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Properties of edible biofilm manufactured from yellowfin tuna (*Thunnus albacares*) skin gelatin

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Abstract–We investigated the functional properties of gelatin biofilms obtained from yellowfin tuna skin and developed an edible bioflim from fish byproducts to replace mammalian sources. For the biofilms, tensile strength and elongation were 48.57 MPa and 15.2%, respectively. The color difference and yellow index values of the biofilms were higher than those of porcine films. The opacity of the biofilms was higher than that of porcine films. In water, the biofilms were stable at pH 4-7. Water vapor and oxygen permeability of the biofilms were 5.3 $cm^3/m^2 \cdot day$ and 110 $g/m^2 \cdot day$, respectively. The glass transition temperature and of the thermal stability of the biofilms was 56.30 °C and ~260 °C, respectively.

Key words: Yellowfin Tuna, Gelatin, Biofilm, Functional Properties, Byproduct

INTRODUCTION

Biofilms are widely used in food and medicine as environmentally friendly, biodegradable materials and occur as biofouling agents [1]. Biofilms can protect food from water, gas, odor, and lipid oxidation, and help to preserve and improve food quality [2]. They are usually made of proteins, polysaccharides, lipids, or combinations of such compounds. Biofilms made of proteins are the most attractive edible films and are unlike those of lipid and polysaccharide films; protein films have excellent gas-blocking effects as well as excellent physical properties [3]. Gelatin obtained by partial degradation of collagen contains rich raw materials that supply nutrients and is biodegradable; thus, it has received attention as an edible film [4]. Because of gelatin's properties, including biocompatibility, thermoreversible formation of sols and gels, water-holding capacity, binding capacity, emulsifying ability, and viscosity, it has been widely used in food, medicine, and photography [5]. Also, gelatin has good mechanical properties and forms an excellent film [6].

In current research on gelatin-based biofilms, Bigi et al. [7] studied the structure of combined films made of gelatin and hydroxyapatite and showed that mechanical properties were improved by cross-linking with glutaraldehyde. Sobral et al. [8] reported on water permeability and mechanical and thermal properties of edible films made of bovine hide and porcine skin gelatin using sorbitol. Cho et al. [9] studied the preparation and physical properties of combined films made of soy protein isolate and gelatin. Haug et al. [10] reported the physical properties and characteristics of gelatin extracted from marine products and mammals. Carvalho and Grosso [11] investigated the characteristics of gelatin film treated with transglutaminase, glyoxal, and formaldehyde. Vanina et al. [12] studied the effect of plasticizers on gelatin-based films and the related thermal and functional characteristics. Gómez-Guillén et al. [13] studied edible films made from tuna gelatin with antioxidant extracts of two different murta ecotypes leaves (*Ugni molinae* Turcz). Yoon et al. [14] optimized the adhesive strength between gelatin processed from yellowfin tuna dorsal skin (*Thunnus albacares*) and plywood.

Industrial raw materials for gelatin include extracts of cartilage, bone, and mammalian skin. Gelatin extracted from mammals exhibits enhanced physical properties, such as gel strength and viscosity, compared with marine products. Most gelatin currently on the market is extracted from cows and pigs. Because pigs and cattle are subject to bovine spongiform encephalopathy (BSE) and foot and mouth disease, there are problems related to safety and a stable supply of raw materials. To solve such problems, it is necessary to develop alternative resources that are safer.

To replace mammalian resources, gelatin manufactured from marine products has been studied. There have been many studies on gelatin extracted from marine animals, including cod, tuna, and tilapia [15], Alaska pollack [16], scorpion fish [17], tilapia [18], eel [19], and halibut, cod, hake, and squid [13]. Additionally, methods for obtaining gelatin from harp seal (*Phoca groendlandica*) have been reported Arnesen and Gildberg [20], and Muyonga et al. [21] studied the physicochemical characteristics of gelatin from the skin and bones of Nile bass (*Lates niloticus*). Giménez et al. [22] extracted gelatin from the skin of soles (*S. vulgris*) with lactic acid. However, gelatin extracted from the byproducts of marine animals typically exhibits suppressed physical properties, such as gel intensity, versus gelatin extracted from mammals. Thus, the industrial use of marine-derived gelatin has been limited. However, gelatin extracted from the skin of tuna shows superior physical properties versus that extracted from mammals [23]; thus, potential uses of tuna-skin gelatin should be further investigated.

