Heliyon 8 (2023) e12596

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Impact of gamma radiation, potassium sorbate and low temperature on shrimp *(Penaeus monodon)* preservation

Md. Shajadul Islam [b], Arzina Hossain [a], Mahfuza Islam [a], M. Kamruzzaman Munshi [a], Mohammad Shakhawat Hussain [a], Keshob Chandra Das [c], Irfan Ahmed [c], Md. Shafiqul Islam Khan [b], Roksana Huque [a,*]

[a] *Food Safety and Quality Analysis Division (FSQAD), Institute of Food and Radiation Biology, Atomic Energy Research Establishment, GPO Box-3787, Savar, Dhaka, 1000, Bangladesh*
[b] *Department of Food Microbiology, Patuakhali Science and Technology University, Patuakhali, 8602, Bangladesh*
[c] *Molecular Biotechnology Division, National Institute of Biotechnology, Savar, Ashulia, Bangladesh*

ARTICLE INFO

Keywords:
Shrimp
Gamma irradiation
Potassium sorbate
Low temperature
Microbiology
PCR

ABSTRACT

The objective this study was to assess the effect of gamma radiation and 2% potassium sorbate with low temperature (4 °C) for shrimp (*Penaeus monodon*) preservation. Fresh samples were prepared for treatment with gamma radiation at 1.0 and 1.5 kGy doses and potassium sorbate (2%) soaked for 30 s (PS 30 s) and 60 s (PS 60 s). Organoleptic score (OS), biochemical composition and microbiological analysis were performed to evaluate the shelf-life extension and quality changes during the storage periods. Data showed that combination treatment of gamma irradiation at 1.5 kGy with low temperature was the most effective in extending shelf-life of shrimp. The isolated bacteria associated with shrimp samples were identified through PCR technique. Antibiotic sensitivity test was examined using ten commonly used antibiotics against these pathogenic isolates. Gentamicin and Imipenem showed up to 50% resistance on Gram-positive (*Bacillus cereus* and *Staphylococcus aureus*). This study indicates that gamma irradiation treatment with low temperature was most effective way for shelf-life extension of shrimp which might reduce the wastage of this important nutritional source.

1. Introduction

In Bangladesh, there are 401 species of marine fish and ranked as the fifth-largest aquaculture producing nation [1]. Fish farming has shown rapid growth with a critical role in Bangladesh's economy, becoming the second-largest export industry after garments [2]. The shrimp culture contributes 71.4 % to the total national production [3] which is one of the key exports items of Bangladesh and makes up about 70% of the total agricultural exports. That is why shrimp is usually referred to as "White Gold" in Bangladesh. Total shrimp and prawn production has been increased from 1,60,000 metric ton in 2002-03 to 2,41,000 metric ton in 2019-20 and its growth rate is 0.59%. At present, the world market demand for shrimp is increasing day by day. A significant area of the southern part is under cultivation of shrimp culture, contributing 3.78% of GDP [4]. Various methods have been proposed to preserve shrimp such as, chilling and ice storage, organic acids, modified atmosphere packaging [5], and ionizing radiation [6]. In order to preserve fish and to extend their shelf-life effectively, new technologies must be developed. Gamma irradiation is used as a safe and effective treatment for fish preservation as basic compositions are not significantly affected at radiation doses up to 10 kGy [7]. Use of gamma irradiation with low temperature might be useful to extend the shelf life and to improve the quality of shrimp. Generally, food preservatives are used for prevents mold and yeast growth and ultimately increase the shelf-life of the products. Potassium sorbate has been widely used as a food preservative that does not affect color, taste, or flavor of the food samples. It has been reported as an effective chemical preservative against yeasts, molds, and selected bacteria to extend the shelf-life of certain seafood [8, 9].

On the other hand, refrigeration processes slow down the enzymatic activities of the spoilage organisms and decrease their biochemical reactions [10]. Freezing temperature kills some but not all of the microorganisms. Therefore, the combination of irradiation, potassium sorbate and low temperature treatments for shrimp preservation may result in synergistic effects on microbial status. The purpose of this study was to

* Corresponding author.
E-mail address: roksanahuque@yahoo.com (R. Huque).

https://doi.org/10.1016/j.heliyon.2022.e12596
Received 31 December 2021; Received in revised form 16 August 2022; Accepted 16 December 2022

understand the effect of gamma radiation and potassium sorbate at low temperature on the sensory quality, biochemical composition, tyrosine value (TV) and microbial load to extend the shelf-life of shrimp. This study was also included isolation, PCR identification as well as antibiotic sensitivity tests to characterize isolated spoilage pathogenic bacteria from the tested shrimp sample.

2. Materials and methods

2.1. Collection and preparation of samples

The shrimp (*Penaeus monodon*) sample was from a local market in Patuakhali, District at 22.3542°N, 90.3181°E.. All the samples were brought in to the laboratory of Food Safety and Quality Analysis Division (FSQAD), Institute of Food and Radiation Biology (IFRB) in a sterile icebox under aseptic condition. After that, all the shrimp samples were cleaned, de-headed, peeled out the shell and removed intestines and tails. Then total shrimp samples were allotted into five different groups (control, 2% potassium sorbate treated for 30 and 60 s and irradiated with 1.0 and 1.5 kGy) and packed in a zip lock poly bag. Each bag contains 10–12 pieces (approx. 60–65 gm) of shrimp. One part of the samples were irradiated such as 1.0 kGy and 1.5 kGy. Another parts were applied with 2% potassium sorbate dipping processes with 30 s and 60 s. All treated and control samples were stored at 4° C. The applied radiation doses were selected from reference articles [11].

Biochemical, chemical, microbiological and organoleptic analyses were carried out at weekly intervals.

2.2. Irradiation

The irradiation process was carried out at the Gamma Source Division, Institute of Food and Radiation Biology (IFRB), AERE, Savar, Dhaka. To administer 1.0 and 1.5 kGy gamma radiation, shrimp samples were placed in an irradiation box (20 × 8 × 18) cm^3, covered with ice packs. These does were selected based on previous studies that showed irradiation with low doses were effective to extend shelf-life of fish and fishery products [12]. The box was taken to the irradiation room and subjected to irradiation at one side due to its low density.

Ceric-Cerous dosimetry system was used and the doses were measured by Potentiometer [13]. The dose rate was 8.34 kGy/hrs and the dose uniformity ratio was 1.04.

2.3. Proximate analysis

2.3.1. Estimation of moisture

Moisture content was determined by AOAC, 2005 [14]. About 5.0 gm of sample was taken into petri dishes dried in a hot air oven (Noberthem, 30–300° C) maintained at 105 °C for about 6–8 h till two successive weighing gives a constant weight. The moisture content (%) was calculated by the following equation Eq. (1):

$$Percentage\ \left(\%\right)\ of\ \ moisture\ \ = \frac{Initial\ \ weight - Final\ \ weight}{Sample\ \ taken} \times 100 \tag{1}$$

2.3.2. Estimation of protein

The protein content of shrimp sample was determined by using the Micro-Kjeldahl technique. This involves the oxidation of the sample with sulfuric acid in the presence of a catalyst and then the formation of ammonium salts and amines from the nitrogen components of the sample. The solution thus obtained in alkaline containing amines and ammonia and is distilled into standard acid. The solution is then back titrated with standard alkali and the amount of nitrogen of ammonia was calculated. The nitrogen value is multiplied by 6.25 to give the value for crude protein.

