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Biodegradable films based on tilapia collagen (*Oreochromis sp*): improvement of properties with PLA and PCL bilayers with potential use in sustainable food packaging

Películas biodegradables a base de colágeno de tilapia (*Oreochromis sp*): mejora de las propiedades con bicapas de PLA y PCL con potencial uso en envases de alimentos sostenibles

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Abstract
The study aimed to develop biodegradable films from collagen (Col) obtained from tilapia by-products (*Oreochromis Sp*) in combination with polylactic acid (PLA) and polycaprolactone (PCL). For collagen extraction, 0.1 N NaOH was used in a solid-liquid ratio of 1:20 (w/v), the fat was removed from the tilapia skin using 10% butyl alcohol, and for the scale's decalcification with 0.5 M EDTA. The yield of the collagen obtained in both by-products was determined and characterized by the UV-VIS and FTIR (Fourier transform infrared spectroscopy) spectra compared to commercial collagen. As a result, there was a notable reduction in water content of less than 20%, and F3+PLA obtained a lower solubility percentage than the other bilayers. Regarding the contact angle, the bilayers obtained values greater than 100°. In mechanical tests, PCL presented the highest percentage of elongation at the point of break, which was 1067%, which could be related to greater flexibility and stretchability before breakage. The permeability values in the bilayers decreased significantly; F2+PLA indicated better control in the film as a barrier against water vapor, 0.55 g-mm/kPa-h-m^2. Therefore, mixing a hydrophilic and a hydrophobic layer could improve the characteristics, providing higher stability and obtaining films with optimal properties to produce food packaging.
Keywords: Eco-friendly packaging, Tilapia by-products, Collagen extraction, Bilayers.

Resumen
El estudio tuvo como objetivo desarrollar películas biodegradables a partir de colágeno (Col) obtenido de subproductos de tilapia (Oreochromis Sp) en combinación con acido poli láctico (PLA) y policaprolactona (PCL). Para la extracción de colágeno se utilizó NaOH 0,1 N en una relación sólido-líquido de 1:20 (p/v), la grasa de la piel de tilapia se eliminó utilizando alcohol butílico al 10% y para la descalcificación de las escamas con EDTA 0,5 M. El rendimiento del colágeno obtenido en ambos subproductos se determinó y caracterizó mediante los espectros UV-VIS y FTIR (Espectroscopía infrarroja por transformada de Fourier) en comparación con el colágeno comercial. Como resultado, hubo una reducción notable en el contenido de humedad de menos del 20%, y F3+PLA obtuvo un porcentaje de solubilidad menor que las otras bicapas. En cuanto al ángulo de contacto, las bicapas obtuvieron valores superiores a 100°. En las pruebas mecánicas, el PCL presentó el mayor porcentaje de elongación en el punto de rotura, el cual fue de 1067%, lo que podría estar relacionado con una mayor flexibilidad y estirabilidad antes de la rotura. Los valores de permeabilidad en las bicapas disminuyeron significativamente; F2+PLA indicó un mejor control en la película como barrera contra el vapor de agua, 0.55 g-mm/kPa-h-m2. Por lo tanto, mezclar una capa hidrófila y otra hidrófoba podría mejorar las características, proporcionando una mayor estabilidad y obteniendo películas con propiedades óptimas para producir envases de alimentos.
Palabras clave: Empaque ecológico, Subproductos de Tilapia, Extracción de colágeno, Bicapas.

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1 Introduction

The fact that the annual world production of plastic increased considerably from 2 million tons produced in 1950 to 381 million tons only in 2015, shows that plastic is a highly used material throughout the world; its use encompasses many sectors. This is due to its great variety, and that the same time, it is a versatile product, becoming almost indispensable. In the food industry, it is mainly essential in product packaging because it is fluid, moldable, thermo-sealable, and can be integrated into production processes. Considering its characteristics, its use is essential, so much so that data reveal that in Europe alone, approximately 40% of plastic packaging is used to contain food, and the more it would represent its use worldwide (Kan and Miller, 2022).