In this study, gelatin extracted from the skin of yellowfin tuna was used to prepare biofilms as an alternative to gelatin extracted from mammals. Yellowfin tuna biofilm was selected because of its tensile strength, elongation, chromaticity, opacity, stability in water, degree of swelling, oxygen permeability, and thermal characteristics.

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EXPERIMENTAL SECTION

1. Materials

Edible biofilms were prepared using gelatin extracted from the skin of yellowfin tuna according to the method reported by Cho et al. [23]. Porcine gelatin was prepared to compare the physical characteristics of the two types of biofilms (EC No 232-554-6, Type A: from porcine skin, Sigma, USA).

2. Methods

2-1. Preparation of Edible Biofilms

A gelatin solution (21 mL of 3% w/v) extracted from the skin of yellowfin tuna was placed in a 10×10×0.4-cm acrylic frame. The gelatin was dried at 25±1 °C and 50±1% relative humidity (RH) for 24 h before preparation of 0.1 mm thick films.

2-2. Film Thickness

Biofilm thickness was measured to within 0.001 mm with a hand-held micrometer (Teclock Co., Japan).

2-3. Tensile Strength and Elongation

Tensile strength and elongation of films (1×8 cm) were measured by a universal testing machine (Instron 1011, USA) according to ASTM (D 822-97, 1999) standards. Tensile strength and elongation were calculated using Eqs. (1) and (2), respectively.

$$\text{Tensile strength (kg·f/cm}^2\text{)} = \text{Maximum tensile force/cross-sectional area} \quad (1)$$

Maximum tensile force (kg·f) is the maximum force applied until a film is cut. Cross-sectional area (cm^2) is the average area of the film (average thickness×width, cm^2).

$$\text{Elongation (\%)} = (\Delta L/L) \times 100 \quad (2)$$

where L is the distance (mm) between the initial grips of the universal testing machine, and ΔL is the distance (mm) between the grips after the film was cut. Tensile strength and elongation measurements were repeated three times under the same conditions to calculate averages and standard error.

2-4. Stability in Water

To determine film stability in water, we modified the method reported by Kim et al. [24]. Prior to experiments, 1×1-cm films were dried at 105 °C for 24 h before measuring the initial weight of the film. To minimize error, each film was soaked in vials containing equal amounts of water (10 mL) under two different pH conditions (pH 4 and 7). Weight changes were measured in 5-day intervals between day 1 and day 15 at an ambient temperature. Any remaining water was removed from the vial, and each film was dried for three days at 50±1 °C and 50±1% RH. The water solubility of the films was calculated using Eq. (3), based on the weight change before and after soaking in water.

$$S\ (\%) = W_2/W_1 \times 100 \quad (3)$$

where S is the weight percent of the film remaining after the experiment, W_1 is the weight before the film was soaked, and W_2 is the weight of the film after it was soaked. Film stability in water was measured three times to calculate averages and standard error.

2-5. Film Chromaticity

Film chromaticity was measured using a color-difference meter (JC801, Color Techno System Co., Japan) in terms of L (brightness), a (redness), and b (yellowness), based on the Hunter scale. The L-value is the level of brightness, ranging from L=0 (black) to L=100 (white). The a-value indicates green and red coloration, ranging from a=−80 (green) to a=100 (red). The b-value indicates blue and yellow coloration, ranging from b=−80 (blue) to b=70 (yellow). Chromaticity measurements were made by using the color coordinates of L=93.83, a=−0.12, and b=0.11 for film samples on the calibration plate. Three films with the same combinations were measured three times to calculate averages and standard errors. Using the values of L, a, and b, based on the Hunter scale, the total color difference (ΔE) and yellow index (YI) on the calibration plate were calculated using Eqs. (5) and (6).

$$\Delta E = [(L_{film} - L_{standard})^2 + (a_{film} - a_{standard})^2 + (b_{film} - b_{standard})2]^{0.5} \quad (5)$$

$$YI = 1.4286 \times b/L \quad (6)$$

2-6. Film Opacity

Film opacity was measured by adjusting the method reported by Han et al. [25]. Films were cut into squares and placed on quartz cells for spectral analysis based on light penetration. Using a spectrophotometer (UV-160A, Shimazu, Japan), light penetrated the field with wavelengths of 400-800 nm to measure optical density. The level of opacity was calculated using Eq. (7).

$$\text{Opacity} = A_{600}/\chi \quad (7)$$

A_{600} is the optical density at 600 nm, and χ is film thickness. Film opacity experiments were repeated three times under the same conditions to calculate the averages and standard error.

