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Cold water fish gelatin films: Effects of cross-linking on thermal, mechanical, barrier, and biodegradation properties

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ABSTRACT

Gelatin was extracted from Alaska pollock (*Theragra chalcogramma*) and Alaska pink salmon (*Oncorhynchus gorbuscha*) skins and cast into films. The fish gelatin films' tensile, thermal, water vapor permeability, oxygen permeability, and biodegradation properties were compared to those of bovine and porcine gelatin films. In addition, fish gelatin films were cross-linked with glutaraldehyde. Pollock and salmon gelatin films had comparable tensile properties, but had lower tensile strength and percent elongation than mammalian gelatin films. The lower strength and elongation might have been due to lower structural gelatin levels present in fish gelatin films. The addition of cross-linkers had little effect on tensile properties and melting temperatures of fish gelatin films. Pollock gelatin films had the lowest water vapor and oxygen permeability values, whereas mammalian gelatin films had the highest permeability values. Cross-linking resulted in lower water vapor permeability for salmon gelatin films and higher oxygen permeability for pollock gelatin films. However, all fish gelatin films had better water vapor and oxygen barrier properties than mammalian gelatin films. Also, fish gelatin films degraded faster than mammalian gelatin films.

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1. Introduction

Gelatin is readily available and has good film forming properties. For gelatin films cast below gelation temperature of the gelatin solution, triple helical structures form and become locked in place as water evaporates from the sample. For films cast above gelation temperature, less triple helical structures form and films might be completely amorphous, depending on cast temperature. Gelatin, in its film form, has been widely used to produce soft and hard capsules for encasing drugs in the pharmaceutical industry. Gelatin films have also been envisioned as coatings for food products [1], as wound dressings [2,3], and as packaging materials [4]. Gelatin films generally have good oxygen barrier properties. However, gelatin films also have relatively poor water barrier and mechanical properties, which limit their potential applications.

Several methods have been used to improve gelatin film properties. One method involves blending gelatin with other proteins or polysaccharides, such as casein [5], chitosan [6], starch [7], and gellan [8]. Various plasticizers have also been added to improve mechanical properties of gelatin films [9,10]. Another method to improve mechanical and water barrier properties involves adding cross-linkers, such as transglutaminase [5,11,12], glutaraldehyde [13,14], genipin [15], glyoxal [12,16], formaldehyde [12,16], ferrulic acid [17], and tannin acid [17].

Most research on gelatin films have focused on films derived from mammalian sources, such as bovine and porcine. One reason is that mammalian gelatin films generally have superior mechanical properties to other types of gelatin films. Recently, there has been more interest in using alternate sources of gelatin, such as those from fish, due

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to religious considerations or fear of bovine spongiform encephalopathy (BSE). Several studies on fish gelatin films have used gelatin from bigeye snapper [18–20], brownstripe red snapper [18–20], Baltic cod [21,22], talapia [23], and tuna [24]. Most of these studies have focused on warm water fish gelatins, with only a few on cold water fish gelatins. The main difference between mammalian and fish gelatin is their gelation temperature. Fish gelatin, especially those from cold water species, has much lower gelation temperature than those of other species. This is due to fish gelatin having lower concentrations of proline and hydroxyproline.

One potential source of fish gelatin might include Alaska pollock and Alaska pink salmon by-products generated from the Alaskan fishing industry. These two fishes comprised approximately 73% of the Alaskan marine finfish catch in 2000 [25]. It has been estimated that over a million tons of fish by-products are generated each year in Alaska [25]. Some of these by-products are converted into fish meal and oil, but a large percentage are not utilized and are dumped back into the ocean [25]. The by-products include substantial quantities of fish skin, which is a good source of gelatin.

In this study, we extracted gelatin from Alaska pollock and Alaska pink salmon skins. We then cast fish gelatin films and compared their tensile, thermal, water vapor permeability, oxygen permeability, and biodegradation properties to those of bovine and porcine gelatin films. We also added glutaraldehyde cross-linkers to fish gelatin films and examined their effects on film properties.