In Colombia, 12 million tons of garbage are produced annually, of which plastic represents 10.78% in all sectors and is considered the second leading group producing solid waste. In addition, a consumption of 1.2 million tons of plastic packaging is estimated, of which 56% is obtained from PET packaging (Zapata Bravo *et al*., 2021). The per capita consumption of plastics in Colombia is 24 kg per year. Until 2010, the use and consumption of plastics went from 1,075 tons per day, equivalent to 392,482 tons per year, to 3,424 tons/per day, increasing 1,250,000 tons produced per year only until 2019, which has generated an annual growth rate of 24.2% (Rangel-Buitrago *et al*., 2021).

Most basic plastics are developed based on olefin. These polymers are difficult to recycle, and when they can be recycled, they are subjected to chemical processes because their polymer chains comprise C-C covalent bonds. Consequently, high temperatures and high-performance catalysts are needed for its decomposition, and other alternatives have been observed to mitigate the production of synthetic plastics, which is why the use of natural polymers (cellulose, chitosan (Chi), starch, whey protein, and gelatin) has been evaluated. These biopolymers are abundant in nature and renewable, and their use allows better degradation of their compounds. As in the case of PLA, a polymer made from starch, once transformed by the action of microorganisms, creates lactic acid, which serves as a base to produce PLA through condensation polymerization or ring-opening polymerization. This polymer leads to the production of products that can decompose and degrade more easily (Andrade *et al*., 2023). Among synthetic polymers, polycaprolactone (PCL), a biodegradable polymer with a low melting point (around 60º C) and is one of the few that can completely biodegrade. Its combination with other biopolymers is a promising option for creating composite materials with improved characteristics (Thakur *et al*., 2021).

Combining biopolymers with synthetic polymers offers numerous benefits, as this blend enhances material properties. Therefore, their biodegradability and biocompatibility are reinforced while maintaining thermal and mechanical properties with significant cost reductions (Rodríguez-Sepúlveda and Orrego-Alzate, 2016). When the purpose is to obtain materials with a faster biological degradation, the combinations can be composed of the biopolymers mainly used, such as collagen, gelatin, chitosan, and starch, applied with synthetic polymers such as PLA, PCL and polyglycolic acid (PGA) (Rodríguez-Sepúlveda and Orrego-Alzate, 2016; Zapata-Luna *et al*., 2023).

The importance of using bilayer materials lies in the fact that the mechanical and barrier properties of the individual layers can complement each other. In this way, monolayers of rigid materials can improve the properties of monolayers with low rigidity, or materials with a high-water vapor barrier can be combined with materials with a high oxygen barrier, giving rise to materials with high performance (Ortega-Toro et al., 2015a).

This innovative approach also extends to the use of tilapia byproducts Tilapia (*Oreochromis sp*.) fillets represent approximately 30% to 33% of the fish, leaving about 70% remaining unused for human consumption. These nutrient-rich byproducts can be transformed into biodegradable materials with packaging applications that include head, carcass, viscera, fins, skin, and scales, providing a good source of macro and micronutrients (Peñarubia *et al*., 2023).

The use of biopolymers represents many advantages in addition to biodegradable properties, and this is because they can be combined with plasticizers to improve their mechanical, barrier, and light transmission properties. A plasticizing additive is a substance that, when incorporated into a polymeric material, alters its characteristics, providing a more manipulable, flexible material with greater fluidity and softness, allowing this polymer to have a variety of formations through molding processes. Glycerol is one of the most used plasticizers because it gives higher softness and flexibility in the production of films (Cedeño Sares *et al*., 2023; Sharafi-Badr *et al*., 2023).

The main objective of this work was to develop biodegradable films from collagen obtained from tilapia by-products in combination with biopolymers such as PCL and PLA. It is essential to evaluate the properties of sustainable food packaging films, focusing on improving mechanical strength, thermal stability, and water content and vapor barrier performance. The study optimized collagen extraction using NaOH and butyl alcohol and evaluated film characteristics using UV-VIS and FTIR analysis. It highlighted moisture reduction, solubility, and

flexibility improvements, particularly in PCL-enhanced formulations.

2 Methodology

2.1 Raw material

The company Playa Pez S.A.S. provided the tilapia waste. The biopolymers that were used were PLA and PCL.

