

Effect of Gamma Irradiation on the Sensory Quality and Amino Acid Yield of Antarctic Krill De-oiled by Supercritical CO²

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Abstract

Research has demonstrated the advantages of radiation treatment in preserving and enhancing the microbial quality of seafood. Additionally, previous studies have illustrated the sensitivity of amino acids to radiation. Consequently, our study aimed to examine the impacts of gamma irradiation on the nutritional, bacteriological, and sensory state of krill. Applied doses consisted of 0 (control), 1, 5, and 10 KGy. Processed samples were initially de-oiled using supercritical $CO₂$ to enhance the efficacy of hydrolysis through sub-critical water. The experiment was conducted within a temperature series of 200 to 280°C, with a material-to-water ratio of 1:50 for hydrolysis, and for less than 5 minutes of reaction time to minimize amino acid decomposition.

The study examined the sensory and bacteriological quality of irradiation-treated krill compared to untreated samples. The hydrolysate of all treated samples yielded the highest amount of amino acids at 280ºC. The total concentration of amino acids was similar in both treated and untreated samples. Sensory evaluation showed no differences among all samples directly after irradiation. After 50 days of storage at 5°C, the sensory quality of irradiated krill was satisfactory; nevertheless, the quality of untreated krill deteriorated. The thiobarbituric acid values remained unchanged, regardless of the dose of irradiation and duration of storage.

Introduction

Dried fish forms a major source of protein in many equatorial countries. Around 80% of dried fish, which is tainted by both insects and toxic insecticides, is classified as unfit for human consumption. These losses result in a decrease in the amount of nutrients available, and leads to a decrease in consumer satisfaction and market prices. Witches affect both the quantity and quality of the product (Singh et al., 2018)

Microbiologists, engineers, and technicians have the challenging responsibility of developing effective strategies to prevent the introduction of microorganisms into dairy products, eliminating any bacteria that do manage to enter along with their enzymes, and inhibiting the growth and functions of any microorganisms that survive processing treatments (Rawat and Mushtaq, 2015). Probably, sun-drying is the primary and most frequently used method in the modern world. It is a straightforward, cost-effective, and often efficacious method. Other commonly used preservation techniques include salting, cooking, smoking, canning, freezing, and the use of chemical preservatives (Sarmah et al., 2022).

The most recent addition to this list is the irradiation of food products, which involves exposing them to carefully measured amounts of ionizing radiation (Arapcheska et al, 2020). Research and practical applications over several decades have shown that irradiation delays food spoilage and reduces infestation by insects, or contamination by microorganisms, especially the agents responsible for food poisoning (Singh and Singh, 2020).

When compared to other commercial pest management technologies, irradiation offers clear benefits. In addition to being less energy-intensive than fumigation, it also prevents the presence of harmful residues in the product. Empirical evidence suggests that a dosage of 0.5 kGy is highly successful in eradicating pests from dried fish and fishery products that have moisture content lower than 40% (Singh et al., 2018).

Antarctic krill, known scientifically as *Euphausia superba* have a critical function in the Antarctic ecosystem, acting as a key link for the transfer of substantial energy and nutrients within the region. These krill are essential as a primary food source for various Antarctic fauna, including seals, penguins, whales, and sea birds (Trathan and Hill, 2016; Bairstow et al., 2022). Additionally, commercial fisheries greatly appreciate krill for its use as fish feed in aquariums and aquaculture. It is subject to extensive research; yet significant doubts remain about crucial aspects of its biology (Kawaguchi and Nicol, 2020).

Also, due to their substantial size, significant biomass, and regular vertical movements, they play a crucial role in the transportation and conversion of vital nutrients, stimulation of primary productivity, and impact on the carbon sink. Compared to the 100 million tonnes of the world's commercial fishery, krill's high biomass resources make it a promising new source of food for the fishing industry. This immense biomass and dominant position of krill in the food chain make it a significant contributor to biogeochemical cycles (Warwick‐Evans et al., 2022).

