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Research Article

Effect of gamma irradiation and modified atmosphere packaging on the shelf-life of white shrimp (*Metapenaeus affinis*)

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Abstract

This study attempts to investigate the variations in pH, thiobarbituric acid (TBA), total volatile basic nitrogen (TVB-N), free fatty acids (FFA), colorimetric parameters (L*, a*, b*), total viable bacterial count (TVC) and textural profile analysis as functions of storage time to evaluate the effect of gamma irradiation (0, 1, 3, 5, 7 kGy) and modified atmosphere packaging (MAP) on the shelf life of white shrimp (*Metapenaeus affinis*) stored for 15 days at 4 ºC. Compared to the control group, increasing radiation dose (up to 7 kGy) and storage time (up to 15 days) led to an increase in pH (p<0.05). TBA, FFA and TVC increased with higher radiation dose and storage time. Prolong the storage time, increased the TVB-N to the end of the fifteenth day, while higher gamma doses from 0 to 7 kGy reduced the TVB-N content (p< 0.05). According to the results obtained from comparing colorimetric parameters, increasing radiation dose did have an influence on L*, a* and b* indices of shrimp samples. Furthermore, increasing the storage time reduced L* and b* index while increased a* (p<0.05). Increasing the gamma radiation and storage time reduced the hardness and chewiness treated shrimp samples (p<0.05). Based on the microbiological and chemical results, the optimal storage time for shrimp under various radiation doses was measured in 7 kGy treatment and on day 12. This treatment could improve the storage time by 6 days compared to the control treatment.

Keywords: Gamma irradiation, Shelf-life, Modified atmosphere packaging, Metapenaeus affinis

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Introduction

Aquatic animals play important role in the human diet. Intensive growth of aquaculture cause contamination of natural aquatic environments and effect safety concerns with regard to aquatic animal used for human consumption (Krizek *et al*., 2018). Despite the development of new food preservation technologies in recent decades, still a great amount of food is wasted and foodborne illnesses are the main cause of casualties and deaths around the world. It is estimated that 76 billion cases of foodborne pathogens illness occurs annually in the U.S. of these 325000 people are hospitalized and 5000 people lose their lives. In addition to the human costs, foodborne diseases also impose a substantial cost on the society. Thus, producers are obliged to use proper methods of food preservation and production (Velusamy *et al*., 2010).

Due to high protein content, sea-food is considered to be perishable which change in color and taste as a result of microbial activity. Therefore, food preservation and packaging methods are of greatest importance (Novoslavskij *et al*., 2016). The simplest method for protection is packaging. In response to the customer needs, and also the tendency to produce foods with higher shelf-life and quality, food packaging technology is advancing. Modified atmosphere packaging is to enclose foods in a modified gas blend (whose composition differs from atmosphere) using a high gas barrier film in order to reduce the respiration rate and microbial growth, and postpone the enzyme-induced corruption which eventually leads to higher shelf-life (Borda *et al*., 2017).

During the recent years, irradiation has been proposed as a method for extending the shelf-life of food. Irradiation is a process in which foods are exposed to ionizing radiation to kill poisoning bacteria like *Salmonella*, *Campylobacter* and *Escherichia coli*, viruses, and insects (Guerrero-Beltran and Barbosa-Canovas, 2004). Three kinds of ionizing radiation are used on an industrial scale for food industries: high energy gamma rays, X-rays, and accelerated electrons. Gamma rays are form of electromagnetic radiation emitted from the exited nuclei. Since the ray-producing elements are product of atomic decomposition and kind of atomic waste, this is the cheapest form of ray to preserve food which is also highly penetrating. Because of high energy, the intake of radiation in food can cause fat oxidation and amino acid decomposition, which causes bad smell in meat. For this reason, it is recommended to use irradiation with other methods of storage in seafood (Josephson and Peterson, 2018).

Various studies have been conducted on the effects of different rays, preserving substances, and packaging methods on food. The newest studies conducted in relation to irradiation are: improving the quality of silver carp fish fillets by gamma irradiation and coatings containing rosemary oil

(Abdeldaiem *et al*., 2018); effect of UV-C radiation on shelf life of vacuum-packed refrigerated pirarucu (*Arapaima gigas*) fillets (Santos *et al*., 2018); effects of electron beam irradiation on the biochemical properties, structure of myofibrillar protein from *Tegillarca granosa* meat (Lv *et al*., 2018); and modified atmosphere packaging and UV-C radiation on shelf life of rainbow trout (*Oncorhynchus mykiss*) (Rodrigues *et al*., 2016). There is no information available regarding the effects of irradiation on shelf-life of *M. affinis*. Therefore, the aim of this study is to investigate the effects of combined use of gamma irradiation and MAP packaging on the shelf-life of *M. affinis* stored at refrigerator temperature.

Materials and methods

Preparation of shrimp

The fresh shrimp was purchased from Abadan (Khuzestan Province, Iran), transported to the laboratory under chilled condition then it was rinsed and the muscles were separated. Shrimp were washed with 75% alcohol solution to eliminate initial microorganisms. Fillets of shrimp were cut into the pieces of 5g. Shrimp fillets were separated into the 5 groups.

