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Microbiological Quality and Shelf Life of Collagen-Coated *Sillago sihama* During Frozen Storage

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ABSTRACT

The study aims to evaluate the use of collagen coatings for inhibiting microbial growth, extending the shelf life of fish and protecting it from spoilage. The present study was conducted on *Sillago sihama* fish obtained from Al-Faw City, Basra Province. The fish were coated with collagen extracted from fish-processing by-products, specifically the skins of *Liza subviridis* (Mullet fish), and then frozen at –18°C for 120 days. Microbiological analyses were performed to determine the total bacterial count, psychrotrophic, proteolytic, lipolytic, and coliform bacteria. The results showed that the bacterial counts in uncoated frozen fish were higher than those in coated frozen samples. The highest total bacterial count in uncoated frozen fish reached 100.4 CFU/g, followed by psychrotrophic bacteria (36.4 CFU/g), proteolytic bacteria (28.8 CFU/g), lipolytic bacteria (21.4 CFU/g), and coliform bacteria (2.8 CFU/g). In contrast, the lowest bacterial counts were recorded in coated frozen fish, with total bacterial counts of 54.8 CFU/g, succeeded by psychrotrophic bacteria with 19 CFU/g, proteolytic bacteria with 17.6 CFU/g, lipolytic bacteria 11.8 CFU/g, and coliform bacteria with 1 CFU/g. In conclusion, the study demonstrated that biodegradable collagen coatings effectively inhibited microbial growth, extended the shelf life of fish, and protected it from spoilage.

INTRODUCTION

Fish represents one of the most important and widely consumed sources of animal protein worldwide, largely due to its relatively low cost and high nutritional value (**Allam *et al*., 2020; Maulu *et al*., 2020; Jalal & Wael, 2024**). Insufficient eating of animal protein, particularly among children, can lead to impaired development or even growth retardation due to the essential physiological functions of dietary proteins (**Schonfeldt & Hall, 2012; Al-Haider *et al*., 2019; Jabbar *et al*., 2019)**. Despite the high nutritional value of fish, they can also serve as a potential source of foodborne illnesses since they may harbor pathogenic microorganisms. Several factors contribute to this issue, including improper fishing, handling, and marketing practices, as well as the inherently delicate biochemical composition of fish tissue and the activity of endogenous enzymes, all of which make fish highly perishable (**Sulaiman & Hassan, 2017; Kadhim**

***et al.*, 2023**). Therefore, proper preservation methods must be employed from the time of capture until consumption to maintain the quality and safety of fish (**Imran *et al*., 2019; Sone *et al*., 2019**). Preservation and processing techniques are essential not only to ensure food safety but also to reduce economic losses and post-harvest waste in the fisheries sector. Common preservation methods include canning, smoking, drying, chilling, pickling, freezing, and salting (**Wang & Xie, 2021; Essa *et al*., 2025**). During recent years, various packaging methods have been developed to extend shelf life of fish and preserve its quality attributes. Among these innovations, active packaging has gained increasing attention. This system goes beyond the traditional functions of packaging which include protection from gases, moisture, and contaminants by incorporating active compounds that maintain product quality and extend shelf life through antimicrobial and antioxidant effects (**Ahmed *et al*., 2022; Al-Hilphy *et al*., 2022; Al-Shammari *et al*., 2024**). Edible coatings and films are particularly effective in preserving meat and fish products, as they contain bioactive components that improve packaging performance by delaying microbial spoilage, retarding lipid and protein oxidation, inhibiting microbial growth, reducing moisture loss, and maintaining the product's sensory acceptability for longer periods (**Umaraw *et al*., 2020; Noomi *et al*., 2025**). Collagen has been widely used in development of edible biodegradable films and coatings, serving as an effective material to protect fish and meat products from spoilage (**Cuq *et al*., 1997; Sultan *et al*., 2019**). The study aims to evaluate the effect of collagen-based edible biofilms in preserving fish quality and extending shelf life of *Sillago sihama* through inhibiting its microbial growth and extending its shelf-life of during frozen storage.

MATERIALS AND METHODS

Fish samples

Fresh *Sillago sihama* fish specimens were obtained from the marine fish market (Al-Naq'ah) in Al-Faw City, Basra Province, during December 2024. The average length and weight of the fish were 17.83cm and 41.75g, respectively, with a total sample weight of 15kg. The fish samples were transported in insulated polystyrene containers filled with crushed ice and then washed with clean water to remove any adhering materials in the laboratory. Random samples were taken for preliminary qualitative analyses while still fresh.