2.2 Collagen extraction and characterization

This process was carried out according to the methodology reported by Nagai and Suzuki (2000) with modifications that took place. For collagen extraction, 0.1 N NaOH was used in a solid: liquid ratio of 1:20 (w/v) for 24 h to remove non-collagenous proteins, then washed with distilled water.

Eliminating the fat in the tilapia skin was necessary; for this, 10% butyl alcohol was used, and the non-insoluble matter could be eliminated using 0.5 M acetic acid. Collagen was precipitated by adding 0.8 and 2.6 M NaCl at 7.0 pH. In the case of the scales, decalcification was carried out with 0.5 M ethylenediaminetetraacetic acid (EDTA) at a pH of 7.4. The fat was then removed with 10% butyl alcohol. After that, collagen was precipitated using 0.8 and 2.6 M NaCl at 7.0 pH. For the characterization of collagen, the methodology proposed by Bhuimbar *et al*. (2019) was followed. In this, the extraction yield was determined by comparing the weight of the dry final product and the dry initial raw material. On the other hand, the UV-Vis spectrum of the collagen samples extracted from the different parts of the tilapia was determined using a Shanghai MAPADA Instrument Co. UV-1800 PC spectrophotometer. The comparison was made with commercial collagen spectra. Also, the Fourier transform infrared spectroscopy spectrum of the extracted collagen was performed between 400 and 4000 cm^{-1}, comparing them with commercial collagen. For this determination was used a Thermo iS50 instrument equipped with a diamond crystal.

2.3 Obtaining biodegradable films

A source of collagen was selected according to the physicochemical properties studied and the production yield. Bilayer films were made, where one layer was formed from a mixture of collagen and chitosan, and the other layer was made of a more hydrophobic polymer type biodegradable polyester (PLA and PCL).

The biodegradable polyester layer was obtained using the pellets of each polymer subjected to a compression molding process according to its melting temperature.

Table 1. Unbalanced factorial design of two factors to obtain active biodegradable films.

Factors	Levels		
Collagen: chitosan ratios	0.25: 1	0.5: 1	1 :1
Biodegradable polyester monolayers	PLA	PCL	

Table 2. Formulations for monolayers based on collagen (Col) and chitosan (Chi).

Formulations	Col (g)	Chi (g)
F1	0.81	3.24
F2	1.35	2.7
F3	2.03	2.03

The monolayer of collagen and chitosan was formed through the casting process because the thermal properties of collagen do not allow it to be processed by extrusion or compression molding without thermal degradation (at the process conditions). This film-forming solution was obtained according to the methodology of Cano *et al*. (2021) with some modifications. Solubilized the chitosan (2% w/w), and a 0.5% aqueous solution of glacial acetic acid at a pH close to 3 was used. Collagen (0.5%, 1%, 2% w/w) was added to this solution. Glycerol, at 25% respect to polymers, was used as a plasticizer, and they were mixed using an Ultra-Turrax (IKA T-25 digital) at 13500 rpm for 5 min. The pouring was carried out on 20 cm x 20 cm Teflon plates and allowed to dry at room temperature. Once dry, the films were removed and conditioned in desiccators with supersaturated magnesium nitrate solutions until characterization. Table 1 shows the experimental design for the development of bilayer films and Table 2 shows the formulations for monolayers based on collagen and chitosan.

An unbalanced two-factor factorial design was used to obtain the different formulations. Collagen was used in 3 percentages respect to chitosan, and two monolayers of biodegradable polyesters were used. PLA and PCL layers were made using 4 g of commercial pellets. The monolayers F1, F2, and F3 were subjected to compression molding, combining them with PLA or PCL to obtain the bilayers. The pressing temperature was 160 °C for PLA and 80 °C for PCL for 1 min at 130 bars. This temperature difference is due to the polyesters' softening temperature. As a result, six types of bilayer films were obtained. The films obtained were conditioned and characterized.

2.4 Evaluation and characterization of the physicochemical properties of the selected films

2.4.1 Water content

The water content of the films previously conditioned at 53% R.H. was determined. For this, a convection oven (Tecnodom S.P.A. 201824397F, Italy) was used at 60 °C, until constant weight was obtained. Water content was calculated as the ratio of wet weight to dry weight. This procedure was performed in triplicate.