The food industry has given permission to use gamma irradiation (<10 kGy) in food packaging and food processing sites (Chen *et al*., 2016). The first untreated group was used as control (0 kGy), the second group was treated with 1 kGy gamma radiation, the third group was treated with 3kGy, the fourth group was treated with 3 kGy, the fifth group was treated with 5 kGy and the sixth group was treated with 7 kGy gamma radiation. Samples were packed in low-density polypropylene trays, sealed with PE/PP/EVOH/PP film and packed under modified atmosphere (50% CO_2, 45% N_2 and 5% O_2) using a Meca 500 machine (France), then transported into the Atomic Energy Organization of Iran for irradiation. In order to expose samples to radiation, a Gammacell-3 (PX-30) instrument (Russia) was used with a maximum height of 20 cm, diameter of 12 cm, and 0, 1, 3, 5, 7, kGy doses. The absorbed doses were measured by alanine dosimeter. After all the samples were irradiated, they were stored under 4 ºC and analyzed during 15 days of storage. All analyses were performed in triplicate groups.

Total volatile bases (TVB-N)

The values of total TVB-N were measured by Kjeldahl method and by placing 10 g of sample plus 2 g of magnesium oxide and adding 300 mL distilled water in a flask. This was conducted by recovering the TVB-N in a solution containing 2% boric acid (20 cc) and methyl red as an indicator and then titration the resultant discolored solution with 0.1 n sulfuric acid till the appearance of a purple color and TVB-N were expressed as mg N 100 g^{-1} meat from the following equation (EEC, 1995):

$$TVB\text{-}N = A \times 1.4 \times 100 / B$$

where A: used 0.1 mol equi/L H_2SO_4 (mL) and B: weight of sample (g).

Thiobarbituric acid (TBA)

The measurement of TBA was conducted using a method suggested by Kirk and Sawyer (1991). The reaction of various samples with TBA and their absorption at 535 nm was measured by a spectrophotometer. TBA index was expressed as mg MDA kg^{-1}.

Peroxide value

80-100 g of minced meat of shrimp was mixed with 200 mL of chloroform solvent for 30 seconds. Then, the resultant blend was filtered using a filter paper and 25 mL of that was immediately transferred to a 150 mL previously weighed beaker. The existing chloroform in the first beaker (150 mL) was changed in a water bath with nitrogen circulation and it was placed in an oven with a temperature of 100°C for a few minutes (until constant weight was reached) and then dried (the weight of oil obtained was considered as the oil level of trial). The peroxide value was determined in the total lipid extracts and calculated as follow:

PV (meq peroxide/kg) = (S-B) × F× N× 1000/W

where S: titration amount of sample; B: titration amount of blank; F: titer of 0.01 N sodium thiosulfate. N: normality of sodium thiosulfate and W: weight of sample (g) (AOCS, 1998).

pH

In order to measure pH, after the preparation of samples, 5 g of each was poured in distilled water at 1:2 (w/ v) ratio and then totally homogenized using an electric mixer (AOAC, 2005). Then, pH of samples was measured using a meat digital pH-meter (Hanna instruments).

Free fatty acid (FFA)

In order to measure FFA, about 5 g of sample was weighed and blended with a sufficient amount of chloroform in the mixer, then it was passed from the filter paper and the filtered solution was passed again on another filter paper impregnated with dried sodium sulfate. Following the evaporation of chloroform, the FFA was titrated with 0.1 N NaOH (the ratio of fat in solvent). The FFA analysis was expressed as g oleic acid/100 g fat (AOCS, 1997).

Microbiological analysis

A 1 cm^2 of shrimp meat was transferred to sterile container containing 9 mL of physiological serum and stirred well (initial suspension with 0.1 dilutions). Then, it was allowed to stand a few minutes till the particles precipitate. Subsequently, using a sterile pipette, 1 mL of initial suspension was transferred to sterile tubes containing 9 mL diluter (physiological serum) thereby dilution series of 0.001 and 0.00001 were obtained. A plate count agar was used to count total bacteria and psychrophilic bacteria in the prepared samples. After obtaining the sample and decimal dilutions, the required dilutions were cultured on the plate count agar (Merck, Germany) via pout plate method. In order to count, the cultured plates were counted related to total viable count bacteria after 48 hours of incubation at 37°C and plates of psychrophilic

bacteria were counted after 10 days of incubation at 7°C (APHA, 1992).

Texture analysis

Textural profile analysis was conducted using a creep meter (Japan) equipped with a cylindrical plunger of 12 mm in diameter at a speed of 3 mm/s and at a penetration 2.5 mm into the flesh. Eventually, the indices related to textural evaluation were recorded from the obtained information. The typical texture analysis parameters were: hardness, adhesiveness, springiness, cohesiveness, gumminess and chewiness.

Color measurements

To evaluate color features a Hunter Lab machine (Chroma Meter CR-400) was used. For that purpose, each sample was placed in Hunter Lab apparatus and L (lightness), a* (redness) and b* (yellowness) indices were determined and recorded (CIE, 1978).

Statistical analysis

The experiments were performed in a completely randomized design with three replicates. The results of physical, chemical, and microbiological experiments were studied to find significant difference between data through one-way ANOVA using SPSS22 software. To compare between treatments means, Duncan's multiple range tests at 5% significance level (p<0.05) was used.

Results

pH

The pH variations of shrimp samples treated with various doses of gamma ray and stored in MAP under refrigerator conditions are shown in Table 1. According to the results, pH variations were dependent upon storage time and gamma-ray dosage (p<0.05). By increasing the radiation dose and storage time, pH of samples also increased (p<0.05).

Peroxide value

Peroxide index variations in MAP packaged shrimps treated with gamma radiation are presented in Table 2. By increasing the storage time from the first day to the fifteenth day, a significant increase (p<0.05) is observed in the peroxide value. By comparing the effect of various dosage of ray on peroxide index, it was found that increasing the radiation dose from 1 to 7 kGy resulted in a decrease in peroxide (p<0.05).

