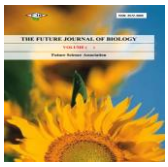

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An Innovative Methods for Preserving Tilapia (*Oreochromis niloticus*) Fish Fillets: Gamma Irradiation with Bioactive Gelatin Coatings

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Future Science Association

Available online free at www.futurejournals.org

Print ISSN: 2572-3006

Online ISSN: 2572-3111

DOI: 10.37229/fsa.fjb.2025.05.15

Received: 14 April 2025
Accepted: 10 May 2025
Published: 15 May 2025

Abstract: The main issues with seafood marketing and distribution are its high perishability, which is mostly caused by infection from harmful bacteria and spoiling. Using one or more treatments to reduce, destroy, or inhibit microbial contamination may result in effective preservation techniques. Utilizing fish like tilapia to make nutritious fillets and other waste products with economic and nutritional value is a promising industry for Egypt and the rest of the globe. Processing tilapia could yield significant quantities of by-products each year. This study examined the effects of low-dose gamma-irradiation and an edible coating made of bioactive gelatin that was derived from the skin of Nile tilapia (*Oreochromis niloticus*) on the chemical, microbiological, and sensory qualities of tilapia fillets that were kept at 4 ± 1 ℃. Gelatin from tilapia skin was removed and combined with essential oils of cumin, garlic, and chitosan to create a 6% (w/v) coating. Fillets were submerged in the coating solution, drained, and then wrapped before being exposed to γ-irradiation at 0, 1, 2, or 3 kGy. Samples were examined for total volatile basic nitrogen (TVB-N), trimethylamine-nitrogen (TMA-N), thiobarbituric acid (TBA), pH, total bacterial counts (TBC), mold and yeast, psychrophilic bacteria, *Staphylococcus aureus, Escherichia coli,* and sensory characteristics (texture, appearance, and odor) at 0, 4, 8, 12, 16, 20, and 24 days. The findings demonstrated that by day 12, the untreated control fillets had achieved spoiling limits (TVB-N ≥ 35 mg/100 g; TMA-N ≥ 10 mg/100 g; TBA ≥ 4.5 mg MDA/kg; TBC ≥ 10^6 CFU/g). However, γ-irradiation at 1, 2, and 3 kGy did not affect sensory scores (staying above 7 on a 9-point hedonic scale), extending the chemical and microbiological acceptability to days 16, 20, and 24, respectively. The 3 kGy treatment produced the optimum pH and lipid-oxidation levels throughout storage and the largest reduction in microbial loads, making psychrophilic bacteria, *Staphylococcus aureus*, and *E. coli* undetectable right after treatment. It could be concluded that using the dose of 3 kGy gamma-irradiation treatment in conjunction with a tilapia-skin gelatin bioactive coating (6 %) provides a promising method to lower post-harvest losses in aquaculture processing by successfully extending the refrigerated shelf life of tilapia fillets up to 24 days while maintaining their chemical integrity and sensory quality.

Key words: Gelatin, Tilapia, gamma rays, Edible coating, shelf life, and quality attributes.

1. Introduction

Fish and seafood preservation is a crucial concern for the food industry, as consumers demand products that are fresh and minimally processed **(Marzieh *et al*, 2019**). Fish and seafood are excellent culture media for microorganisms due to intrinsic and extrinsic factors, such as high nutritional composition, water content, and pH, which favor microbial growth, **t**hus, the fishing industry has great difficulty in maintaining fish and seafood quality, often due to the considerable distances between consumers and harvesting or production areas, offering opportunities for microbial growth and recontamination **(Skara *et al.,* 2012**). In addition to contamination by natural microbiota, fish and seafood can be easily contaminated during processing, both by spoilage microorganisms and by important pathogens that cause foodborne outbreaks, such as *Escherichia coli*, *Salmonella* spp and Staphylococcus aureus (**sheng and wang, 2021**).