Moreover, Krill is a remarkable source of highquality protein that distinguishes itself from other animal proteins through its low-fat content and abundance of omega-3 fatty acids. Krill exhibits higher antioxidant levels compared to fish, suggesting potential advantages in combating oxidative damage (Sun et al., 2017). The proximate analysis of whole krill reveals that the moisture content ranges from 77.9% to 83.1%, total lipids range from 0.5% to 3.6%, crude protein ranges from 11.9% to 15.4%, ash content is 3%, and chitin and glucides make up 2% (Janet et al., 2008).

Nevertheless, the interest in utilizing krill as a viable food source for human consumption is anticipated to grow due to the advent of technical advancements and the development of new products. However, the extent to which krill becomes widely accepted as a component of the human diet will rely on how consumers perceive it to be both nutritious and healthy (Janet et al., 2008).

In this context, Krill as a vital component of marine ecosystems, has gained significant attention for its nutritional value and potential health benefits. However, maintaining its hygienic quality during processing and storage presents a challenge due to the risk of microbial contamination. Gamma irradiation treatment emerges as a potential solution to ensure the safety and quality of krill products. This study aims to evaluate the effectiveness of gamma irradiation in enhancing the hygienic quality of krill, but also, to investigate its impact on the nutritional and sensory qualities.

Material and Methods

Material

Krill (*Euphausia superba*) was collected from Dongwon F & B Co, South Korea. The krill blocks were stored at -80°C before experimental use. Carbon dioxide (99.99% purity) was supplied by KOSEM, South Korea. All other chemicals used in the various analyses were of analytical quality suitable for HPLC.

Methods

Gamma Irradiation

Krill samples were packaged using a sterile biaxially oriented polypropylene (BOPP) film weighing 300±5 g, in preparation for treatment. Gamma irradiation was carried out at the Institute of Atomic Energy in Korea. Using the 60 Co gamma irradiation facility (150 TBq capacity, ACEL, MDS Nordion Inc., Ottawa, Ontario, Canada); irradiation was carried out at a rate of 10 KGy/h. In order to determine the actual absorbed dose, alanine dosimeters were attached to the upper, middle, and lower sections of each box prior to irradiation and were analyzed using an electron paramagnetic resonance analyzer (e-scan alanine analyzer, Bruker Instruments). The difference between the target and actual absorbed doses was within 5%. The doses applied were 0 (Control group), 1, 5, and 10 KGy as shown in Figure 1. After completion of the treatment, the samples were stored at 5°C until use.

Approximate Composition

Moisture, ash, and crude protein levels were analyzed following AOAC (1990) guidelines. Lipid content was measured using a conventional soxhlet apparatus with hexane as the solvent for 12 hours. Nonprotein content was estimated by deducting the combined weight of moisture, ash, protein, and lipid from the total weight.

Sensory Evaluation

Colour

To comprehensively assess color changes, we not only examined the treated raw krill samples; but also studied potential color changes in oils extracted from these samples.

The krill samples' color assessment involved a 10 member panel using the method outlined by Civille and Szczesniak in 1973. Unirradiated krill served as the reference (control). The initial evaluation occurred upon receiving the treated samples, and the second assessment took place after a 50-day storage period. Panelists provided scores in triplicate on a 5-point scale: 1, very poor; 2, poor; 3, common; 4, good; 5, very good.

The oils in the samples underwent color measurement in triplicate using reflectance spectra (Lovibond spectrophotometer, USA). Two analyses were conducted on samples treated with various doses (0, 1, 5, and 10 kGy). The first analysis occurred immediately after irradiation, and the second took place after 50 days of storage.

The samples were positioned in a white cup enclosed with optical glass. Then,CIE color coordinates (L*), (a*), and (b*)were calculated with considering standard illuminant D65 and the 10° observer. Color changes were assessed based on luminosity (L*) and the green-red (a*) and blue-yellow (b*) coordinates.

Smell

Krill sample odor evaluation involved the color assessment panel utilizing the Civille and Szczesniak (1973) methodology and notation. The ranking scale ranged from 1 (very poor) to 5 (very good) to ensure uniformity in the assessment process.

