Biodegradable films derived from devil fish skin collagen (*Pterygoplichthys pardalis*)

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ABSTRACT

Objective: This study aimed to evaluate the feasibility of using collagen extracted from *Pterygoplichthys pardalis* skin to develop biodegradable films.

Design/methodology/approach: Collagen was extracted by assessing the effects of the acid-to-skin ratio and extraction time using a 2^2 factorial experimental design. The recovered collagen was characterized by SDS-PAGE, Fourier-transform infrared spectroscopy (FTIR), and ultraviolet-visible (UV-Vis) spectroscopy. Subsequently, films were formulated using a 2^2 factorial design with collagen concentrations (1 and 2%) and glycerol levels (10 and 20 mL), incorporating arabic gum. Mechanical performance including tensile strength, elongation at break, and Young's modulus was determined, alongside thermal properties (glass transition temperature, Tg; denaturation temperature, Tm) and biodegradability.

Results: The maximum collagen extraction yield reached 54%. The resulting films exhibited an average tensile strength of 1.26±0.17 MPa, elongation at break of 15.99±0.07%, and Young's modulus of 22.09±0.078 MPa. Tg ranged from −13 °C to −17 °C, while Tm varied between 140 °C and 158 °C. The biodegradability index attained 77.74±6.76%.

Limitations on study/implications: The work was performed at laboratory scale; therefore, additional studies are required to evaluate process scalability and the prospective industrial applicability of the films.

Findings/conclusions: Collagen derived from *P. pardalis* skin represents a viable alternative feedstock for producing biodegradable biomaterials with promising mechanical and thermal characteristics, supporting fish-waste valorization and the development of more sustainable materials.

Keywords: Fish waste; biodegradability; texture; spectroscopy; SDS-PAGE.

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INTRODUCTION

For decades, polymers such as polyvinyl chloride, polyethylene, polypropylene, and polystyrene have been extensively used in the manufacture of plastic packaging owing to their low weight, water resistance, and adequate mechanical performance (Jones *et al.*, 2020). Nevertheless, their non-biodegradable nature and improper disposal have driven the excessive accumulation of plastics across multiple ecosystems, resulting in

substantial and long-lasting environmental impacts (Esterhuizen & Kim, 2022). In response, packaging-material research has increasingly focused on developing films and materials derived from biomolecules including proteins, polysaccharides, and lipids as sustainable alternatives to petroleum-based materials (Amin *et al.*, 2021). In particular, agro-industrial and fishery residues have gained prominence as renewable sources of structural compounds most notably cellulose, chitosan, and collagen due to their availability, low cost, and strong valorization potential (Teixeira-Costa & Andrade, 2022). Within this context, collagen has become one of the most widely utilized proteins for biomaterial fabrication because of its bioactivity, biocompatibility, and biodegradability, as well as its ability to form polymeric networks with tunable mechanical and functional properties (Gallo *et al.*, 2022; Yamaura *et al.*, 2022). Structurally, collagen consists of polypeptide chains characterized by the repeating amino-acid motif [Gly-X-Y]n, which gives rise to its distinctive triple helix. This architecture directly governs its thermal stability, intermolecular interactions, and bioactivity (Zeugolis & Raghunath, 2011). Fish represent a relevant source of collagen, predominantly type I, which is highly demanded by the pharmaceutical, biomedical, and cosmetic industries (García-Sifuentes *et al.*, 2019; Atay *et al.*, 2022; Irastorza *et al.*, 2021; Boland *et al.*, 2018; Tacias-Pascacio *et al.*, 2020; Valbuena *et al.*, 2020). Moreover, more than 70% of the biomass generated by the fishing industry is considered waste, which has encouraged the use of skins, scales, and skeletons as feedstocks for collagen recovery, often achieving yields comparable to or higher than those obtained from traditional mammalian sources (Erasmus *et al.*, 2021; Venugopal, 2021; Wijaya & Juanito, 2021). This approach not only reduces the environmental burden associated with fishery by-products, but also mitigates risks related to transmissible diseases and circumvents religious restrictions commonly associated with terrestrial collagen sources (Rodriguez-Salvador & Dopico, 2020). Most studies to date have focused on the extraction and characterization of collagen from high-commercial-value species, such as tilapia (*Oreochromis niloticus*), salmon, and cod (Hoyer *et al.*, 2012; Raftery *et al.*, 2016; Lovett *et al.*, 2020). In contrast, a knowledge gap persists regarding the utilization of collagen from invasive or non-commercial species, particularly with respect to its application in forming biodegradable films and packaging materials. *Pterygoplichthys pardalis* is a widely distributed invasive species in diverse aquatic ecosystems, whose proliferation generates negative ecological and economic impacts. The skin of this species constitutes a promising collagen source with a relevant hydroxyproline content an amino acid associated with triple-helix stability and enhanced intermolecular interactions which may translate into materials with improved thermal and mechanical performance (Nurubhasha *et al.*, 2019; Sun *et al.*, 2021). However, studies exploring collagen from invasive species for the development of films or functional biomaterials remain limited.