2.3.3. Estimation of lipid

The lipid content was determined using AOAC 2005 [14] method. Five grams of the sample with an adequate number of powders were ground gently by a pestle. Ten milliliter of chloroform-methanol (2:1) mixture was added to the above sample and homogenized properly. The sample was then collected into a pre-weighted test tube after being filtered with a filter paper. Subsequently, 4% $CaCl_2$ (1 ml) solution was added and kept it overnight. Then the supernatant was separated from the upper portion of the test tube and kept in the oven till up to drying of the mixture. After drying out the solution, the test tube was again weighted. The lipid content was calculated by using the following Eq. (2):

$$Percentage\ \left(\%\right)\ of\ Lipid\ = \frac{Initial\ weight - Final\ weight\ of\ the\ test\ tube}{Weight\ \text{of}\ sample\ taken} \times 100 \tag{2}$$

2.3.4. Estimation of ash

Ash was determined by incineration of shrimp at 600 °C for 4–5 h, depending on the method used [15]. About 5 gm of the sample was weighed into a pre-weighed crucible over a low flame till completely churned. Then the churned sample was kept in an electric muffle furnace for 3–5 hrs at about 600 °C to ash completely white or grayish-white in color. Then, cooled in a desiccator and weighted again. This was repeated until two consecutive weights for each sample were the same in weight. The ash content was calculated by using the following Eq. (3):

$$Ash\ \ content\ \ \left(\frac{gm}{100\ \ gm}\right)\ \ = \frac{Weight\ \ of\ \ the\ \ ash}{Weight\ \ of\ \ the\ \ sample\ \ taken} \times 100 \tag{3}$$

2.4. Preparation of mineral solution

The ash (obtained from the previous experiment) in the crucible was moistened with 1 ml of distilled water added with 4 ml of HCl. In a hot water bath, the mixture was then evaporated until dry. Another 4 ml of HCl was added and the solution was evaporated to dryness as before then 4 ml of HCl and a few ml of distilled water were added and warmed the solution over a boiling water bath. Then, the solution was filtered into a 100 ml volumetric flask. After cooling the volume is made up to 100 ml with distilled water. For the estimation of calcium and phosphorus, this mineral solution was used.

2.4.1. Estimation of iron

Iron determination in the shrimp sample, was measured by converting the iron to ferric compound. Sample (5 ml) was taken with 0.5 % concentrate sulfuric acid, then 1 ml potassium persulphate, 2 ml potassium thiocyanate and 6.5 ml water were added which made the volume at 15 ml in total. Then the final solution was measured of iron density at 480 nm [16] and calculated by using the following Eq. (4):

$$Iron\ \ \left(\frac{gm}{100\ \ gm}\right)\ \ = \frac{OD\ \ of\ \ sample\ \ \times\ \ 0.1\ \ \times\ \ Total\ \ volume\ \ of\ \ ash\ \ solution}{OD\ \ of\ \ standard\ \ \times\ \ 5\ \ \times\ \ weight\ \ of\ \ sample\ \ taken\ \ for\ \ ashing} \times 100 \tag{4}$$

2.4.2. Estimation of calcium

Calcium was determined by precipitating it as calcium oxalate and following by titration the solution of oxalate in dilute sulfuric acid against standard potassium permanganate [16]. At the first, 25 ml solution was diluted in 100 ml of distilled water. Subsequently, 2 drops of methyl red indicator were added, and mixed with neutralizing agent until the pink color changed into yellow color. After that 5 ml of 6% ammonium oxalate was added and allowing boiling for a short while, then added few drops of glacial acetic acid until became yellow to pink. The mixture was kept in a warm place to settle down the precipitation. Then precipitation was filtered through filter papers and rinsed in warm water until the oxalate was removed. A drop of ammonium oxalate solution was added to the supernatant to ensure that the precipitation was completed. Then precipitate was filtered and transferred into a beaker and diluting about 10 ml (2 N) H_2SO_4. The solution was heated to about 70^0C and titrated against N/100 $KMnO_4$ solution to deep brown color. The calcium content was calculated by using the following Eq. (5):

$$Calcium\ \ content\ \ \left(\frac{gm}{100\ \ g}\right) = \frac{Titrating\ \ value\ \times\ 0.1\ \times\ Total\ \ volume\ \ of\ \ ash\ \ solution}{Volume\ \ taken\ \ for\ \ estimation\ \times\ \ wt.\ \ of\ \ sample\ \ taken\ \ for\ \ ashing} \times 100 \quad (5)$$

2.4.3. Estimation of phosphorus

One milliliter of mineral solution was taken and was added to 1 ml ammonium molybdate, 1 ml hydroquinone and 1 ml of Na_2SO_3 solution. Then, the solution was mixed well by shaking. The volume is then made up to 15 ml with distilled water and mixed properly. After 30 min, the optical density of the solution was analyzed by using a photoelectric colorimeter, at 660 nm. The phosphorus content was calculated with a standard phosphate curve which ranges from 0.01–0.1 mg.

2.5. Organoleptic score (OS)

Organoleptic study protocol was checked and approved by the Research Ethical Committee (REC), Department of Food Microbiology, Patuakhali Science and Technology University, Bangladesh (Project No: FMB:01/07/2017:0011). Sensory analysis to assess the freshness or shelf life of the preserved shrimp and consumer's acceptance was determined by five skilled food scientists and performed through nine point's hedonic scale according to the Qualitative Content Analysis of QDA method [17]. The samples were presented to them without being informed about the treatments and requested to scoring them according to their physical characteristics such as appearance, color, odor, and texture. Finally, average data of acceptability by the panelists on individual attributes were determined. The hedonic scale was as follows: 1—Dislike extremely; 2—Dislike very much; 3—Dislike moderately; 4—Dislike slightly; 5—Neither like nor dislike; 6—like slightly; 7—like moderately; 8—like very much; and 9—Like extremely, where the limit of acceptability was set as a 5 (Table 1).

2.6. Estimation of tyrosine value (TV)

Tyrosine value was used to evaluate the degree of autolysis and bacterial proteolysis in shrimp. Tyrosine value was calculated using the method of Wood [18]. Two gram of sample homogenized with 40 ml of 5% trichloro acetic acid solution. After homogenization for 2 min, the macerated mixture was filtered through Whatman filter No. 1. Then, 10 ml of 0.5 (N) sodium hydroxide and 3 ml of diluted Folin Ciocalteau's reagent was taken in to 5 ml of diluted filtrate mixture. Following, 5 min of waiting, the optical density (OD) was measured at 660 nm. The TV value was calculated from the standard curve as mg/l00 g.