Hence, using gamma rays along with MAP packaging under chilled conditions will change oxidation trend. Then, as the results show, the combined application of MAP packaging made the increase in radiation dose to decrease the peroxide index in this study (p<0.05).

Table 1: Changes in pH of *M. affinis* treated with different treatments on storage.

Storage (day)	0	3	6	9	12	15
0 kGy	5.56 ± 0.01^{cA}	5.61 ± 0.06^{cA}	5.97 ± 0.03^{cB}	6.09 ± 0.01^{bC}	6.20 ± 0.01^{bD}	6.39 ± 0.04^{bE}
1 kGy	5.71 ± 0.06^{bA}	5.72 ± 0.04^{bA}	6.00 ± 0.02^{bcB}	6.12 ± 0.03^{abC}	6.12 ± 0.03^{bD}	6.42 ± 0.04^{bE}
3 kGy	5.71 ± 0.02^{bA}	5.80 ± 0.08^{abB}	6.04 ± 0.08^{abcC}	6.13 ± 0.01^{abC}	6.28 ± 0.02^{aD}	6.42 ± 0.01^{bE}
5 kGy	5.80 ± 0.02^{aA}	5.80 ± 0.03^{abA}	6.08 ± 0.03^{abA}	6.13 ± 0.01^{abA}	6.31 ± 0.06^{aB}	6.53 ± 0.01^{aC}
7 kGy	5.80 ± 0.03^{aA}	5.83 ± 0.02^{aA}	6.10 ± 0.05^{aB}	6.15 ± 0.02^{aB}	6.30 ± 0.01^{aC}	6.53 ± 0.05^{aD}

a- c lowercase letters indicate a significant difference (p<0.05) in each column and A-E uppercase letters indicate a significant difference (p<0.05) in each row.

Table 2: Changes in peroxide variations (meq O_2/kg) of *M. affinis* treated with different treatments on storage.

Storage (day)	0	3	6	9	12	15
0 kGy	0.59 ± 0.002^{aA}	1.72 ± 0.02^{aB}	2.86 ± 0.23^{aB}	3.52 ± 0.06^{aB}	4.79 ± 0.07^{aC}	4.57 ± 0.05^{aC}
1 kGy	0.59 ± 0.04^{aB}	1.65 ± 0.04^{abA}	2.61 ± 0.10^{aAB}	3.28 ± 0.10^{bB}	4.37 ± 0.02^{bB}	4.65 ± 0.08^{aBC}
3 kGy	0.57 ± 0.02^{aA}	1.54 ± 0.10^{bA}	2.58 ± 0.13^{aAB}	3.29 ± 0.06^{bA}	3.65 ± 0.06^{cAB}	4.39 ± 0.04^{bE}
5 kGy	0.59 ± 0.02^{aA}	1.29 ± 0.08^{abA}	2.26 ± 0.05^{abA}	2.65 ± 0.15^{abA}	3.30 ± 0.04^{aB}	4.13 ± 0.04^{aC}
7 kGy	0.56 ± 0.02^{aA}	1.18 ± 0.06^{cBC}	1.99 ± 0.03^{cBC}	2.42 ± 0.04^{dBC}	3.28 ± 0.04^{dBC}	3.90 ± 0.06^{dA}

a- c lowercase letters indicate a significant difference (p<0.05) in each column and A-E uppercase letters indicate a significant difference (p<0.05) in each row.

Thiobarbituric acid

Increasing the gamma radiation dosage resulted in a decrease in thiobarbituric acid content (p<0.05). The highest and lowest values of this parameter were measured at day 15 of storage in the control treatment (3.650± 0.030 mg MA kg^{-1}) and 7 kGy treatment (2.570±0.100 mg MA kg^{-1}), respectively (Table 3).

Table 3: Changes in TBA (mg MA kg^{-1}) of *M. affinis* treated with different treatments on storage.

Storage (day)	0	3	6	9	12	15
0 kGy	0.50 ± 0.01^{aE}	0.88 ± 0.007^{aD}	1.57 ± 0.04^{aC}	2.16 ± 0.04^{aC}	2.82 ± 0.07^{aB}	3.65 ± 0.05^{aA}
1 kGy	0.48 ± 0.01^{aE}	0.89 ± 0.06^{aD}	1.59 ± 0.13^{aC}	2.18 ± 0.07^{aC}	2.72 ± 0.26^{aB}	3.55 ± 0.06^{aA}
3 kGy	0.49 ± 0.01^{aF}	0.84 ± 0.007^{aE}	1.47 ± 0.12^{abD}	1.97 ± 0.09^{bC}	2.48 ± 0.02^{bB}	2.98 ± 0.22^{bA}
5 kGy	0.50 ± 0.01^{aF}	0.70 ± 0.006^{bE}	1.31 ± 0.05^{bD}	1.49 ± 0.04^{cC}	2.03 ± 0.09^{cB}	2.41 ± 0.07^{cA}
7 kGy	0.47 ± 0.02^{aF}	0.70 ± 0.02^{bE}	1.01 ± 0.13^{cD}	1.31 ± 0.15^{dC}	1.89 ± 0.06^{cB}	2.57 ± 0.11^{cA}

a- c lowercase letters indicate a significant difference (p< 0.05) in each column and A-E uppercase letters indicate a significant difference (p< 0.05) in each row.