2.4.2 Water solubility

It was determined by immersing the films in distilled water at a ratio of film to water of 1:10 for 48 h. This assay was carried out in triplicate for each formulation. Subsequently, the samples were transferred to a natural convection oven for four hours at 60 °C to remove free water and were weighed again. The solubility of the films was estimated from the initial and final weights. The data obtained were used to estimate the W.S. for each sample using the following formula:

$$WS(\%) = (Wi - Wf)Wi \times 100$$

2.4.3 Contact angle

The contact angle of distilled water on the analyzed films was measured by studying the shape of a drop of water (0.01 mL) after 60 s using a digital camera with a white background. The distance was 50 cm from the camera lens to the water drop. The analysis of the images is carried out using the Goniotrans Pro Software (Ortega-Toro *et al*., 2015b; Gómez-Contreras *et al*., 2021).

2.4.4 Mechanical properties

The ASTM D 882 standard guidelines carried out the procedure. The stress at breakage and the percentage of elongation were determined. For this, a universal testing machine (model 43, MTS Advantage TM) was used with a cell of 500 N and a head speed of 10 mm/min. Before determining these properties, the samples were cut and kept at a relative humidity of 53 % at 25 °C for 48 h. The thickness measurement of the samples was carried out by taking five measurements using a micrometer. These determinations were carried out using five repetitions for each formulation.

2.4.5 Water Vapor Permeability (WVP)

It was determined according to the protocol reported by Ortega-Toro *et al*. (2014), following the standard ASTM E96-95 method with some modifications, a humidity gradient of 53% R.H. was used to 100% at 25 °C.

2.4.6 Structural properties

The surface sections of the biodegradable films were studied using an optical microscope (Zeiss, Primostar, Germani) integrated with a high-definition camera. The photomicrographs obtained were processed using Image Pro-Plus software version 5.1.

2.4.7 Statistic analysis

All results were analyzed using Statgraphics Plus for Windows 5.1 software. An analysis of variance (ANOVA) was carried out, and the averages were evaluated using Fisher Least Significant Differences (LSD) test with 95% confidence.

3 Results and discussion

3.1 Characterization of collagen obtained from tilapia skin and scales

3.1.1 Collagen performance

It is essential to mention that the collagen extraction process was carried out in batches of 1 kg of byproduct. Considering this, the yields obtained from tilapia skin and scales were 36.5% and 2.52%, respectively. Given that the collagen extracted from the skin obtained a higher yield, it was presented as the best option for processing. In a study by Gómez-Contreras *et al*. (2023), collagen was extracted using the skin, scales, fins, and heads, byproducts of the freshwater fish *Prochilodus magdalenae*. This analysis showed that the highest yield of collagen extraction was obtained from the skin, 55.6%, and from the scales, it was 16.1%. The differences in performance present in the previous study and this research may be due to various influential factors that affect the usefulness of the collagen obtained, such as the species, the age of the fish, and the extraction methods, among others.

Figure 1 shows tilapia scale by-products and tilapia skin by-products, where it is noted that the tilapia scales are thin, flat, and translucent with a flaky appearance, and on the other hand, the tilapia skin is thicker and denser.

As seen in Figure 2A and B, the collagen obtained presented a grayish visual appearance, which could be due to the presence of natural pigments from said byproducts. Also, it showed a fibrous texture. In similar studies, tilapia byproducts have been used to extract collagen, as is the case of Velarde-Rodríguez *et al*. (2015). To eliminate the grayish tone of tilapia skin, they used 0.525% sodium hypochlorite as a whitening agent (v/v). It resulted in a pearl white protein mixture (Figure 2C).