2. Experimental

2.1. Film preparation

Gelatin films were prepared by first dissolving 5% (w/w) gelatin in deionized water at 60 °C for 1 h. The solution was allowed to cool at room temperature (23 °C) for 50 min before being cast on a flat Mylar sheet placed over a glass plate. The gelatin film was formed when water evaporated overnight at room temperature. Cross-linked fish gelatin films were prepared by adding 0.25%, 0.50%, and 0.75% (w/w) glutaraldehyde (Sigma–Aldrich) to the gelatin solution after the cooling period and mixing with a stir bar for 5 min. The samples were then cast on the Mylar sheet. Porcine gelatin was obtained from Gelita (250A) and bovine gelatin was obtained from Kraft (250B). Alaska pollock (*Theragra chalcogramma*) and Alaska pink salmon (*Oncorhynchus gorbuscha*) gelatins were extracted from skins obtained from a commercial fish processing plant in Alaska. The extraction procedures were detailed elsewhere [26].

2.2. Extent of reaction

The extent of reaction was determined by measuring the amount of free or unreacted amino groups in each gelatin film using the method of Offner et al. [27]. In short, the 0.05 mm thick film was mixed with 4% (w/w) sodium bicarbonate ($NaHCO_3$) (Sigma–Aldrich) and 0.5% (w/w) trinitrobenzenesulfonic acid (TNBS) (Sigma–Aldrich) at 40 °C for 4 h. Afterwards, 6 N HCl (Fisher) was added to the mixture and the sample was heated at 120 °C for 1 h in an oven. The sample was then extracted with ethyl ether (Fisher). A Pharmaspec UV-1700 double beam spectrophotometer was used to measure the absorbance of the aqueous phase at 346 nm. The moles of amino groups per gram of gelatin were then calculated from the absorbance data. The extent of reaction was defined as $1\text{-}A_C/A_O$, where A_C is moles of amino groups per gram gelatin in the cross-linked film and A_O is moles of amino groups per gram gelatin in the film without glutaraldehyde.

2.3. Differential scanning calorimetry

A TA Instruments DSC 2910 was used to measure the thermal properties of the gelatin films. The sample amount used was 4.5 ± 0.1 mg of 0.05 mm thick films and each sample was heated from 30 °C to 140 °C at a rate of 5 °C/min. The sample chamber was purged with nitrogen gas at a flow rate of 75 cm^3/min. At least four replicates were tested for each sample.

2.4. Tensile tests

An Instron 5500R universal testing machine was used to measure the tensile properties of gelatin films at 23 °C. The tests were performed according to the ASTM D6380-00 method. Prior to each test, the 0.05 mm thick films were conditioned in a 50% relative humidity chamber for 48 h. A 100 N load cell was used and the extension rate was set at 10 mm/min. At least 10 replicates were tested for each sample.

2.5. Water vapor permeability

Water vapor transmission tests were performed according to the ASTM E96-80 method and to the method of McHugh et al. [28]. The gelatin films were mounted in polymethyl methacrylate test cells that had 50.8 mm diameter openings and the bottom of each cell was filled with 6 ml of deionized water. The test cells were placed in a cabinet, kept inside an incubator maintained at 25 °C, that contained anhydrous calcium sulfate to ensure 0% relative humidity on the outside of the cells. The films were allowed to equilibrate for 1 h before the cells were initially weighed. Thereafter, each cell was weighed seven more times over a 30 h period. Eight replicates were tested for each sample.

2.6. Oxygen permeability

An Ox-Tran 2/20 modular system (Modern Controls Inc.) was used to measure oxygen transmission rates through 0.05 mm thick gelatin films according to the ASTM D3985 method. Each film was placed on a stainless steel mask with an open testing area of 5 cm^2. The mask was then placed in a test cell and exposed to 98% N_2 + 2% H_2 flow on one side and 100% oxygen flow on the other. The film was allowed to equilibrate for 10 h before measurements were taken. Oxygen transmission rates were mea-

sured at 23 °C and 55% ± 1% relative humidity. Oxygen permeability was calculated by dividing oxygen transmission rate by oxygen partial pressure difference and multiplying by average film thickness. Four replicates were evaluated for each film.

2.7. Biodegradation

A Micro-Oxymax Respirometer System (Columbus Instruments Inc.) was used to monitor biodegradation of the gelatin films. Each gelatin sample (0.2 g) was mixed with 20 g of compost and kept in a 250-mL sample chamber connected to a fully computerized, closed-circuit system. Carbon dioxide evolution from each sample was measured every 8 h for a total of 64 days at room temperature. Triplicates were performed for each sample.