2-7. Water Permeability

Permeability of the films was measured by using the cup method according to the Korean Industrial Standard (2001). By storing the film in a thermo-hygrostat at 23±2 °C and 50±5% RH, the amount of water vapor penetrating the film layer during 24 h was used to calculate permeability according to Eq. (8).

$$\text{Water permeability (g/m}^2\text{·24 h)} = (\text{Film weight after 24 h (g)} - \text{Initial film weight (g)})/\text{permeated area of the cup (m}^2\text{)} \quad (8)$$

2-8. Oxygen Permeability

Oxygen permeability was measured using the standard method of ASTM [26] and an OX-Tran 2/61 (Mocon, USA).

2-9. Differential Scanning Calorimetry (DSC)

The glass transition temperature of the fabricated films was measured using DSC (Q200, TA, USA). The measurement was carried out under sufficient nitrogen flow (50 mL/min) at 10-120 °C.

2-10. Thermogravimetric Analysis (TGA)

TGA analysis, which was used to determine the thermo-stability and thermo-behavior of the fabricated films, was performed with a thermogravimetric analyzer (SDT Q600, TA, USA). Measurements were carried out under nitrogen flow (100 mL/min) at 20-500 °C.

2-11. Statistical Analyses

The average and standard error for each sample was calculated. After performing variance analysis, we verified significant differences for each group using to Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

1. Tensile Strength and Elongation of the Biofilms

Biofilms are influenced by external physical forces when used

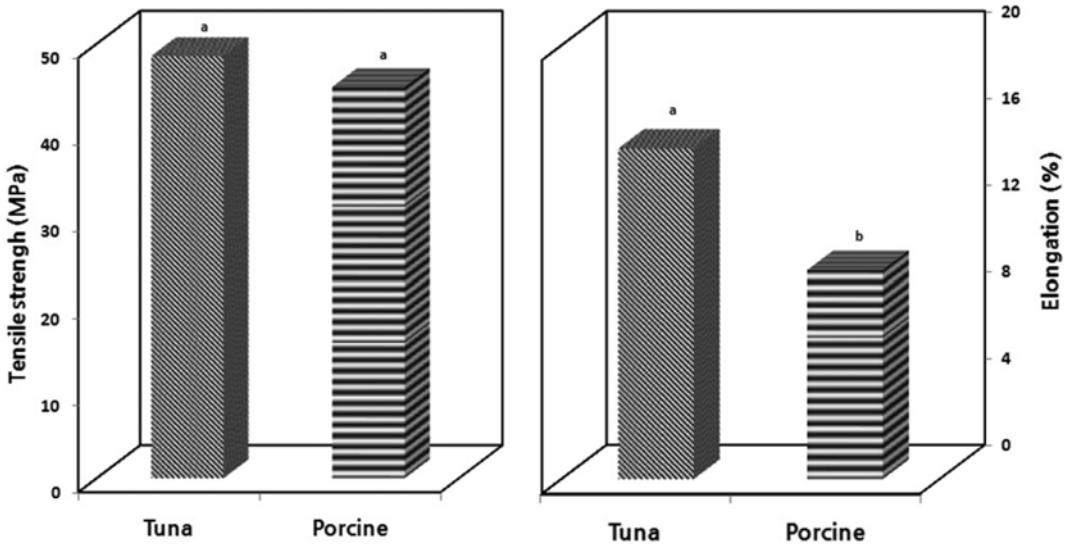

Fig. 1. Comparison of tensile strength and elongation of tuna and porcine gelatin biofilms. Means with the same letter are not significantly different (p<0.05) by Duncan's multiple range test.

as internal packing materials for food or medicine and as biological adhesives. Of the factors that determine film quality, physical properties are of particular importance. Most films are subjected to high tensile stress during use, so it is important to have high tensile strength, related to thickness and function.