Against this backdrop, the present work aims to produce and characterize a biodegradable material based on collagen extracted from *P. pardalis* skins via an acid-based method, in order to assess its technological potential as a film for packaging applications. Implicitly, we hypothesize that the hydroxyproline content of this collagen promotes the formation of polymeric networks with greater thermal stability and superior

mechanical properties, thereby supporting the development of a sustainable alternative to conventional plastics and enabling the valorization of invasive biomass.

MATERIALS AND METHODS

Sample collection and skin pretreatment

Fish were obtained from the Peñitas Reservoir, in the municipality of Ostuacán, Chiapas, Mexico (latitude 17.843, longitude −93.487). A total of 68 adult *P. pardalis* individuals (mean weight: 389.96±99.61 g) were collected and transported in coolers under refrigerated conditions. Approximately 2381.2±129.45 g of fresh skin were excised, washed, and stored under refrigeration. The skin was dried at 60 °C for 72 h; these conditions do not compromise type I collagen integrity, as type I collagen from fish skin has been reported to exhibit a denaturation temperature of approximately 97.8 °C (Majeed *et al.*, 2025). Subsequently, the dried skin was ground and sieved to a particle size of 0.6 mm to maximize the available surface area.

Pretreatment of raw skins was carried out according to the methodology proposed by Sun *et al.* (2021), with minor modifications. Briefly, 100 g of dry raw material (skin) were mixed with 0.1 M NaOH at a 1:10 (w/v) ratio for 6 h at 4 °C, replacing the NaOH solution every 3 h to remove non-collagenous proteins and skin pigments. The skin was then washed with distilled water until neutral pH (7.0-7.5) was reached. Next, the skin was defatted using 10% butanol at a 1:10 (w/v) ratio for 24 h and subsequently washed three times with distilled water.

Acid-soluble collagen extraction

Collagen extraction was performed following the methodology described by Atef *et al.* (2020). A completely randomized 2^2 factorial design was applied with two factors: acid-to-skin ratio (10:1 and 20:1, v/w) and extraction time (24 and 72 h). Each treatment was conducted in triplicate (Table 1).

The samples were mixed with 0.5 M acetic acid as the extraction solvent, given its reported effectiveness for mild collagen extraction. The mixture was maintained at 4 °C, filtered, and neutralized with 0.1 M NaOH to pH 7.0 (monitored using a potentiometer). Subsequently, the suspension was centrifuged at 4000 rpm (Eppendorf model 5810R, Hamburg, Germany) for 20 min at 4 °C to separate soluble collagen in the supernatant from the precipitated fraction. The supernatant was then lyophilized at 0.520 mBar for 48 h (Labconco, Kansas, USA) and stored at 4 °C until further use.

Table 1. Experimental design for acid-soluble collagen extraction.

Treatment	Acid:skin ratio (v:w)	Extraction time (h)
I	10:1	24
II	20:1	24
III	10:1	72
IV	20:1	72

v:w=volume:weight ratio.

Characterization of the extracted collagen

Extraction yield

Extraction yield was determined as the ratio between the mass of the lyophilized product and the mass of the initial material (dry skin weight), as described in Equation 1:

$$Yield\,(\%) = \frac{Collegen\,(g)}{Dry\,skin\,weight\,(g)} \times 100$$

UV-Vis spectroscopy

Lyophilized collagen was dissolved in 0.5 M acetic acid at a 1:100 (w/v) ratio. The solution was transferred to a 1 cm quartz cuvette and scanned from 190 to 1100 nm to identify the wavelength of maximum absorbance using a DR 5000 spectrophotometer (HACH, Loveland, USA). The baseline was established using 0.5 M acetic acid.

