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Quality changes of sous-vide cooked and blue light sterilized Argentine squid (*Illex argentinus*)

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ABSTRACT

The present work was carried out to investigate the quality changes and shelf life of blue light (Blu-ray) irradiated sous-vide cooked (SVC) Argentine squid (Illex argentinus) during storage at 0, 5, and 10 °C. Sensory evaluation, color, shear force, lipid oxidation levels, total viable counts (TVC), and psychrophilic bacterial count were used to study the changes in storage quality of SVC squid at different temperatures. Results showed that the high-quality endpoints of Blu-ray irradiated Argentine squid were 360, 144, and 72 h, and the shelf-life endpoints were 504, 240, and 120 h during storage at 0, 5, and 10 °C, respectively. The redness values of irradiated squid did not differ significantly (p >0.05) during the storage, the brightness and yellowness values of irradiated squid showed an increasing trend, and the sheer force initially increased and then decreased. The thiobarbituric acid reactive substance of each squid stored at low temperature increased with the extension of the storage period, indicating that they exhibited fat oxidation with the extension of the storage period. The TVC and the number of Psychrobacter species increased with the storage period. The correlation analysis suggested that TVC and Psychrobacter count as indicators of quality changes in Argentine squid during low-temperature storage were in good agreement with sensory scores (R^2 >0.9). Additionally, our results showed that Blu-ray sterilization played a positive role by inducing photosensitive oxidation and decreasing TVC and the total number of Psychrobacter than the control group during storage of SVC squid after Blu-ray irradiation. This study provides a theoretical basis for applying Blu-ray sterilization in aquatic product processing.

Keywords: blue light sterilization, sous-vide cooking, squid, storage period, quality changes

INTRODUCTION

The squid belongs to the family of molluscs and is a member of class Cephalopoda. At present, the main species of squid processed are Japanese common squid (*Todarodes Pacificus*), Argentinean squid (*Illex argentinus*), Peruvian squid (*Dosidious gigas*), and New Zealand squid (*Nototodaras Sloane*) **[1]**, **[2]**. Squid has high economic value; its edible part is 80%, nearly 20% higher than ordinary fish, protein content is 15 – 20%, and fat content is 1 – 2%. Additionally, it is rich in essential amino acids and contains high taurine levels, which can relieve fatigue, restore vision, and improve liver function. It is also abundant in calcium, phosphorus, iron, selenium, and other trace elements **[3]**, **[4]**. In addition, squid is rich in vitamins; every 100 g of fresh squid contains 35, 20, 60, 1600, and 600 µg of vitamin A, thiamine, riboflavin, niacin, and vitamin E1, respectively **[5]**. Therefore, squid is consumed as an essential source of protein.

For easier and better consumption of squid, it can be made into ready-to-eat squid using sous-vide cooking, which is a heat treatment method. The raw materials are placed in a vacuum bag and heated at temperature and time **[6]**. This method reduces water loss from the food during the cooking process, maintains tenderness and juiciness, preserves the color of raw materials, prevents oxidation during the heating process, hampers secondary contamination during storage, and extends the shelf-life **[5]**. Gonnella et al. **[7]** addressed that compared to traditional cooking methods (boiling, steaming, and microwaving), ready-to-eat asparagus prepared using sous-vide microwave cooking increased green color and reduced chlorophyll content, resulting in better consumer satisfaction, and created a positive impact on the ready-to-eat vegetable industry. Renna et al. **[8]** indicated that sous-vide combined with microwave cooking could control naturally occurring thermophilic aerobic bacteria, yeasts, and moulds in vacuum-packed vegetables for up to 30 days and reduced *Escherichia coli* and *Listeria monocytogenes* levels on chicory by more than 5 LG CFU/g. Furthermore, Cui et al. **[5]** showed that SVC squid's appearance, texture, and preference were better than those prepared using traditional cooking methods (boiling and steaming), along with good quality feasibility for industries processing squid.