2.7. Estimation of microbial count

To evaluate microbial loads, 10 g of shrimp samples were transferred into 90 ml of (0.9% NaCl) normal saline solution and homogenized. Serial dilutions were performed from 10^{-1} to 10^{-8} by transferring 1 ml of each sample to 9 ml of NaCl solution (0.9%). There were three different selective agar media were used to isolation of microbial loads such as Nutrient agar for TBC, MacConkey agar for TCC, and Mannitol Salt Phenol Red Agar media for TSC were prepared following manufacturers' recommendations. Hundred µl or 0.1 ml from the dilution was inoculated onto specific culture media and incubated plates at 37 °C for 24 h to 48 h. After incubation, individual colonies had been counted and multiplied with the average number of colonies through the dilution factor to calculate the microbial load. Total counts were determined by standard spread plate technique described by Cappuccino and Sherman [19]. All analysis was done in triplicates and expressed as $\log_{10}$ (cfu/g).

2.8. Physiological and biochemical identification of bacterial isolates

For proper identification of the isolated bacteria, different biochemical tests were applied. The Catalase test for catalase enzyme activity, Methyl Red test, Voges-Proskauer test for determination of fermentation pathway where glucose was used in the mixed acid fermentation pathway process, and Citrate utilization test were performed to determine the organism's ability to utilize citrate as its carbon source. Indole test was applied for determining the ability of the organism to convert tryptophan into indole, Carbohydrate fermentation test for utilized certain types sugar, Nitrate reduction test was used for differentiating bacterial species according to their capacity to convert nitrate to nitrite or nitrogenous gases, Starch Hydrolysis test used for to observed starch degradation ability, and Hydrogen Sulfide (H_2S) production test was used for determination the sulfide compound during their metabolisms [19].

2.9. Molecular identification of bacterial species

2.9.1. DNA extraction and purification

Bacterial genomic DNA was extracted from found bacterial isolates (1.50 ml) using the Phenol: Chloroform: Isoamyl alcohol (25:24:1) extraction of boiling method [20]. Obtained pellet was completely dried and then added 50 µl TE buffer and 7 µl of RNAse which produce a total volume of 57 µl. Extracted DNA was stored at -20 °C until required for PCR. DNA concentration was measured by using of a NanoDrop™ 2000

Table 1. The hedonic scale (Nine-points) for assessment of organoleptic score of food samples.

Sl. No.	Grade	Score
1	Like extremely	9
2	Like very much	8
3	Like moderately	7
4	Like slightly	6
5	Neither like nor dislike	5
6	Dislike slightly	4
7	Dislike moderately	3
8	Dislike very much	2
9	Dislike extremely	1

Table 2. Biochemical compositions of collected shrimp.

Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Iron (mg/100 g)	Calcium (mg/100 g)	Phosphorus (mg/100 g)
78.0 ± 00	22.93 ± 0.23	0.86 ± 0.23	1.0 ± 0.00	3.83 ± 0.67	728.0 ± 43.10	567.5 ± 9.2

spectrophotometer (Thermo Scientific™) at 260 nm wavelength. Rratio of absorbance at 260 nm– 280 nm was used to measure isolated DNA purity.

2.9.2. PCR amplification

Polymer chain reaction (PCR) was performed for all of the extracted DNA samples using a thermocycler (Eppendorf, Germany). The universal primer 27F (5′-AGAGTTTGATCATGGCTCAG-3′) as forward and 1492R (5′-GGTTACCTTGTTACGACTT-3′) as a reverse primer was used for the amplification of DNA fragment for nine isolated bacteria. PCR was performed in volumes of 20 μL, containing PCR master mix 10 μL, nuclease-free dH_2O 6 μL, 1.0 μL of forward primer, 1.0 μL reverse primer, and 2 μL DNA template. PCR was running following the setup as initial denaturation for 5 min at 95 °C; 30 cycles of denaturation for 30 s at 95 °C, annealing for 1 min at 57 °C, and extension for 3 min at 72 °C; the final extension at 72 °C for 15 min, followed by cooling at 4 °C until the sample was recovered. PCR products were separated in the 2% gel electrophoresis and finally DNA bands were visualized with ethidium bromide by using *UV transilluminator*. A Promega PCR clean-up kit was used to remove primers and extra nucleotides from the amplified DNA. .

2.9.3. Sequencing of B. cereus 16S rRNA

BigDye Chain Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems, USA) and ABI PRISM 310 automated sequencer (Applied Biosystems, USA) used to detect the species specific sequencing regions of PCR products according to the manufacturers' instructions. In brief, a master-mix was prepared in a tube and mixed by a vortex machine. A volume of 2.0 μL of 5X sequencing buffer and 1.0 μL of BigDye ready reaction mixture and 0.32 μL of individual primer (forward or reverse), 3.5 μL of PCR products and 13.18 μL of nuclease-free water was added into the volume and make 20 μL in total. Samples were vortexed and centrifuged and then PCR strip was placed in a cycle sequencing machine (Mastercycler® gradient, USA Scientific) and the PCR cycling profile were pre-denaturation at 94 °C for 1 min; 25 cycles of denaturation at 94 °C for 10 s, annealing at 58 °C for 5 s and extension at 60 °C for 4 min; and a final extension at 60 °C for 10 min. The tubes were subjected to capillary electrophoresis through (denaturing) POP-6TM polymer sequencing machine (ABI PRISM® 310 Genetic Analyzer).

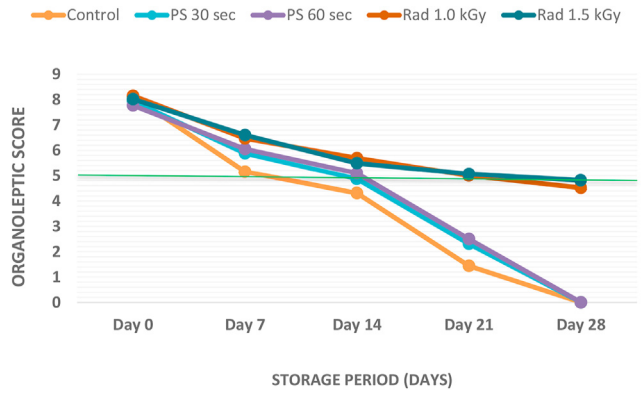

Figure 1. Organoleptic score (OS) of control, irradiation (1.0 kGy and 1.5 kGy) and potassium sorbate (PS 30 s and PS 60 s) treated samples during storage at 4 °C.

2.10. Antibiotic sensitivity test

All isolated bacteria were tested for antimicrobial susceptibility tests on Mueller–Hinton agar through using a standard disc diffusion method. The protocol was performed as follows: a 24 h bacterial culture broth was swabbed in Mueller-Hinton agar's whole surface with a sterile cotton swab and then antibiotic disks were placed in to the specific placed on the media. After that all plates were incubated at 37° C for 18–24 h. A Standard scale was used measure the clear zones of growth inhibition surrounding each antimicrobial agent disk in millimeters. Each bacterial species which was cultured on specific culture media was classified as Sensitive (S) and Resistant (R) in Mueller-Hinton agar against commercially available antimicrobial (10) sensitivity discs including gentamycin (30 μg), ampicillin (10 μg), erythromycin (30 μg), amoxicillin (10 μg), ciprofloxacin (10 μg), amikacin (30 μg), chloramphenicol (20 μg), penicillin (6 μg), tetracycline (30 μg), and imipenem (10 μg).