Fig. 1 shows different levels of tensile strength and elongation for films made of yellowfin tuna gelatin and porcine gelatin. The tensile strength and elongation of tuna gelatin films were 48.57 MPa and 15.23%, respectively, while those of porcine gelatin films were 45.02 MPa and 9.62%, respectively. Tuna gelatin films showed the same level of physical intensity as porcine gelatin films, which are used commercially. The tensile strength and elongation of gelatin are closely related to gelatin formation, or gelation, which occurs due to the formation of a three-dimensional network. Small fields, produced from three polypeptide chains that return to a triple-helix structure as gelatin dissolves in an irregular coil of the heating solution, form cross-linking sites [5,26]. Because tuna gelatin films form denser structures when water molecules, generated after gelation, are lost in drying, tensile strength and elongation are higher than those of other biofilms.

Generally, gelatin made from marine products displays inferior physical properties, such as gel strength, compared with mammalian gelatin. As a result, commercial applications of marine gelatin have been limited. However, tuna gelatin shows higher gel strength than porcine or cow gelatins [23]. Tuna gelatin shows high tensile strength and elongation because it has enhanced gelation properties compared with mammalian gelatins.

Of the factors that determine high-molecular weight film quality, physical properties are very important. Thus, knowledge of the physical properties of tuna gelatin is needed to assess tuna gelatin

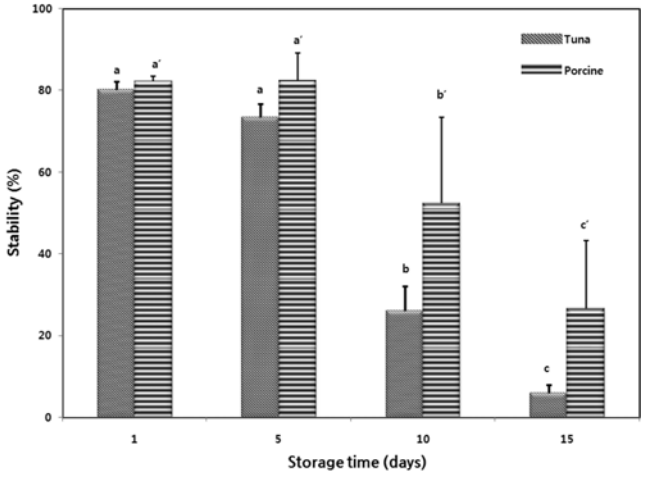

Fig. 2. The stabilities of tuna and porcine gelatin biofilms in the aqueous solutions of pH 7. Different letters (a, a′, a″, …) indicate significant difference (p<0.05) by Duncan's multiple range test.

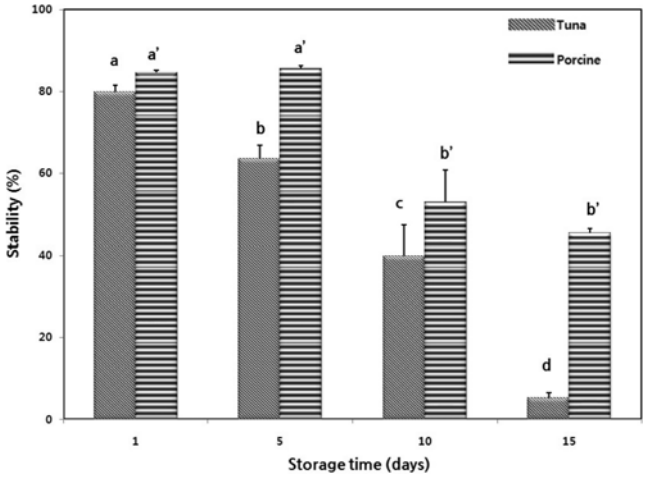

Fig. 3. The stabilities in water of tuna and porcine gelatin biofilms in the aqueous solutions of pH 4. Different letters (a, a′, a″, …) indicate significant difference (p<0.05) by Duncan's multiple range test.

Table 1. Hunter values (L, a, and b), total color differences (ΔE), and yellow index (YI) of tuna and porcine gelatin biofilms

Type of film	L	a	b	ΔE	YI
Tuna	87.121±0.2429^b	−0.151±0.1391^b	22.499±0.3238^a	23.346±0.3741^a	36.896±0.6276^a
Porcine	95.810±0.0964^a	−0.066±0.0069^a	0.641±0.0193^b	2.147±0.0928^b	0.956±0.0293^b

Means with the same letter are not significantly different ($p<0.05$)

Table 2. Absorbance of tuna and porcine gelatin biofilms

Type of film	Wavelength				
	400 nm	500 nm	600 nm	700 nm	800 nm
Tuna	0.303±0.002^a	0.147±0.001^a	0.090±0.001^a	0.067±0.001^a	0.056±0.000^a
Porcine	0.058±0.001^b	0.053±0.003^b	0.047±0.001^b	0.046±0.004^b	0.043±0.001^b

Means with the same letter are not significantly different ($p<0.05$)

biofilms as replacements for mammalian gelatin.

