Role of Edible Collagen Coating in Preserving the Chemical Quality of Hasoom (*Sillago sihama*) Fish During Frozen Storage

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Abstract

The study had Sillago sihama fish obtained from the Al-Faw city, situated at the southern boundary of Basra province. The fish were treated with collagen derived from the fish residue, the skin of Scombromorus commerson, before being frozen at -18°C for a period of 120 days. Chemical analysis of the fish, done after the freezer storage, entailed the assessment of pH, volatile nitrogenous bases, levels of free fatty acids, thiobarbituric acid, and fluid loss. The findings were indicative of an increase in the pH level of the coated and uncoated fish, although the latter registered a higher percentage at 13.6% compared to the former at 10.24%. The highest content of volatile nitrogenous bases was found in the coated fish at 74.404%, higher than the uncoated fish at 74.193%. The free fatty acid percentage for the uncoated and coated fish stands at 83.827% and 58.333%, respectively. (TBA) values also showed the highest increase in uncoated fish 85.610 % and the lowest in coated fish 63.390%, with significant differences (p<0.05) between freezing periods 60, 90, and 120 days in uncoated samples. Drip loss increased in uncoated frozen fish, reaching 44.920 %, while coated frozen fish showed a lower drip loss of 24.241%. In conclusion, the current study demonstrated the potential of collagen edible coatings in reducing oxidative changes, extending shelflife, and preserving the quality of frozen fish.

Keywords: *Sillago sihama*, Collagen, Chemical Characteristics, Frozen Fish.

Introduction

Fish are considered one of the most important and widely consumed sources of animal protein, and they remain affordable for most people worldwide (Allam et al., 2020; Maulu et al., 2020). Fish hold great importance in developing countries, where they represent up to 75% of animal protein intake and serve as an essential food source for many populations. They also play a significant role in the labor market (Mansour et al., 2021). Fish are nutritionally rich in essential nutrients, including high-quality proteins, amino acids, lipids, and various vitamins and minerals (FAO, 2020). Fish consumption varies depending on geographical regions and cultural and social customs. (Obeed and Al-Noor, 2025). Fish are characterized by their rapid spoilage, which is due to their chemical composition and active enzymes that lead to autolysis, microbial activity, and fat oxidation (Al-Hamdani and Al-Noor, 2024) For safe consumption, proper storage is essential to maintain quality (Sone et al, 2019) Preservation methods are very important, and the most important of these is freezing, which prevents chemical and physical damage and microbial growth during storage. (Malik et al., 2021) Frozen fish may undergo several undesirable changes, including altered flavor, bitterness, unwanted rancidity, reduced water retention capacity and consistency, all of which affect its appearance, texture, and color (Tocher et al., 2019). These changes can be minimized through suitable packaging techniques (Al-Noor et al., 2013), This includes optimal active packaging systems for preserving seafood quality, containing natural antimicrobials, and is safe for use with food (Kamau et al.,2025). Edible biofilms derived from natural materials have been widely used for preserving meat and fish due to their ability to inhibit enzymatic activity and prevent discoloration (AlNoor et al., 2013; Al-Busalimi et al., 2022). Therefore, this study was carried out to evaluate the role of edible collagen coating in preserving the chemical quality of silver sillago (Hasoom) Sillago sihama during frozen storage.

Materials And Methods

Fresh *Sillago sihama* (Silver sillago; Hasoom) were obtained from the marine fish market (Al-Nak'ah) in Al-Faw City, southern Basra Governorate, during December 2024. Fish average length and weight were 17.83 cm and 41.75 g, respectively. A total of 15 kg of fish samples was collected The samples were transported in ice-cooled insulated containers, washed thoroughly upon arrival at the laboratory, and a random samples was taken for initial quality assessment.

pH Measurement

The pH value was measured by homogenizing 5 g of fish muscle with 10 ml of distilled water for 5 minutes, following the method of Wong *et al.* (1991) using a pH meter.

Total Volatile Nitrogen (TVN)

TVN was determined by mixing 10 g of the sample with 2 g of magnesium oxide, 300 ml distilled water, and glass beads. The distillate was collected in 25 ml of 2% boric acid





with methyl red indicator, distilled for 25 minutes, and titrated with 0.1 N sulfuric acid according to (Egan *et al.*, 1988).