2.11. Statistical analysis

Descriptive statistics were done for all of the data obtained. All investigations were performed three times with triplicates of each sample and expressed as average with a standard deviation (SD). One way-ANOVA was done to see the differences among the samples. Significant differences among the averages were performed by the Duncan test and least significant difference (LSD) with a significance level of $P < 0.05$. Correlation coefficients among the entire samples were performed using Pearson's correlation with the help of Microsoft software SPSS version 25 (SPSS Chicago, IL).

All methods are completely supervised under all the authors' full supervision and contributions. So, it is informed consent was obtained from all participants for our experiments.

3. Results and discussion

3.1. Proximate analysis of shrimp samples

Proximate composition of raw shrimp was performed. Data is shown in Table 2.

Moisture content of shrimp was found to be 78.00 ± 00%. It has been reported previously that fresh shrimp contains up to 75–80% moisture [21]. Similar results were reported in other experiments with moisture values ranging from 73.91 to 78.2% [22, 23]. Nisa and Sultana (2010) were found 74–77% moisture in the muscle of matured shrimp (*Fenneropenaeus penicillatus*) [24]. In contrast, Abdel-Salam et al. (2013) observed the moisture content near about 42.88 and 40.68 ± 2.28% in male and female of *P. indicus* respectively [25].

The Protein content was found to be 22.93 ± 00.23% in the tasted shrimp. In previous studies, the protein content of shrimp reported to be ranges from 17% to 21% depending on their species [21, 26]. Similarly, 17.70–18.71% of protein were found in four different species of shrimp [27] In contrast, Liu et al., (2021) was found lower protein content (12.33–15.09%) in five different species of shrimp [28]. High crude protein content in shrimp indicates its premium quality. Enhanced protein synthesis during the active growth period of shrimp might be responsible for the higher protein content [29, 30].

Meanwhile, the lipid content was found to be 0.86 ± 00.23%. Fresh water prawns are regarded as very low fat contained seafood (less than 5%). Bragagnolo and Rodriguez-Amaya (2001) found lipid levels ranging from 0.9% to 1.0% of shrimp [32]. Whereas, the higher level of lipid 1.78% was found in *E. annandalei* species of shrimp [33] compared to the lipid content obtained in the present study. On the hand, lower level of lipid (0.10%–0.30%) was found in shrimp (*A. antennatu*) [34]. The lipid content of shrimp could vary due to their unique food consumption and environmental conditions [33].

In the present study, 1.0 ± 00% ash was recorded. Yanar and Celik et al. (2006) obtained 0.95 and 1.47% of ash in black tiger and white

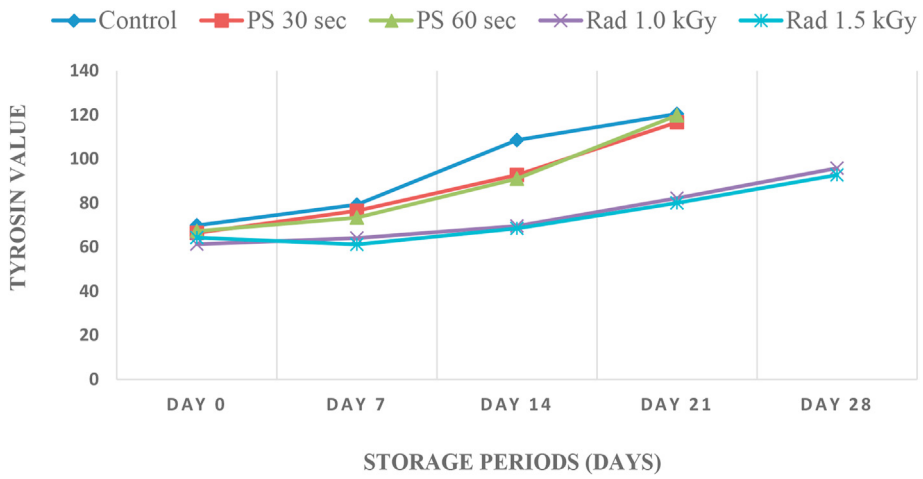

Figure 2. Tyrosine value of control, radiation ion (1.0 kGy and 1.5 kGy) and potassium sorbate (PS 30 s and PS 60 s) treated samples during storage at 4 °C.

shrimps respectively [21]. While, Nguyen et al. (2019) found 1.35 ± 0.05% in male and 1.46 ± 0.21% in female freshwater prawn [31]. In *L. vannamei* and *L. stylirostris* contained higher level of ash such as 2.26 ± 1.66% and 2.30 ± 0.71% respectively [35]. The wide range of ash content is likely related to the inclusion of bone or shell as edible parts in some species [23].

In our study, calcium (Ca) content was found to be as 728.0 mg/100g in shrimp muscle and shell which was much higher than other research findings [23, 36, 37]. However, Ca quantity may vary according to their sex, size, eating habits, molting stage as well as reproductive stage of the life cycle within and in between species to species [34].

The phosphorus (P) content was found to be 567.5 mg/100g in shrimp muscle and shell. P content of *L. vannamei* and *F. indicus, the P ontent* were found to be 215.39 to 251.46/100g respectively in tissues [36]. But P ranges 82.4 mg/100 g to 91.5 mg/100 g in shell and flesh of *P. indicus species* [38].

In this study, the iron content was found to be 3.83mg/100g in tested shrimp. Previously, in the study of Bogard et al. (2015) observed the iron content 2.6 mg/100 g in *Harpiosquilla raphidea*. Iron contents in shrimp might vary due to accumulation of iron based on different environmental conditions [23].

3.2. Organoleptic score (OS)

The organoleptic score of control, PS 30 s and PS 60 s, as well as irradiated (1.0 kGy and 1.5 kGy) samples were investigated during the storage periods based on hedonic scores. Overall acceptability was determined through a 9-point hedonic scale, as appearances, colors like reddish white to fade blue-green color; odours changes from no odour to strong off-odour, textures (from firm to soft) were evaluated. Hedonic scale data is shown in Table 1. Scores below 5 points were considered unacceptable. Initially, all the samples showed higher OS, during the storage period increases the organoleptic score values were decreased significantly due to the autolysis, and proteolytic changes of shrimp as a result off-color, and off-odour. For instance, the highest OS was found in 1.5 kGy treated sample, whereas the lowest score was obtained in the control (Figure 1).

The OS was significantly different for both treatments [4.99 ($p = 0.009$)] and days [33.62 ($p < 0.001$)]. The OS evaluation thereby revealed that the irradiated samples had more acceptability than other samples which was supported by Sheuty et al. [8]. The OS of potassium sorbate treated shrimp was less than the irradiated samples. This could be as a result of protein and lipid related chemical interactions that resulted in negative modifications to the nutritional and sensory qualities [39,40].

The acceptable limit of the sensory score is 5 for storage periods, according to Miyachi et al. [41]. In the case of shrimp, control, radiation (1 kGy and 1.5 kGy) and potassium sorbate (PS 30 s and PS 60 s) treated samples were remained acceptable for up to 7, 21, 21, 14 and 14 days of storage at 4 °C, respectively.

Table 3. Average scores (mean ± SD) of microorganisms of shrimp (*Penaeus monodon*) during storage. Duncan test was performed according to a,b,c,d,e = Day wise, A,B,C,D,E = Treatment wise. Different superscript letters indicate statistically significant ($p < .05$) result.