Blue light (Blu-ray) sterilization is a non-pharmacological technology and has been widely studied as an alternative to traditional antibiotics **[9]**. This sterilization technology uses Blu-ray for light sterilization at a wavelength of $4.05\times10^{-7} – 4.70\times10^{-7}$ m. Blu-ray receptors induce gram-positive and Gram-negative bacteria and fungi to cause physiological reactions **[10]**. Since Blu-ray sterilization does not exhibit any thermal effect and is effective in sterilization, researchers in the food field observed that after Blu-ray irradiation (4.13×10^{-7} m, <2 h, 7.20×10^{5} J/m^2), all *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella Typhimurium*, and *Mycobacterium fortuitum* presented a 5-log inactivation in milk **[11]**. Moreover, Blu-ray irradiation (4.60×10^{-7} m) inhibited Salmonella inoculated on the surface of fresh-cut pineapples without any food additives **[12]**. A previous study found that SVC squid irradiated with Blu-ray was superior to the non-Blu-ray irradiated control group in terms of sensory attributes, shear force (SF), and fat oxidation.

In this study, the sensory evaluation, color, shear force, total viable count (TVC), and the number of *Psychrobacter* were used as indicators to quantify the sensory, physicochemical, and microbiological characteristics of SVC Argentine squid to explore and analyze their quality changes and shelf-life during storage at 0, 5, and 10 °C using Blu-ray sterilization. Furthermore, the consistency of each indicator was investigated to provide essential information for better research on the feasibility of making ready-to-eat squid using SVC and optimizing the squid cold chain.

Scientific Hypothesis

Blu-ray is an anti-sterilization technology. This study assumed that Blu-ray exposure would affect the quality of SVC squid and play a positive role during the storage process. According to the results obtained, the SF of the SVC squid was not affected by SVC. The SVC squid obtained after Blu-ray was different from the control group in brightness and yellowness. In addition, the TVC and *Psychrobacter* count in the SVC squid after Blu-ray exposure were lower than those in the control group.

MATERIAL AND METHODOLOGY

Samples

Argentinian squid (about net weight 0.4 kg) was chosen the same size and purchased from an aquatic products market in Qingdao city, China's Shandong Province.

Chemicals

The chemical reagents are of analytical grade and purchased in Sinopharm Chemical Reagent Beijing Co., LTD, China. Ethanol, magnesium acetate solution, glucose solution, anthrone reagent, sulfuric acid solution, boric acid solution, sodium hydroxide solution, phosphate buffer solution, trichloroacetic acid, 2-thiobarbituric acid, casein solution (Sinopharm Chemical Reagent Beijing Co., LTD, China).

Animals and Biological Material

Argentinian squid (*Illex argentinus),* China's Shandong Province.

Instruments

Vacuum sealer (DZ-260, Dajiang Holding Group Electric Co., LTD, China).
Sous Vide machine (A3.2-120V, Anova Culinary, United States of American).
Spectrophotometer (7200, Unico Shanghai Instruments Co., LTD, China).
Digital light meter (Lutron-LX-101A, Lutron electronic enterprise Co., LTD, Taiwan, China).
Colorimeter (CR-400, Konica Minolta Holding Company, Japan).
Texture meter (TA.XTC, Shanghai Baosheng Industrial Development Co., LTD, China)

Laboratory Methods

According to the International Standards and Chinese National Food Standards, the study was carried out.

The sensory evaluation was determined according to ISO 11136:2014 **[13]**. The sensory evaluation group consisted of 15 trained panellists aged 18 and 25. The color, odor, body mucus, and muscle elasticity of squid were evaluated and scored, with 20 being the best quality, 12 being the high-quality period endpoint, and 4 being the endpoint quality.

The squid samples‘ colour (control and irradiated) was measured using a colorimeter. The color was described with the CIELAB color space scale: lightness (L*), green to red hue (a*), and blue to yellow hue (b*).

The thiobarbituric acid reactive substances were determined according to GB/T 35252-2017 **[14]**. For extraction, the sample (0.02 kg) was first mixed with 25 ml of aqueous 20% trichloroacetic acid (TCA) solution and 15 ml of distilled water. The mixture was homogenized and allowed to stand at room temperature (25 °C) for one h. After centrifugation at 3000 rpm for 10 min, the filtrate was diluted with distilled water to 50 ml. Next, 2 ml of the fresh filtrate was mixed with 2 ml of 0.02 M aqueous 2-thiobarbituric acid (TBA) solution, placed in a water bath in a cuvette containing a stopper at 95 °C for 30 min, and then cooled under running water. The spectrophotometer was calibrated at 5.32×10^{-7} m with distilled water, and then the sample absorbance was measured. The colorimetric absorbance obtained from the spectrophotometer was converted to mg malonaldehyde/kg meat to represent TBA content.