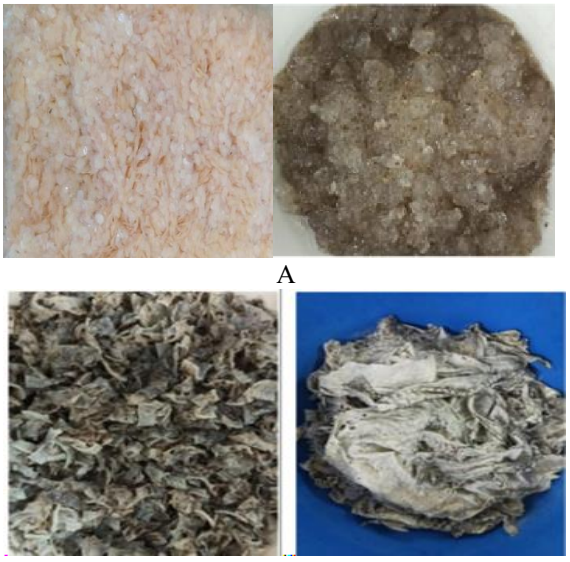

Figure 1. A) Tilapia scale by-products and B) Tilapia skin by-products.

Figure 2. Collagen extracted from tilapia skin and scales.

3.1.2 *UV-Vis spectrum of extracted and commercial collagen*

UV-Vis spectra of the collagens extracted from skin and scales were obtained and compared with commercial collagen. The analysis was carried out from 190 to 360 nm (Figure 3). In curve A (collagen from skin), a peak is observed at a wavelength of around 220 nm, with an absorbance value of approximately 1.543. In curve B (collagen from scales), a peak is observed at a wavelength around 215 nm, with an absorbance value of approximately 1.273. In curve C (commercial collagen), a peak is observed at a wavelength around 225 nm, with an absorbance value of approximately 2.095.

The differences in the position of the absorbance peaks (220 nm for A, 150 nm for B, and 225 nm for C) suggest that the samples have different molecular structures. This fact could be due to variations in collagen content, different functional groups, or the structural conformation of collagen and biodegradable polymers (He *et al*., 2019). Commercial collagen is hydrolyzed, so it is normal to expect some differences with extracted collagens whose original structure is largely preserved.

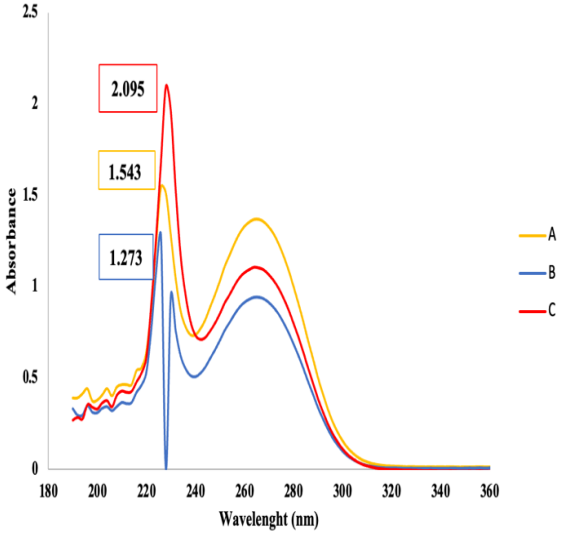

Figure 3. UV-Vis spectra of collagen: A) extracted from the skin; B) extracted from the scales; C) commercial collagen.

On the other hand, the height of the peaks (2.095 for A, 1.543 for B, and 1.273 for C) indicates the concentration of absorbent species present in the samples. A higher absorbance suggests a higher concentration or higher light absorption efficiency at that specific wavelength.

The curves also present a broad band at 275 nm (approximate absorbance of 0.8), 265 nm (approximate absorbance of 0.7), and 270 nm (absorbance of 0.75), respectively, for curves A, B, and C. The analysis utilizing UV-VIS in extracted collagens has been reported in different investigations with values similar to those obtained in this work, as is the case of the one carried out by Santiago-Gutierrez, (2020) for which was used extracted collagen of the devil fish (*Pterygoplichthys pardalis*) for analysis, the obtained spectrum of maximum absorbance occurred at a wavelength of 220 nm, they also indicate that typically proteins in the ultraviolet region generate a maximum absorbance at wavelengths close to 280 nm, especially in the case of amino acids such as tryptophan, phenylalanine and tyrosine.

3.1.3 *FTIR spectrum of extracted and commercial collagen*

FTIR is a technique used to identify the functional groups present in a sample. Bonds and groups of bonds vibrate at characteristic frequencies. For proteins such as collagen, amides are particularly interesting in this analysis (Nashchekina *et al*., 2020). In Figure 4, absorbance peaks can be seen at specific wavenumber.