Protein quantification

Protein concentration was determined using the Lowry method, employing a bovine serum albumin (BSA) calibration curve. Briefly, 2 mL of collagen sample solutions (1 g/mL in 0.5 M acetic acid) were mixed with 5 mL of Lowry reagent, and UV absorbance was measured at 500 nm (DR 5000, HACH, Loveland, USA) (Song *et al.*, 2021).

Fourier-transform infrared spectroscopy (FTIR)

Functional groups present in *P. pardalis* collagen were identified following the procedure reported by Zhang *et al.* (2020). FTIR spectra were recorded using a Nicolet iS10 FT-IR spectrometer (Thermo Fisher Scientific Inc., Madison, USA). Lyophilized collagen was placed on an attenuated total reflectance (ATR) cell at ambient temperature. Signals were collected over 64 scans in the 4000-650 cm^{-1} range at a resolution of 2 cm^{-1} and compared with a background spectrum acquired from a clean, empty cell at room temperature (25 °C).

Molecular-weight profile of the extracted collagen

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970), with minor modifications. The resolving gel was prepared at 8% and the stacking gel at 5%. Gels were stained with 1% Coomassie Brilliant Blue. Electrophoresis was conducted at 80 V for 20 min followed by 120 V for 50 min, using 1x running buffer (25 mM Tris-Cl/250 mM glycine/0.1% SDS). Type I collagen extracted from rat tail was used as the standard (Sigma-Aldrich, USA). Electrophoresis was carried out in a Mini-PROTEAN system (Bio-Rad, Hercules, USA).

Preparation of collagen films

Films were formulated using a dispersion of collagen, gum arabic, and glycerol. First, gum arabic (1.5%) was hydrated for 24 h using triple-distilled water. Glycerol was then added, followed by the gradual addition of collagen. Formulations were prepared according

to a 2^2 experimental design with two central points and two axial points, using collagen concentration (1 and 2%) and glycerol (10 and 20%) as factors.

Dispersions were held for 24 h at room temperature, heated to 70 °C, and stirred, then cast into 5 cm-diameter Teflon-coated aluminum molds and dried at 23 °C for 24 h, as described by Andonegi *et al.* (2020).

Film analysis

Mechanical properties

Prior to mechanical testing, films were equilibrated at 25 °C and 75% relative humidity for 48 h. Mechanical properties tensile strength (TS), elongation at break (EAB), and Young's modulus (E) were determined according to ASTM D882 (American Society for Testing and Materials, 1996) using a TA.XT Plus texture analyzer (Stable Micro Systems, London, UK), with a 20 mm initial grip separation, a crosshead speed of 5 mm/min, and 80% deformation. Films were cut into 50×10 mm strips, and all measurements were performed in triplicate.

EAB was calculated as the ratio between the change in film length at rupture (L) and the initial gauge length (L_0), as described in Equation 2:

$$EAB = L\frac{L}{Lo} \tag{2}$$

Cutting force (F) was obtained using Exponent software v6.1.16.0 (Stable Micro Systems, London, UK) at the moment of film rupture. Tensile strength (TS) was estimated using Equation 3, where F denotes the cutting force and A corresponds to the film cross-sectional area.

$$TS = \frac{F}{A} \tag{3}$$

Young's modulus (E) was calculated as the ratio between the increase in applied stress (TS) and the corresponding increase in elongation (EAB) of the tested material, using Equation 4:

$$E = \frac{TS}{EAB} \tag{4}$$

Differential scanning calorimetry (DSC)

The glass transition temperature (Tg) and denaturation temperature (Tm) of the films were determined in triplicate (n=3) using a Q-2000 differential scanning calorimeter (TA Instruments, New Castle, USA), following the methodology of Valencia *et al.* (2019). Approximately 10 mg ± 0.002 mg of sample were placed in aluminum pans under a dry nitrogen purge (50 mL/min). Films were cooled to 0 °C and subsequently heated from −80 to 200 °C at a heating rate of 20 °C/min. This analysis provided

indirect information regarding the thermally stable structural integrity of collagen within the films.

Biodegradability index

The biodegradability index (BI) was determined according to the oxygen-consumption methodology described in OECD 301 (Organization for Economic Cooperation and Development, 1992), which is based on the ratio between chemical oxygen demand (COD) and biochemical oxygen demand (BOD_0), as expressed in Equation 5. All analyses were performed in triplicate.

$$BI = \frac{DBO_5}{DQO} \times 100 \quad (5)$$

Chemical oxygen demand (COD) was estimated using the micro-reflux method described in the HACH manual for chemical reactors. An aqueous solution of skin collagen (1:10,000, w/v) was prepared and digested at 150 °C for 2 h. Subsequently, the sample was transferred to a quartz cuvette, and absorbance was measured at 620 nm using a UV-Vis spectrophotometer (DR-5000, HACH, Loveland, USA).