TVNB mg N/100g fish= (ml $0.1N H_2SO_4 \times 14$)

Free Fatty Acids (FFA)

FFA were determined following Wong *et al.* (1991) by homogenizing 10 g of the sample with 50 ml of neutralized ethanol (95%) containing phenolphthalein, then titrating with 0.1N NaOH.

FFA (%)= (ml NaOH \times molarity \times 28.2)/ sample weight

Thiobarbituric Acid (TBA)

TBA values were determined according to Egan et al. (1988) using distilled samples reacted with TBA reagent, heated in a water bath for 35 minutes, cooled, and read spectrophotometrically at 538 nm.

TBA= Absorbance \times 7.8 (mg malondialdehyde/ kg fish)

Drip Loss Measurement

A fish fillet portion was weighed, suspended at 4°C for 48 hours, reweighed, and drip loss was calculated according to (Rasmussein and Mast, 1989):

Drip loss (%)= (Initial weight – Final weight)/ Initial weight × 100

Statistical analysis

The data were statistically analyzed using ANOVA through the SPSS software using the LSD method, the least significant factors were tested at the 0.05 level and a completely randomized design (CRD) was used.

Results and Discussion

Table (1) shows the effect of packaging on pH values and freezing storage duration for hasoom fish. The results indicate a gradual increase in pH values in both packaged and unpackaged fish, with the largest increase observed in unpackaged fish (10.24%) compared to packaged fish (6.13%). The difference between the two treatments was observed to increase towards the end of the study period, reaching its highest point at 0.31 during the 120-day storage period. Regarding the overall average, the pH was higher in unpackaged frozen fish (6.484) and lower in packaged frozen fish (6.306). The increase in the overall average pH in unpackaged frozen fish was attributed to the formation of nitrogenous compounds produced by microorganisms and the activity of enzymes that lead to protein degradation (Badee *et al.*, 2013). The decrease in the overall average pH was attributed to For frozen, packaged fish, this may be due to the role of packaging, which inhibits the growth of microorganisms and protein breakdown. Ammonia accumulation leads to a decrease in pH. Statistical analysis results showed significant differences





(p<0.05) in unpackaged frozen fish at 0, 90, and 120 days. For packaged frozen fish, there was no significant difference across all periods.

Concerning the effect of frozen storage duration on pH values, the results show a progressive increase in pH as storage time increased. In uncoated frozen fish, the highest value 6.83 was recorded at 120 days, while the lowest value occurred at day zero 6.13, followed by 6.33 at 30 days, with further increases at 60 and 90 days, reaching 6.46 and 6.67, respectively. Similarly, coated frozen fish exhibited higher pH values at later stages of storage, recording 6.52 at 120 days. The lowest value was observed at day zero 6.12, followed by gradual increases during subsequent storage periods, reaching 6.21, 6.29, and 6.39 at 30, 60, and 90 days, respectively. The overall increase in pH during frozen storage may be attributed to microbial degradation of proteins, producing amino acids and releasing nitrogenous compounds such as ammonia, which contribute significantly to pH elevation (Souza et al., 2019). Malik et al. (2021) also reported increasing pH values with prolonged storage, with pH ranging from 5.74 Sin (O. niloticus) to 6.24 in (B. bayad) during storage periods up to 45 days. pH increased significantly (p≤0.05) from the initial period to day 15, then continued to rise through day 30 and the final storage period. Li et al. (2013) suggested that increases in pH may result from endogenous enzymatic activities in fish, including deamination and decarboxylation of proteins, leading to the production of alkaline compounds such as amines and ammonia. The findings of the present study are consistent with those of Liu et al. (2023), who reported that pH values in frozen Pagrus major coated with collagen and chitosan were significantly lower than those in uncoated samples throughout storage, except on day zero.

Table (1): Effect of Collagen Coating and Frozen Storage (-18°C) on pH Values of *Sillago* sihama

Treatment		Stor	age Pe	riods	Overall	% Final	
Treatment	0	30	60	90	120	Mean	Difference
Uncoated Frozen	6.13 ^a	6.33 ^a	6.46a	6.67 ^{ab}	6.83 ^b	6.484	10.24 ^a
Coated Frozen	6.12 ^a	6.21 ^a	6.29 ^a	6.39 ^a	6.52a	6.306	6.13 ^b
Overall Mean (Storage Periods)	6.125	6.27	6.375	6.53	6.675	6.395	
Difference Between Durations	0.01 ^a	0.12 ^a	0.17 ^a	0.28ab	0.31 ^b		

^{*}Means in the same row with different superscripts are different at p≤0.05.