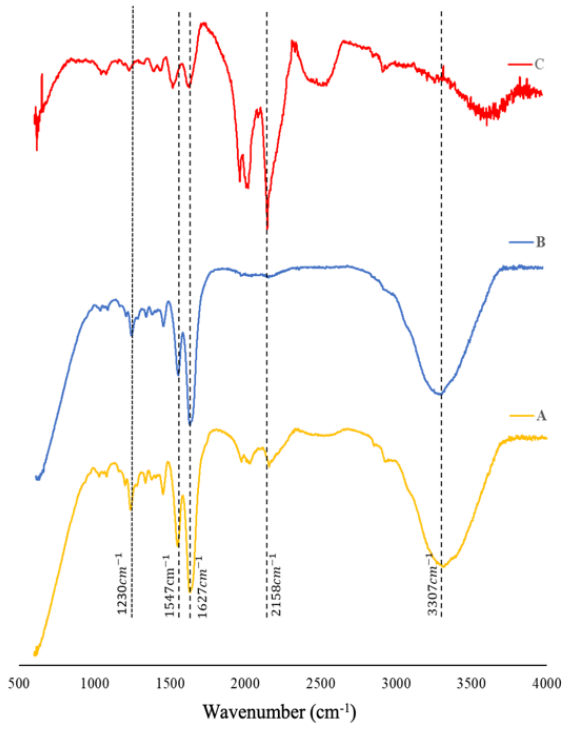

Figure 4. FTIR spectrum for collagen samples extracted from A) skin and B) scales, compared to C) commercial collagen.

Absorbance was recorded at a wavelength of 3307 cm^{-1}, characteristic of Amide A, which is commonly associated with the stretching vibration of NH. However, it should be noted that the wide band observed at 3307 cm^{-1} could also indicate the presence of hydroxyl groups. A minor peak was observed at a wavelength of 2158 cm^{-1}; inferred as Amide B; this behavior could be due to a symmetric CH_2 stretching. The characteristic absorption wave number in the Amide I bond is usually in the range of 1600-1700 cm^{-1}; as seen in Figure 4, the Amide I band had a value of 1627 cm^{-1}; which is generated by the stretching vibration of C=O in the polypeptide backbone of the protein. The Amida II peak is generally responsible for the combination of in-plane curvature of NH and stretching vibration of CN; it was found at a wavelength of 1547 cm^{-1}. Finally, Amide III bands with a wavenumber of 1230 cm^{-1} were found. This peak is complex, with intermolecular interactions in collagen consisting of components of CN stretching and NH planar bending from amide bonds and absorptions arising from CH_2 vibrations (Vitale *et al*., 2019).

The chemical composition of the different types of extracted collagens determined by FTIR showed a high correlation with the results obtained by (Slimane and Sadok, 2018), where they observed that the spectrum of amide A was generated at a wavenumber of 3293.57 and 3296.16 cm^{-1}. However, this type of bond is generally observed in a range of 3400 to 3440 cm^{-1}. Amide B, related to the methylene groups (-CH_2) present in amino acids such as proline, showed an absorption peak from 2942 to 3092 cm^{-1} Likewise, Amides I, II, and III peaks were observed at wavenumbers of 1629 cm^{-1},1548 cm^{-1} and 1454 cm^{-1}, respectively. Likewise (Jeong *et al*., 2013) obtained similar results in the FTIR analysis of collagen extracted from big-eyed tuna (*Thunnus obesus*), with a wavelength range between 3352 and 3446 cm^{-1} for Amide A, values between 2880 to 2927 cm^{-1} for Amide B, Amide I with values of 1645 cm^{-1}, Amides II and III presenting values of 1558 and 1263 cm^{-1}. These specific regions of the collagen protein, called amides, are directly related to the ability to form a triple helix structure, where the structural changes that occur in the peptide bonds produce a stretch that, depending on the nature, significantly affects the stability of collagen.