Head, Skin, bones, fins, and scales as by-products from fish account for 30 % to 70% of the total weight of some fish **(Pearubia *et al*., 2023)** The manufacturing of marine by products is now getting prominence due to the abundance of fish skins and bones **(Ahmed *et al*., 2021).** Gelatin is a denatured fibrous protein that has been partially hydrolyzed from collagen The main commercial Gelatins are derived from pig skin and collagen (46%), mammalian skin and bones (29%), and certain other source materials (1.5%) **(GME, 2020**). When compared to other sources, pig's gelatin is desirable due to its low cost and remarkable functional as well as physical qualities but with limited use in some parts of the world. Recently; gelatin generated from fish and marine resources is in high demand **(Grand View Research, 2020**). The avoidance of infections and the fact that it is permissible in some counties are two advantages of marine gelatin. Recently, there has been a lot of interest in the use of gelatin in the biofilm industries. Because of its remarkable film-forming properties, gelatin is regarded as one of the most advanced bio-polymers to be incorporated into biofilm production. This property enables it to function as film for packaging surface, protecting food from excessive light, heat, and oxygen. The two types of gelatin-based coating that could be developed are active and smart edible coating. In order to maintain food quality, active packaging is designed to extend shelf life, improve safety, or improve sensory characteristics **(Xylia *et al*., 2022)**. While smart packaging is a type of packaging that monitors the packed food state in order to deliver food quality information to producers, retailers, and customers. Gelatin is widely utilized as an antioxidant barrier, antibacterial prevention, and lipid oxidation protection. Furthermore, there is a growing demand for fresh fish fillet packaging. Packing might delay the degradation of fresh fish fillets during storage and transport. In general, packaging and coating with different materials has been widely used is required to protect food products from pathogens and extend the shelf life **(Abdulla *et al*., 2023**). One method is to apply a coating or film prepared using a gelatin gel that is stable at cold temperatures, which is an excellent method of coating preparation. The characteristics of gelatin molecules make this substance particularly well suited to the making of flexible coatings and films, while the remaining properties will vary depending on gelatin type, coating composition, and application procedures **(Sobral and Habitane, 2001 and Sobral *et al*., 2001**). Fish skin gelatin is a realistic solution to the more widely used mammalian gelatin for coating seafood products. The properties of fish gelatin vary depending on the raw material, i.e. the source species and processed parts of the body, as well as the production process. As a result, a combination of fish gelatin and chitosan, both derived from marine sources, appears to be particularly suitable for use in the production of seafood items **(Gomez-Guillen *et al*., 2000 and Gomez-Guillen *et al*., 2002). Mohamed, *et al.* (2022**) indicated that there are large quantities of tilapia and catfish by-products to be used to produce healthy fish fillets and different nutrient materials with good nutritional quality which attracts business for both tilapia and catfish in Egypt. Limited studies have been found on utilizing this fish gelatin as an edible coating for fish fillets preservation.

Therefore, the objective of the present study was to (i) extract the gelatin from Egyptian Fish Tilapia *(Oreochromis niloticus)* and measure different properties of the extract. (ii) The extracted gelatin was used as an edible coating either alone or in combination with chitosan; garlic oil and cumin essential oils, to extend the shelf life of fish fillets during cold storage. (iii) the present study was to evaluate the effects of gamma irradiation on the microbiological, chemical, and organoleptic qualities of Tilapia fish fillet.

2. Materials and Methods

2.1. Fish gelatin extraction

Fish gelatin was extracted based on a reported method **(Arpi *et al*., 2016 and Apri and Novita, (2018).**

2.2. Fish samples preparation

Fresh Tilapia fish *(Oreochromis niloticus)* were obtained from the production ponds in Central Laboratory for Aquaculture Research, Abbassa, Abu-Hammad, Sharkia (Egypt) and immediately transported under refrigerated conditions (4°C) to the laboratory. The whole Bolti fish were immediately washed by tap water to remove the surface slime, dirt, then the fish were filleted (skin was removed from the fillet) and washed again with tap water to remove any residual blood. The fillets were trimmed, cleaned with distilled water

2.3. Gelatin-Chitosan coating solution preparation

The gelatin (6%, w/v) coating solutions were made using the procedure described by **(Gómez-Estaca *et al*.,2009**) with some modifications, by dissolving (6 g) of tilapia skin gelatin in 100 mL of distilled water and then heated to 70 °C for 30 min, while stirring continuously. 1.5 g of cumin and garlic essential oil in a ratio of 25% by volume per mass of gelatin was added and then, 0.22 g of egg albumin in a ratio of 15% by volume per mass of essential oil was added as a stabilizer. Chitosan solution was prepared by dissolving 2% (w/v) chitosan in 1% (v/v) acetic acid, and then mixture was stirred for 24 h. At room temperature, pH value of chitosan solution was adjusted to 6.0 and was then filtered to remove residues. As a plasticizer, glycerol (1.5 g) was added in a 25% by volume per mass of gelatin ratio, and the solution was warmed and disturbed at 45 °C for 15 min. A gelatin chitosan-solution with a ratio (1:10) 2% chitosan and 6% gelatin was prepared and heated at 45°C for 30 min with continuous stirring. Individually, the fillets were immersed in gelatin edible coating solution for 1 min. After draining (to remove the excess coating), the fillets were suspended for 1 min before being individually packaged in a polyethylene bags, wrapped in retractile film, and stored at cold storage 4 ºC ± 1. Tilapia fillets were divided into four experimental groups and subjected to different doses of gamma irradiation (0 (control); 1;2 and 3 kGy):

1) Control (Fillets with gelatin bioactive coating without gamma irradiation).
2) Fillets with gelatin bioactive coating subjected to gamma irradiation by dose 1 kGy
3) Fillets with gelatin bioactive coating subjected to gamma irradiation by dose 2 kGy
4) Fillets with gelatin bioactive coating subjected to gamma irradiation by dose 3 kGy

Tilapia fillets were randomly removed and analyzed after 0, 4, 8, 12, 16,20 and 24 days of during cold storage at 4 ±1ºC.