Microbiological analyses

Microbial counts were determined using serial dilutions. One gram of fish muscles tissue was homogenized with 9mL of sterile physiological saline to prepare a 10^{-1} dilution. Subsequent dilutions were prepared by transfering 1mL from previous dilution at 9mL of saline until the desired dilution levels were obtained. For all microbiological examinations, the pour plate method was used. One milliliter of each dilution was inoculated into sterile, empty Petri dishes, followed by the addition of the appropriate culture medium (cooled to 35°C). Plates were gently rotated to ensure uniform distribution before medium solidification, and then incubated in invert position at 37°C for 24–48 hrs. Bacterial growth was expressed as colony-form units per gram (CFU/g), calculated by multiplying number of colony by the reciprocal of the dilutions factors, according to method described by **Andrews (1992)** and **Galo *et al*. (2025)**.

Total plate count (TPC)

The culture medium used was Nutrient Agar (28 g/L) dissolved in distilled water, adjusted to a total volume of one liter. After sterilization by autoclave at 121°C and 15psi for 15min, the medium was poured into Petri dishes and incubated at 35°C for 24–48 hours. Colony counts were determined following procedure outlined by **Andrews (1992)**.

Proteolytic bacteria count

Nutrient Agar supplemented with 10% skim milk was used as the culture medium. After incubated at 35 °C to 24 – 48 hours, colonies surrounded by clear zones (indicating casein hydrolysis) were counted as proteolytic bacteria, according to the method described by the American Public Health Association (**APHA, 1992**).

Lipolytic bacteria count

Nutrient Agar containing Tween 80 was used as the medium. Plates were incubated at 35 °C for 24–48 hrs. Colonies exhibiting a soapy or opaque appearance were recorded as lipolytic bacteria, following the procedure of **APHA (1992)**.

Psychrotrophic bacteria count

Plates containing Nutrient Agar were inoculated and incubated at 7°C for 5–7 days, as recommended by **APHA (1992**), to determine the counts of cold-loving (psychrotrophic) bacteria.

Coliform bacteria count

Mac Conkey Agar medium was used for coliform enumeration. The plates were incubated at 37°C for 24–48 hours, and characteristic colonies were counted as described by **Andrews (1992)**.

RESULTS AND DISCUSSION

Total plate count (TPC)

The results illustrated in Fig. (1) show the effect of frozen storage duration on the mean total bacterial count of *Sillago sihama* fish. As shown in Fig. (1), uncoated frozen fish samples exhibited the highest mean value of 100.4 CFU/g, whereas coated frozen fish showed the lowest mean bacterial count of 54.8 CFU/g. The reduction in microbial counts in coated samples may be attributed to inhibitory effect of bioactive compound present in collagen coating, which can disrupt bacterial cell walls and inhibit microbial growth, particularly for Gram-positive bacteria. The antimicrobial activity of collagen-based and other active films has been previously reported, as these coatings often contain active ingredients that suppress microbial proliferation (**Genskowsky *et al*., 2015; Jumma, 2024**). **Al-Hamdani (2024)** also observed a decrease in total bacterial counts in frozen fish, which he attributed to the destructive effect of large ice crystals that rupture bacterial cell walls, leading to cell death. Similarly, **Maghami *et al*. (2019)** reported a decline in bacterial growth in *Huso huso* fish fillets coated with chitosan containing essential oils, suggesting that the combination of chitosan and essential oils exerted a synergistic antimicrobial effect. Microbial assessment is a key indicator of fish and seafood quality since such products are higher susceptible to microbial contaminations and spoilage (**Joukar *et al*., 2023; Jumma, 2024**). The duration of frozen storage had a gradual increasing effect on bacterial count which was observed with prolonged storage. In uncoated frozen fish, the lowest bacterial count was recorded at day 0 (16 CFU/g), followed by 43 CFU/g after 30 days, 84 CFU/g after 60 days, 155 CFU/g after 90 days, and a maximum of 204 CFU/g after 120 days of storage. In coated frozen fish, similar trends were observed but with significantly lower bacterial counts. The lowest count was recorded at day 0 (12 CFU/g), increasing to 27 CFU/g after 30 days, 41 CFU/g after 60 days, 83 CFU/g after 90 days, and 111 CFU/g after 120 days of frozen storage. These results are consistent with those of **Khorami *et al*. (2024)**, who reported reduced bacterial counts in chitosan-coated fish fillets compared to uncoated samples due to the antimicrobial properties of chitosan-based coatings.