Sample	Count of viable microorganism (cfu/g)*	Storage period (Days)				
		0	7	14	21	28
Control	TBC	4.07^{aE}	5.11^{aE}	6.32^{aC}	7.47^{bC}	8.67^{cE}
	TCC	2.00^{aD}	2.74^{aD}	3.50^{aC}	3.69^{bE}	4.71^{cE}
	TSC	2.49^{aC}	2.65^{aD}	3.81^{bE}	3.84^{cD}	4.57^{dD}
Rad 1.0kGy	TBC	3.49^{aB}	4.56^{aB}	5.30^{aA}	6.39^{bA}	7.23^{cB}
	TCC	1.49^{aA}	1.77^{aA}	2.25^{aA}	2.63^{bB}	3.46^{cB}
	TSC	1.23^{aA}	1.83^{aA}	2.49^{bB}	2.85^{cB}	3.77^{dB}
Rad 1.5kGy	TBC	3.25^{aA}	4.17^{aA}	5.20^{aA}	6.32^{bA}	7.11^{cA}
	TCC	1.00^{aA}	1.60^{aA}	2.00^{aA}	2.27^{bA}	3.00^{cA}
	TSC	1.04^{aA}	1.82^{aA}	2.11^{aA}	2.69^{bA}	3.74^{cA}
PS 30 sec	TBC	3.90^{aD}	4.84^{aD}	5.39^{aB}	6.46^{bB}	7.54^{cD}
	TCC	1.60^{aC}	2.11^{aC}	2.81^{bB}	3.17^{bD}	3.46^{cD}
	TSC	1.38^{aB}	1.97^{aC}	2.79^{bD}	2.99^{cC}	3.86^{dD}
PS 60 sec	TBC	3.77^{aC}	4.74^{aC}	5.34^{aB}	6.43^{bB}	7.47^{cC}
	TCC	1.47^{aB}	2.00^{aB}	2.77^{bB}	3.07^{cC}	3.92^{dC}
	TSC	1.59^{aB}	2.07^{aB}	2.87^{bC}	3.14^{cC}	3.96^{dC}

Duncan test was performed according to a,b,c,d,e = Day wise, A,B,C,D,E = Treatment wise.
*TBC = Total bacterial count; TCC = Total coliform count; TSC = Total staphylococcal count; PS 30 s = 30 s dip in 2% potassium sorbate; PS 60 s = 60 s dip in 2% potassium sorbate; Rad 1.0 = Radiation dose 1 kGy; Rad 1.5 = Radiation dose 1.5 kGy.

3.3. Chemical (tyrosine value) change of shrimp

Tyrosine value (TV) is an indicator of protein degradation in fish samples. Therefore, we conducted this experiment to assess protein degradation (autolytic and bacterial proteolysis) during preservation of shrimp. Data is shown in Figure 2. The tyrosine content was found to increase with the increasing of storage periods. It was observed that the increasing of tyrosine value was lower in irradiated sample (1.5 kGy). Tyrosine concentrations could have increased as a result of shrimp hydrolysis caused by bacterial proteolysis and inborn tissue enzymes [42].

Table 4. Correlation among OS, TV, TBC, TCC and TSC of Shrimp (*Penaeus monodon*) at different treatments during storage at 4 °C temperature.

		Control					PS 30 s					PS 60 s					1 kGy					1.5 kGy				
		OS	TV	TBC	TCC	TSC	OS	TV	TBC	TCC	TSC	OS	TV	TBC	TCC	TSC	OS	TV	TBC	TCC	TSC	OS	TV	TBC	TCC	TSC
Control	OS	1																								
	TV	−.830**	1																							
	TBC	−.937**	.971**	1																						
	TCC	−.948**	.961**	.998**	1																					
	TSC	−.750*	.990**	.933**	.919**	1																				
PS 30 s	OS						1																			
	TV						−.951**																			
	TBC						−.994**	.964**	1																	
	TCC						−.960**	.996**	.975**	1																
	TSC						−.959**	.997**	.974**	1.000**	1															
PS 60 s	OS											1														
	TV											−.975**	1													
	TBC											−.995**	.962**	1												
	TCC											−.942**	.923**	.966**	1											
	TSC											−.935**	.919**	.960**	.997**	1										
1 kGy	OS																1									
	TV																−.868**	1								
	TBC																−.971**	.959**	1							
	TCC																−.914**	.983**	.975**	1						
	TSC																−.956**	.959**	.988**	.991**	1					
1.5 kGy	OS																					1				
	TV																					−.748**	1			
	TBC																					−.945**	.920**	1		
	TCC																					−.914**	.918**	.977**		
	TSC																					−.891**	.925**	.974**	.989**	1

Abbreviations: OS: Organoleptic score, TV: Tyrosine value, TBC: Total bacterial count, TCC: Total coliform count, TSC: Total *Staphylococcus* count.

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

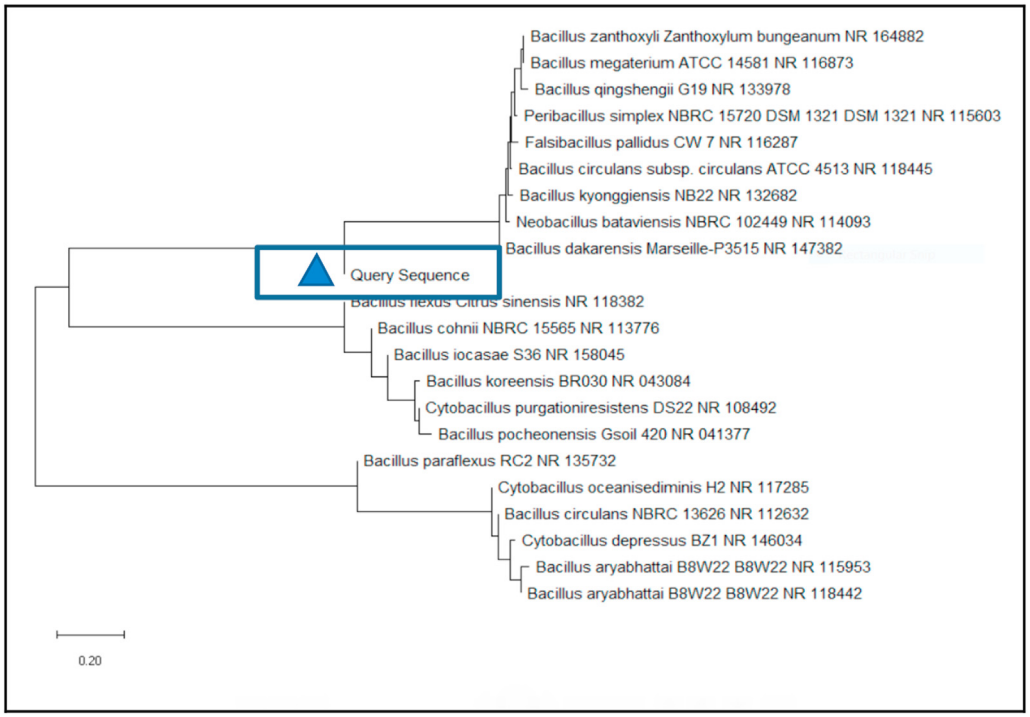

Figure 3. Phylogenic tree of *B. cereus*.