3.2 Characterization of the physicochemical properties of the selected films

3.2.1 Water content of films

The film's water content indicates its hydrophobicity, meaning that hydrophilic films contain high water content. Table 3 shows that the monolayers containing collagen and chitosan (F1, F2, F3) presented the highest water content due to their great affinity to water. The opposite occurred with the PLA and PCL films; the water content in these was low, with values of 0.50 ± 0.05 and 19.92 ± 31.29 (g of water of dry film), respectively, which could be attributed to the fact that these polymers are hydrophobic and their interaction with water is limited. On the other hand, the bilayers showed a water content of less than 16% compared to the monolayers; this indicates that joining two layers with different characteristics contributed to a significant reduction of water present in the films. Furthermore, interactions with chitosan generate a network that prevents water molecules from being retained. It is essential to mention that the plasticizer used could also have influenced the results obtained because glycerol, being highly hygroscopic, films formed with it can experience an increase in humidity.

The results obtained by Andonegi *et al*. (2020) showed very similar average water content values, around 12%; the collagen films showed a lower water content than the films made from collagen-chitosan; the molecular weight of chitosan did not contribute to the increase or the decrease in water content, since for films with LMW (low molecular weight) and HMW (high molecular weight) chitosan the water contents were (11.97% ± 0.51) and (11.53 % ± 0.50) respectively.

Table 3. Mean values and standard deviation of water content (Xw, g of water/g of the dry film), water solubility (Sw, g of soluble film/g of the dry film), water vapor permeability (WVP, g*mm/kPa*h*m^2), and contact angle (CA,°) obtained in monolayer and bilayers.

Formulation	Xw	Sw	WVP	CA
F1	32.2 ± 0.2^{a}	0.31 ± 0.13^{fg}	1.4 ± 0.3^{a}	$77 \pm 5e$
F2	29.7 ± 0.4^{b}	0.17 ± 0.02^{g}	1.28 ± 0.06^{a}	68 ± 1^{e}
F3	29.3 ± 0.5^{b}	0.14 ± 0.02^{g}	0.87 ± 0.11^{b}	60 ± 17^{e}
PLA	0.50 ± 0.05^{g}	0.28 ± 0.02^{f}	0.27 ± 0.04^{c}	125 ± 2^{b}
PCL	0.8 ± 0.2^{g}	0.48 ± 0.02^{e}	0.6 ± 0.3^{b}	95 ± 5^{d}
F1+PCL	10.1 ± 0.8^{f}	2.05 ± 0.04^{a}	0.7 ± 0.2^{b}	133 ± 5^{a}
F2+PCL	12.5 ± 1.0^{de}	1.04 ± 0.02^{b}	0.79 ± 0.25^{b}	129 ± 6^{a}
F3+PCL	11 ± 3^{def}	0.89 ± 0.03^{c}	1.4 ± 0.2^{a}	131 ± 6^{a}
F1+PLA	12.1 ± 0.4^{e}	0.85 ± 0.05^{c}	0.59 ± 0.08^{b}	133 ± 2^{a}
F2+PLA	13.8 ± 0.5^{d}	0.87 ± 0.02^{c}	0.55 ± 0.05^{b}	117 ± 2^{c}
F3+PLA	15.6 ± 0.2^{c}	0.74 ± 0.02^{d}	0.65 ± 0.24^{b}	125 ± 3^{b}

Different superscript letters between the columns indicate significant differences ($p < 0.05$) between the formulations.

3.2.2 Solubility in water

Films for packaging foods with high humidity must have low solubility to prevent them from dissolving and compromising storage. In Table 3, the monolayer films showed low solubility, with PCL at 0.48%. The bilayer F1 + PCL, which contains chitosan, had 2.05% solubility since chitosan is soluble in water due to its polar groups, although not more than 45% (Yadav and Dutta, 2024). F3 + PLA had the lowest solubility (0.74%), the most viable option due to its higher water resistance.

The study by Blanquicet *et al*. (2015) reported 24.6% and 7.03% solubility percentages in films developed from chitosan and whey. In this analysis, the concentration of chitosan was the main factor that influenced the variation in solubility levels since the lowest percentage of solubility was observed in films with a higher concentration of chitosan as the amount of chitosan increased. The solubility of said films decreased considerably.

Slimane and Sadok (2018) observed that for films with pure collagen, the solubility was 32.14%, and the addition of chitosan to the film solution produced a decrease in their solubility, with the partial replacement of collagen by chitosan to 50-75% respectively.

