4.66±0.51^{ab}	4.48±0.51^{ab}	4.00 ±0.51^{a}	3.5±0.50^{a}
	P*5kGy	±00.5 0.00^{a}	4.40±0.45^{ab}	4.00±0.71^{a}	4.00±0.71^{a}	±00.30.00^{b}	1.66±0.51^{c}

a,b,c…Means with different small letters in the same row represent significant difference at $P<0.05$.

sensory score was gradually reduced over time. The control sample was assigned the lowest score. It was for 8th, 12th, 16th, and 20th day of storage that the control reached limit of sensory acceptability. Differences in overall acceptability of any samples were noticeable after irradiation ($P<0.05$). The best sensory quality was retained in the samples treated by 3 kGy and Tio_2 nanocomposite ($P<0.05$). Our results in irradiated samples confirm those reported by Sayed, Alam, Khan, Nilla and Mustafa (2013). Also, Ahmed *et al* (2009) estimated that sensory score went down gradually by irradiation in Chinese pomfret (*Pampus chinensis*) samples. It can be stated that with the increase of storage period, the organoleptic score were rapidly decreasing in the control fish than those of irradiated fish. The lowest scores regarding all assessed characteristics were assigned to sample irradiated at 5 kGy. This is likely because of higher lipid and protein oxidation. Sulphur-volatiles like dimethyl sulphide, dimethyl disulphide, and dimethyl trisulphide resulted in off-odor effect in the samples. Meat odor and color both affected negatively with increasing dose of irradiation. This is supported by results obtained from analyses performed on the microbial and chemical quality.

Microbial Assay

The results of total microbial count showed that microbial load of Tio_2 nanocomposite, irradiated samples (at 3 and 5 kGy) and combination of two technology, were significantly ($P<0.05$) lower than controls throughout the storage period (Figure 6). Irradiation and Tio_2 nanocomposite treatment was reported more effective than each treatment alone at decreasing microbial load.

The initial TVC ranged 3.54 - 8.75 at the end of storage in control group. TVC count at the end of storage for irradiated at 3 kGy, Tio_2 nanocomposite+3kGy, at 5 kGy and Tio_2 nanocomposite became 4.12, 2.27, 1.91 and 6.41 logs CFU/g respectively. Similar result reported by Oraei, Motalebi, Hoseini and Javan (2010); Mohamed *et al* (2009) and Moini *et al* (2009). They concluded that irradiation can reduce survival of TVC in fish fillet. TVC bacteria were not detectable at day 0 of refrigrator storage in Tio_2 nanocomposite irradiated by 5 kGy. Microbial load of irradiated Tio_2 nanocomposite at 5 kGy until the end of the storage was below detection level. Gamma irradiation mainly reduces TVC (Loaharanu, 1995). A 90% reduction of most vegetative cells can be accomplished with 1–1.5 kGy (Brewer, 2009). Morever, in this study Tio_2 nanocomposite caused a significant ($P<0.05$) reduction effect on microbial count. Irradiation and packaging treatment retained good quality in case of freshwater fish based on the recommended limits in literature.

The major group of aerobic microorganisms spoiling fresh fish in storage is Gram-negative psychrotrophic bacteria (PTC) at chilled temperatures (Gram & Huss 1996; Sallam 2007). PTC count increased significantly in control and Tio_2 nanocomposite samples from 0 day and after 10 days for irradiation treatment at 3 kGy and Tio_2 nanocomposite+ 3kGy. Results revealed lower PTC in the samples treated by irradiation and Tio_2 nanocomposite. Furthermore the results indicated that irradiated at 5 kGy and 5 kGy+Tio_2 nanocomposite samples are highly efficient inhibiting PTC bacterial growth. The result of Tio_2 nanocomposite effect on PTC bacteria count of groups are consistent with the results of another researcher (Bodaghi *et al*, 2013) and irradiation effect (Moini *et al*, 2009; Oraei *et al*, 2010).

LAB is not detectable throughout the storage, creating spoilage microflora along with lower numbers of *Enterobacteriaceae* which are not considered as belonged to the aquatic environments. LAB counts of the control increased slightly by the end of storage ($P<0.05$). LAB bacteria of irradiation and Tio_2 nanocomposites with more antibacterial effect was lower than control sample of rainbow trout fillet ($P<0.05$). LAB bacteria in irradiated samples at 5 kGy and Tio_2 nanocomposites+5 kGy were not detectable. Riebroy *et al* (2007) reported these results for irradiated Som-fug produced from bigeye snapper. In agreement our result, Liu and Yong (2003) demonstrated antibacterial effect of Tio_2 nanocomposite on *Lactobacillus helveticus*. The nanocomposite packaging conditions affected shelf life of samples. On the other hand, gram-positives like LAB and *Micrococcus* are more irradiation sensitive than gram-negative bacteria (Lebepe, Molins, Charven, Farrar, & Skowronski, 1990).

Figure6 depicts results for *Enterobacteriaceae* with significant differences between all treatments. Highest microbial load was recorded in control sample and there were no bacteria in the sample treated by Tio_2 nanocomposite when treated with combined 5 and 3 kGy gamma irradiation. It indicates conservation potential of technologies. Tio_2 nanocomposite effect on bacteria has shown in some researches. (Hashemabad, Shabanpour, Aziz, Ojagh & Alishahi, 2017; Bodaghi *et al* 2013). Antibacterial mechanism of Tio_2 is correlated to hydroxyl radicals and reactive oxygen species generated on the illuminated Tio_2 surface. It plays an effective role in inactivating microorganism. The hurdle technology of preservation; Tio_2 nanocomposite and gamma irradiation, extends the shelf life of refrigerated rainbow trout.