Bacteriological Evaluation

The bacteriological analysis involves isolation, identification (qualitative aspect), and enumeration (quantitative aspect). For inoculum solutions, 25 grams (g) of each sample (0, 1, 5, and 10 kGy) were taken and placed in a Stomacher-type homogenizer bag with a previously sterilized solution of tryptone sell. This 250 g mixture was homogenized for 30 seconds. The resulting solution termed the stock solution, was allowed to stand for 30 minutes to ensure the resuscitation of bacteria stressed by homogenization. The solution was then diluted to 10⁻¹for further analysis. This preparation is carried out every 10 days during the 50-day storage period at 5°C.

The total number of microorganisms was determined using standard techniques as described by Speck (1976). 1 mL of each dilution was taken and introduced aseptically into Petri dishes. Super optimum culture medium was prepared as nutrient-rich bacterial growth medium. Then, the plates were incubated for 2 days at 25±1°C, and the colonies were subsequently counted.

1 KGy tl KGy 5 KGy 10 KG7

Figure 1. Photographs of samples irradiated at doses of 0 (control), 1, 5, and 10 KGy. (Original photo)

The percentage survival is presented as $N/N_0\%$, where N denotes the number of colonies in the treated sample and N_0 is the number in the untreated sample. This value is the average of three replicates.

Extraction by Supercritical Carbon Dioxide (SC-CO2)

Krill samples, with an average body length of 5.20 cm and an average body weight of 2.07 g, underwent a 72-hour freeze-drying process. Subsequently, the samples were ground and passed through a 700 μm mesh. The EFS procedure used in the experiment, shown in Figure 2, employed various equipment including a pump (ILSHIN measurement, Korea), a water bath (DW-15 S, Dongwon Scientific system), a chiller (DW-N30L, Dongwon Scientific system, Korea), an extraction cell, and a wet gas meter (WNK-1A, Sinagawa Corp, Tokyo).

For the extraction process, 35 g of each krill sample, including untreated samples and those irradiated at doses of 1, 5 and 10 KGy, were placed in a 200 mL stainless steel extraction vessel. The vessel was lined with a thin cotton layer at the bottom and top. Subsequently, a high-pressure pump injected $CO₂$ into the vessel under controlled conditions until it reached a pressure of 25 MPa. The vessel was steady at a constant temperature of 45°C.

Gas flow rates and volumes fleeting over the device were measured with a gas flow meter set to a constant $CO₂$ flow rate of 22 g/min for each extraction condition. The oil extracted by SC-CO² was collected in the cyclone separation vessel, and the extraction process lasted for 2.5 hours. The temperature and pressure conditions were optimized according to the findings of Ali-Nehari et al (2012) to improve extraction yield.

Value of Thiobarbituric Acid (TBA)

The TBA technique measures the free radicals existing after peroxide oxidation. The TBA rate was determined using the Ottolenghi (1959) method. Samples were treated with doses of 0, 1, 5, and 10 KGy with an interval of 10 days throughout the 50-day storage period. In brief, 1 mL of the emulsion solution was mixed with 2 mL of 20% trichloroacetic acid and 2 mL of 0.67% 2-thiobarbituric acid. Then, the mixture was placed in a water bath at 100°C for 10 minutes.

After cooling, the mixture was spun at 3000 rpm for 20 minutes. The absorbance of the supernatant was determined at 532 nm using a UV/visible spectrophotometer (UVIKON 933, Kontron Instruments).

Hydrolysis of Residues by Subcritical Water

The subcritical water hydrolysis unit diagram is shown in Figure 3. Hydrolysis occurred in an 80 mL batch reactor with temperature control and stirring. To ensure homogeneity, de-oiled residues from samples treated with radiation doses (0, 1, 5, and 10 KGy) were individually prepared with deionized water.

The reactor, filled with the chosen reaction atmosphere (nitrogen or air) at 0.20 MPa, received the solution with a concentration of 2 g/100 mL. An electric heater achieved the needed temperature (between 200 to 280°C) before sealing the reactor. The sample was stirred at 140 rpm, with a short reaction time (5 min) allocated to prevent significant amino acid decomposition.