2.4. Irradiation process

The prepared Tilapia fillets were placed in sterile polyethylene bags either treated with lemon grass or not treated and subjected to gamma irradiation at a dose of Tilapia fillets was divided into four experimental groups and subjected to different doses of gamma irradiation (0 (control); 1;2 and 3 kGy using a Cobalt 60 Russian Chamber (dose rate 272 Gy / h) belonging to Cyclotron Project, Nuclear Research Center, Egyptian Atomic Energy Authority, Cairo, Egypt**.**

2.5. Physiochemical quality attributes for coated tilapia fillets

The total volatile basis nitrogen and trimethyleamine were determined according to the method of **AMC (1979).** Thiobarbituric acid was determined according to the method described by **Hekmat and McMahon (1997),** the pH was assessed using a pH meter as described by **Abdel-Naeem *et al*. (2021).**

2.6. Microbiological quality

Total bacterial (TBC); and psychrophilic bacterial counts were determined as recommended by the **APHA (1992).** Mould and yeast counts were determined as described by **Oxoid Manual (2006**) using Oxytetracycline Glucose Yeast Extracts Agar (Oxoid CM 545) medium *Stahylococcus aureus* was counted on Baird parker agar media according to **Oxoid manual (2006).** The count of *Escherichia coli* was determined on Macconkey agar medium after incubation at 37 ºC for 20-24 h as described by **Feng *et al.* (2002)**. The results were calculated as log $_{10}$ cfu/g. All the biosafety instructions / precautions were implemented and strictly followed all over the experimental work procedures. At the end of the experiment all bacterial cultures, isolate and remains of potential biological hazards were safely disposed via autoclaving

2.7. Sensory Evaluation

Sensory attributes, including appearance, odor, and texture, were evaluated by a panel of trained judges using a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely). The samples were evaluated both at the initial time (zero time) and throughout the cold storage period at 4±1°C. Samples were rejected based on the total bacterial count exceeded 1×10^6 or if unacceptable changes in appearance or odor were detected. **(NFSA,2021)**

2.8. Statistical analysis

One-way analysis of variance (ANOVA) was used and means comparison was performed by Duncan's multiple range test **(Steel and Torrie, 1980**). Statistical analysis was carried out using SPSS statistic program (Version 10.0) for Windows (SPSS Inc. Chicago, IL).

3. Results and discussions

Quality Characteristics and Microbiological Aspects of gelatin bioactive coating Tilapia fillets subjected to (0;1;2 and 3 kGy) during cold storage

The Egyptian standards (EOS) (2005 a) recommended that the value of 35 mg TVBN/100 gm fish meat is the limit of acceptance for fishes. TVBN of fish samples were 9.82 ±0.036; 9.75 ±0.02a; 9.72± 0.012; and 9.67±0.014 mg/100g for gelatin bioactive coating Tilapia fillets treated with gamma irradiation doses (0; 1;2 1and 3 kGy, respectively. (Table 1), this means that the fish was in good quality. There was a significant increment ($p < 0.05$) in TVB-N value of all samples under investigation during cold storage period4 ±1 °C , wherever it could be notice that irradiation reduce the rate of increment reached to the maximum limit of TVBN at 12; 16;20 and 24th day of cold storage for gelatin bioactive coating Tilapia fillets treated with gamma irradiation doses (0; 1;2 1and 3 kGy,, the increase of TVBN contents might be due to the breakdown of nitrogenous substances due to microbial and enzymatic activity **(Özogy *et al.*, 2006).**

Table (1). Effect of gamma irradiation at dose 0,1, 2 and 3 kGy and gelatin bioactive coating on the TVBN mg/100g of tilapia fillets during cold storage at 4 ±1°C

Storage period (day)	Control	Gelatin Bioactive Coating and Gamma rays (kGy)		
		1	2	3
0	9.82 ±0.036 aC	9.75 ±0.02aBC	9.72±0.012 aAB	9.67±0.014aA
4	17.45 ± 0.026 bD	14.42 ±0.032bC	11.84±0.026 bB	10.49±0.026bA
8	28.72± 0.017 cD	20.82 ±0.017cC	15.19±0.02 cB	12.86±0.03cA
12	40.43 ±0.008 dD ®	26.65 ±0.023dC	19.49±0.032 dB	16±0.023dA
16		38.81 ±0.021eC ®	28.67±0.028 eB	21.94±0.026eA
20			36.48±0.012 fB ®	26.72±0.02fA
24				35.66±0.04g ®