Biochemical oxygen demand was determined as the difference between the initial dissolved oxygen and the dissolved oxygen remaining after five days of incubation at 20 °C. Incubation was conducted using a BOD Trak I respirometric analyzer (HACH, Loveland, USA), with PolySeed® as the inoculum. Although this method is not specific to solid polymers, it provides a preliminary indicator of the intrinsic biodegradability of collagen as an exploratory stage of the study.

Statistical analysis

All experiments were performed in triplicate. One-way ANOVA was used to assess the effects of the factors and their interactions on extraction yield at a 95% confidence level. Mean differences were evaluated using confidence intervals with Tukey's test. Statistical analyses were conducted using Statgraphics software (version XV).

RESULTS AND DISCUSSION

Fish skin is a valuable collagen source, as reported by Virtanen *et al.* (2017). Collagen recovery yields from *P. pardalis* skin are presented in Table 2, where a statistically significant difference ($p<0.05$) was observed among all treatments. In this experiment, yields ranged from 37.4% to 57.05% and were significantly higher than those reported for eel skin (Govindharaj *et al.*, 2019), elasmobranch by-products (Seixas *et al.*, 2020), and swim bladders from Atlantic cod (*Gadus morhua*) (Sousa *et al.*, 2020). The pronounced variability in collagen yields is attributed to differences in collagen architecture among the distinct by-products and species evaluated (Wu *et al.*, 2016). The highest yield was achieved using an acid-to-skin ratio of 1:20 (v/w) and an extraction time of 24 h.

Analysis of variance showed that the acid-to-skin ratio (v/w) exerted a statistically significant positive effect ($p<0.05$) on extraction yield, whereas the interaction between

Table 2. Extraction yields of acid-soluble collagen from *P. pardalis* skin.

Ratio (mL g^{-1})	Time (h)	Yield (%)
10:1	24	37.40d
20:1	24	57.05a
10:1	72	41.16c
20:1	72	51.88b
LSD		3.68

LSD=Least significant difference. Different letters within the same column indicate statistically significant differences among treatments ($p<0.05$).

both factors had a statistically significant negative effect on the response variable (Figure 1). The positive influence of the acid-to-skin ratio has been previously described for collagen extraction processes (Dhakal *et al.*, 2018) and is attributed to an enhanced hydrolysis-driven solubilization mechanism without inducing collagen degradation (Arumugam *et al.*, 2018).

Regarding the statistically significant negative effect of the interaction between both factors, this outcome may be explained by the fact that increasing both solvent concentration and extraction time can promote collagen denaturation, thereby reducing the amount of collagen recovered (Surasani *et al.*, 2019). In this respect, it is crucial to establish whether a critical solvent concentration exists beyond which further increases no longer enhance protein extraction. Otherwise, prolonged extraction times may intensify solvent-collagen interactions, potentially leading to deprotonation of functional groups that are not exclusively associated with cross-links between collagen α-helices, ultimately destabilizing and denaturing the protein (Tan & Chang, 2018).