2.8. Statistical analyses

Data were analyzed by one-way analysis of variance and Tukey's multiple comparison tests at 95% confidence level using Minitab version 14.12.0 statistical software (Minitab Inc.).

3. Results and discussion

3.1. Extent of reaction

Both pollock and salmon gelatin films had comparable extents of reaction after cross-linking. These results are shown in Fig. 1, where we plot extent of reaction as a function of glutaraldehyde concentration for fish gelatin films. Glutaraldehyde reacts with amino groups in gelatin, such as those on lysine, to produce imine linkages. Both gelatin films had comparable initial concentrations of amino groups. The addition of 0.25% (w/w) glutaraldehyde resulted in both gelatin films having an extent of reaction of 0.56. After adding 0.50% (w/w) glutaraldehyde, pollock gelatin films had an extent of reaction of 0.87 and salmon gelatin films had an extent of reaction of 0.94. A further increase to 0.75% (w/w) glutaraldehyde led to only a slight increase in extent of reaction for both samples. These results indicated that adding 0.50% (w/w) glutaraldehyde is sufficient to react most of the amino groups. It should be noted that there are initially between 22 (0.25% w/w glutaraldehyde) and 67 (0.75% w/w glutaraldehyde) times more aldehyde groups than amino groups in samples containing glutaraldehyde. The presence of some unreacted amino groups in films indicated that the films might have dried too quickly for complete reaction to occur.

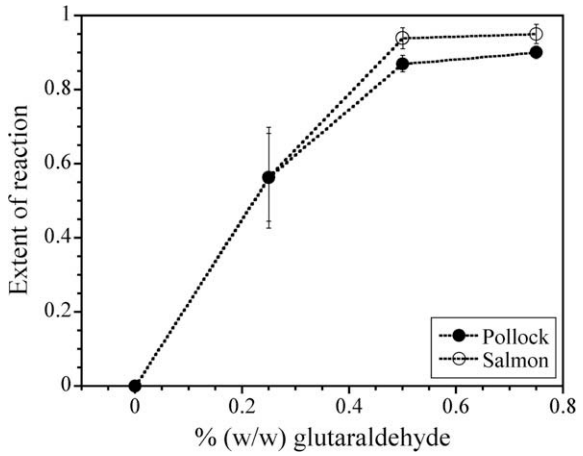

Fig. 1. Extents of reaction in fish gelatin films as a function of glutaraldehyde concentration. The lines only serve as eye guides.

3.2. Mechanical and thermal properties

Pollock and salmon gelatin films had comparable tensile properties. They also had comparable modulus, but lower strength and percent elongation values compared to mammalian gelatin films. This is shown in Table 1, where we present tensile properties of the gelatin films. These results might be related to the different levels of renaturation developed during the film forming process. Previous studies had shown that porcine gelatin films with higher renaturation levels had higher stress at break values [13,29,30]. For mammalian gelatin, film drying occurred at a temperature below their gelation temperature. This allowed the gelatin chains to partially revert back to a triple helical structure in solution. The chains then became locked into this structure as the solution dried into a film. In contrast, film drying for pollock and salmon gelatin occurred at a temperature above their gelation temperature. The gelatin chains initially remained as random coils in solution and began interacting more intensely as water evaporated from the sample. Ultimately, the chains might form triple helical structures as well as structures not entirely triple helical in nature. A previous study [31] had shown that mammalian gelatin films dried above their gelation temperature still formed structural gelatin that could be detected as a melting peak in a DSC scan. However, the amount of structural gelatin, represented by the melting enthalpy, was much less than that produced for films dried below their gelation temperature. In this study, pollock and salmon gelatin films had melting enthalpies of 5.7 ± 0.5 and 5.9 ± 0.6 J/g, respectively, whereas bovine and porcine gelatin films had enthalpies of 21.3 ± 1.5 and 22.7 ± 2.0 J/g, respectively. The enthalpy values for bovine and porcine films were comparable to bovine [12,32] and porcine [14,15] values found in literature. The larger enthalpy values for mammalian gelatin films indicated that they had higher renaturation levels than fish gelatin films, leading to improved strength and percent elongation values.