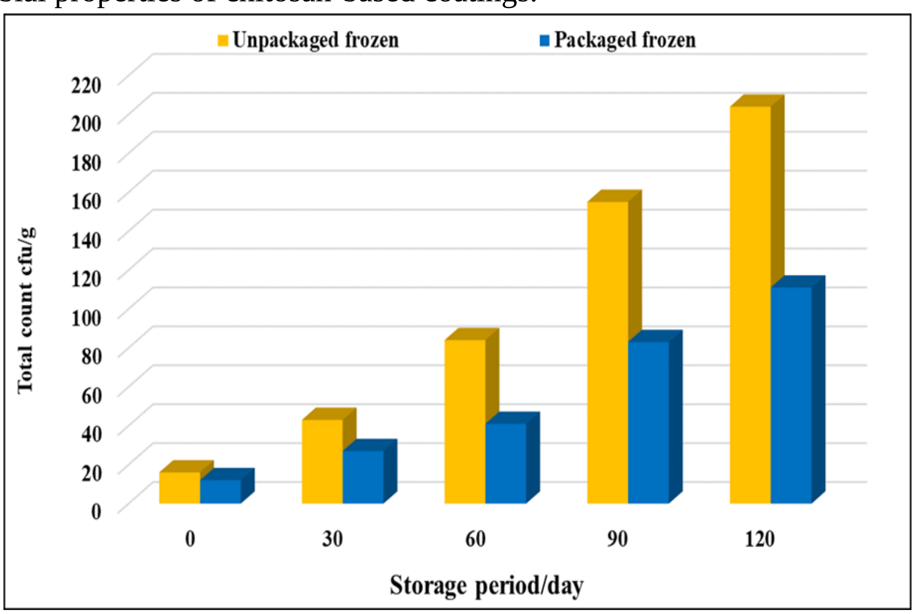

Fig. 1. Effect of packaging and frozen storage duration (-18°C) on the total bacterial count in *Sillago sihama* fish

Psychrotrophic bacteria

The results presented in Fig. (2) illustrate effects of coating and frozen storage duration on psychrotrophic bacterial count of *Sillago sihama* fish. A gradual elevation in bacterial counts was observed during storage period. The highest overall mean count was recorded in the uncoated frozen samples (36.4 CFU/g), while the lowest was observed in the coated frozen fish (19 CFU/g). This reduction may be attributed to the ability of the collagen-based coating to act as a physical barrier, limiting oxygen permeability and inhibition bacteria growth (**Khorami *et al*., 2024**). Psychrotrophic bacteria are among the most significant spoilage microorganisms responsible for the deterioration of fish during chilled or frozen storage (**Sallam, 2007**). **Chamanara *et al*. (2013)** reported similar findings in *Oncorhynchus mykiss* (rainbow trout) fillets coated with chitosan, where the growth of psychrotrophic bacteria was considerably reduced compared to uncoated samples. Likewise, **Ebadi *et al*. (2019)** observed decreased psychrotrophic count in *Nemipterus japonicus* fillets coated with chitosan nanoparticles combined with beeswax extract, confirming the antimicrobial and oxygen-barrier effects of biopolymer coatings. Regarding the impact of frozen storage duration, a progressive increase in bacterial counts was detected in both coated and uncoated samples. In uncoated frozen fish, the initial counts at day 0 was 6 CFU/g, which increased to 19 CFU/g after 30 days, 33 CFU/g after 60 days, 51 CFU/g after 90 days, and peaked at 73 CFU/g after 120 days. In contrast, coated frozen fish exhibited a slower growth rate. At day 0, the psychrotrophic bacterial count was 4 CFU/g, rising to 9 CFU/g after 30 days, 18 CFU/g after 60 days, 24 CFU/g after 90 days, and reaching 41 CFU/g after 120 days of frozen storage. These results clearly indicate that the application of collagen-based edible coatings effectively delayed the growth of psychrotrophic bacteria, thereby contributing to improved microbiological stability and extended the shelf life of frozen *Sillago sihama* fish.