Total Volatile Nitrogen (TVN)

Table (2) illustrates the effect of collagen coating and freezing storage duration on the average values of volatile nitrogenous bases in hassom fish. The results show that both uncoated and collagen-coated frozen fish gradually increased their nitrogenous base levels during the storage period. The highest percentage increase was observed in the coated fish, reaching 74.404%, while the lowest percentage increase was observed in the uncoated





fish, reaching 74.193 %. The greatest difference between the two transactions appeared at the 120-day storage period, reaching 4.9mg nitrogen/100g fish. However, the highest overall average value of nitrogenous bases was 13.02 mg nitrogen/100 g fish in unwrapped frozen fish, and the lowest overall average was 10.1 mg nitrogen/100 g fish in wrapped frozen The variation in nitrogenous base values in our current study may be attributed to enzyme activity and bacterial spoilage (Amin et al., 2023). These results are consistent with Alparslan et al. (2014)'s study on the effects of edible gelatin-based films enriched with bay essential oil on the quality of rainbow trout (Oncorhynchus mykiss) fillets during refrigerated storage. Lopez-Caballero et al. (2005) found that the protective chitosan layer in gelatin reduced TVN volatile nitrogen levels, thus slowing spoilage. Ahmad et al. (2012) demonstrated that the inhibitory effects of a gelatin film containing lemon essential oil reduce and delay the production of degradation products that lead to microbial growth, as the gelatin film inhibits microbial growth. Other similar studies have reported that fish fillets encased in edible films containing essential oils and extracts showed a decrease in TVN values. Volatile nitrogenous bases and Coban 2018, (Fadiloglu et al., 2014; Alparslan et al., 2012; Ahmad et al., 2012), and the results of the statistical analysis showed a significant difference (p<0.05) in unpackaged frozen fish between storage periods of 0, 90, and 120 days.

Regarding the effect of storage duration on volatile nitrogen levels, the results table shows an increase in volatile nitrogen levels with increasing storage time. The highest average was recorded in the last month of the study, reaching 21.7 mg nitrogen/100g of fish. During a 30-day storage period, the average was 9.8 mg nitrogen/100g of fish, while during a 60-90-day storage period, it was 12.6 mg nitrogen/100g of fish and 15.4 mg nitrogen/100g of fish, respectively. However, the lowest average values were recorded in the zero-day period, reaching 5.6 mg nitrogen/100g of fish in frozen fish not coated with collagen. In fish coated with collagen, the average values of volatile nitrogen levels also increased with increasing storage duration, reaching the highest value in the last month of the study at 16.8 mg nitrogen/100g of fish. Lower values were observed at earlier stages: 4.3mg nitrogen/100g (day 0), 7mg nitrogen/100g (30 days), 8.4mg nitrogen/100g (60 days), and 14 mg nitrogen/100g (90 days). The increase in volatile nitrogen (TVN) levels may be attributed to the accumulation of alkali compounds, including trimethylamine and ammonia. These compounds are a byproduct of protein breakdown by enzymes (Duarte et al., 2020). Wang et al. (2022) indicated that total nitrogen levels in fish are relatively acceptable when they are less than 20 mg of nitrogen/100 g of fish, while levels above 20 mg of nitrogen/100 g of fish result in a loss of freshness.

Table (2): Effect of Collagen Coating and Frozen Storage (-18°C) on TVN Values of *Sillago* sihama

Treatment		Sto	rage Pe	Overall	% Final		
Treatment	0	30	60	90	120	Mean	Difference
Uncoated Frozen	5.6a	9.8a	12.6ab	15.4 ^b	21.7 ^b	13.02	74.193 ^a





Coated Frozen	4.3a	7.0a	8.4 ^a	14.0 ^{ab}	16.8b	10.1	74.404 ^a
Overall Mean (Storage Periods)	4.95	8.4	10.5	14.7	19.25	11.56	
Difference Between Durations	1.3ª	2.8a	4.2 ^{ab}	1.4ª	4.9 ^b		

^{*}Means in the same row with different superscripts are different at p≤0.05.