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Table (2) show Effect of gamma irradiation at dose 0,1, 2 and 3 kGy and gelatin bioactive coating on the TVBN mg/100g of tilapia fillets during cold storage at 4 ±1ºC, the results indicated that at the beginning of cold storage at 4 ±1ºC the TMA-N values of Tilapia fillets coated with gelatin bioactive compound and subjected to gamma irradiation with (0; 1; 2 and 3 kGy) were 4.58±0.029; 4.51±0.012; 4.49±0.029 and 4.47±0.026 mg/100 g, respectively, these results within the permissible level (10 mg/100g) recommended by **Egyptian standards (EOS, 2005)** it is apparent that significant gradual increases in TMA contents were observed in all samples during cold storage but also at lower rate of increase with increasing the irradiation dose. The levels of TMA contents exceeded the maximum acceptable level (10 mg/100g) according to the Egyptian standards **(EOS, 2005)** on day 12, 16, 20, and 24th for of Tilapia fillets coated with gelatin bioactive compound and subjected to gamma irradiation with (0; 1; 2 and 3 kGy) (the time of the rejection of samples), These results clearly showed that irradiated has an important effect of lessening volatile amine contents, which may be explained by the diminished total viable count and the account of surviving flora of the irradiated fish in generally non-proteolytic. Therefore, the production of free volatile amines is reduced relative to that of non-irradiated fish.

Table (2). Effect of gamma irradiation at dose 0,1, 2 and 3 kGy and gelatin bioactive coating on the TMAN mg/100g of Tilapia fillet during cold storage at 4 ±1ºC

Storage period (day)	Control	Gelatin Bioactive Coating and Gamma rays (kGy)		
		1	2	3
0	4.58±0.029aB	4.51±0.012aAB	4.49±0.029aAB	4.47±0.026aA
4	5.86±0.02bC	4.97±0.029bB	4.73±0.020bA	4.67±0.023bA
8	8.92±0.024cD	6.67±0.020cC	5.43±0.023cB	5.03±0.045cA
12	12.78±0.028dD ®	8.1±0.014dC	6.57±0.014dB	6±0.017dA
16		11.02±0.039eC®	7.96±0.020eB	7.11±0.020eA
20			10.8±0.023fB®	8.88±0.020fA
24				10.28±0.020g®

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EOS (2005) recommended that the value of TBA values must not be more than 4.5 malonaldehyde /Kg fish meat. As shown in Table (3) similar levels of TBA were observed for all samples at zero time of storage which were 0.198±0.006; 0.25±0.023; 0.38±0.011 and 0.49±0.026 for Tilapia fillets coated with gelatin bioactive compound and subjected to gamma irradiation with (0; 1; 2 and 3 kGy), respectively, similar results were obtained by **Mohamed *et al,* (2009)** which found that Irradiated fish meat produce higher TBA than non-irradiated samples and the amounts of TBA showed positive correlations with the applied dose. Cold storage at 4 ±1ºC significantly increased the contents of TBA in all samples but at lower rate of increase with increasing of the irradiation dose. The levels of TBA exceeded the maximum acceptable level reported by the **Egyptian standards (EOS, 2005),** (4.5 mg malonaldehyde/Kg) on days 12, 16, 20, and 24 of cold storage for Tilapia fillets coated with gelatin bioactive compound and subjected to gamma irradiation with (0; 1; 2 and 3 kGy), respectively.

The pH values for the fish range between 6.0 and 6.8, according to the species, diet, catching stress level, season, and muscle type **(Khalafalla *et al*., 2015).** The changes in pH values were determined in all sample's during cold storage at 4± 10C (table 4), Samples of Tilapia fillets coated with gelatin bioactive compound and subjected to gamma irradiation with (0; 1; 2 and 3 kGy), had similar initial pH value at Zero time of cold storage. Cold storage, however induced slight gradual increase in the pH values for all samples under study. The high pH value with prolonged storage time may be due to the decomposition of proteins and endogenous or microbial enzymes convert additional nitrogen-containing components to volatile bases **(Li *et al*., 2012a,b).** However, the rate of increase was lower with increasing irradiation doses as shown in (table 4). Nearly similar findings were obtained by **Mohamed *et al.* (2009).**

Table (3). Effect of gamma irradiation at dose 0,1, 2 and 3 kGy and gelatin bioactive coating on the TBA mg/kg of Tilapia fish fillet during cold storage at 4 ±1ºC

Storage period (day)	Control	Gelatin Bioactive Coating and Gamma rays (kGy)		
		1	2	3
0	0.198±0.006aA	0.25±0.023aA	0.38±0.011aB	0.49±0.026aC
4	1.65±0.023bA	1.76±0.023bB	1.88±0.014bC	1.26±0.023bD
8	2.99±0.026cA	3.15±0.023cB	2.45±0.026cC	2±0.017cD
12	4.62±0.017dD®	4.21±0.026dC	3.15±0.021dB	3.03±0.017dA
16		4.97±0.020eC®	4.35±0.023eB	3.9±0.023eA
20			5.2±0.020fB®	4.43±0.021fA
24				5.55±0.029g®