The determination of shear force was carried out referring to the method of Baublits et al. **[15]**. The cooked samples were divided into 0.02 × 0.02 × 0.005 m cubes, and samples were sheared along the muscle fibers vertically using a texture meter at 20 °C. The force required to shear the samples were recorded in Newton (N).

The total viable count was determined according to ISO 4833-1:2013 **[16]**. The samples were placed in 10 ml of phosphate-buffered saline (PBS) (10 mM, pH = 7.4, NaCl 8 g, KH_2PO_4 0.00024 kg, $Na_2HPO_4 \cdot 12H_2O$ 0.00363 kg, KCL 0.0002 kg, distilled water 1 L) in a centrifuge tube and sonicated for 5 min, centrifuged (5000 rpm for 5 min), the supernatant removed, and resuspended in 200 µl PBS. The bacterial solution was then diluted in 96-well plates in a gradient ($10^0 - 10^7$) and incubated in Luria-Bertani medium (pH = 7.4 − 7.6, tryptone 10, yeast extract 0.005 kg, NaCl 0.01 kg, agar .0.015 kg, distilled water 1 L) (37 °C, 7 – 9 h) and counted to calculate the TVC.

The samples were placed in 10 ml of phosphate buffer saline (10 mM, pH=7.4, NaCl 0.008 kg, KH_2PO_4 0.0024 kg, $Na_2HPO_4 \cdot 12H_2O$ 0.00363 kg, KCL 0.0002 kg, distilled water 1 L) in a centrifuge tube and sonicated for 5 min. They were then centrifuged at 5000 rpm. The supernatant after gradient dilution ($10^0 - 10^7$) was taken for 5 min and incubated (37 °C, 7 − 9 h) in Luria-Bertani medium (pH = 7.4 − 7.6, tryptone 10, yeast extract 0.005 kg, NaCl 10 g, agar 0.015 kg, distilled water 1 L) and counted. The number of *Psychrobacter* was determined after incubating the plates at 5 °C for 72 h.

Description of the Experiment

Sample preparation: The squid specimens were kept refrigerated with flake ice inside polystyrene boxes provided with a lid and holes for drainage and transported to the laboratory at -18 °C.

Number of samples analyzed: 402.

Number of repeated analyses: 5.

Number of experiment replication: 3.

Design of the experiment: Just before cooking, squid specimens separated into the head, foot (wrist), and ketone body with scissors after thawing and washing. The average weight of the ketone body of squid was 0.02 ±0.004 kg (n = 16), respectively. Each squid specimen's length, width, and thickness of the ketone body of squid were 0.04 ±0.01 m, 0.04 ±0.01 m, and 0.001 ±0.0004 m. The samples were in a plastic vacuum bag (nylon/polyethylene, 0.03 mm, 121 °C/249.8 °F) and sealed using a vacuum sealer and using SV machine heated in water baths, timed experiment time. Next, they were placed in water maintained at a temperature of 60 °C and heated for 30 min until further use **[5]**. After heating, the samples were cooled to 4 °C in cold water and placed in the refrigerator for testing. All samples were randomly divided into the control group and the Blu-ray irradiated group. All the samples were irradiated by Blu-ray at the dose of 2.16×10^5 J/m^2. Then the irradiated and control samples were sent for storage. The storage temperatures were controlled at 0 ±0.1 °C, 5 ±0.1 °C, and 10 ±0.1 °C. According to the pre-experiment results, the samples were randomly taken at appropriate intervals (adjusted based on the spoilage rate at different storage temperatures, and the frequency was increased at a later stage according to the spoilage rate) for physicochemical and microbiological tests.

Statistical Analysis

Origin 2021 software (OriginLab Corporation, Massachusetts, USA) was used for data analysis. All assays were repeated at least three times independently, and the experimental data were represented as mean ± standard deviation. The means were compared by Tukey's multiple range test at p <0.05.

RESULTS AND DISCUSSION

Changes in sensory attributes of squid during storage

The time required to reach the end of the high-quality period (control group and irradiated group) was 360, 144, and 72 h. The sensory score was 12, while those taken to reach the end of shelf-life (control group and irradiated group) was 504, 240, and 120 h, and the sensory score was 4 during storage at 0, 5 and 10 °C, respectively. The quality of squid at different temperatures varied considerably (Figure 1).