Proteolytic bacteria

The results depicted in Fig. (3) demonstrate effects of coating and frozen storage duration on proteolytic bacterial count of *Sillago sihama* fish. A gradual higher value in proteolytic bacterial numbers was observed during storage. The highest overall mean count was recorded in uncoated frozen fish (28.8 CFU/g), whereas the lowest mean value was observed in coated frozen samples (17.6 CFU/g). This reduction in proteolytic bacteria in coated samples can be attributed to the protective role of the collagen-based coating, which inhibits microbial activity and preserves the nutritional components of fish by forming a semipermeable barrier that reduces oxygen transfer and moisture loss. The results further showed that number of proteolytic bacterial increased with storage time. In uncoated frozen fish, the initial bacterial count was 5 CFU/g at day 0, which gradually increased to 10 CFU/g after 30 days, 22 CFU/g after 60 days, 44 CFU/g after 90 days, and reached the highest level of 63 CFU/g after 120 days of frozen storage. In contrast, coated frozen fish exhibited a significantly slower increase. The lowest count was 2 CFU/g at day 0, followed by 8 CFU/g after 30 days, 17 CFU/g after 60 days, 22 CFU/g after 90 days, and 39 CFU/g after 120 days. These findings suggest that the collagen coating played an effective role in inhibiting proteolytic bacterial growth during frozen storage. The results corroborate those reported by **Elkassas *et al*. (2020)**, who evaluated the quality of *Oreochromis niloticus* (the Nile tilapia) fillets coated with chitosan and nano-chitosan during refrigerated storage at 4°C for 15 days. They observed a gradual increase in proteolytic bacterial counts with storage duration, but the increase was significantly lower in coated samples compared to uncoated controls. Thus, the current study confirms that collagen-based edible coatings act as an antimicrobial and protective layer, effectively reducing proteolytic bacterial activity and contributing to the microbiological stability of frozen fish.

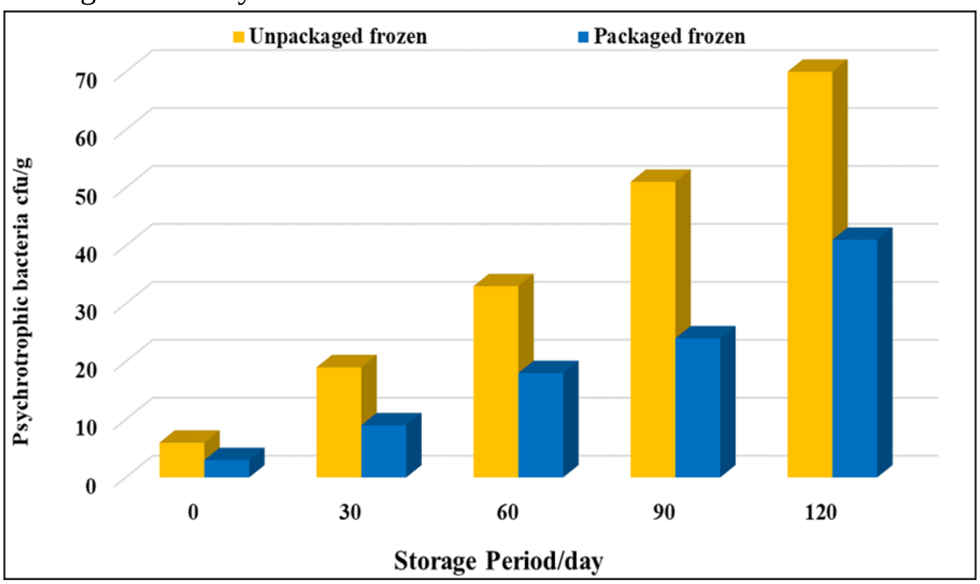

Fig. 2. Effect of packaging and frozen storage duration (-18°C) on the psychrotrophic bacteria in *Sillago sihama* fish