Total volatile bases (TVB-N)

According to the obtained results, changes in TVB-N of samples treated with gamma radiation during the storage time were dependent on the dose of gamma radiation applied and also the time-period stored in refrigerator and increasing the radiation dose decreased the TVB-N substances (p< 0.05). Nonetheless, increasing the storage time increased the TVB-N index (p< 0.05) (Table 4). The highest and lowest values of this parameter were measured at day 15 in control treatment (0.77±54.46 mg N/100g sample) and 7 kGy treatment (38.91±0.24 mg N/100 g), respectively (Table 4).

Table 4: Changes in TVB-N (mg N/ 100 g) of *M. affinis* treated with different treatments on storage.

Storage (day)	0	3	6	9	12	15
0	10.00 ± 0.44^{aE}	14.59 ± 0.08^{aD}	21.48 ± 1.02^{aC}	24.61 ± 0.06^{aC}	47.58 ± 1.69^{aB}	54.46 ± 0.77^{aA}
1	10.00 ± 0.01^{aE}	14.26 ± 0.31^{aD}	21.03 ± 0.59^{aC}	2.09 ± 0.51^{aC}	43.45 ± 1.16^{aB}	53.92 ± 0.33^{aA}
3	9.85 ± 0.41^{aF}	13.42 ± 0.84^{aE}	20.39 ± 1.21^{abD}	22.41 ± 1.08^{bC}	28.00 ± 0.67^{bB}	35.68 ± 0.67^{bA}
5	9.78 ± 0.51^{aF}	12.59 ± 0.82^{cdE}	19.74 ± 0.31^{bD}	21.17 ± 0.32^{cC}	29.15 ± 2.39^{cB}	32.00 ± 2.55^{cA}
7	9.74 ± 0.51^{aF}	11.89 ± 0.28^{dE}	15.90 ± 0.31^{cD}	19.15 ± 0.32^{dC}	22.38 ± 0.89^{dB}	29.91 ± 0.24^{dA}

a- c lowercase letters indicate a significant difference (p<0.05) in each column and A-E uppercase letters indicate a significant difference (p<0.05) in each row.

Free fatty acid

According to the results (Table 5), it was found that free fatty acid changes during storage time (p<0.05) depended on the storage time period and the applied gamma radiation dose. By increasing the storage time, free fatty acids content increased from 0.364±0.018 to 3.265±0.100 mg/g and also by increasing the gamma radiation dose, the free fatty acids content decreased (p<0.05) so that at the end of the storage time, by increasing the radiation dose, the free fatty acids content decreased from 3.265 to 2.27 mg/g.

Table 5: Changes in FFA (mg/g oleic acid) of *M. affinis* treated with different treatments on storage.

Storage (day)	0	3	6	9	12	15
0	0.36 ± 0.008^{aA}	0.66 ± 0.003^{aB}	1.03 ± 0.02^{aB}	1.61 ± 0.07^{aB}	2.40 ± 0.05^{aC}	3.21 ± 0.04^{aBC}
1	0.36 ± 0.002^{aA}	0.66 ± 0.009^{aA}	1.04 ± 0.01^{aAB}	1.58 ± 0.07^{abB}	2.44 ± 0.12^{aB}	3.26 ± 0.10^{aC}
3	0.36 ± 0.01^{aB}	0.59 ± 0.02^{bA}	0.86 ± 0.003^{bAB}	1.40 ± 0.12^{bcAB}	2.05 ± 0.05^{bAB}	2.58 ± 0.03^{bAB}
5	0.38 ± 0.01^{aA}	0.54 ± 0.01^{cA}	0.86 ± 0.04^{bBC}	1.26 ± 0.14^{cA}	2.04 ± 0.02^{bBC}	2.27 ± 0.04^{cC}
7	0.36 ± 0.02^{aA}	0.49 ± 0.01^{dBC}	0.80 ± 0.06^{bA}	1.07 ± 0.06^{dBC}	1.73 ± 0.10^{cA}	2.35 ± 0.05^{cA}

a- c lowercase letters indicate a significant difference (p<0.05) in each column and A-E uppercase letters indicate a significant difference (p<0.05) in each row.

Microbiological assay

Total microorganisms count showed that by increasing radiation dose, the number of viable cells decreased significantly (p<0.05). On the other hand, from the first day to 15th day, their number increased in all treatments which were statistically significant (p<0.05) (Table 6).

Color evaluation

Results obtained from changes in color parameters (L*, a*, b*) of shrimp samples treated with gamma rays and stored in MAP packages and also their comparison based on Duncan's multiple range test are shown in Table 7. By increasing the radiation dose to shrimp samples, lightness index (L*) increased, and by expansion the storage time of samples, lightness index decreased (p<0.05). Assessment of changes in redness (a*) and yellowness (b*) indices showed that by raising the radiation dose applied and also the storage time, these indices exhibited a significant increase (p<0.05).

Table 6: Changes in TVC (log cfu g^{-1}) of *M. affinis* treated with different treatments on storage.

Storage (day)	0	3	6	9	12	15
0	2.69 ± 0.07^{aF}	3.59 ± 0.03^{aE}	6.43 ± 0.11^{aD}	6.83 ± 0.05^{aC}	7.81 ± 0.11^{aB}	9.65 ± 0.20^{aA}
1	2.00 ± 0.02^{aF}	2.49 ± 0.24^{aE}	3.38 ± 0.18^{aD}	4.48 ± 0.37^{bC}	5.66 ± 0.12^{aB}	6.61 ± 0.09^{aA}
3	2.00 ± 0.16^{aF}	2.07 ± 0.03^{bE}	2.71 ± 0.54^{bD}	3.89 ± 0.02^{cC}	4.87 ± 0.04^{bB}	5.78 ± 0.16^{bA}
5	2.00 ± 0.19^{bF}	1.77 ± 0.02^{cE}	2.65 ± 0.09^{bD}	3.00 ± 0.18^{cC}	3.68 ± 0.08^{bB}	4.00 ± 0.31^{bA}
7	2.00 ± 0.06^{bF}	1.71 ± 0.02^{cE}	2.47 ± 0.15^{cD}	2.66 ± 0.25^{dC}	3.15 ± 0.45^{cB}	3.20 ± 0.13^{cA}

a- c lowercase letters indicate a significant difference ($p< 0.05$) in each column and A-E uppercase letters indicate a significant difference ($p< 0.05$) in each row.