3.4. Microbiological analysis

Standard plating methods were used to determine the TBC, TCC, and TSC loads. The obtained data is shown in Table 2. The TBC value was found to be 4.07 log cfu/gm in control at zero-day. After 28 days of storage TBC were increased and excessed the acceptable range as 8.67 log cfu/gm of control. Meanwhile, the treated samples of both radiation and potassium sorbate with low temperature showed lower count which were 3.49 log cfu/gm (1.0 kGy) and 3.25 log cfu/gm (1.5 kGy), 3.90 log cfu/gm (PS 30 s) and 3.77 log cfu/gm (PS 60 s). The results showed that TBC were reduced gradually in both irradiated and potassium sorbate treated samples than that of control (untreated) sample which has similarity with previous studies [42, 43]. For the purpose of extending the shelf-life of fresh fish, radiation doses between 1 and 3 kGy have been proposed [44]. The bacterial count of irradiated fish samples were lower than non-irradiated samples at 4 °C storage times, which is similar result of previous study [45]. The antibacterial effect of potassium sorbate, which prevents bacterial spore production in fish fillets, may account for the decreased count of TBC in the samples [45].

On the other hand, TCC values of 2.0, 1.60, 1.47, 1.0 and 1.49 log cfu/gm was observed in control, PS 30 s, PS 60 s, 1.0 kGy and 1.5 kGy respectively. During 21 days of storage, TCC values were increased gradually for all samples. TCC values were significantly different among the treatments [3.44 ($p = 0.023$)]. Obtained results in this study revealed that irradiation has good impact on the elimination of TCC. The decreasing effect of radiation on TCC load in shrimps is in agreement with several studies [46, 47].

In this study, potassium sorbate treated samples also reduced TCC values than control samples. Similar data of potassium sorbate treated samples observed in reducing the growth of TCC after 7 days storage time [48]. According to ICMSF (1986) guideline, acceptable total coliform count for fish is less than 500 cfu/g [49].

In this study, the TSC values were 1.23 log cfu/gm and 1.04 log cfu/gm for irradiated samples of 1.0 kGy and 1.5 kGy, respectively. Meanwhile, 1.3 log cfu/gm and 1.59 log cfu/gm were found for PS 30 s and for PS 60 s respectively (Table 3). The TSC value was significantly different among the tested treatments [3.04 ($P = 0.037$)] for shrimp preservation. All of the treated samples remained acceptable up to 21 days of storages

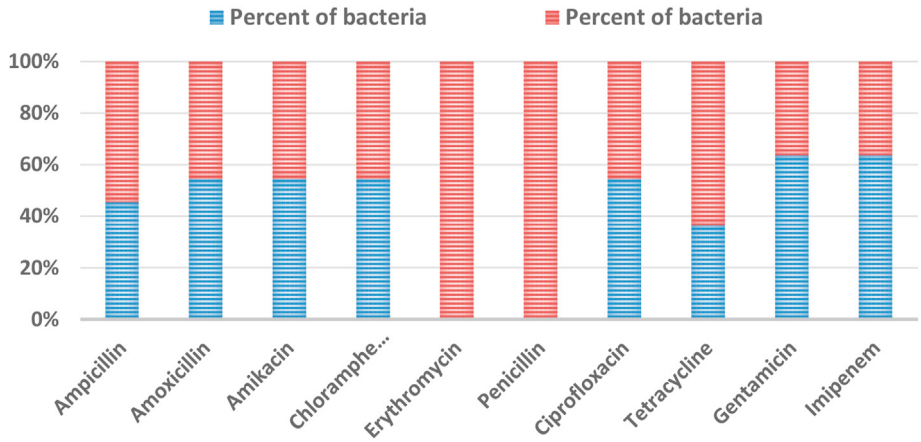

Figure 4. Antibiotic Sensitivity Test against isolated bacterial strains from shrimp.

in case of TSC. These data was in agreement with previous studies that reduced TSC level and complete reduction during irradiation treatment [50].

3.5. Correlation analysis

Correlation coefficients among all the treated and untreated samples were performed by the Pearson's correlation. A strong significant correlation among OS, TV, TBC, TCC and TSC were found during storage (Table 4). OS was negatively correlated with TV. All the samples showed an increased level of TV with the decreased level of OS that might be due to protein oxidation. A positive correlation among TV, TBC, TCC and TSC were found in all the samples. Furthermore, TBC, TCC and TSC have been showed a strong negative correlation with OS ($p > 0.01$) which leads to quality degradation of shrimp.

3.6. Polymerase chain reaction (PCR) assay

In this study, molecular technique including PCR was performed to identify isolated strains responsible for shrimp spoilage. PCR data is shown in supplementary SF Table 1. Gel electrophoresis image showed a 1492 bp length DNA amplified PCR product in Lanes 1–9 for isolated bacterial strains.

3.6.1. 16S rRNA sequence of for conform identification of strains

A total of 11 bacterial isolates had initially been identified using 16S rRNA molecular markers. These identified genera, there was a wide range of spoilage bacteria such as Gram-negative rods, cocci as well as Gram-positive cocci including *Lactococcus lactis, Klebsiella pneumonia, E. coli, B, cereus, Enterobacter, Staphylococcus aureus, Pseudomonas, Salmonella, Shigella, Micrococcus, and Alcaligenes*. Each strain yielded a set of high-quality 16S sequences, for example 1495 bp for *B. cereus* (Figure 3).

3.7. Antibiotic sensitivity test

In this study, antibiotic sensitivity test was conducted to evaluate the resistance of isolated bacteria associated with shrimp for their further disease formation as well as treatment management. Obtained data is shown in Figure 4. Results showed that more than half of the Gram-positive isolated strains showed up to 75 % resistance to Ampicillin, Amoxicillin, and Amikacin. All of them are second-third generation antibiotics (Figure 4). In contrast, all of the Gram-negative bacterial isolates gave 75% sensitivity to these antibiotics. However, only Erythromycin and Penicillin were found to be 100% resistant to all Gram-positive and Gram-negative bacteria.

In the previous study, all of the Gram-negative strains isolated from shrimp showed resistance to Ampicillin and Amoxicillin, Chloramphenicol and Tetracycline [51, 52].

4. Conclusions

P. monodon is a nutritionally enriched fish containing low-fat, good protein as well as minerals. In this study, the best method for increasing shelf-life of shrimp was found to be irradiation (1.5 kGy) with low temperature (4 °C). Irradiated samples stored at low temperature were acceptable up to 21 days based on OS and Tyrosine values as well as microbial data. Shrimp samples showed the presence of pathogenic bacteria. Antibiotic sensitivity test data showed resistance for all of the Gram-positive isolates with third-fourth generation antibiotics. This study revealed that irradiation treatment with low temperature was effective for shrimp preservation in terms of safety and quality perspective. However, further research should be done to evaluate cost effective analysis of these techniques for commercial shrimp preservation in large quantity.

Declarations

Author contribution statement

Md. Shajadul Islam, Arzina Hossain: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools and data; Wrote the paper.

Mahfuza Islam, Irfan Ahmed: Analyzed and interpreted the data.

M. Kamruzzaman Munshi: Conceived and designed the experiments; Contributed reagents, materials, analysis tools and data.

Mohammad Shakhawat Hussain, Md. Shafiqul Islam Khan: Conceived and designed the experiments.