2. Water Stability of the Biofilms

Humidity must be considered when storing food or medicine. In low-temperature storage conditions, the film-water contact rate, a function of the humidity of the storage room, increases compared with higher temperature storage. Thus, film stability in water is very important. For edible films, it is more important to consider film stability in water than plastic film packaging materials. Film stability in water is related to resistance to water, in contrast to permeability, and is determined by the chemical structure of the film.

Figs. 2 and 3 show the changes in weight after soaking each film in solutions of pH 4 and 7, respectively, for 15 days. For tuna gelatin, there was little difference between day 1 and day 5 at pH 7. However, a rapid decrease in weight occurred after 5 days. At pH 4, weight decreased gradually between day 1 and day 15. The porcine gelatin film showed little difference between day 1 and day 5 at pH 4 or 7. However, a rapid decrease in weight occurred after 5 days. Porcine gelatin films were more stable than tuna gelatin films at pH 3-11.

3. Chromaticity of the Biofilms

Chromaticity is an important characteristic that influences the appearance of the films. Chromaticity values for Hunter L, a, b, ΔE, and YI are listed in Table 1. The 'L' value, indicating color brightness, of the porcine gelatin film was higher than that of the tuna gelatin film. The 'a' value, indicating green and red coloration, showed little difference between the porcine and tuna gelatin films. For the 'b' value, indicating blue and yellow coloration, the tuna gelatin film had a higher value than the porcine gelatin film. Based on the values of Hunter L, a, and especially b, ΔE showed that the total color difference was ~11 times higher in the tuna gelatin film than the porcine gelatin film, and the YI value was ~36 times higher for the tuna gelatin film. Because colors such as yellow and brown high ultraviolet-ray blocking capabilities, the tuna gelatin films, with a high proportion of yellow, have potential applications in commercial products for ultraviolet-ray blocking.

4. Opacity of the Biofilms

The results for 400-800-nm wavelength light are shown in Table 2. All films showed maximum optical density at 400 nm. As the wavelength increased, optical density decreased. Opacity calculations based on the Han equation [25] are shown in Table 3.

Tuna gelatin films had the highest opacity value at 600 nm. This was because the 'L' value was lower than previous chromaticity estimates. Film opacity is particularly important when the film is used on the surface of food. As the opacity value increased, the amount of light absorbed decreased, preventing the deterioration of food, medicine, and product quality, which could be caused by light penetration.

Table 3. Opacity and absorbance (A_{600}/mm) of tuna and porcine gelatin biofilms

Type of film	Absorbance at 600 nm	Opacity
Tuna	0.090±0.001^a	0.900±0.013^a
Porcine	0.047±0.001^b	0.470±0.013^b

Means with the same letter are not significantly different ($p<0.05$)

5. Water Vapor Permeability of the Biofilms

The permeability of films plays an important role in the expiration date of packing materials for food and medicine. Permeability through a film occurs due to differences between water vapor pressures on the two sides of the film. When there are micropores or small cracks, water vapor is transported by capillary diffusion [28]. Permeability in high-molecular weight films is influenced by structural and chemical characteristics, molecular orientation, the level of crystallization, and the level of cross-linking [29]. Factors that influence the permeability of films are the amount of raw materials, the solvent used for the fabrication of film solution, and the kind and density of the plasticizer.

The permeabilities of gelatin biofilms are shown in Table 4. The tuna gelatin film had a permeability of 112.5 g/m^2·day, while the porcine gelatin film had a permeability of 175.1 g/m^2·gday. The results show that the tuna gelatin film was more resistant to relative humidity than were the porcine gelatin films. Also, the tuna gelatin film showed a low level of permeability compared with the porcine gelatin film. Thus, the tuna gelatin film showed enhanced stability

Table 4. Water vapor permeability and oxygen permeability of tuna and porcine gelatin biofilms

Type of film	Water vapor permeability (g/cm^2·day)	Oxygen permeability (cm^3/cm^2·day)
Tuna	112.5±7.87^b	5.3±0.36^b
Porcine	175.1±12.25^a	7.4±2.71^a

Means with the same letter are not significantly different ($p<0.05$)

in humid conditions compared with the porcine gelatin film. Consequently, tuna gelatin films can be used as moisture-proof biofilms.