Table7: Color measurements of *M. affinis* treated with different treatments of radiation on storage.

Storage (day)	0	3	6	9	12	15
L^*						
0	76.95 ± 0.02^{eA}	75.24 ± 0.02^{eB}	72.65 ± 0.02^{eC}	71.64 ± 0.02^{eD}	68.27 ± 0.02^{eE}	66.37 ± 0.03^{eF}
1	77.23 ± 0.02^{dA}	75.45 ± 0.01^{dB}	72.78 ± 0.02^{dC}	71.88 ± 0.03^{dD}	68.32 ± 0.02^{dE}	67.24 ± 0.02^{dF}
3	77.38 ± 0.02^{cA}	75.96 ± 0.02^{cB}	73.28 ± 0.01^{cC}	72.35 ± 0.02^{cD}	70.16 ± 0.02^{cE}	68.43 ± 0.03^{cF}
5	77.44 ± 0.03^{bA}	76.35 ± 0.01^{bB}	74.24 ± 0.02^{bC}	73.19 ± 0.02^{bD}	70.29 ± 0.03^{bE}	68.57 ± 0.03^{bF}
7	77.64 ± 0.01^{aA}	76.59 ± 0.14^{aB}	74.58 ± 0.03^{aC}	73.51 ± 0.01^{aD}	72.43 ± 0.02^{aE}	70.15 ± 0.02^{aF}
a^*						
0	1.59 ± 0.005^{dE}	1.62 ± 0.01^{cD}	1.64 ± 0.01^{dC}	1.67 ± 0.01^{dB}	1.69 ± 0.01^{cA}	1.70 ± 0.01^{eA}
1	1.61 ± 0.005^{cE}	1.63 ± 0.011^{cD}	1.66 ± 0.005^{cC}	1.69 ± 0.001^{cB}	1.70 ± 0.01^{cB}	1.72 ± 0.02^{dA}
3	1.65 ± 0.005^{bD}	1.68 ± 0.01^{bC}	1.69 ± 0.005^{bC}	1.72 ± 0.00^{bB}	1.74 ± 0.005^{bA}	1.75 ± 0.01^{cA}
5	1.67 ± 0.005^{aE}	1.67 ± 0.01^{bE}	1.70 ± 0.005^{abD}	1.72 ± 0.01^{bc}	1.74 ± 0.01^{bB}	1.77 ± 0.01^{bA}
7	1.67 ± 0.00^{aF}	1.69 ± 0.01^{aE}	1.71 ± 0.01^{aD}	1.74 ± 0.005^{aC}	1.76 ± 0.01^{aB}	1.79 ± 0.005^{aA}
b^*						
0	5.83 ± 0.01^{eF}	5.35 ± 0.01^{dE}	4.65 ± 0.02^{eD}	4.38 ± 0.02^{eC}	3.47 ± 0.01^{eB}	2.53 ± 0.01^{eA}
1	5.70 ± 0.02^{dF}	5.31 ± 0.01^{dE}	4.46 ± 0.002^{dD}	4.29 ± 0.01^{dC}	3.15 ± 0.01^{dB}	2.29 ± 0.01^{dA}
3	5.64 ± 0.02^{cF}	5.19 ± 0.01^{cE}	4.09 ± 0.005^{cD}	4.15 ± 0.02^{cC}	2.91 ± 0.01^{cB}	1.64 ± 0.01^{cA}
5	5.51 ± 0.005^{bF}	4.67 ± 0.01^{bE}	3.92 ± 0.005^{bD}	3.22 ± 0.01^{bC}	2.65 ± 0.01^{bB}	1.37 ± 0.01^{bA}
7	1.67 ± 0.00^{aF}	1.69 ± 0.01^{aE}	3.75 ± 0.01^{aD}	3.09 ± 0.02^{aC}	2.56 ± 0.01^{aB}	1.21 ± 0.02^{aA}

a- c lowercase letters indicate a significant difference ($p<0.05$) in each column and A-E uppercase letters indicate a significant difference ($p<0.05$) in each row.

Texture profile analysis

Results of comparing data means based on Duncan's multiple range test in terms of changes in hardness, chewiness, gumminess, cohesiveness and elasticity of shrimp samples treated with various doses of radiation in MAP are presented in Table 8. It was found that changes in hardness and chewiness of treated shrimp samples ($p<0.05$) are dependent on the applied radiation dose and their storage time. Increasing the radiation dose and also the storage time of shrimp samples decreased their rigidity ($p<0.05$) and by intensifying the applied radiation dose and storage time of treated shrimp samples ($p<0.05$), their chewing ability also decreased.

Table 8: Texture profile analysis of *M. affinis* treated with different treatments of radiation on storage.