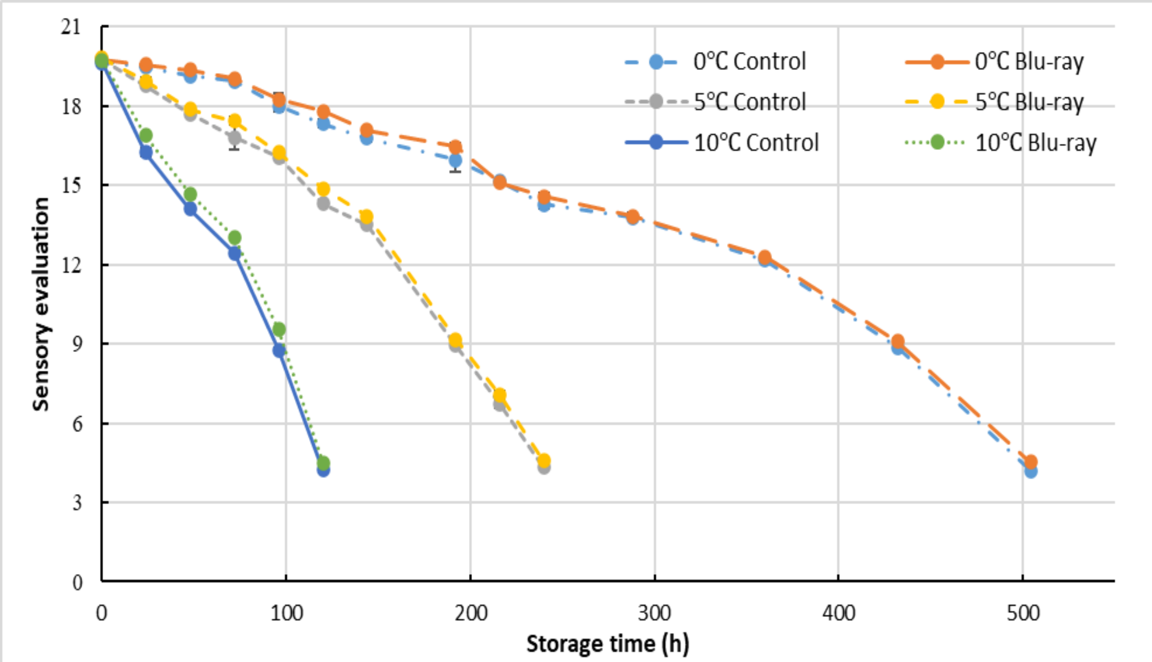


Figure 1 Sensory evaluation of squid during storage.

Other than this, there was no significant difference in sensory scores between the Blu-ray treated and control groups. However, the sensory scores of the Blu-ray group were higher than those of the control group.

Changes in the color of squid in storage

The brightness (L^*), redness (a^*), and yellowness (b^*) values of squid were measured using a LAB colorimeter as recommended by the International Commission on Illumination. The *squid's* L^* *and* b^* *values* at different storage temperatures showed an increasing trend. The trend of a^* was not noticeable; except for a more significant change at 10 °C, the other values were more stable. With an increase in storage time, the color of squid displayed increased brightening and yellowing (Table 1).

Table 1 The value of L^*, a^*, b^* of squid during storage.

Storage temperature	Time	L^*	a^*	b^*
0 °C Control	Initial time	75.61 ±0.24[b]	-1.58 ±0.43[a]	2.32 ±0.35[a]
	The end of high-quality period	78.37 ±0.75[a]	-2.04 ±0.57[ab]	2.40 ±0.43[a]
	The end of shelf period	79.69 ±0.57[a]	-2.62 ±0.12[b]	2.50 ±0.47[a]
0 °C Blu-ray	Initial time	78.79 ±0.72[b]	-1.24 ±0.09[a]	1.32 ±0.46[a]
	The end of high-quality period	79.39 ±0.47[ab]	-1.69 ±0.36[a]	2.30 ±0.34[a]
	The end of shelf period	80.32 ±1.19[a]	-2.42 ±0.26[b]	2.33 ±1.28[a]
5 °C Control	Initial time	79.55 ±1.32[a]	-1.47 ±0.28[a]	1.54 ±0.11[c]
	The end of high-quality period	81.05 ±0.36[a]	-1.61 ±0.06[a]	4.02 ±0.42[b]
	The end of shelf period	81.57 ±0.17[a]	-1.62 ±0.43[a]	6.42 ±1.08[a]
5 °C Blu-ray	Initial time	78.03 ±0.67[b]	-0.65 ±0.13[a]	0.11 ±0.26[b]
	The end of high-quality period	78.42 ±0.23[b]	-0.74 ±0.02[a]	3.56 ±0.16[a]
	The end of shelf period	80.46 ±0.25[a]	-0.78 ±0.16[a]	3.68 ±0.20[a]

Table 1 Continue.