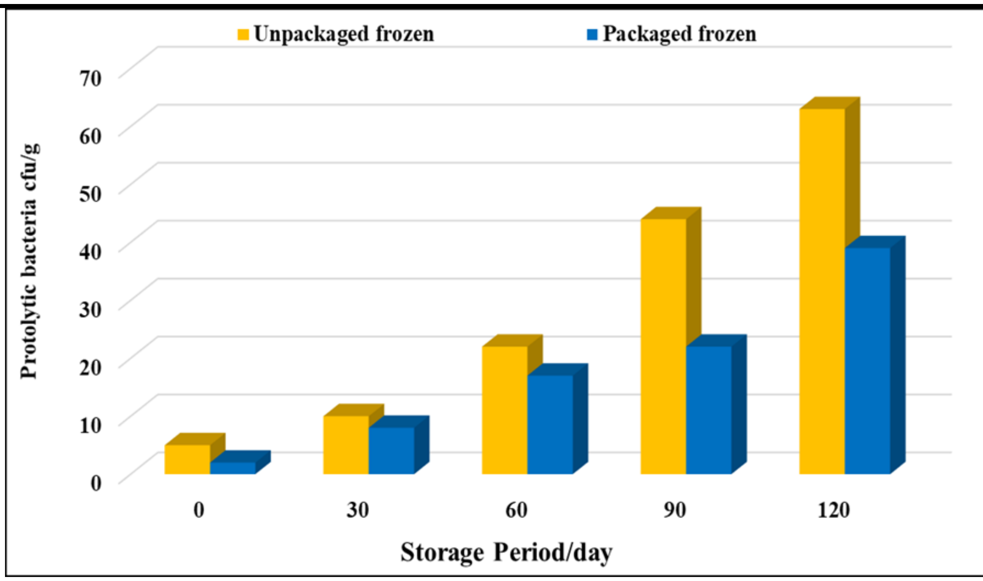

Fig. 3. Effect of packaging and frozen storage duration (-18°C) on the proteolytic bacteria in *Sillago sihama* fish

Lipolytic bacteria

The results illustrated in Fig. (4) show the effect of coating and frozen storage duration on the lipolytic bacterial count of *Sillago sihama* fish. A gradual increase in lipolytic bacteria was observed with increasing storage time. The highest overall mean count was recorded in uncoated frozen fish (21.4 CFU/g), whereas the lowest mean value was observed in coated frozen samples (11.8 CFU/g). The lower counts in coated fish may be attributed to the antimicrobial and oxygen-barrier properties of the collagen coating, which restricts bacterial access to lipids and thus reduces the rate of lipid hydrolysis and microbial growth. With respect to storage time, the results showed a clear increase in lipolytic bacterial counts as storage progressed.

In uncoated frozen fish, the initial count was 3 CFU/g at day 0, increasing to 9 CFU/g after 30 days, and 21 CFU/g after 60 days, 31 CFU/g after 90 days, and reaching 43 CFU/g after 120 days of frozen storage. In contrast, coated frozen fish exhibited lower bacterial counts throughout storage. The initial count was 1 CFU/g at day 0, which increased to 5 CFU/g after 30 days, and 12 CFU/g after 60 days, 17 CFU/g after 90 days, and 24 CFU/g after 120 days. Similar results were reported by **Elkassas *et al*. (2020)**, who evaluated the quality of *Oreochromis niloticus* (the Nile tilapia) fillets stored at 4°C with chitosan and nano-chitosan coatings. Their results showed that while lipolytic bacterial counts increased with storage duration, the coated samples exhibited significantly lower bacterial loads compared to uncoated fish. These results suggest that collagen-based edible coatings can effectively inhibit lipolytic bacterial growth by limiting oxygen diffusion and microbial enzymatic activity, thereby slowing down lipid degradation and improving the overall microbial stability of frozen fish products.

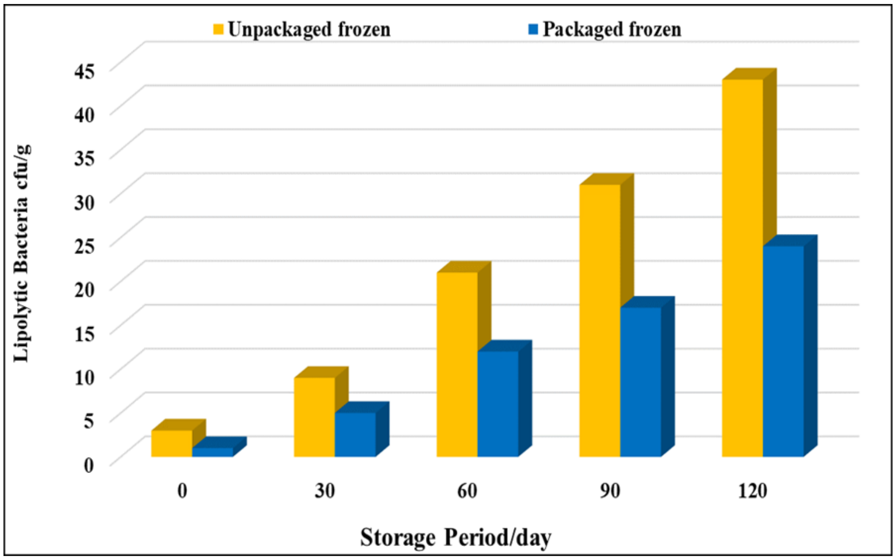

Fig. 4. Effect of packaging and frozen storage duration (-18°C) on the lipolytic bacteria in *Sillago sihama* fish