Storage (day)	0	3	6	9	12	15
Hardness (N)						
0	165.54±0.03^{aA}	161.22±0.03^{aB}	156.90±0.03^{aC}	141.80±0.03^{aD}	132.45±0.03^{aE}	117.43±0.06^{aF}
1	165.21±0.02^{bA}	159.59±0.02^{bB}	155.21±0.03^{bC}	139.73±0.03^{bD}	128.43±0.03^{bE}	115.94±0.07^{bF}
3	164.95±0.03^{cA}	157.54±0.03^{cB}	152.41±0.04^{cC}	137.76±0.02^{cD}	126.46±0.03^{cE}	113.21±0.06^{cF}
5	164.88±0.01^{dA}	155.62±0.03^{dB}	147.83±0.02^{dC}	132.73±0.03^{dD}	124.19±0.03^{dE}	108.95±0.03^{dF}
7	164.27±0.02^{eA}	153.95±0.02^{eB}	145.22±0.03^{eC}	130.87±0.02^{eD}	123.59±0.04^{eE}	108.66±0.04^{dF}
Chewiness (N× mm)						
0	86.12±0.04^{aA}	80.15±0.03^{aB}	73.93±0.07^{aC}	61.49±0.07^{aD}	53.29±0.06^{aE}	37.84±0.07^{aF}
1	85.99±0.03^{bA}	79.22±0.03^{bB}	70.14±0.08^{bC}	60.27±0.08^{bD}	51.54±0.07^{bE}	37.13±0.08^{bF}
3	85.60±0.02^{cA}	77.69±0.04^{cB}	68.28±0.06^{CC}	58.22±0.06^{cD}	50.47±0.06^{cE}	36.49±0.08^{cF}
5	85.45±0.02^{dA}	72.33±0.03^{dB}	66.93±0.06^{dC}	57.70±0.06^{dD}	49.67±0.05^{dA}	30.57±0.06^{dF}
7	85.21±0.02^{eA}	72.14±0.03^{eB}	65.84±0.04^{eC}	56.88±0.07^{eD}	49.18±0.04^{eE}	29.32±0.06^{eF}
Gumminess (N)						
0	117.46±0.01^{eF}	105.44±0.04^{dE}	89.57±0.05^{eD}	80.45±0.04^{eC}	73.70±0.05^{eB}	67.91±0.07^{eA}
1	117.28±0.03^{dF}	102.47±0.05^{dE}	87.76±0.04^{dD}	77.21±0.04^{dC}	71.43±0.05^{dB}	65.85±0.07^{dA}
3	117.26±0.02^{cF}	98.54±0.04^{cE}	86.27±0.05^{cD}	73.49±0.06^{cC}	65.37±0.05^{cB}	55.43±0.04^{cA}
5	116.94±0.04^{bF}	95.24±0.06^{bE}	79.62±0.05^{bD}	68.90±0.05^{bC}	55.50±0.05^{bB}	50.67±0.06^{bA}
7	116.26±0.03^{aF}	93.68±0.05^{aE}	77.33±0.04^{aD}	64.58±0.04^{aC}	52.67±0.04^{aB}	47.60±0.05^{aA}
Cohesiveness (ratio)						
0	0.41±0.00^{aA}	0.38±0.00^{aC}	0.40±0.00^{aAB}	0.37±0.00^{aC}	0.41±0.00^{aA}	0.38±0.00^{aC}
1	0.41±0.00^{aA}	0.37±0.00^{abB}	0.40±0.00^{bA}	0.38±0.00^{aB}	0.40±0.00^{aA}	0.37±0.00^{abB}
3	0.40±0.00^{aA}	0.36±0.00^{bcC}	0.38±0.00^{cB}	0.35±0.00^{bC}	0.38±0.00^{bB}	0.36±0.00^{bcC}
5	0.41±0.00^{aA}	0.35±0.00^{cdC}	0.35±0.00^{dC}	0.34±0.00^{bcC}	0.37±0.00^{bBC}	0.36±0.00^{bcBC}
7	0.38±0.00^{bA}	0.34±0.00^{cdBC}	0.34±0.00^{dBC}	0.33±0.00^{cC}	0.37±0.00^{bA}	0.35±0.00^{cB}
Elasticity (mm)						
0	0.65±0.00^{abA}	0.57±0.00^{cB}	0.54±0.00^{cC}	0.47±0.00^{cD}	0.43±0.00^{cE}	0.38±0.00^{aF}
1	0.64±0.00^{bA}	0.56±0.00^{cB}	0.52±0.00^{dC}	0.48±0.00^{cD}	0.43±0.00^{cE}	0.37±0.00^{abF}
3	0.65±0.00^{abA}	0.57±0.00^{cB}	0.56±0.00^{bB}	0.51±0.00^{bC}	0.46±0.00^{bD}	0.36±0.00^{bE}
5	0.66±0.00^{aA}	0.60±0.00^{bB}	0.58±0.00^{aC}	0.52±0.00^{abD}	0.46±0.00^{bE}	0.36±0.00^{bf}
7	0.65±0.00^{abA}	0.62±0.00^{aB}	0.58±0.00^{aC}	0.53±0.00^{aD}	0.50±0.00^{aE}	0.34±0.00^{cF}

a- c lowercase letters indicate a significant differences (p<0.05) in each column and A-E uppercase letters indicate a significant differences (p<0.05) in each row.