Hydrolysis conditions, set based on Ali-Nehari et al (2011), identified 280°C and 0.20 MPa as optimal. After

Figure 2. Schematic diagram of extraction by SC-CO₂ (Ali-Nehari et al, 2012).

cooling by immersion in cold water, the hydrolysate was filtered and analyzed for proteins and amino acids. All tests were conducted in duplicate.

Measuring the Protein Content of Hydrolysates

The protein content of different hydrolysates was measured using bovine serum albumin (BSA) as a standard according to the method outlined by Lowry et al (1951). The hydrolysate protein content was measured by referencing a calibration curve for the BSA standard, depicted in Figure 4.

Amino Acid Analysis of Hydrolysates

The samples' hydrolysates were diluted with 0.02 N HCl to a protein concentration of 0.25 mg/mL. Samples were filtrated and analyzed using an amino acid analyzer (Hitachi L-8900, Tokyo) located in Pukyong National University's central laboratory.

Statistical Analysis

All sample extractions and analyses were performed in triplicate in random order and means were considered. Data were assessed by Duncan's multiple range test with SAS 9.1 (SAS Institute Inc., Cary, NC, USA) to assess differences in mean values. The significance level was set at P<0.05.

Results and Discussion

Approximate Composition

In order to investigate the impact of gamma irradiation on the nutritive value of krill samples the composition of krill was evaluated before and after treatment. The composition of the various samples is presented in Table 1. For lipid content, the almost identical values were obtained through hexane extraction for all the samples subjected to various doses

Figure 3. Schematic diagram of hydrolysis by subcritical water (Ali-Nehari et al, 2011).

of irradiation and for the untreated samples (approximately 16%). As like marine organisms, krill is considered to be the unique source of polyunsaturated fatty acids.

The same results were also noted for protein content. No significant changes between treated and untreated samples were observed. Almost 62 % of protein content was found for all samples analyzed. Similar to other animal foods, the protein derived from krill is a complete protein, as indicated by the presence of all nine of the indispensable amino acids for adults required by FAO/WHO/UNU (Nicol et al., 2000).

Also, the results indicated slight variations between the different samples for moisture, and ash content (Almost 3.3% for moisture and 4.6% for ash).

In fact, similar results were reported by Lee et al (2002) in their study on the impact of gamma irradiation on the hygienic quality stability of Pacific saury fish. It is worth mentioning that the raw krill content values obtained are consistent with other research, which indicates that krill analysis typically ranges from 45% to 80% protein, 7% to 30% total lipids, and 8% to 20% total ash (Ali-Nehari et al., 2012).

Extraction by SC-CO²

The quantity of krill oil obtained after 2.5 hours was determined by weight (g) using a balance with a precision of ± 0.001 g. The oil extracted from the krill and the residues obtained by extraction of the $SC-CO₂$ are shown in Figure 5. The oil extracted from the krill was fluid and bright red due to the existence of astaxanthin (Xie et al., 2019). The extraction yield obtained was close to 12% for all the samples used. The best yield was 4.16±0.08 g / 35 g of krill irradiated at 10 KGy at a temperature of 45°C and a pressure of 25 MPa. This confirms the results of the approximate composition of the different samples.

Nevertheless, another study has shown that, depending on the experimental conditions, the extraction yield of krill oil varied from 4.1 to 12.2% (Ali-Nehari et al., 2012). Zaidul et al (2007) mentioned that the pressure of SC-CO² affects both the yield and solubility of palm oil. Oil obtained from marine resources is rather non-volatile and thermally sensitive (Panadare et al., 2021). Consequently, and to avoid the activity of bioactive compounds in both extracts and extracted residues, extractions were carried out at a low temperature (45°C). Sun et al (2018) reported that up to 45°C, the quantities of extracted oils were almost constant despite the temperature and pressure studied.

The oils obtained were used to evaluate color changes and TBA during the storage period.

Sensory Evaluation

Results from Table 2 show the sensory evaluation of gamma-treated krill during storage. No significant organoleptic differences were found between treated and untreated samples during the first few days of storage. Similar results have been found for other marine resources (Lee et al., 2002).

Figure 4. BSA calibration curve for protein estimation.