Storage temperature	Time	*L**	*a**	*b**
10 °C Control	Initial time	75.49 ±0.24[c]	-2.39 ±0.12[b]	0.76 ±0.53[c]
	The end of high-quality period	78.49 ±0.72[b]	-2.11 ±0.10[ab]	5.73 ±0.24[b]
	The end of shelf period	80.84 ±0.46[a]	-1.91 ±0.04[a]	9.57 ±1.08[a]
10 °C Blu-ray	Initial time	77.48 ±0.26[b]	-4.10 ±0.37[c]	1.47 ±0.08[c]
	The end of high-quality period	77.75 ±0.17[b]	-1.84 ±0.30[b]	4.34 ±0.09[b]
	The end of shelf period	83.25 ±0.53[a]	0.35 ±0.02[a]	5.69±0.14[a]

Note: Results are mean ± standard deviation (n = 3), values within a column with different superscript letters are significantly different (p <0.05).

Although the skin of the squid samples was peeled, the surface was still protected by a film, and their oxidation rate was low. Therefore, the change in redness was small. Moreover, the mucus moisture produced during the storage period covered the surface layer, resulting in progressive production of specular reflection by the film and thus increasing its brightness (Figure 2).

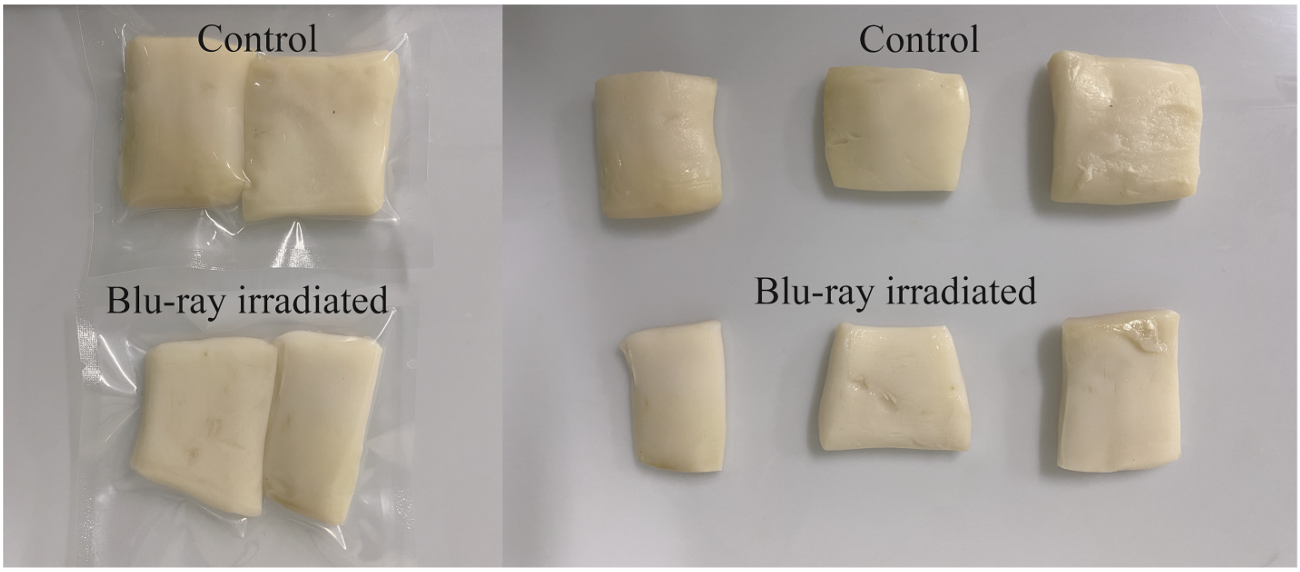


Figure 2 Appearance of squid samples.

Ramirez-Suarez et al. **[17]** found a similar gradual yellowing of color during storage (0 °C, for 15 days) of squid (*Dosidicus gigas*). Our results, to some extent, indicated that with the prolongation of time, the squid surface mucus increased, and the quality decreased, which was consistent with the sensory evaluation **[18]**, **[19]**.