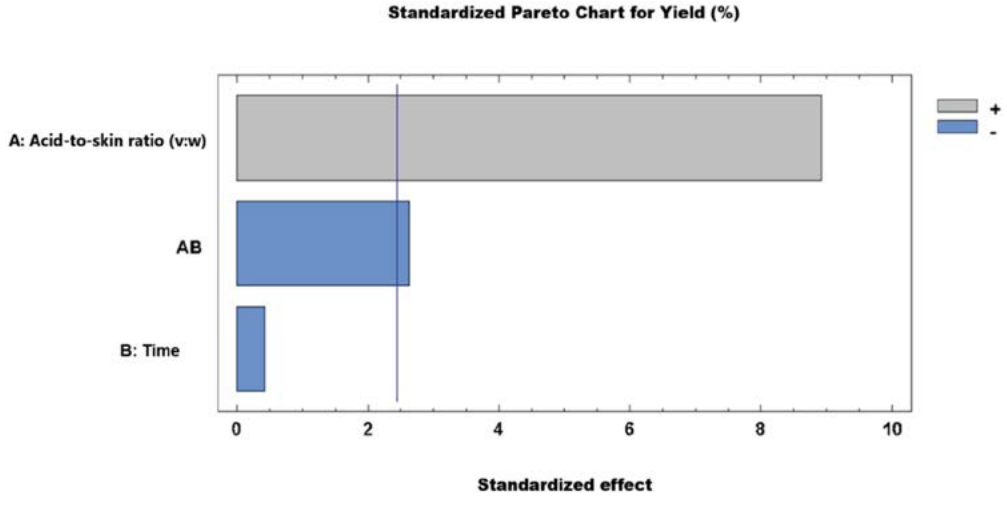

Figure 1. Standardized Pareto chart for collagen extraction yield obtained via acid extraction from *P. pardalis* skin. A represents the raw-material-to-extraction-solution ratio (w/v), and B represents extraction time (h).

Spectroscopic characterization of collagen

Fourier-transform infrared spectroscopy (FTIR)

The IR spectrum of *P. pardalis* skin collagen exhibited absorption bands at 2930, 1640, 1550, 1415, and 1019 cm^{-1}. The band at 2930 cm^{-1} corresponds to the asymmetric stretching of the Csp^3-H bond (secondary amine) (Moula Ali *et al.*, 2017). Bands in the 1600-1700 cm^{-1} region have been associated with C=O stretching vibrations and hydrogen bonding coupled with COO^-, corresponding to amide I (S. S. Wang *et al.*, 2019). The absorption at 1550 cm^{-1} arises from N-H bending coupled with C-N stretching of amide II. Finally, amide III was represented by the absorption band at ~1410-1415 cm^{-1}, attributed to Csp^3-H vibrations (Bi *et al.*, 2019). This band is related to the degree of molecular order in collagen and participates in the formation of the triple-helix structure (Chen *et al.*, 2016). In addition, a band near 1000 cm^{-1} corresponding to the Csp^3-O bond characteristic of hydroxyproline was observed. Overall, the IR signals were consistent with those reported for collagen extracted from *P. pardalis* (Nurubhasha *et al.*, 2019) and from other fish species (El-Rashidy *et al.*, 2015; Sionkowska *et al.*, 2015).

UV-Vis spectroscopy

To obtain the UV-Vis spectrum of the protein extract, a spectral scan from 190 to 1100 nm was performed, revealing a maximum absorbance at 220 nm. Because fish collagen contains low levels of tryptophan, tyrosine, and phenylalanine and due to the presence of C=O, -COOH, and $CONH_2$ groups the maximum wavelength of acid-soluble collagen (ASC) is shorter (λmax=220 nm) than that of many other proteins (Veeruraj *et al.*, 2015), whose absorption maxima are typically near 280 nm (Wang *et al.*, 2018). Comparable results have been reported for collagen extracted from Pacific cod (*Gadus macrocephalus*) skin (Sun *et al.*, 2017), catla (*Catla catla*), and rohu (*Labeo rohita*) (Pal & Suresh, 2016).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

To identify the collagen type extracted from *P. pardalis*, SDS-PAGE was used to separate protein fragments. Bands with molecular weights of 243 kDa, 122 kDa, and 110 kDa were detected (Figure 2). Although SDS-PAGE does not allow unequivocal assignment of the characteristic α- and β-chains of collagen, the observed banding pattern is consistent with the type I collagen profile previously reported for chemically extracted acid-soluble collagen from Nile tilapia skin (Hu *et al.*, 2017), as well as from tilapia and grey mullet (Shalaby *et al.*, 2020). Moreover, SDS-PAGE confirmed the effectiveness of the extraction method, as no bands attributable to non-collagenous proteins were observed.

Tensile strength, elongation at break, and Young's modulus of the films

Tensile strength values ranged from 0.23 to 2.92 MPa across all treatments, and were significantly lower than those reported by Erciyes & Ocak (2019) for collagen-hydrolysate films. According to conventional benchmarks, the tensile strength of the films developed in this study falls below recommended values (Hosseini *et al.*, 2016). Figure 3 illustrates the effects of collagen concentration and glycerol content, as well as their combined influence,

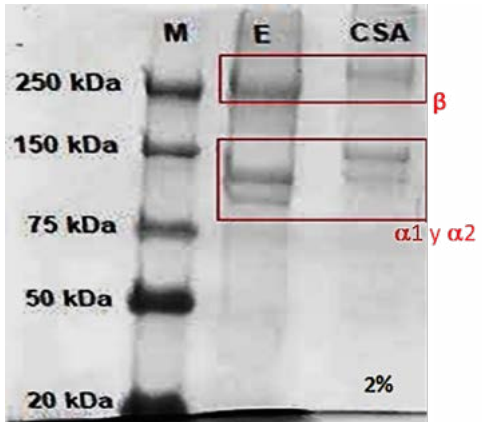

Figure 2. Electrophoretic profile of collagen extracted from *Pterygoplichthys pardalis* skin. M=molecular-weight marker; E=type I collagen standard; ASC=acid-soluble collagen.