Free Fatty Acids (FFA)

Table (3) shows the values of free fatty acid levels and the effect of freezing storage duration for collagen-coated and uncoated Hasoom fish. The results indicate a gradual increase in free fatty acid values during the freezing storage period. The highest increase was observed in uncoated fish, reaching 83.827%, while the lowest increase was observed in coarsely coated fish, reaching 58.333%. The table also shows that the highest difference between the two treatments during a storage period of (120) days was 0.454. The highest overall free fatty acid content was observed in unwrapped frozen fish at 0.3948, while in collagen-coated frozen fish it was 0.2054. Collagen coating significantly reduced free fatty acid levels, as evidenced by the free fatty acid levels during storage periods. Unwrapped frozen fish had the highest free fatty acid content at the end of the study period, reaching 0.742 after 120 days, followed by 0.493 after 90 days. The lowest free fatty acid content was observed at 0.12 after 30 days, followed by 0.253 after 30 days. Free fatty acid levels increased during storage in unwrapped frozen fish, while collagen-coated frozen fish also showed an increase, albeit at a lower rate than unwrapped fish. The highest free fatty acid levels were observed in the frozen, coated fish during the storage period. The fat content was 0.288 after 120 days of storage, followed by 0.253 after 90 days. However, the lowest values were observed in the initial storage periods, reaching a low of 0.12 at day zero, followed by 0.141 after 30 days. Fatty acid ratios reflect the hydrolysis index of fats. Alparslan et al. (2014) noted in their study an increase in free fatty acid values. Their study also observed that gelatin coating had a better effect on the quality of trout (Oncorhynchus mykiss) fillets compared to uncoated fish. Nguyen et al. (2024) demonstrated that low temperatures (-25 ± 2°C) delay fat breakdown and oxidation, resulting in a decrease in free fatty acid (FFA) values. It is also believed that freezing increases the free fatty acid content, and this increase may lead to enzymatic hydrolysis. Using phospholipase and lipase enzymes, the statistical analysis revealed significant differences (p<0.05) in uncoated fish between storage periods of 0, 60, 90, and 120 days.

Table (3): Effect of Collagen Coating and Frozen Storage (-18°C) on FFA Values of *Sillago* sihama

Treatment		Sto	orage Pe	Overall	% Final		
Treatment	0	30	60	90	120	Mean	Difference
Uncoated Frozen	0.12 ^a	0.253 ^a	0.366ab	0.493 ^{ab}	0.742 ^b	0.3948	83.827 ^a
Coated Frozen	0.12 ^a	0.141 ^a	0.225 ^a	0.253 ^a	0.288a	0.2054	58.333 ^b





Overall Mean (Storage Periods)	0.12	0.197	0.2955	0.373	0.515	0.3001	
Difference Between Durations	O ^a	0.112 ^a	0.111 ^a	0.240 ^{ab}	0.454 ^b		

^{*}Means in the same row with different superscripts are different at p≤0.05.

Thiobarbituric Acid (TBA)