Furthermore, in our study, the color of the samples treated with Blu-ray irradiation had some differences from the control group. Moreover, the samples after Blu-ray irradiation were whitish compared to the control group from the sensory observation. Thus, these changes might be caused by the loss of riboflavin in the squid samples after Blu-ray irradiation **[11]**, **[20]**. Besides, Ghate et al. **[21]** showed that the color of orange juice changed after exposure to Blu-ray (4.60×10^{-7} m). However, the samples irradiated using Blu-ray did not reduce the sensory characteristics of the squid.

SF changes in squid during storage

SF value is widely used to measure the tenderness of aquatic food products, and it reflects the internal structure of the meat; the structural properties of various proteins in the muscle determine the tenderness of the meat and average meat shear value **[22]**. The SF at all three temperatures increased and then decreased, indicating that the flesh quality of squid initially decreased due to muscle tenderness caused by stiffness and then because of storage time, wherein the squid flesh softened due to decomposition (Figure 3).

The storage of squid at 10 °C exhibited the fastest rise and fall in SF value, suggesting that storage in ice could maintain the meat quality. However, the SF at 0 °C was the highest, with a value larger than the initial SF, probably because the meat was slightly harder than fresh squid after storage at 0 °C. Squid is rich in protein and has high elasticity, and as stiffness occurs, it can increase various indicators such as elasticity, hardness, and cohesion. In this study, the temperature of heat treatment was only 60 °C. Therefore, the endogenous enzymes in the squid might not have been wholly inactivated and still functioned in the subsequent low-temperature storage. With the prolongation in storage time, the squid cell structure, muscle tissue, and protein stereo-structure were gradually destroyed by microorganisms, resulting in less SF **[17]**, **[23]**, **[24].** Our results showed that the Blu-ray irradiation treatment had little effect on the SF of the squid compared to each control group during storage.

A B C

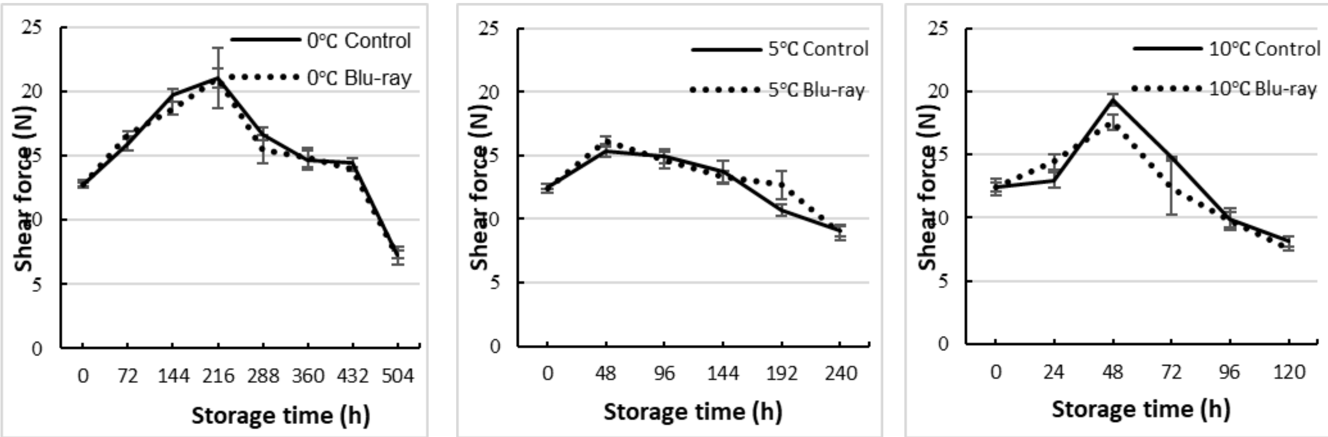


Figure 3 Shear force of squid changes during storage at (A) 0 °C, (B) 5 °C, and (C) 10 °C.

Changes in TBARS in squid storage

The lower the TBARS value, the lower the degree of fat oxidation and the better the quality of the product **[25]**, **[26]**. Lipid degradation products can cause off-flavours in fresh fish during storage **[27]**, **[28]**, **[29]**. As shown in Figure 4, the values of TBARS in squid in both the control and treatment groups showed an increasing trend as the storage time increased.