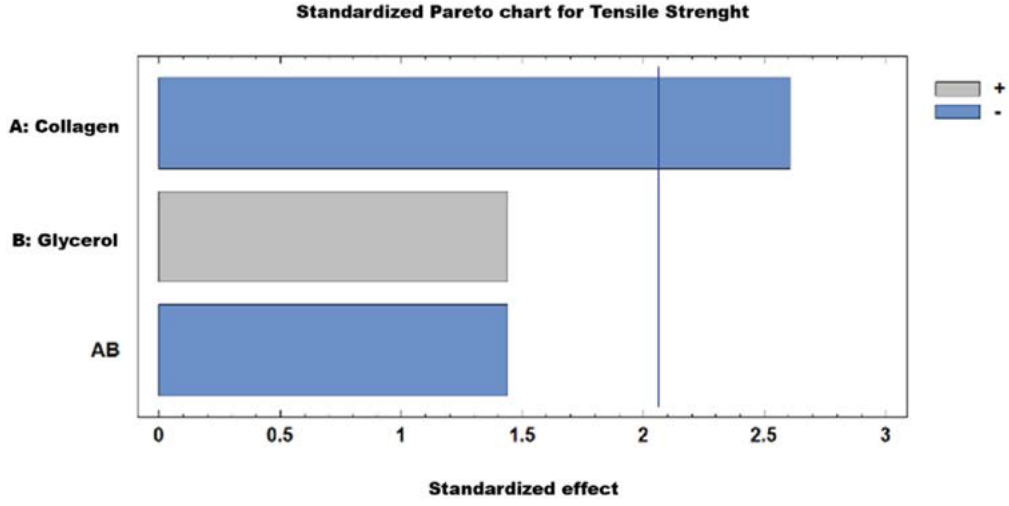

Figure 3. Effect of collagen (%) and glycerol (%) concentrations on the tensile strength of collagen films obtained from *P. pardalis* skin.

on the mechanical properties of the films (tensile strength, elongation at break, and Young's modulus).

Figure 4 shows a statistically significant negative effect of collagen concentration on tensile strength. This indicates that, as collagen concentration increased, film tensile strength decreased. This behavior may be explained by the fact that, rather than forming an effectively cross-linked network with gum arabic and/or glycerol, collagen molecules may slide relative to one another (Gómez-Guillén *et al.*, 2011; Tian *et al.*, 2021), potentially due to partial loss of mechanical functionality during the extraction process (Vergne *et al.*, 2018). In contrast, no statistically significant effect of the factors on film elongation at break was detected ($p>0.05$). Nevertheless, a slight trend toward higher elongation at break with increasing collagen concentration was observed, a phenomenon commonly attributed to the hydrophilic character of collagen, which promotes film hydration (Slimane & Sadok, 2018).