6. Oxygen Permeability of the Biofilms

Generally, the permeability of high-molecular weight films is greatly influenced by the density and amount of the molecules, the level of crystallization, humidity, film thickness, and the kind and amount of the plasticizer. To be used commercially, a film must be impermeable to external humidity and oxygen [24]. Oxygen permeability of the tuna gelatin films is shown in Table 4. The tuna gelatin films had an oxygen permeability of 5.3 $cm^3/m^2 \cdot day$, while that of porcine gelatin films was 7.4 $cm^3/m^2 \cdot day$.

The tuna gelatin film was a better oxygen barrier than the porcine gelatin film (Table 4). The results show that tuna gelatin films can be used as packing materials for food that must be protected against oxidation or oxygen-induced quality deterioration.

7. Thermal Properties of the Biofilms

The glass transition temperature indicates the thermal characteristics of high-molecular weight substances. A high glass transition temperature means that the melting temperature is high. Fig. 4 shows the glass transition temperatures of various films, measured by DSC. Tuna gelatin had a glass transition temperature of 56.30 °C, while that of the porcine gelatin was 54.24 °C. Thus, tuna gelatin had better heat resistance compared with porcine gelatin. Other thermal properties also indicated that tuna gelatin and porcine gelatin films had similar glass transition properties.

To identify the thermal stability of each film, TGA analysis measured the weight changes and level of pyrolysis for each film with increasing temperatures (Fig. 5). The tuna gelatin film exhibited decomposition of the main chains at ~260 °C, while the porcine gelatin film decomposed at ~270 °C. Generally, rapid decomposition does not occur above 370 °C. It is thought that complete combustion would occur above 500 °C. Based on these results, the porcine gelatin film had enhanced thermal stability compared with tuna gelatin films.

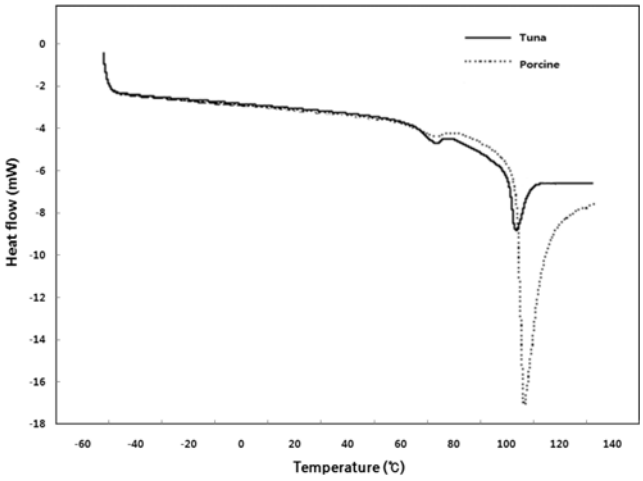

Fig. 4. Differential scanning calorimetry (DSC) profiles of the tuna and porcine gelatin biofilms.

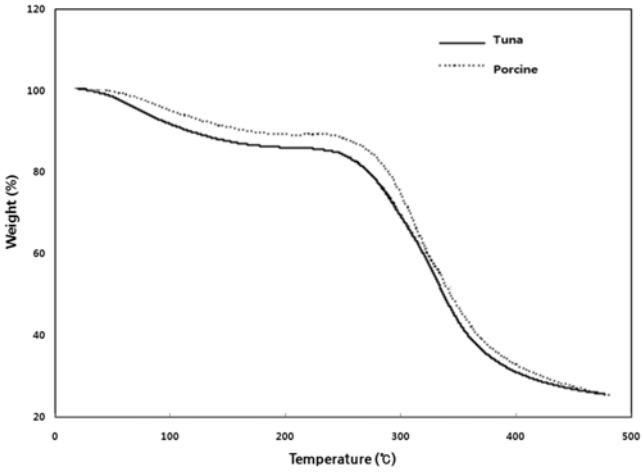

Fig. 5. Thermogravimetric analysis (TGA) profiles of the tuna and porcine gelatin biofilms.

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