Keshob Chandra Das, Roksana Huque: Contributed reagents, materials, analysis tools and data.

Funding statement

This work was supported by Ministry of Science and Technology of the Goverment of Bangladesh (NST) Grant (847, 01/07/2017-18).

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2022.e12596.

Acknowledgements

The authors would like to thank Dr. Md. Salimullah Director-General of the National Institute of Biotechnology, Dhaka, Bangladesh for assisting PCR and16S rRNA sequencing analysis.

References

[1] M.T. Uddin, A. Goswami, M.S. Rahman, A.R. Dhar, How can governance improve efficiency and effectiveness of value chains? An analysis of pangas and tilapia stakeholders in Bangladesh, Aquaculture 510 (2019) 206–215.

[2] M.A.R. Hossain, M.R. Hasan, An Assessment of Impacts from Shrimp Aquaculture in Bangladesh and Prospects for Improvement" 618, FAO Fisheries and Aquaculture, 2017, p. 96. Technical Paper No.

[3] BBS, Yearbook Of Agricultural Statistics - 2017, Statistics and Informatics Division (SID) Ministry of Planning, Government of the People's Republic of Bangladesh, Dhaka, 2017.

[4] DoF, Yearbook of fisheries statistics of Bangladesh, 2018-19, in: Fisheries Resources Survey System (FRSS) 36, Department of Fisheries, Bangladesh: Ministry of Fisheries and Livestock, 2019, p. 135.

[5] P. Masniyom, S. Benjakul, W. Visessanguan, Shelf-life extension of refrigerated sea bass slices under modified atmosphere packaging, J. Sci. Food Agric. 82 (2002) 873–880.

[6] I.N. Savvaidis, P. Skandamis, K. Riganakos, N. Panagiotakis, M.G. Kontominas, Control of natural microbial flora and *Listeria monocytogenes* in vacuum-packaged trout at 4 and 10^0C using irradiation, J. Food Protect. 65 (2002) 515–522.

[7] V.J. Sinanoglou, A. Batrinou, S. Konteles, K. Sflomos, Microbial population, physicochemical quality, and allergenicity of molluscs and shrimp treated with cobalt-60 gamma radiation, J. Food Protect. 70 (4) (2007) 958–966.

[8] T.F. Sheuty, M. Kamrujjaman, M. Islam, M.A. Hossain, R. Haque, Effect of potassium sorbate and gamma irradiation on the shelf-life of Hilsa shad, *Tenualosailisha*(Hamilton, 1822) at low temperature, Jahangirnagar Univ. J. Biol. Sci. 6 (2) (2017) 67–73.

[9] O. Remisha, K.B. Biji, S.K. Gupta, J.P. James, S. Mathew, Applications of Potassium Sorbate dip treatment for the extension of shelf life of Indian Mackerel (*Rastrelligerkanagurta*) during chill storage, Imp. J. Interdiscip. Res. (IJIR) 2 (11) (2016) 1874–1883.

[10] M. Zarei, A. Fazlara, D. Jamaledin, M. Mojtahedi, Microbial and chemical quality of vacuum-packed frozen fillets of narrow-barred Spanish mackerel