Discussion

Shrimp is an aquatic animal with high protein content, therefore by increasing the gamma radiation and also the storage time, some amine and basic compounds are produced as a result of protein decomposition which leads to an increase in pH of treated shrimp samples during the storage time (AMC, 1979). On the other hand, given the fact that the control treatment had a higher microbial count during the storage time, some acid was produced as a result of their activity. Therefore, the slope of pH rise in the control treatment was lower compared to other treatments. Results obtained from this study were in agreement with the results of Al-

Bachir (2016). The initial pH for shrimp was 5-6 and pH values ranged from 6.50 to 6.70. Results showed that MAP and radiation could slow down the increase in pH values by preventing bacterial activity.

Peroxide index represents the oxidation rate and primary products of lipids oxidation. Yet, this index is not a reliable way of monitoring the oxidation since the peroxides resulted from lipids oxidation are highly unstable and rapidly convert to the secondary products (Al-Bachir, 2016). Among the food compounds lipids are the most sensitive to irradiation and using this method for full fat foods will lead to lipids oxidation. Taking into the account that shrimp and sea-food products contain long-chain unsaturated fatty acids, using gamma rays for irradiation and also increasing the storage time may cause lipids to oxidize and thus increasing their peroxide index (FDA, 1997), but MAP packaging could slow down the increase. Probably due to low values of available oxygen in MAP packaging process, conditions are unfavorable for the formation of peroxides (primary products of lipids oxidation) and this causes the peroxide index to decrease as the ray dose increases. However, expanding the storage time may provide lipids with trace amounts of oxygen available which in turn makes the peroxide index to increase by storage time. Mexis and Kontominas (2009) found that PV increased with an increase in irradiation dose, which is disagree with our result, which could be due to the lack of packaging.

In order to determine the secondary products of oxidation, thiobarbituric acid index was considered. The thriobarbituric acid test is based on the formation of a pink color due to the reaction between thiobarbituric acid and MDA which is a by-product of reaction. As a result of oxidation reaction, secondary products of oxidation such as ketones, aldehydes, and peroxides grow which increase the formation of MDA and this leads to an increase in thiobarbituric acid index, therefore the development of lipids oxidation rate will be shown using thiobarbituric acid index (Fernandez *et al*., 1997). According to the obtained results, it was found that the thiobarbituric acid index was in range of 0.478 to 3.65 mg MA kg^{-1}. It is recommended that values of TBA<5 mg MA kg^{-1} are indicative of good shrimp meat (Krizek *et al*., 2018).

If enough oxygen is available for the oxidation process, the application of gamma rays for foods will produce free radicals which react with unsaturated fatty acids in shrimp during the storage time and produce various products of oxidation whose decomposition generates secondary products of oxidation and the thiobarbituric acid index of shrimp samples will increase during the storage time (Zhao *et al*., 2018). Using the MAP packaging under chilled conditions, however, may lower the available oxygen content for the chain oxidation reactions to occur which lessens the decomposition of primary products (peroxides) and

production of secondary ones (Lv *et al*., 2018). Concomitant use of gamma rays and MAP packaging made the thiobarbituric acid index to reduce and the radiation dose to increase which prevented the enhancement of chain oxidation reactions compared to the control sample. Nonetheless, the higher storage time increased the thiobarbituric acid index which was probably due to enough available time for the reaction of existing oxygen with fatty acids of shrimp. Findings of this study are in line with that of other studies. Chouliara *et al.* (2008) evaluated using the combination of gamma rays and MAP packaging on extending the shelf-life of chicken meat. Their results showed that simultaneous application of gamma rays and MAP packaging led to a significant decrease in TBA index compared to the control sample. Przybylski *et al*. (1989) also noted that as a result of irradiation and MAP packaging, thiobarbituric acid index of catfish samples increased with higher storage time.

Volatile nitrogenous compounds are produced as a result of proteins and non-protein nitrogenous compounds which mainly include ammonia and primary and secondary type amines. In fisheries products, the TVB-N index in the range of 35-40 mg N/100 g represents a suitable quality and a TVB-N of 50 mg N/100 g indicates a low quality due to degradation of tissue proteins (Lakshmanan *et al*., 1999). TVB-N index generally includes trimethylamine (the result of bacterial spoilage), dimethylamine (a product of enzyme's self-digestion), ammonia, and other amine volatile compounds associated with the spoilage of fisheries products (Ryou *et al*., 2016). Therefore, probably due to higher doses of gamma rays and using MAP packaging method, bacterial and enzyme activity in the treated samples is reduced which lessens the formation of volatile nitrogenous substances. On the other hand, with the growth of microbial count during the storage time, the content of volatile nitrogenous substances increases by increasing the storage time. These results were in agreement with that of the other studies. Mbarki *et al.* (2009) studied the effect of low irradiation dose on the formation of volatile nitrogenous substances in chub mackerel. Based on their results it was found that there is a reverse relationship between increasing radiation dose and the formation of volatile nitrogenous substances and a direct relationship between the storage time and the formation of volatile nitrogenous substances. It should be noted that the treated samples after 15 days with 7 kGy of gamma rays which was stored in MAP packages under chilled conditions had a TVB-N index of less than 50 mg N/100 g.