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Table (4). Effect of gamma irradiation at dose 0,1, 2 and 3 kGy and gelatin bioactive coating on the pH of Tilapia fish fillet during cold storage at 4 ±1ºC

Storage period (day)	Control	Gelatin Bioactive Coating and Gamma rays (kGy)		
		1	2	3
0	6.35±0.005 aD	6.29±0.008 aC	6.24±0.00 aB	6.17±0.012 aA
4	6.42±0.008 bD	6.34±0.008 bC	6.29±0.01 bB	6.21±0.012 bA
8	6.61±0.029 cC	6.41±0.012 cB	6.36±0.012 cB	6.28±0.015 cA
12	6.79±0.018 dD®	6.47±0.012 dC	6.41±0.014 dB	6.35±0.005 dA
16		6.52±0.00 eB®	6.51±0.00 eB	6.46±0.012 eA
20			6.63±0.008 fA®	6.61±0.0083 fA
24				6.68±0.008 gA®

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Total bacterial counts (TBC) of treated tilapia fillets during 24 days of refrigerated storage is presented in Table (5). The initial total bacterial count of control tilapia fillets in this study was 4.71±0.026 log CFU/g wherever samples treated with gamma irradiation (1;2 and 3 kGy) had lower TBC specially by increase the dose which were reached to be undetected at dose 3 kGY. These results are in agreement with those obtained by **Moslemi and Naderi (2022)** which indicated that the unirradiated Salmon samples had an initial TBC count of 2.8 log CFU microorganisms per gram, in samples irradiated to 2, 3 and 4 kGy, the initial bacterial count was reduced to 1.11, 0.5, and 0.1 log CFU organisms per gram, respectively .Regarding, cold storage period there was a significant increment during cold storage period reaching to the maximum level of TBC at 12;16; 20 and 24 th day, for Tilapia fillets coated with gelatin bioactive compound and subjected to gamma irradiation with (0; 1; 2 and 3 kGy), respectively.

Table (5). Effect of gamma irradiation at dose 0,1, 2 and 3 kGy and gelatin bioactive coating on the total bacterial count (TBC) of Tilapia fillets during cold storage at 4 ±1°C

Storage period (day)	Control	Gelatin Bioactive Coating and Gamma rays (kGy)		
		1	2	3
0	4.71±0.026aC	3.22±0.023aB	2±0.020aA	ND
4	5±0.051bC	3.29±0.040aB	2.21±0.029bA	ND
8	5.88±0.017cD	4.12±0.027bC	3±0.023cB	1.81±0.024aA
12	6.65±0.020dD®	5.66±0.014cC	4.24±0.023dB	2.13±0.025bA
16		6.54±0.026dC®	5.35±0.026eB	3.57±0.055cA
20			6.29±0.031fB®	4.96±0.023dA
24				6.11±0.035e®

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The changes in psychrophilic bacteria count of Tilapia fillets coated with gelatin bioactive compound and subjected to gamma irradiation with (0; 1; 2 and 3 kGy in Table (6) it could be noticed that the samples after subjected to gamma irradiation with different doses at the initial time of cold storage, the psychrophilic bacterial count in the control sample was recorded as 2.84±0.020 log CFU/g, whereas all irradiated samples showed no detectable bacterial counts (0.00 ± 0.000 log CFU/g). This significant reduction suggests that gamma irradiation effectively eliminated psychrophilic bacteria immediately after treatment. The total $\log_{10}$ psychrophilic counts for all treatments of Tilapia fillets either control or irradiated samples with 0; 1;2 and 3 kGy, respectively, were significantly increased during the cold storage period reached 5.24±0.020; 4.27±0.018; 4.55±0.024 and 3.92±0.014 $\log_{10}$ CFU/g at 12; 20; 24 and 24th day of storage period, respectively. **Moslemi and Naderi (2022)** found that Pseudomonaceae count of Salmon fish was not detected immediately after irradiation, and increased during the cold storage time but it was greater in unirradiated samples ($P< 0.05$) and irradiation with 3 and 4 kGy reduced Pseudomonaceae counts as there were not significant differences between 3 and 4 kGy.