The addition of glutaraldehyde had little effect on the tensile properties of pollock and salmon gelatin films. This is shown in Table 1. All fish gelatin films had comparable modulus, strength, and percent elongation values except for the salmon gelatin film containing 0.50% (w/w) glutaraldehyde. However, even this film had marginally greater modulus, strength, and percent elongation values compared to the others. Previous studies on gelatin films had found divergent results on how cross-linkers affected tensile properties. For samples without plasticizers, the mod-

Table 1
Tensile and thermal properties of gelatin films

Gelatin type	Glutaraldehyde %(w/w)	Modulus (MPa)	Strength (MPa)	% Elongation	Melt temperature (°C)
Pollock	0	2077 ± 80a	50.1 ± 4.9a	3.44 ± 0.25a	57.5 ± 1.9a
	0.25	1991 ± 47a	49.0 ± 6.7a	3.43 ± 0.43a	57.7 ± 0.8a
	0.50	2091 ± 95a	47.3 ± 7.4a	3.24 ± 0.42a	57.0 ± 0.8a
	0.75	2035 ± 108a	45.9 ± 6.3a	3.23 ± 0.33a	55.3 ± 2.5a
Salmon	0	2043 ± 97a	51.2 ± 4.7a	3.57 ± 0.32a	57.2 ± 2.6a
	0.25	2050 ± 97a	49.6 ± 9.1a	3.37 ± 0.45a	59.8 ± 4.1a
	0.50	2245 ± 164b	60.0 ± 10.9ab	3.80 ± 0.55ab	58.2 ± 1.8a
	0.75	2175 ± 101ab	49.7 ± 8.2a	3.36 ± 0.48a	54.1 ± 1.4a
Bovine	0	1978 ± 73a	69.6 ± 9.7b	5.27 ± 1.05b	87.4 ± 3.7b
Porcine	0	2008 ± 70a	84.8 ± 15.5c	8.10 ± 2.94c	89.4 ± 4.3b

Different letter within a column indicate significant difference at $p < 0.05$.

ulus of porcine gelatin films showed an increase in value with addition of glutaraldehyde [14,30] and genipin [15]. The tensile strength of porcine and bovine gelatin films also generally increased after adding tannin acid [17], ferulic acid [17], and glutaraldehyde [14]. However, the tensile strength of porcine gelatin films remained constant after adding genipin [15]. Also, percent elongation of porcine and bovine gelatin films showed a decrease in value after adding various cross-linkers [14,15,17]. The tensile properties of gelatin films containing plasticizers exhibited an even wider range of behavior with addition of cross-linker. Modulus values had been shown to increase for fish gelatin films cross-linked with glutaraldehyde [33], to remain constant for gelatin films cross-linked with glyoxal [3], and to decrease for waste gelatin films cross-linked with glutaraldehyde [34]. Tensile strength of gelatin films also showed an increase in value [12,16,33–35] or remained constant [3,5,12] after addition of cross-linkers. In addition, percent elongation of gelatin films exhibited an increase [5,16,34], remained constant [3,12], or showed a decrease in value [12,33,35] after adding cross-linkers.

Mammalian gelatin films had higher melting temperatures than pollock and salmon gelatin films. This is shown in Table 1. The melting temperatures of bovine and porcine gelatin films are comparable to bovine [31,32] and porcine [9,14,15] values determined in other studies. The greater thermal stability of mammalian gelatin films might be related to the presence of more complete triple helical structures. Mammalian gelatins had higher concentrations of proline and hydroxyproline than fish gelatins, which enabled them to more readily form helical structures in solution. In this study, bovine and porcine gelatins had total proline and hydroxyproline concentrations of 22.9 and 24.3 mol%, respectively, whereas pollock and salmon gelatins had total concentrations of 15.4 and 16.4 mol%, respectively [26]. In addition, mammalian gelatin films were cast below their gelation temperature, which enabled triple helical structures to form before complete drying of the sample. In contrast, pollock and salmon gelatin films were cast above their gelation temperatures. In this case, triple helical structures as well as incomplete helical structures formed only when enough water evaporated and gelatin chains became sufficiently close together. A previous study had shown that films cast above gelation temperature contained some similar structures to those cast below gelation temperature [36]. However, as far as we know, a complete characterization of structures present in gelatin films does not exist in literature.