Production of free fatty acids in fisheries products which are rich in unsaturated fatty acids matters from various aspects. The presence of fatty acids may provide the required conditions for chain oxidation reactions and production of unfavorable products which diminish the nutritional value of food products. On the other hand, the

existence of free fatty acids and their unfavorable reactions may create an unpleasant taste and smell in fisheries products. Therefore, preventing the formation and controlling the amount of free fatty acids is of greatest importance for food products. Fatty acids may be produced as a result of lipase enzyme activity and degradation of triglycerides. Hence, the growth of microorganisms in the shrimp samples may increase the production of lipase enzyme which causes triglycerides to degrade and free fatty acids to be produced (Arvanitoyannis *et al*., 2009). Irradiation causes radiolysis of water, leading to the formation of free radicals. The amount of lipids affected the rate of lipid oxidation in irradiated fisheries products (Kristinsson, 2014). Perhaps by increasing the radiation dose, the activity of lipase-producing microorganisms is deactivated or lowered in the irradiated samples which reduce the amount of free fatty acids in treated samples compared to the control sample. On the other hand, probably by increasing the storage time, the activity of microorganisms promotes the release of free fatty acids. This increase in the storage time period was higher in the control sample than the treated samples. Ahn *et al.* (2013) found that by applying gamma radiation, microbial count and following that, the amount of free fatty acids in poultry meat are reduced. Bari *et al*. (2000) reported that by increasing the storage time an increase occurred in the amount of free fatty acids in fish cutlets. For example, irradiation was reported increase oleic and linoleic acids in tilapia and the amount of stearic and oleic acids in Spanish mackerel (Al-Kahtani *et al*., 1996).

Microbial spoilage is one of the limiting factors of food shelf-life. The proliferation of microorganisms decomposes the nutrient compounds and the production of unfavorable compounds creates unpleasant smell and taste. As a result of these activities, the product's nutritional value is reduced and, eventually, the corruption may expand to some extent that the food product could no longer be used. Therefore, controlling and creating conditions to annihilate microorganisms or postpone their growth and reproduction can prevent bacterial spoilage and its unfavorable effects (Oraei *et al*., 2011). TVC of shrimp was increased in control group from 2.69 log cfu g^{-1} at the beginning to 9.65 log cfu g^{-1} at the end of storage. During the final day of storage, TVC was high (9.65±0.20 log cfu g^{-1}) for control whereas it was only 3.20 log cfu g^{-1} for 7 kGy irradiated samples. These results indicate that the combined use of gamma rays (7 kGy) and MAP packaging under refrigerated conditions can well reduce the microorganisms count in the shrimp samples and thus increase their shelf-life. Paari *et al*. (2012) observed that even by using 10 kGy of gamma rays, there was still 2.70 log cfu g^{-1} of microorganism in fish samples. Yang *et al*. (2014) studied the effects of e-beam irradiation combined with vacuum packaging on the microbial properties of salmon fish

fillet. Their results showed that the TVC was 4.70 log cfu g^{-1}which rapidly reached 9.20 log cfu g^{-1}at the end of storage. Despite that, their count was equal to 5.50 log cfu g^{-1} at the end of storage in samples treated with 3 kGy of gamma rays. Synergistic effect of freezing and irradiation on bonito fish indicated that fewer bacterial counts were detected in sample irradiated as compared with un-irradiated sample (Altan and Turan, 2016).

Considering the increase of lipids oxidation during the storage time and by increasing the ray dose, lightness index reduced significantly over time. The effect of irradiation dose was statistically insignificant for parameter L*, while parameters a* and b* statistically significant. The results of Mexis *et al*. (2009) showed a decrease in L*, while a* and b* unaffected. Furthermore, the results of evaluating redness (a*) index showed that by increasing the ray dose and also the storage time in MAP, these indices increased significantly. As a result of lipids and proteins oxidation, more carbonyl groups are likely to be produced which react with amine groups and lead to a maillard reaction with a brownish yellow color (Zha *et al*., 2015). Reibroy *et al*. (2007) noted that by applying gamma rays at low dose (2 kGy) during the storage time, lightness index of Som-fug (prepared from fish surimi) decreased and also the yellowness and redness indices increased significantly. However, according to Yang *et al*. (2014), at the beginning of the storage, it was found that increasing irradiation doses by 0.5 to 3 kGy for Atlantic salmon fillets resulted in a decrease of a* value. Naveena *et al.* (2006) showed that application of bioactive agents produced a significant increase in the value of a* at 4ºC, indicating an improved redness, which is an important factor in the visual appeal of meat.

Rigidity is a texture index which represents the highest force required to change the object's shape under applied force. Chewiness is one of the textural parameters that it associated with sensory evaluation. Chewiness is a parameter dependent on hardness. Therefore, its value follows the same trend as hardness. Chewiness is the amount of energy required to chew solid food to be easily swallowed. Probably the intensified oxidation of proteins and lipids present in the treated shrimp samples is associated with inferior textural properties. Oxidized proteins cannot form a homogenous network, thus, the heterogeneous network leads to a lower water storage capacity, a fact which becomes more apparent during the storage time when more oxidation happens and water storage capacity lessens subsequently (Yoon, 2003). Studies of fisheries products show that the effect of irradiation depends upon the dose, storage time and packing method. The sensitivity of different species of fish proteins and the effects of irradiation on meat of muscle texture differs (Ehlermann, 1976; Desrosiers, 1989; Eustice and Brubn, 2006). Diehl *et al.*

(1995) reported that following the breakdown of peptide bonds and also breaking disulfide bonds, the viscosity of treated protein solution can decreased. Riebroy *et al*. (2007) reported that by increasing the storage time and also the radiation dose, the hardness and chewiness indices of Som-fug reduced which is compatible with findings of the current study. Hultmann and Rustad (2004) reported there were no significant changes in texture properties of salmon in this case.

Results of the current study indicated the positive effects of gamma irradiation and MAP packaging on microbiological, biochemical, and sensory evaluation of shrimp (*Metapenaus affinis*) stored in refrigerator. Furthermore, based on the results obtained, the best storage time for shrimp under various doses was associated with 7 kGy treatment and on day 12. This treatment could improve the food storage time by six days compared to the control.

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