Table (6). Effect of gamma irradiation at dose 0,1, 2 and 3 kGy and gelatin bioactive coating on the Psychrophilic bacteria log $_{10}$ CFU/g of Tilapia fillet during cold storage at 4 ±1°C

Storage period (day)	Control	Gelatin Bioactive Coating and Gamma rays (kGy)		
		1	2	3
0	2.84±0.020 a	ND	ND	ND
4	3.12±0.012 b	ND	ND	ND
8	4.35±0.026 cB	1.14±0.020 aA	ND	ND
12	5.24±0.020 dC	1.76±0.017 bB	1.16±0.008 aA	ND
16		4.27±0.014 cB®	2.36±0.020 bC	1.38±0.02 aA
20			4.55±0.011cB®	2.38±0.011 bA
24				3.92±0.014 cA®

Means with the same capital letter in the same rows are not significantly different, Means with the same small letter in the same columns are not significantly different**.** ® Rejected

Table (7). Effect of gamma irradiation at dose 0,1, 2 and 3 kGy and gelatin bioactive coating on the Mold and Yeast counts log $_{10}$ CFU/g of Tilapia fillet during cold storage at 4 ±1ºC

Storage period (day)	Control	Gelatin Bioactive Coating and Gamma rays (kGy)		
		1	2	3
0	2.37±.021 a	1.18±.012 a	0.00±.000 a	0.00±.000 a
4	2.72±.009 b	1.34±.012 b	1.14±.021 b	0.00±.000 a
8	3.54±.026 c	2.47±.026 c	1.20±.020 b	1.11±.042 b
12	4.40±.023 d ®	3.76±.021 d	1.57±.046 c	1.19±.009 c
16		4.00±.020 e	2.44±.018 d	1.51±.022 d
20		4.67±.026 f ®	3.88±.032 e	1.88±.010 e
24			4.21±.026 f ®	3.56±.009 f ®

Means with the same capital letter in the same rows are not significantly different, Means with the same small letter in the same columns are not significantly different. ® Rejected

The effects of different gamma irradiation dosages (1, 2, and 3 kGy) combined with a gelatin bioactive coating on mold and yeast counts (log_{10} CFU/g) in Nile tilapia fillets throughout a 24-day period of refrigeration at 4 ± 1°C are shown in Table (7). Mold and yeast count in the control group (gelatin-coated fillets without irradiation) increased gradually and significantly during cold storage, going from 2.37 ± 0.021 log_{10} CFU/g at day 0 to 4.40 ± 0.026 log Day 12 CFU/g,

The use of gamma irradiation in combination with gelatin bioactive coating, on the other hand, dramatically decreased microbial multiplication; the effect became more noticeable as the dose increased. The counts of the 1 kGy-treated fillets remained below 4 log_{10} CFU/g until day 16, and by day 20, they significantly increased to 4.67 ± 0.026 log_{10} CFU/g. Microbial growth was further delayed by the 2 kGy treatment; counts remained below 3 log_{10} CFU/g until day 16 and increased to just 4.21 ± 0.026 log_{10} cfu /g by day 24. The highest gamma irradiation dose (3 kGy) had the strongest antibacterial effect, mold and yeast were not detected until day 4 and continued at very low levels throughout the 24-day storage period, reaching just 3.56 ± 0.009 log_{10} CFU/g by the end. This may be due to Gamma irradiation destroys microbial DNA and metabolic activity, and when combined with a gelatin-based bioactive coating (presumably enhanced with antimicrobial substances like essential oils or polyphenols), it offers a twofold barrier to contamination. The combination of gamma irradiation, especially at 2 and 3 kGy, with gelatin bioactive coatings is very successful in lowering the growth of mold and yeast in Nile tilapia fillets kept in cold storage. The synergistic antibacterial action of radiation and gelatin coatings was also demonstrated by **Mingkang *et al.* (2011).**

Table (8). Effect of gamma irradiation at dose 0, 1, 2 and 3 kGy and gelatin bioactive coating on the *Staphylococcus aureus* log $_{10}$ CFU/g of Tilapia fillet during cold storage at 4 ±1ºC

Storage period (day)	Control	Gelatin Bioactive Coating and Gamma rays (kGy)		
		1	2	3
0	1.38±0.026a	ND	ND	ND
4	1.78±0.029b	ND	ND	ND
8	2.14±0.005c	ND	ND	ND
12	2.54±0.024d®	ND	ND	ND
16		ND®	ND	ND
20			ND®	ND
24				ND®

Means with the same capital letter in the same rows are not significantly different, means with the same small letter in the same columns are not significantly different**.** ® Rejected

Gamma irradiation with different doses 1;2 and 3 kGy in (Table 8) completely destroy *Staphylococcus aureus* count and remain not detected during cold storage period these results were in good agreement with those obtained by **Hossain *et al*. (2008) and Mohamed *et al.* (2023)** who stated that gamma irradiation as a cold process is an attractive technology for solving the problem arising from fish-borne pathogens. whereas the *Staphylococcus aureus* count for control samples significantly increased during cold storage reached to it maximum limit at the day 12th of cold storage, according to Egyptian standards **(EOS, 2005),** the acceptability of *Staphylococcus aureus* must not exceed 10^3 cfu/g for chilled and frozen fish according to the permissible limit (PL).