A B C

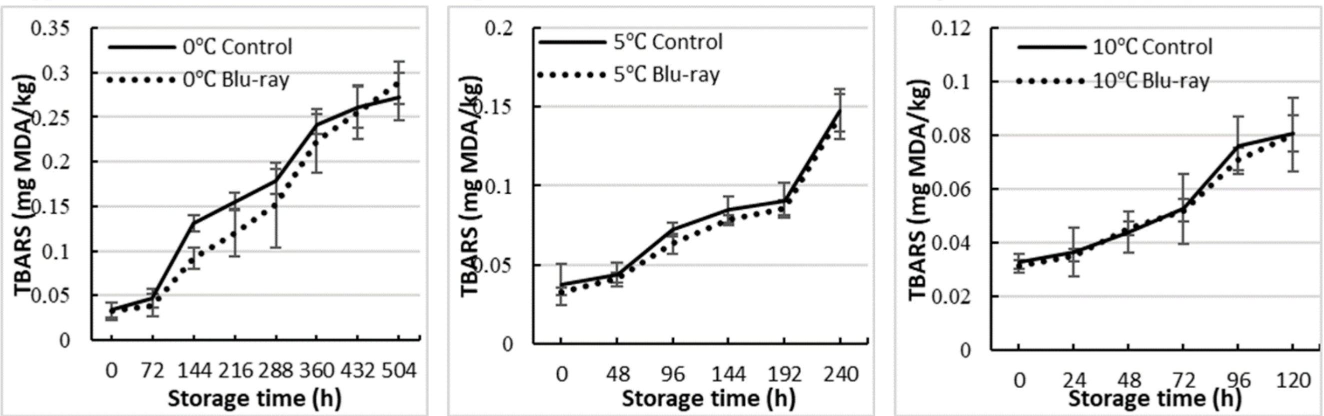
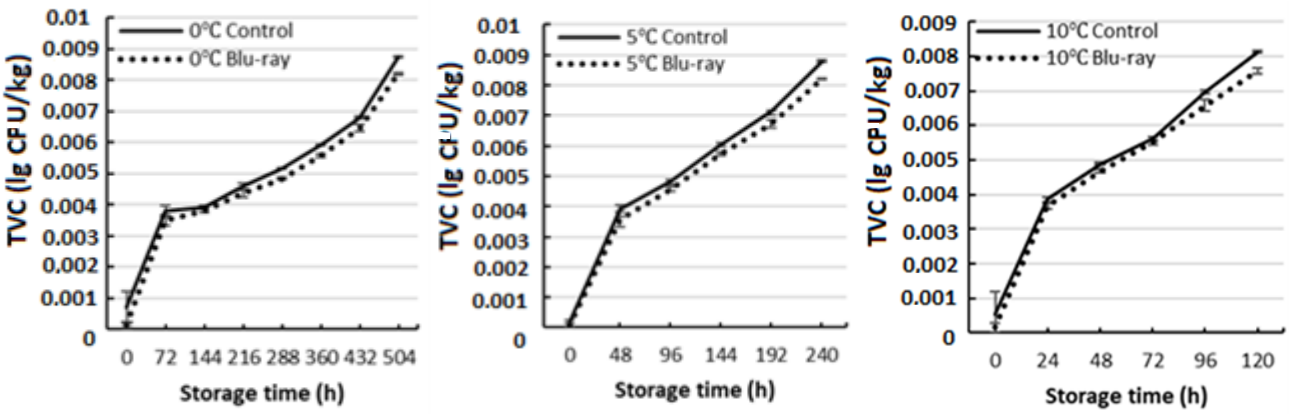


Figure 4 The TBARS changes in squid during the storage at (A) 0 °C, (B) 5 °C, and (C) 10 °C. Note: The increasing trend of TBARS of stored squid at 0, 5, and 10 °C was consistent.

Furthermore, Blu-ray treatment did not induce photosensitive oxidation in squid. It was noteworthy that TBARS increased rapidly after 144 h in control and treated groups at 0 °C. The reason for this is to be investigated in future studies. However, compared with the control group, Blu-ray irradiation reduced the fat oxidation of the squid stored at low temperature, enhanced the anti-bacterial effect, and resulted in a longer shelf-life.