Microorganisms like bacteria, yeasts, and molds are affected by irradiation. Irradiation generates lesions in the cell genetic material and causes it to be unable in carrying out the biological processes. The charged particles generated from irradiation can break deoxyribonucleic acid (DNA) of bacteria. Therefore, irradiation can be responsible for reducing initial microbial level and can retard microbial growth of

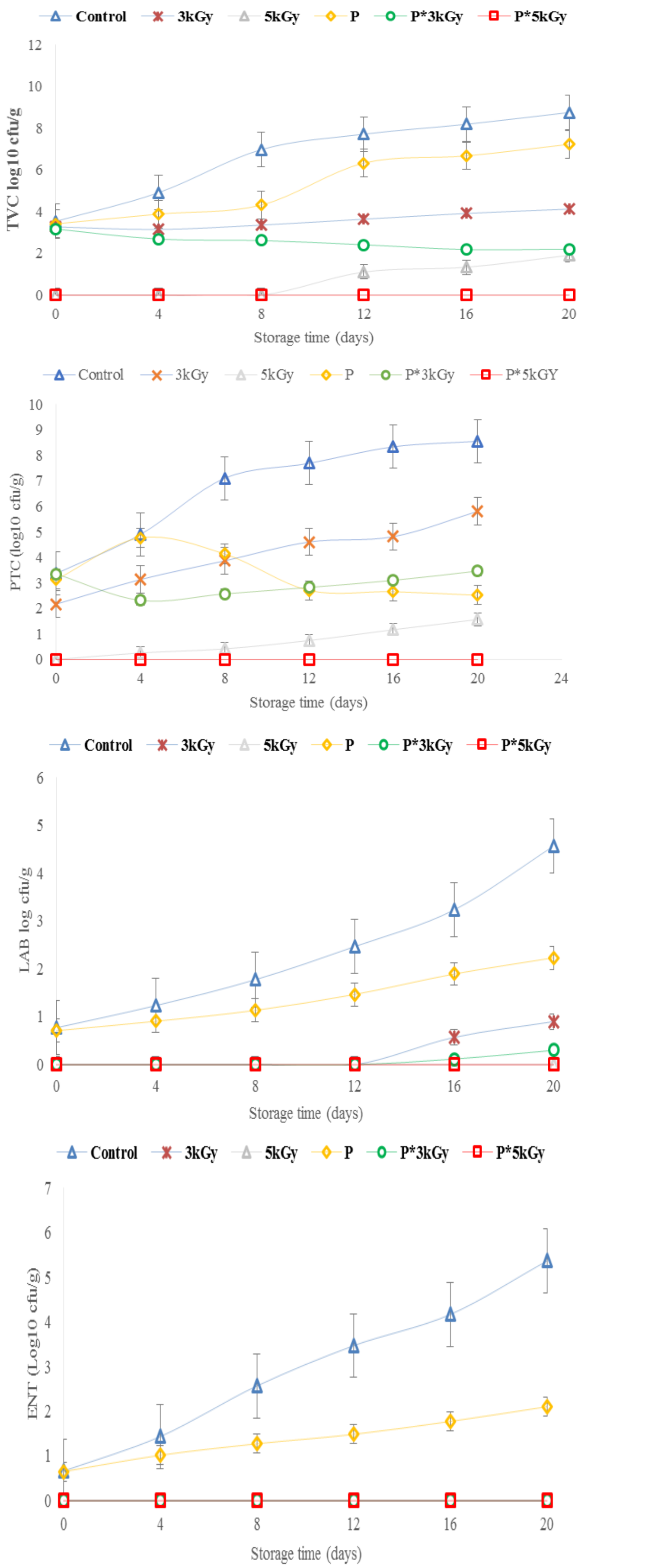

Figure 6. Changes in microbial load ((TVC) (PTC), (LAB), (ENT)) of fish samples during 20 days at +4°C SE standard error significant differences (P<0.05).

fish fillet in storage.

Low-dose (0.5–3 kGy) irradiation can extend the shelf life of fishery products. This is done by killing bacteria while nutritional value or quality of products is maintained (Venugopal, Doke, & Thomas, 1999). Meanwhile, Tio_2 nanocomposite has more advantages because it reduces the extent of product oxidation. Furthermore, chilling storage is an effective means of preserving seafood products that can be protected effectively and stops bacteria growth and chemical reactions.

Conclusions

Some researchers predict that irradiation may become important well in improving the hygienic status of lumpy, solid foods like meat or fish, as is heat pasteurization of liquid foods like milk. Moreover, future researches can deal application of nanocomposites to improve barrier properties. Our results revealed that Tio_2 nanocomposite is a high potential film to be used in the active food packaging industry, because it is capable to extend shelf life of highly perishable foods like fish products. This study revealed that a mixture of treatments (irradiation at 3 kGy and Tio_2 nanocomposite) was effective in extending shelf life of refrigerated rainbow trout fillet by maintaining chemical, microbial and sensory characteristics throughout storage. This treatment could extend trout fillet shelf life during 20 days at 4°C without any significant loss of texture and nutritive of fish. The current study showed the synergistic effect of two preservation methods, Tio_2 nanocomposite lead to reduce the oxidation effect of irradiation and support antimicrobial effect of irradiation in low dose, extending the shelf-life of rainbow trout fillet. Finally, it can be concluded that the combination of aforementioned methods is more effective than single method that can be applied for the long term preservation of fish meat and other food.

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