Table (9). Effect of gamma irradiation at dose 0,1, 2 and 3 kGy and gelatin bioactive coating on the *E coli* log $_{10}$ CFU/g of Tilapia fillet during cold storage at 4 ±1ºC

Storage period (day)	Control	Gelatin Bioactive Coating and Gamma rays (kGy)		
		1	2	3
0	1.24±0.021 a	ND	ND	ND
4	1.5±0.018 b	ND	ND	ND
8	2.12±0.012 c	ND	ND	ND
12	2.52±0.014 d®	ND	ND	ND
16		ND®	ND	ND
20			ND®	ND
24				ND®

Means with the same capital letter in the same rows are not significantly different, Means with the same small letter in the same columns are not significantly different. ® Rejected

Similarly, for the effect gamma irradiation with different doses (0;1;2 and 3 kGy) on *E coli* count of Tilapia fillets coated with gelatin bioactive compound presented in Table (9) It is clear that the *E coli* counts of irradiated samples were undetectable at zero time and during the whole period of cold storage. While it was 1.24±0.021 log $_{10}$ cfu/g for control sample at the initial and significant increase was noticed during cold storage reached to 2.52±0.014 log $_{10}$ cfu/g at the 12th day of cold storage**. Mohamed *et al*, (2009),** found that a dose of 3 kGy was enough to eliminate Staph. aureus and *E. coli* from the examined samples (imported frozen fish fillets) without affecting the chemical attributes of the samples

Table (10). Effect of gamma irradiation at dose 0,1, 2 and 3 kGy and gelatin bioactive coating on the sensory characteristics *(appearance)* of Tilapia fillet during cold storage at 4 ±1ºC

Storage period (day)	Control	Gelatin Bioactive Coating and Gamma rays (kGy)		
		1	2	3
0	8.9±0.05aA	8.9±0.05aA	8.73±0.14aA	8.76±0.08aA
4	8.7±0.05bA	8.6±0.057bAB	8.5±0.05bB	8.53±0.033bAB
8	6.15±0.02cC	7.95±0.038cB	8.2±0.057cA	8.06±0.07cAB
12	3.16±0.018dC®	7.33±0.015dB	7.6±0.057dA	7.6±0.057dA
16		3.3±0.01eC®	6.45±0.028eB	7.5±0.057dA
20			3.39±0.014eB®	7.09±0.008eA
24				3.40±0.051eB®

Means with the same capital letter in the same rows are not significantly different, Means with the same small letter in the same columns are not significantly different. ® Rejected

Table (11). Effect of gamma irradiation at dose 0,1, 2 and 3 kGy and gelatin bioactive coating on the sensory characteristics *(odor)* of Tilapia fillet during cold storage at 4 ±1ºC

Storage period (day)	Control	Gelatin Bioactive Coating and Gamma rays (kGy)		
		1	2	3
0	8.93±0.06 aA	8.85±0.02 aA	8.77±0.037 aA	8.9±0.057aA
4	8.73±0.033 aA	8.6±0.028 bB	8.58±0.044 bB	8.51±0.04 bB
8	6.63±0.120 bB	8.16±0.122 cA	8.21±0.044 cA	8.22±0.192 cA
12	2.93±0.088 cC®	7.42±0.014 dA	7.30±0.052 dAB	7.18±0.07 dB
16		3.51±0.018 eB®	7.23±0.044 dA	7.15±0.02 dA
20			3.09±0.008 eA®	7.06±0.035 dA
24				3.00±0.008 eB®

Means with the same capital letter in the same rows are not significantly different, Means with the same small letter in the same columns are not significantly different. ® Rejected

Table (12). Effect of gamma irradiation at dose 0,1, 2 and 3 kGy and gelatin bioactive coating on the sensory characteristics *(texture)* of Tilapia fillet during cold storage at 4 ±1ºC

Storage period (day)	Control	Gelatin Bioactive Coating and Gamma rays (kGy)		
		1	2	3
0	8.93±0.06 aA	8.85±0.02 aA	8.77±0.037 aA	8.9±0.057aA
4	8.73±0.033 aA	8.6±0.028 bB	8.58±0.044 bB	8.51±0.04 bB
8	6.63±0.120 bB	8.16±0.122 cA	8.21±0.044 cA	8.22±0.192 cA
12	2.93±0.088 cC®	7.42±0.014 dA	7.30±0.052 dAB	7.18±0.07 dB
16		3.51±0.018 eB®	7.23±0.044 dA	7.15±0.02 dA
20			3.09±0.008 fA®	7.06±0.035 dA
24				3.00±0.008 eB®

Means with the same capital letter in the same rows are not significantly different, Means with the same small letter in the same columns are not significantly different. ® Rejected

Data in Tables (9; 10 and 11) show the mean values of sensory scores including appearance, odour and texture of Tilapia fillets coated with gelatin bioactive compound and subjected to gamma irradiation with (0; 1; 2 and 3 kGy). It is obvious that the control samples were completely rejected by the panelists at 12th day of cold storage due to the putrefaction smell and flabby texture. While application of gamma irradiation with different doses prolonged the shelf life of Tilapia fish fillets to 16; 24 and 24th day of cold storage, these samples were rejected by the panelists due to the appearance of mould these results agree with those obtained by **Shawki *et al.*, 2012**. Also, **Al-Kuraieef (2021)** Stated that the irradiation of food enhances the safety and the hygienic qualities of fresh and smoked fish products because of its high efficacy for inactivating pathogenic and spoilage microorganisms without deteriorating the quality of the product.