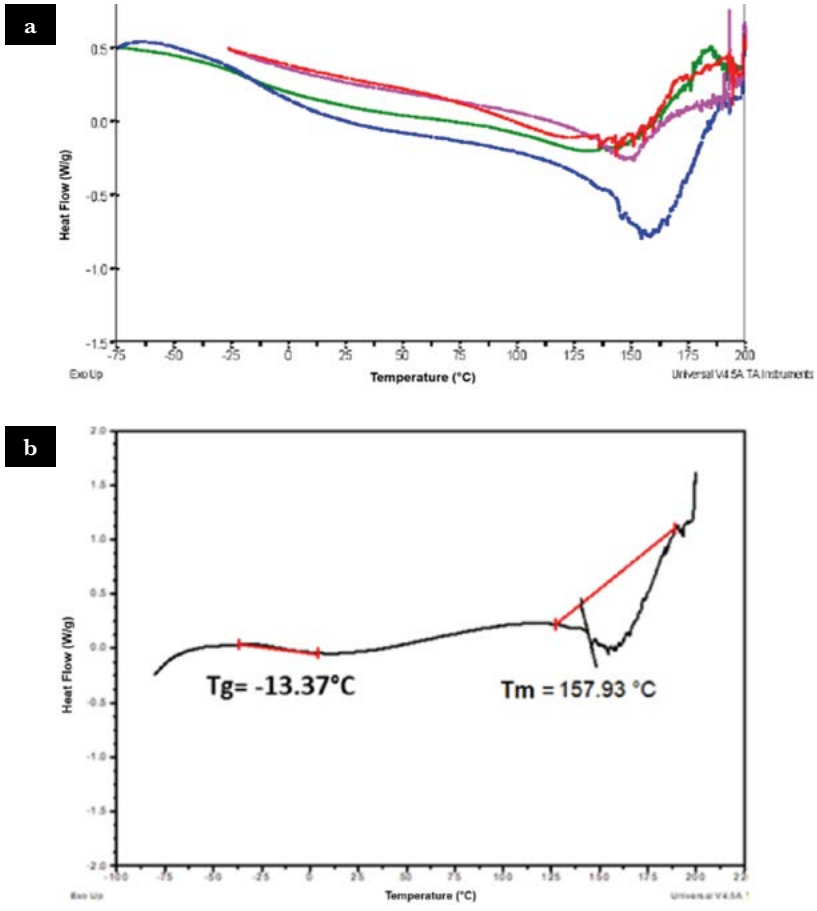

Figure 4. [a] Glass transition (Tg) and [b] denaturation (Tm) temperatures in thermograms of collagen films extracted from *P. pardalis* skin.

Ahmad *et al.* (2016) reported that the glass transition temperature (Tg) is governed by the structural features of polymeric materials, including molecular weight, chain branching, and the degree of cross-linking among the formulation components. Consistent with the tensile-strength trends, the absence of pronounced cross-linking between collagen and gum arabic and/or glycerol may explain why the Tg values of the films evaluated here were higher than those reported by Valencia *et al.* (2019), who obtained Tg values below −50 °C. The endothermic peak around 150 °C was associated with the film denaturation temperature (Chakrapani *et al.*, 2012), as previously described for collagen-based films (Andonegi *et al.*, 2020). The higher denaturation temperatures observed for *P. pardalis* collagen may be attributed to the stability conferred by its elevated hydroxyproline content (Sun *et al.*, 2021), an amino acid that can promote a higher degree of intermolecular cross-linking due to the presence of hydroxyl groups (Tatli *et al.*, 2018).

Biodegradability index

The biodegradability index (BI) is defined by the relationship between chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5). This parameter was calculated after five days of incubating the samples at 20 °C. The films developed in this

study presented COD values of $1.203\times10^{6}\pm3.682\times10^{4}$ mg O_2/L and BOD_5 values of $9.354\times10^{5}\pm5.310\times10^{4}$ mg O_2/L, yielding a BI of 77.74±6.76%. This indicates that 77.74% of the organic matter present in the sample is biodegradable within five days at 20 °C. Accordingly, the films can be classified as biodegradable materials under the criterion reported by Arias *et al.* (2012). The biodegradability index exhibited by collagen films produced from *P. pardalis* skin supports the inference that these films could serve as an alternative to materials such as nylon, given that they displayed a BI higher than that reported by Tachibana *et al.* (2013) for four types of nylon films. The COD, BOD_5, and BI values obtained in this work are particularly relevant, as biodegradability assessments for this type of film have not been previously reported. Atarés & Chiralt (2016) attributed film biodegradability to the organic and biological nature of the components used in their formulation, although this has not been conclusively demonstrated. Based on the present results, it can be stated that films fabricated from acid-extracted *P. pardalis* skin collagen, glycerol, and gum arabic are biodegradable, since most of the organic and inorganic matter present can be oxidized biochemically.

CONCLUSIONS

Collagen extraction from *P. pardalis* skin represents a promising route for the commercial valorization of this invasive species. Under controlled conditions (acid-to-skin ratio 1:20, v/w; extraction time 24 h), an extraction yield close to 60% was achieved. Using this collagen, films with high elongation capacity and a high biodegradability index (>70%) were produced, features that may position this collagen as a valuable feedstock for the biopolymer industry. However, further studies should evaluate alternative extraction strategies such as enzymatic methods which may better preserve the molecular structure and, consequently, the mechanical performance of collagen.

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