^amean value of three repetitions (at least) ± S. E.

Numerous investigations have demonstrated that irradiating spices at a dose of 10 kGy can remove the microbial burden without appreciably changing their organoleptic or chemical properties (Esmaeili et al., 2018; Singh and Singh, 2020).

Color

The red color of krill is due to its high content of astaxanthin, which is a potent carotenoid with an antioxidant capacity 10 times stronger than zeaxanthin, lutein, canthaxanthin, and β-carotene, and up to 500 times stronger than vitamin E (Shimidzu et al., 1996). Possible color changes post-irradiation is closely tied to alterations in the quantity and quality of astaxanthin in various treated samples. To comprehensively assess color changes, we not only examined the treated raw krill samples; but also studied potential color changes in oils extracted from these samples. Given that astaxanthin is lipid-soluble, extracting lipids ensures the retrieval of nearly all quantities of this powerful antioxidant.

In our study, the variations in brightness (L^*) , redness (a*), and yellowing (b*) of the different extracted krill oils are shown in Table 2. Brightness does not appear to be affected by gamma rays, a slight difference in (L*) value was noted amid oils from treated and untreated samples. For the oil from the untreated samples, however, an increase in (L*) value was noted after 50 days of storage.

The most significant changes were observed in the (a*) values, which are related to the color tone, changing from red (a*) to green in the hexane-extracted oil. The characteristic red color of krill oil is indicated by a high value of (b*) which shows a slight increase at the end of the storage period for oil from samples irradiated at 10 KGy; probably related to the irradiation conditions. However, further research into the effect of gamma rays on color tone is required (Pandiselvam et al., 2023).

Odour

The panelists concluded that untreated krill (control) is unsuitable for consumption after 50 days of storage due to its deteriorating hygienic quality. According to the literature, proteins in the krill are attacked by bacterial enzymes, resulting in weakened and softened flesh, and ultimately the formation of urea and the emission of putrid odors (Wang et al., 2021). However, the odor of the treated samples remained relatively stable during the initial few days of storage, and even after 50 days for those exposed to 10 KGy of irradiation.

Figure 5. A) Krill in its raw state; B) Krill oil extracted by SC-CO₂); C) Krill residue after extraction (Original photos).

Brightness (L *), redness (a *), and yellowing (b *). 1, very poor; 2, poor; 3 common; 4, good; 5, very good.¹ Colour of raw krill samples assessed by panelists. ²Color of oils obtained from krill samples assessed by Spectrophotometer.

Microbiological Evaluation

The growth of total aerobic Krill bacteria during the storage period after treatment with gamma irradiation is shown in Table 3. The total bacterial count of the untreated sample (control) was 2.6 to 10^4 CFU/g which augmented to 3.1 to 10^9 or more than 10^5 times after storage for 50 days at 5°C. The results indicate that the microbiological quality of Krill cannot be assured by unusual treatment process such as the combined treatment of vacuum packing and cold storage. However, the level of viable micro-organisms reduced directly after irradiation, depending on the dose absorbed (Boshevska et al., 2024). Colonies were not observed in the sample treated at 10 KGy during the first twenty days of storage. Similarly, the number of colonies detected in the samples irradiated at 1 KGy after 20 days of storage, as well as those irradiated at 10 KGy after 50 days of storage, was lower than that of the control group at the beginning of storage (0 days).

The results of this research on the consequence of gamma irradiation on the bacteriological quality of krill are identical to those obtained by Lee et al (2002).

Gamma irradiation treatment of food is not only effective in reducing pathogenic microorganisms, but it is also a particularly efficient method for controlling them (IAEA, 2000). Thus, the results propose that gamma irradiation can be excellently used to ensure the hygienic quality of Krill.

Value of Thiobarbituric Acid (TBA)

The result showed that in all samples, TBA values increased throughout storage time, regardless of the radiation dose as shown in Figure 6. No statistical difference was observed between the control (0 KGy) and irradiated samples. According to this finding, the various gamma radiation dosages did not affect the lipid oxidation of any sample.