Glutaraldehyde had no effect on melting temperatures of fish gelatin films, as shown in Table 1. Pollock and salmon gelatin films had relatively constant melting temperatures in the 54–60 °C range. Previous studies generally found that incorporating cross-linkers in gelatin films had no effect [12,14] or led to an increase in melting temperatures [12,15,35].

3.3. Water vapor permeability

Fish gelatin films had water vapor permeability values that were approximately half those of mammalian gelatin films, as shown in Table 2. Also, the pollock gelatin film without cross-linker had lower water vapor permeability than the salmon gelatin film without cross-linker. These results indicated that water vapor permeability did not directly correspond to the renaturation level in gelatin films. From DSC results, we determined that mammalian gelatin films had higher levels of renaturation than fish gelatin films. However, this greater amount of helical structures did not lead to better water barrier properties. Previous studies involving fish gelatin films made from bigeye snapper [18–20], brownstripe red snapper [18–20], and a commercial fish gelatin [35] had found comparable permeability values to pollock and salmon gelatin films. However, other studies involving Baltic cod [22] and talapia [23] found permeability values several times higher

Table 2
Water vapor and oxygen permeabilities of gelatin films

Gelatin type	Glutaraldehyde %(w/w)	Water vapor permeability (g mm/m^2 h kPa)	Oxygen permeability (cm^3 μm/m^2 day kPa)
Pollock	0	0.857 ± 0.071a	2.398 ± 0.494a
	0.25	0.795 ± 0.057a	3.526 ± 0.661bc
	0.50	0.756 ± 0.066a	3.608 ± 0.990bc
	0.75	0.728 ± 0.087a	3.404 ± 1.095b
Salmon	0	1.084 ± 0.089b	3.539 ± 0.549bc
	0.25	0.932 ± 0.060ab	3.857 ± 0.272bc
	0.50	0.885 ± 0.052a	4.145 ± 0.536bc
	0.75	0.848 ± 0.047a	3.677 ± 0.120bc
Bovine	0	1.862 ± 0.251c	4.776 ± 0.094c
Porcine	0	1.854 ± 0.122c	4.794 ± 0.243c

Different letter within a column indicate significant difference at $p < 0.05$.

than those in this study. We should note that many of these studies included plasticizers in their films, which might affect permeability values.

The addition of glutaraldehyde reduced water vapor permeability in salmon gelatin films, but had no effect on pollock gelatin films. This is shown in Table 2. Higher concentrations of glutaraldehyde eventually led to salmon gelatin films having comparable permeability values to pollock gelatin films. Previous studies [12,16] had also found that incorporating cross-linkers, such as transglutaminase, formaldehyde, and glyoxal to bovine gelatin films led to a decrease in water vapor permeability. The authors suggested that cross-linking reduced free volume in these films, resulting in better barrier properties. However, others had also found that adding cross-linkers did not affect permeability [17,35], much like the pollock gelatin films in this study.

3.4. Oxygen permeability

Fish gelatin films had lower oxygen permeability than mammalian gelatin films, with pollock gelatin films having the lowest oxygen permeability. This is shown in Table 2. Salmon gelatin films had intermediate permeability values to pollock and mammalian gelatin films. These results might be related to differences in molecular mobilities of gelatin chains in the films. A previous study had used phosphorescent tracers to measure molecular mobility and oxygen diffusion in porcine films [37]. The authors examined hot cast films that were cast above gelation temperature and cold cast films that were cast below gelation temperature. The cold cast films contained triple helical structures, whereas the hot cast films had no structures. The authors found that cold cast films actually had higher molecular mobilities than hot cast films, although they had comparable oxygen diffusion coefficients, except at high relative humidities (75% and 84%). The higher molecular mobilities in films containing more helical structures might in part explain the greater oxygen permeabilities found in mammalian gelatin films compared to fish gelatin films. There have been few studies examining oxygen permeability of gelatin films, but previous studies generally found higher permeability values than those in this study [11,12,35].

The addition of glutaraldehyde increased oxygen permeability in pollock gelatin films, but had no effect on salmon gelatin films. This is shown in Table 2. Even with this increase in oxygen permeability, all fish gelatin films still had lower permeability values than mammalian gelatin films. A previous study involving a commercial fish gelatin film also found an increase in oxygen permeability with addition of transglutaminase cross-linker [35]. In contrast, another study found that addition of transglutaminase, formaldehyde, and glyoxal cross-linkers to bovine gelatin films had no effect on oxygen permeability [12], much like salmon gelatin films in this study.