The average thiobarbituric acid (TBA) value in Table (4) showed different trends. The largest increase in TBA value was recorded in the uncoated fish at 85.610%, and the lowest increase in the collagen-coated group at 63.390%, respectively. In addition, the largest difference among the treatments was recorded after 120 days of storage, and the value reached 2.168 mg of malondialdehyde per kilogram of fish. The highest concentration of malondialdehyde (MDA) was found in unpackaged frozen fish, reaching 1.8232 mg/kg. The lowest concentration was recorded in collagen-coated frozen fish, at 0.9062 mg/kg. The concentration of malondialdehyde was found to be affected by storage duration, and storage duration, in turn, affected the concentration of thiobarbituric acid (TBA), which exhibited an uneven distribution. The thiobarbituric acid concentration was 0.78 mg/kg after 30 days of storage, then decreased to 0.514 mg/kg on the first day. However, this concentration gradually increased over time, peaking at 3.572 mg/kg after 120 days of storage Concomitant with the 60-90-day storage period, the value of thiobarbituric acid (TBA) was 1.762 mg malonaldehyde/kg fish and 2.488 mg malonaldehyde/kg fish. These values indicate the total amount of thiobarbituric acid (TBA) in uncoated frozen fish. The values in the table showing the results indicate that the increase in the amount of TBA in the collagen-coated fish was highest during the storage period of 120 days, amounting to 1.404 mg malonaldehyde/kg fish. The lowest amount was seen in the initial storage periods of the frozen fish, which was 0.514 mg/kg fish at 0 days, 0.624 mg/kg fish at 30 days, 0.858 mg/kg fish at 60 days, and 1.131 mg malonaldehyde/kg fish at 90 days. We observe that the TBA ratios increased with the increase in the freezing storage period; this may be due to oxidation of fats and the formation of aldehydes and malondialdehyde that react with other compounds in the body, such as proteins, amines, nucleic acids, and nucleoids. This reaction may vary according to the fish type (Liu et al., 2010). Similarly, Agustinelli and Yeannes (2015). Khoshnoudi Nia and Mousavi Nasab (2019) observed a substantial rise in the concentration of TBA in trout fillets during the zero to ten-day interval. Thus, the process of reheating and reheating the fish could cause the concentration of TBA to rise (Cheng et al., 2019). The research proved the oxidation and growth of fat within the muscles of the mackerel and the entire fish at -19°C, showing a high level of thiobarbituric acid (TBA) within the tissues. TBA is normally found in foods like fish, although there may be some influence within the levels, for example, oxidation by oxygen or bacterial contamination, among others, cited in (Rathwood et al., 2021). The results of this study are in accordance with Al-Qurashi and Awad (2018) since it indicated that TBA in the first storage period was 0.78 mg malondialdehyde/kg, then increased





gradually. This could be attributed to the oxidation of fish fillets, the dryness of fish, as well as the unsaturated fatty acids. Inanlit $et\ al.$ (2020) proved in his study that chitosan coating can prevent effectively the oxidation of lipids in fish fillets, as well as the increase of the Thiobarbituric acid index in cod ($Gadus\ morhua$). However, the results of the statistical analysis of this study indicated that there were significant differences at p < 0.05 in the uncoated frozen fish for the periods of 60, 90, and 120 days.

Table (4): Effect of Collagen Coating and Frozen Storage (-18°C) on TBA Values of <i>Sillago</i>
sihama

Treatment		Sto	rage Per	riods		% Final	
Treatment	0	30	60	90	120	Mean	Difference
Uncoated Frozen	0.514 ^a	0.780a	1.762a	2.488ab	3.572 ^b	1.8232	85.610 ^a
Coated Frozen	0.514 ^a	0.624a	0.858a	1.131 ^a	1.404 ^a	0.9062	63.390 ^b
Overall Mean (Storage Periods)	0.514	0.702	1.31	1.8095	2.488	1.3647	
Difference Between Durations	O ^a	0.156ª	0.904ª	1.357 ^{ab}	2.168b		

^{*}Means in the same row with different superscripts are different at p≤0.05.

Drip Loss (Water Loss Percentage)

Table (5) presents data on the percentage of fluid loss in uncoated fish, fish coated with collagen, and the effect of freezing time on fluid loss in hasoom fish. Fluid loss in uncoated fish upon freezing shows a loss of 44.920%, while coated fish recorded a loss of 24.241%. Looking at the average values, the highest percentage loss was 3.404% for uncoated fish However, packaged frozen fish recorded the lowest percentage loss at 2.672%. The largest difference was at 120 days, at 1.24%. The differences in the percentage of fluid loss can be attributed to muscle tissue damage resulting from the formation of large ice crystals, which contributes to fiber shrinkage, protein denaturation, and the formation of aggregates as a result of the freezing and thawing process, in addition to cell damage (Xie, 2023). Another possible cause is the level of free water on the surface of the flesh. Weakly bound water is easily removed, and a high level of free water indicates increased moisture loss (Dang et al., 2022). According to Liu et al. (2017), moisture loss in fresh fish leads to the disappearance of water-soluble components, as well as changes in taste and aroma. A high level of moisture loss is considered a key indicator of food quality. The analysis shows a statistically significant difference (p<0.05). Compared to unpackaged frozen fish, no statistically significant differences were observed in packaged frozen fish. Regarding the percentage of liquid loss in frozen fish, the initial freezing process results in a low percentage of liquid loss. This percentage gradually increases over time. The highest percentage was recorded in unpackaged frozen fish, reaching 4.43% after 120 days. The average liquid loss was 3.89% after 90 days. The lowest liquid loss was recorded on day o at 2.44%, followed by 2.84% and 3.42% on days 30 and 60, respectively. Liquid loss was