Changes in TVC in squid during storage

The main factors that cause spoilage of aquatic products are microorganisms, as well as enzymatic and chemical changes, and the degree of spoilage of marine products through the growth of spoilage microorganisms **[30]**, **[31]**, **[32]**; therefore, TVC is known as a conventional indicator of aquatic product quality **[33]**, **[34]**, **[35]**. The total number of bacterial colonies in all samples showed an increasing trend with increased storage time. The growth rate of microorganisms was higher under storage at 10 °C, almost directly entering the logarithmic phase, while there were obvious delay periods during storage at 0 and 5 °C. Since microbial metabolism requires enzyme catalysis and the catalytic rate of enzymes depends on temperature, low temperature causes microbial growth to be delayed **[36]**. There was a good correlation between TVC and sensory scores in control and Blu-ray irradiated groups at 0, 5, and 10 °C. In Figure 5, TVC at the end of the high-quality period was 5.92, 6.05, and 5.61 LG CFU/g in the control group and 5.58, 5.74, and 5.50 LG CFU/g in the Blu-ray irradiated group at 0, 5, and 10 °C, respectively.



a) **b)** **c)**

Figure 5 Changes in TVC during the storage at (a) 0 °C, (b) 5 °C, and (c) 10 °C.

At the end of shelf life, the total number of colonies was 0.00879, 0.00879, and 0.00813 LG CFU/kg in the control group and 0.00821, 0.00822, and 0.00757 LG CFU/kg in the Blu-ray irradiated group, respectively. The microbial counts reached 0.0055 LG CFU/kg or more at the end of the high-quality period in both Control and Blu-ray groups. In microbiological quality guides for ready-to-eat foods in Australia and New Zealand, the microbial limit for Class A food (after heat treatment) is proposed to be LG 0.005 CFU/kg **[37]**. Therefore, in terms of the high-quality sensory period of squid, the lower the temperature, the more likely it causes the illusion of food safety, good sensorial characteristics, or high quality; however, its total bacterial count is high, with the total number of colonies at the end of high-quality periods at 0 and 5 °C more than that at 10 °C. In addition, there was a good correlation ($R^2 > 9$) between TVC and sensory scores at 0, 5, and 10 °C (Table 2).

Table 2 Correlation between the total viable counts and sensory evaluation.

Storage temperature	Correlation between the total viable counts and sensory evaluation (R^2)
0 °C Control	0.95
0 °C Blu-ray	0.95
5 °C Control	0.93
5 °C Blu-ray	0.92
10 °C Control	0.92
10 °C Blu-ray	0.99

Changes in the number of *Psychrobacter* during storage of squid

Typical bacteria grow at 25 to 40 °C, while *Psychrobacter* generally grows best between -15 to 20 °C. The most common species of cold-loving bacteria are *Yersinia pestis*, *Listeria monocytogenes,* and *Pseudomonas spp*. Aquatic products and meat are more vulnerable to contamination of food by these species **[38]**, **[39]**, **[40]**. The squid growing in the ocean depths is more likely to become infected with this type of *Psychrobacter*. From Figure 6, we could see that the growth trend of *Psychrobacter* was similar to that of TVC, and the growth of the number of *Psychrobacter* and TVC was the same in all temperature conditions throughout the storage process, and the correlation between the two was good ($R^2$0.9). During low-temperature storage, the growth of non-*Psychrobacter* might have been inhibited, while the *Psychrobacter* had an advantage over that of non-*Psychrobacter*. Our results suggested that at the initial time and during storage, the Blu-ray treatment group had a lower number of TVC and colonies of *Psychrobacter* than the control group, indicating that Blu-ray sterilization was effective and performed well during the whole storage period.

CONCLUSION

In conclusion, our results demonstrated the positive effect of Blu-ray treatment during the storage of SVC squid by inhibiting microbial growth and reducing fat oxidation. And the end of shelf-life (control group and irradiated group) was 504, 240, and 120 h. Although Blu-ray irradiation affected the color of the squid, especially yellowing, it did not affect the sensory characteristics of the squid. Moreover, our results showed that the quality of squid decreases as the storage time increases. However, storage at lower temperatures could extend the storage time of SVC squid. Our study notes that the evaluation of food quality cannot be done only by sensory evaluation but also requires a comprehensive evaluation concerning other physical and chemical indicators. Future research should focus on the mechanism of Blu-ray for the destruction of specific food-borne micro-organisms and the application of Blu-ray for sterilization of aquatic products.

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