3.5. Biodegradation

Most of the fish gelatin films had comparable biodegradation rates in the first two weeks compared to mammalian gelatin films, but degraded faster thereafter. This is shown in Fig. 2, where we plot carbon dioxide evolution as a function of time for the various films. The bovine and porcine gelatin films had similar degradation rates throughout the experiment. Also, the cross-linked fish gelatin films generally degraded at a slower rate than the films without cross-linker early on, but eventually degraded at a faster rate after 60 days. A previous study, in which weight loss was measured, had shown that porcine gelatin films modified with various cross-linkers degraded faster in soil than the film without any cross-linker [38]. From Fig. 2, there did not appear to be any relationship between glutaraldehyde concentration and degradation rate. The pollock gelatin films containing 0.25% and 0.75% (w/w) glutaraldehyde seemed to track the degradation of the film without glutaraldehyde relatively closely. In contrast, the pollock gelatin film containing 0.50% (w/w) glutaraldehyde degraded faster than the others. In comparison, the salmon gelatin film containing 0.25% (w/w) glutaraldehyde degraded at a slower rate than the film without glutaraldehyde. On the other hand, films containing 0.50% and 0.75% (w/w) glutaraldehyde degraded at a faster rate.

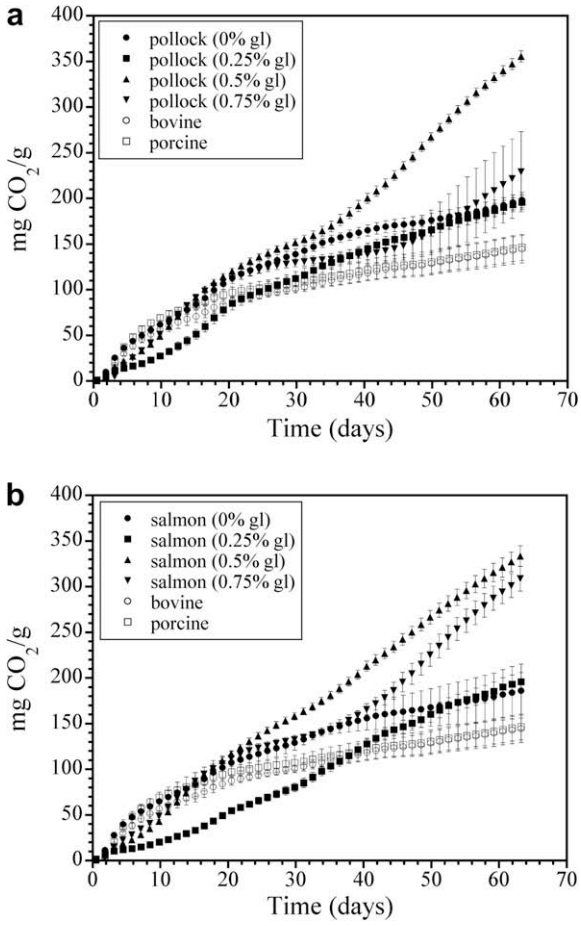

Fig. 2. Carbon dioxide evolution of (a) pollock and (b) salmon gelatin films as a function of time. The term gl in the legend denotes glutaraldehyde.

4. Conclusions

Pollock and salmon gelatin films had comparable tensile properties. They also had comparable modulus values, but lower tensile strength and percent elongation values compared to bovine and porcine gelatin films. This might be due to lower levels of renaturation found in fish gelatin films. Also, incorporating glutaraldehyde cross-linkers in fish gelatin films had little effect on their tensile properties and melting temperatures.

Pollock gelatin films had the lowest water vapor and oxygen permeability values, whereas bovine and porcine gelatin films had the highest values. Adding cross-linkers led to a decrease in water vapor permeability of salmon gelatin films and an increase in oxygen permeability of pollock gelatin films. However, all fish gelatin films still had lower water vapor and oxygen permeability values than mammalian gelatin films.

Fish gelatin films generally degraded faster than mammalian gelatin films. Also, there did not seem to be any relationship between cross-linker concentration and degradation rate.

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