lower in frozen and packaged fish than in unpackaged fish throughout all storage days. The highest liquid loss was recorded in packaged fish after 120 days at 3.19%, followed by 2.78% on day 90. The lowest liquid loss was recorded on day 0 at 2.41%, followed by 2.46% and 2.52% on days 30 and 60, respectively. The recorded values showed a similar pattern to the liquid loss pattern identified by Liu *et al.* (2020), which found that fluid loss in chitosan-coated Pagrus major frozen for 2-10 days. The percentage of moisture loss was higher in unwrapped fish, reaching 7.98% on day 2 and 14.2% on day 10, while it was lower in wrapped fish, reaching 6.63% on day 10. These results indicate that unwrapped fish have a lower capacity for moisture retention, which can be improved by using a wrapper. Bhujba *et al.* (2021) observed that water loss was highest in all treatment groups from day 1 to day 120, ranging from 2.18% on day 120.

Table (5): Effect of Collagen Coating and Frozen Storage (-18°C) on Drip Loss Values of *Sillago sihama*

Treatment		Stora	age Pe	riods		Overall	% Final
Treatment	0	30	60	90	120	Mean	Difference
Uncoated Frozen	2.44 ^a	2.84ª	3.42 ^a	3.89 ^{ab}	4.43 ^b	3.404	44.920 ^a
Coated Frozen	2.41 ^a	2.46a	2.52 ^a	2.78a	3.19 ^a	2.672	24.241 ^b
Overall Mean (Storage Periods)	2.425	2.65	2.97	3.335	3.81	3.038	
Difference Between Durations	0.03 ^a	0.38a	0.9ª	1.11 ^{ab}	1.24 ^b		

^{*}Means in the same row with different superscripts are different at p≤0.05.

Conclusions

This study aimed to highlight the effectiveness of edible collagen coatings in extending the shelf life of frozen fish, reducing spoilage and preserving their nutritional value. Collagen coating demonstrated favorable antioxidant and protective properties, contributing to better quality retention during frozen storage.

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دور غلاف الكولاجين الصالح للأكل في الحفاظ على الجودة الكيميائية لأسماك الحاسوم (Sillago sihama)

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المستخلص

أجريت الدراسة على أسماك الحاسوم Sillago sihama الذي تم الحصول عليها من مدينة الفاو التابعة لمحافظة البصرة. أذ غُلفت الأسماك بالكولاجين المستخلص من المخلفات السمكية المتمثلة بجلود أسماك المزلك، ثم جُمَدت الأسماك عند درجة حرارة -18 درجة مئوية لمدة 120 يوماً، حيث قُيمت خلالها العديد من الخصائص الكيميائية. أظهرت النتائج أرتفاعاً تدريجياً في قيم الرقم الهيدروجيني للأسماك المغلفة وغير المغلفة إلا أن أعلى زيادة سُجَلت في الأسماك غير المغلفة، حيث بلغت 10.24%، مُقارنة بد 61.6% في الأسماك المغلفة أما بالنسبة للنيتروجين الكلي المتطاير (TVN)، فقد سُجَلت أعلى زيادة في الأسماك المغلفة بنسبة 74.404% أما الأحماض الدهنية الحرة (FFA)، فقد سُجَلت أعلى زيادة في الأسماك غير المغلفة بنسبة 83.827% أما الأحماض الدهنية الأسماك المغلفة بنسبة (TBA) أيضاً أعلى زيادة في الأسماك المغلفة بنسبة (TBA) أيضاً أعلى زيادة في الأسماك غير المغلفة (60.85%)، مع وجود اختلافات كبيرة (\$0.05) بين فترات التجميد 60 و 90 و 120 يومًا في العينات غير المغلفة. زاد نسبة السوائل المفقودة في الأسماك المجمدة المغلفة بينما أظهرت الدراسة الحالية إمكانات طلاءات الكولاجين الصالحة للأكل في تقليل التغير ات التأكسدية وإطالة العمر الافتراضي والحفاظ على جودة الأسماك المجمدة. المحمدة. المحمدة. المخلفة المحمدة. المخلفة المحمدة المخلفة المحمدة.