Marine resources contain a high content of unsaturated fatty acids, making them easily oxidized in oxygen and natural light (Rontani and Belt, 2020). However, krill lipids showed a high stability to oxidation, which is clarified by the attendance of the natural antioxidant astaxanthin in these lipids; since krill is a principal source of astaxanthin, which has a high antioxidant activity (Sanchez et al., 2021).

Protein Yield in Hydrolysates of Sample Residues

Other studies have shown that the residue hydrolysate extracted by the $SC-CO₂$ contained more protein than the crude sample hydrolysate (Ali-Nehari et al., 2011). It was therefore decided to remove the lipids from the material before hydrolysis. This could be explained by the raw materials hydrophobic oil content, which prevented it from being as easily hydrolyzed.

For this work, the protein contents in all the hydrolysates from the diverse samples are shown in

Table 3. Effect of irradiation on total aerobic bacteria (N/N₀; CFU / g) of Krill irradiated with gamma rays during storage at 5°C

Figure 6. Changes in TBA values over the storage period for the various samples.

Figure 7.Vedovatto et al (2021) reported that the protein yield from subcritical water hydrolysis of soybean reduced as the temperature augmented from 200 to 220°C. In our study, protein yield was found to increase with increasing temperature in the hydrolysate of all samples. Also, it was found that for each hydrolysis temperature, the protein yields were almost identical in all the hydrolysates from the different samples studied. The highest protein yield in the krill hydrolysates was 548.17±2.71 mg /g at 280ºC. The finding suggests that almost all the protein content could be obtained from the hydrolysates.

Generally, Proteins have a low solubility in water at room temperature. Nevertheless, the solubility of proteins in water improved at higher temperatures. As a result, and based on these results, it is possible to consider that treatment with gamma rays had no impact on the protein content of krill. Similar results were reported by Lee et al (2002).

Amino Acid Yield

Krill protein is considered to be of high quality, and biochemical analysis has shown that the protein improved from krill contains the nine essential amino acids in sufficient quantities to meet FAO and WHO requirements for human adults (Sanchez et al., 2021). Amino acids have a significant biological role in all forms of life. However, they also contribute to the flavor of food.

Amino acids and protein hydrolysates are therefore beneficial additives in the food industry (Hou et al., 2022). In this investigation, to reduce amino acid decomposition, a short reaction time was applied for hydrolysis using subcritical water. On the other hand, a low sample-to-water ratio was used considering a higher hydrolysis efficacy by subcritical water to obtain amino acids. At a similar ratio of material to water, the best yield of amino acids by subcritical hydrolysis has also been found in other investigations of Lamoolphak et al (2008).

The amount of primary amino acids obtained from krill hydrolysates subjected to CO2-SC de-oiling followed by hydrolysis at 280°C is shown in Figure 8. Cheng et al (2008) discovered that most amino acids had a maximum yield at 200-290ºC. The analysis indicated that all different krill sample hydrolysates contained the principal krill amino acids, in almost equal amounts, irrespective of the radiation dose. Arginine, leucine, and lysine are the most commonly occurring amino acids in the profile of other krill amino acids. Alike findings were reported by Gigliotti et al (2008) when working with krill protein concentrates.

Figure 8. Amino acid yield in 280°C hydrolysates.

Conclusion

Gamma irradiation enhanced krill's hygienic and nutritional quality by reducing viable bacteria levels and maintaining lipid stability. The organoleptic quality of irradiated samples exceeded non-irradiated ones, and consistent amino acid yields demonstrated unaffected nutritional quality. Additionally, a novel SC-CO₂ lipid extraction process showcased potential sterilizing effects on biomaterials. Further research is needed to validate CO2's sporicidal activity, especially in complex substrates. Overall, gamma irradiation and $SC-CO₂$ extraction present promising methods for ensuring krill quality and diverse applications.

Ethical Statement

Not applicable.

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Author Contribution

AAN: concept of the manuscript. literature analysis, sampling, data analysis, manuscript writing, review and editing; BSC: literature analysis, data analysis, manuscript writing,; KB: literature analysis, sampling, preparation of illustrations, data analysis. All the authors have seen the final version of the manuscript and provided their approval for the submission.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this article.

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