(*Scomberomoruscommerson*) and silver pomfret (*Pampus argenteus*) marketed in Iran, World J. Fish Marine Sci. 3 (5) (2011) 410–413.
[11] S. Mehrzadeh, L. Roomiani, Effect of gamma irradiation and modified atmosphere packaging on the shelf-life of white shrimp (*Metapenaeus affinis*), Iran. J. Fish. Sci. 20 (2021) 1004–1021.
[12] Md. Kamrunnahar, Enamul Haque, Arzina Hossain, Mahfuza Islam, Md. Kamruzzaman Munshi, Khandoker Asaduzzaman, Roksana Huque, Effects of citric acid and gamma radiation on the shelf life of *Labeo bata* (Hamilton, 1822), Jahangirnagar Univ. J. Biol. Sci. 8 (2) (2019) 25–33.
[13] M.F. Mortuza, L. Lepore, K. Khedkar, S. Thangam, A. Nahar, H.M. Jamil, L. Bandi, M.K. Alam, Commissioning dosimetry and in situ dose mapping of a semi-industrial Cobalt-60 gamma-irradiation facility using Fricke and Ceric-cerous dosimetry system and comparison with Monte Carlo simulation data, Radiat. Phys. Chem. 144 (2018) 256–264.
[14] AOAC, Official Method of Analysis, Association of Official Analytical Chemists, Washington D.C, 2005.
[15] K.J. Carpenter, Biochem. J. 77, 1960, p. 608.
[16] S. Ranganna, Handbook of Analysis and Quality Control for Fruits and Vegetable Products, second ed., Tata McGraw-Hill Pub. Com. Ltd., New Delhi, India, 1986a, pp. 126–127.
[17] D.R. Peryam, F.I. Pilgram, Hadonic assessment and food technology, Food Technol. 11 (1957) 9–14.
[18] A.J. Wood, G.J. Sigurdssas, W.J.D. Year, J. Fish. Res. Board Can. 6, 1942, p. 53.
[19] J.G. Cappuccino, N. Sherman, Microbiology - a Laboratory Manual, tenth ed., The Benjamin/Cummings Pub. Co. Inc., New York, USA, 2014, pp. 161–216.
[20] D. De Medici, L. Croci, E. Delibato, S. Di Pasquale, E. Filetici, L. Toti, Evaluation of DNA extraction methods for use in combination with SYBR green I real-time PCR to detect Salmonella enterica serotype enteritidis in poultry, Appl. Environ. Microbiol. 69 (6) (2003) 3456–3461.
[21] Y. Yanar, M. Celik, Seasonal amino acid profiles and mineral content of green tiger shrimp (*Penaussemisulcatus*, De Haan, 1844) and speckled shrimp (*Metapenausmonoceros,* Fabricus 1789) from the Eastern Mediterranean Se, J. Food Chem. 94 (2006) 33–36.
[22] A.F.P. Moura, A. Tenuta, Effects of processing on free cholesterol and 7-ketocholesterol concentrations in pink-shrimp, Ciência e Tecnologia de Alimentos (Food Science and Technology) 22 (2002) 117–121.
[23] J.R. Bogard, S.H. Thilsted, G.C. Marks, M.A. Wahab, M.A.R. Hossain, J. Jakobsen, J. Stangoulis, Nutrient composition of important fish species in Bangladesh and potential contribution to recommended nutrient intakes, J. Food Compos. Anal. 42 (2015) 120–133.
[24] K. Nisa, R. Sultana, Variation in the proximate composition of shrimp,*Fenneropenaeuspenicillatus*at different stages of maturity, Pak. J. Biochem. Mol. Biol. 43 (3) (2010) 135–139.
[25] H.A. Abdel-Salam, Evaluation of nutritional quality of commercially cultured Indian white shrimp Penaeusindicus, Int. J. Nutr. Food Sci. 2 (4) (2013) 160–166.
[26] P. Sriket, S. Benjakul, W. Visessanguan, K. Kijroongrojana, Comparative studies on chemical composition and thermal properties of black tiger shrimp (*Penaeus monodon*) and white shrimp (*Penaeusvannamei*) meats, Food Chem. 103 (4) (2007) 1199–1207.
[27] S. Wu, M. Zhao, S. Gao, Y. Xu, X. Zhao, M. Liu, X. Liu, Change regularity of taste and the performance of endogenous proteases in shrimp (*Penaens vannamei*) head during autolysis, Foods 10 (2021) 1020.
[28] Z. Liu, Q. Liu, D. Zhang, S. Wei, Q. Sun, Q. Xia, W. Shi, H. Ji, S. Liu, Comparison of the proximate composition and nutritional profile of byproducts and edible parts of five species of shrimp, Foods 10 (2021) 2603.
[29] R. Tanuja, Some Aspects of Biology and Utilization of the Mantis Shrimp *Oratosquillaneppa*from Cochin Waters, Cochin University of Science and Technology, India, 1996, p. 87.
[30] A. Pedrazzoli, C. Molina, N. Montoya, S. Townsend, A. Leon- Hing, Y. Parades, J. Calderon, Recent Advances on Nutrition Research of Penaeusvannameiin Ecuador, Rev. Fish. Sci. 6 (1998) 1–2.
[31] T.M. T Nguyen, T.Y. Chen, C.Y. Shiau, Y.T. Cheng, Y.W. Chang, Study on biochemical divergences of the meat and egg of freshwater prawns (Macrobrachium rosenbergii), Food Sci. Nutr. 7 (2019) 2017–2023.
[32] N. Bragagnolo, D.B. Rodriguez-Amaya, Total lipid, cholesterol, and fatty acids of farmed freshwater prawn (*Macrobrachiumrosenbergii*) and wild marine shrimp *(Penaeusbrasiliensis, Penaeusschimitti, Xiphopenaeuskroyeri*), J. Food Compos. Anal. 15 (2001) 359–369.
[33] Li. Guipu, J. AndrewSinclair, Li. Duo, Comparison of lipid content and fatty acid composition in the edible meat of wild and cultured freshwater and marine fish and shrimps from China, J. Agric. Food Chem. 59 (5) (2011) 1871–1881.
[34] R. Rosa, M.L. Nunes, Nutritional quality of red shrimp, *aristeusantennatus*(risso), pink shrimp, *parapenaeuslongirostris*(lucas), and Norway lobster, *nephropsnorvegicus*(linnaeus), J. Sci. Food Agric. 84 (1) (2003) 89–94.
[35] D. Puga-lopez, J.T. Ponce-palafo, G. Barba-quintero, M.R. Torres-herrera, E. Romero-beltran, J.L. Arredondo-figueroa, M.G. Gomez, Physicochemical, proximate composition, microbiological and sensory analysis of farmed and wild harvested white shrimp *litopenaeusvannamei* (boone, 1931) Tissues, Curr. Res. J. Biol. Sci. 5 (3) (2013) 130–135.
[36] R. Shalini, A.R. Nazar, M.A.B. Haq, S. Shanker, Biochemical changes of *Litopenaeusvannamei* and *Fenneropenaeusindicus*in the different stages of WSSV infection, J. Coastal Life Med. 1 (1) (2013) 63–69.
[37] M.G. Mustafa, M.A. Hossain, M.J. Alam, M.M.R. Khan, S.S. Nilla, M.Z. Alam, Effects of gamma irradiation on the shelf-life of frozen tiger shrimp, *Penaeus monodon* (Fabricius, 1798), Int. J. Nat. Sci. 4 (1) (2014) 10–15.
[38] S. Ravichandran, G. Rameshkumar, A.R. Prince, Biochemical composition of shell and flesh of the Indian white shrimp *penaeusindicus (H.milne*Edwards 1837), Am.-Eurasian J. Sci. Res. 4 (3) (2009) 191–194.
[39] V.T. Pardio, K.N. Waliszewski, P. Zuniga, Biochemical, microbiological and sensory changes in shrimp (*Panaeus aztecus*) dipped in different solutions using face-centred central composite design, Int. J. Food Sci. Technol. 46 (2) (2011) 305–314.
[40] D. Miyachi, M. Eklund, J. Spinelli, N. Stall, Fd Technol 18, 1964, p. 928.
[41] D. Pearson, Application of chemical method for the assessment of beef quality. II. Methods related to protein breakdown, J. Sci. Food Agric. 19 (1968a) 366–369.
[42] I.S. Arvanitoyannis, A.C. Stratakos, Effect of irradiation on fish and sea food, in: Irradiation of Food Commodities Techniques, Applications, Detection, Legislation, Safety and Consumer Opinion", 2010, pp. 287–365. Chapter 9.
[43] M. Michele, M. Giuliana, F. Francesca, S. Grazia, E. Antonio, Official checks by an accredited laboratory on irradiated foods at an Italian market, Food Control 33 (2) (2013) 307–312.
[44] R. Mendes, H.A. Silva, M.L. Nunes, J.M.A. Empis, Effect of low-dose irradiation and refrigeration on the microflora, sensory characteristics and biogenic amines of Atlantic horse mackerel (Trachurustrachurus), J. Europ. Food Res. Technol. 221 (2005) 329–335.
[45] B. Laxmareddy, G. Benarjee, Intestinal histopathology of trematode infected fish. channastriatus, Biolife 1 (1) (2013) 29–31.
[46] M.E. Haque, A.A. Mahin, T. Afroz, H.O. Rashid, Changes of microbial load and protein content of irradiated dried prawn during long-term storage, J. Food Process. Preserv. 37 (2013) 946–951.
[47] A. Noomhorm, P. Vongsawasdi, C. Inprasit, J. Yamprayoon, P. Sirisoontaralak, M.E.A. Ingles, A. Adulpichit, Impact of irradiation on the quality of processed meat and fishery products, in: Radiation Processing for Safe, Shelf-Stable and Ready-To-Eat Food". pp. 167–193, July 10–14, Proceedings of a Final Research Co-ordination Meeting Held in Montreal, 2000. Canada.
[48] B.M. Abu-Ghazaleh, Effects of ascorbic acid, citric acid, lactic acid, NaCl, potassium sorbate and *Thymus vulgaris* extract on *Staphulococcus aureus* and *Escherichia coli*, Afr. J. Microbiol. Res. 7 (1) (2012) 7–12.
[49] International Commission on the Microbiological Specification of Foods (ICMSF), Microorganisms in Food, in: Sampling for Microbiological Analysis: Principles and Specific Applications 2, Univ. Toronto Press, Toronto, Canada, 1986.
[50] M.M. Haque, M.G. Sorrowar, H.U. Rashid, Effects of frozen storage, radiation and their combined treatments on microorganisms of freshwater mola fish AmblypharyngodonMola, J. Bangladesh Acad. Sci. 37 (1) (2013) 21–31.
[51] J.H. Kim, H.J. Ahn, J.W. Lee, H.J. Park, G.H. Ryu, Effects of gamma irradiation on the biogenic amines in pepperoni with different packaging conditions, Food Chem. 89 (2005) 199–205.
[52] A.A.M. Hatha, A.A. Vivekanandhan, G.J. Joice, Antibiotic resistance pattern of motile aeromonads from farm raised freshwater fish, Int. J. Food Microbiol. 98 (2005) 131–134.