Coliform bacteria

The results shown in Fig. (5) illustrate the effect of coating and frozen storage duration on coliform bacteria in *Sillago sihama* fish. The findings revealed that coated frozen fish samples had markedly lower coliform counts than uncoated frozen fish. The overall mean coliform count in uncoated frozen fish was 2.8 CFU/g, whereas in coated frozen fish it was 1 CFU/g. The storage duration had a noticeable impact on coliform growth. In uncoated frozen fish, coliforms were not detected at day 0 but appeared after 30 days of storage with a count of 1 CFU/g, increasing to 3 CFU/g after 60 days, 4 CFU/g after 90 days, and peaking at 6 CFU/g after 120 days. In contrast, coated frozen fish samples showed no detectable coliform bacteria during the first 30 days of storage. Counts began to appear after 60 days (1 CFU/g), increased slightly to 2 CFU/g after 90 days, and remained at 2 CFU/g after 120 days of frozen storage. According to **Al-Hamdani (2024)**, the reduction in coliform counts during frozen storage may be due to the low resistance of coliform bacteria to freezing temperatures, which cause cellular damage and gradual death over time. This observation aligns with result of this study, where collagen coating further enhanced microbial inhibition by limiting environmental exposure and slowing bacterial recovery during thawing. Overall, the application of collagen-based edible coatings effectively suppressed coliform bacterial growth, contributing to improved microbial safety and extended shelf life of frozen *Sillago sihama* fish.

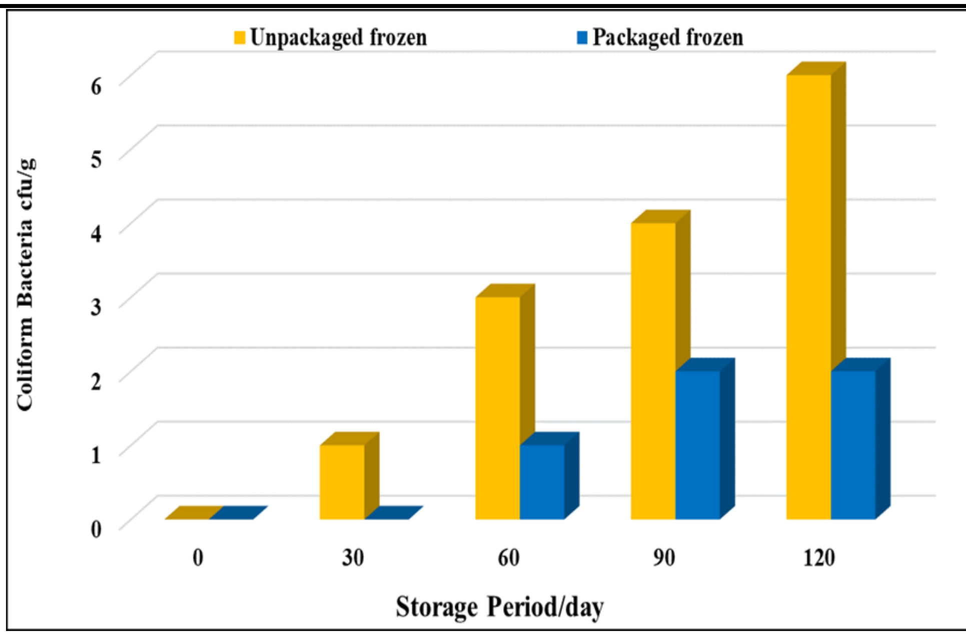

Fig. 5. Effect of packaging and frozen storage duration (-18°C) on the coliform bacteria in *Sillago sihama* fish

CONCLUSIONS

In conclusion, collagen coatings not only enhanced the microbiological safety and storage stability of frozen *Sillago sihama* but also contributed to the valorization of fish-processing waste, promoting more sustainable and eco-friendly practices in seafood preservation.

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