4. Conclusion

Nile tilapia skin was effectively valorized in this work to create an edible gelatin coating that, when paired with low-dose γ-irradiation, significantly increased the refrigerated shelf life and maintained the quality of the tilapia fillets. The use of fish-derived gelatin coatings (6%), in conjunction

with a 3 kGy γ-irradiation treatment presents a promising method for extending the shelf life of fresh fish, which might potentially improve fish safety and reduce waste in aquaculture processing. It could be concluded that the treatment by 3KGy dose combined with bioactive gelatin coating (6%) showed the highest values of all quality of chemical, microbiological and sensory evaluation of refrigerated tilapia fillets.

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طريقة مبتكرة لحفظ شرائح سمك البلطي النيلي : أشعة جاما مع أغلفة حيوية نشطة من الجيلاتين

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الملخص العربى

تُعدّ القابلية العالية للتلف من أبرز المشكلات التي تواجه تسويق وتوزيع المأكولات البحرية، ويرجع ذلك أساسًا إلى التلوث بالميكروبات المسببة للفساد والممرضة. ويمكن أن يؤدي تقليل أو تثبيط أو القضاء على هذه الملوثات الميكروبية، سواء باستخدام طرق منفردة أو مشتركة، إلى تطوير طرق فعالة لحفظ المنتجات البحرية. نظرًا لأهمية المأكولات البحرية من الناحية الصناعية والاقتصادية كمصدر للبروتين الحيواني، يكتسب استغلال الأسماك مثل البلطي في إنتاج شرائح بطي صحية، وكذلك الاستفادة من المنتجات الثانوية ذات القيمة الغذائية والاقتصادية، أهمية متزايدة على مستوى العالم وفي مصر. يتم سنويًا إنتاج كميات كبيرة من المنتجات الثانوية من معالجة مخلفات أسماك البلطي، مما يفتح المجال لاستخدامها في تطبيقات ذات قيمة مضافة. في هذه الدراسة، تم استخراج الجيلاتين من جلود سمك البلطي النيلي (*Oreochromis niloticus*)، وهو أحد أكثر أنواع الأسماك شهرة وتكلفة منخفضة في مصر. حيث تم تقييم جودة الجيلاتين المستخرج واستخدامه كغلاف قابل للأكل لشرائح البلطي، بالإضافة إلى دراسة تأثير جرعات منخفضة من أشعة جاما (0، 1، 2، و3 كيلوجراي) على إطالة العمر التخزيني وتحسين الجودة الكيميائية، الميكروبية، والحسية للشرائح المخزنة في التبريد. أظهرت النتائج أن الجيلاتين المستخرج من البلطي يمكن استخدامه كغلاف قابل للأكل في الحفاظ على جودة الشرائح. بالإضافة إلى ذلك، ساعدت الأغلفة الجيلاتينيه الحيوية على تحسين الخصائص الكيميائية، الميكروبية، والحسية للشرائح مقارنةً بالعينة الكنترول أثناء فترة التخزين البارد. كما بينت الدراسة انخفاضًا طفيفًا في قيم النيتروجين القاعدي الكلي المتطاير-(TVB-N)، والنيتروجين ثلاثي ميثيل الأمين(TMA-N) ، ودرجة الحموضة (pH) في العينات المعالجة بجرعات 1، 2، و3 كيلوجراي، بينما لوحظ ارتفاع طفيف في قيمة حمض الثيوباربيتوريك (TBA) في نفس العينات. ومع زيادة فترة التخزين، شهدت قيم TVB-N، TMA-N، TBA، و pHزيادة ملحوظة، لكنها ظلت ضمن الحدود المسموح بها حتى اليوم الثاني عشر من التخزين في العينات الكنترول بينما وصلت العينات المعالجة بالاشعاع إلى بداية الفساد بعد 16 يوم للجرعة 1 كيلو جراي بينما20 و 24 يوم للجرعات 2 و 3 كيلو جراي علي التوالي من التخزين. أثبتت جميع الجرعات المختبرة (1، 2، و3 كيلوجراي) فعاليتها في تقليل الحمل الميكروبي وإطالة العمر التخزيني لشرائح البلطي دون التأثير سلبًا على تقبلها الحسي. ومع ذلك، كانت جرعة 3 كيلوجراي هي الأكثر كفاءة، حيث أظهرت أعلى القيم في التقييمات الكيميائية، الميكروبية، والحسية، مما يجعلها الخيار الأمثل لتحسين جودة شرائح البلطي المخزنة في التبريد.