Ocak (2018) in this study, high solubility values were found for hydrolyzed collagen films (70.34% ± 1.38) and lower values for chitosan films (46.1% ± 0.58); the mixture of both materials was significantly better in terms of solubility concerning the collagen film (32.46% ± 0.74 to 53.64% ± 1.22), but slightly more soluble than the chitosan film because the interactions of the materials and its rigid structure cause a reduction in the solubility. As can be seen, the development of bilayer materials using a hydrophilic monolayer (F1, F2, and F3) and a hydrophobic monolayer (PLA and PCL) can be a good strategy since you have the option of which of the 2 layers is more convenient to use. in contact with food (depending on the properties of the food).

3.2.3 Water vapor barrier properties

The permeability test was carried out to evaluate the barrier properties against water vapor; the bilayer films were placed in such a way that the layer composed of chitosan and collagen was located at the bottom so that this way interaction with water occurs due to its hydrophilic character. Taking this into account, at the top was the layer of hydrophobic polymer, which has a limited interaction with water.

Water vapor permeability is a fundamental property due to water's essential role in the reactions that deteriorate food. For this reason, low permeability is required to have more higher resistance to the passage of water vapor molecules. Table 3 shows the values obtained when evaluating the barrier properties against water vapor. Monolayers F1 and F2 presented the highest permeability values of 1.43 ± 0.31 and 1.28 ± 0.06 g-mm/kPa-h-m^2, respectively, followed by the F3 with a value of 0.87 ± 0.11 g-mm/kPa-h-m^2. These layers are composed of collagen and chitosan and have poor barriers in terms of water vapor. However, they have better barrier properties against gases such as oxygen.

On the other hand, PLA and PCL films obtained the lowest permeability values, these being 0.27 ± 0.04 and 0.62 ± 0.27 g-mm/kPa-h-m^2. Due to this, being less permeable indicates that there is a more significant barrier against water vapor. When comparing the monolayers with the bilayers, a significant difference could be noted regarding the reduction in the permeability value, especially in the films made up of PLA and F1, F2, and F3. The lowest permeability value was found in F2 + PLA, 0.55 ± 0.05 g-mm/kPa-h-m^2, indicating better control in the said film as a barrier against water vapor. A different case occurred in the bilayer films containing PCL;

F3 + PCL obtained the highest permeability with a value of 1.43 ± 0.52 g-mm/kPa-h-m^2, so its weak barrier against water vapor. This result is curious since PCL is expected to exert its hydrophobic character by preventing water molecules from permeating the film. Possibly at high concentrations of collagen, interactions with PCL are generated that promote intermolecular spaces through which water vapor molecules can pass.

Andonegi *et al*. (2020) did not obtain significant differences regarding the WVTR (water vapor transmission) values with the addition of chitosan to the film-forming solution, the water vapor transmission rate being 1176 and 1174 g m^{-2} day^{-1} and 1172 g m^{-2} day^{-1} for the control (only collagen), on the other hand, the results obtained by Elango *et al*. (2015) showed through the analysis of variance that the effect of chitosan was significantly high for the reduction of WVTR, this is because the protein-chitosan interaction decreases the free space between the polymeric matrices, which results in a decrease in the WVTR of the films.

3.2.4 Contact angle

Table 3 shows the values obtained by measuring the contact angle of the films. This determination gives an idea of the hydrophilicity of the films' surface since the interior of the films can present various interactions with water promoted by the structure, organization, and interactions between the polymers and the additives. As seen in this, the monolayers reflect angles less than 90° in most cases, except the PCL and PLA films, which obtained higher angles of 95.0 ± 5.0 and 125.0 ± 5.00 respectively. F3 presented the lowest value of angle created between the water drop when it met the film, being 60.0 ± 17.0, showing a more hydrophilic surface as the amount of collagen in the chitosan matrix increases. Similar studies indicate that a contact angle of less than 90° in films demonstrates the hydrophilic character of these since the water drop spreads on the surface of the films, showing preference for this substrate. In turn, it is shown that the contact angles greater than 90° do not present as much interaction between the drop and the surface, so it remains as a rim on the surface (Zapata *et al*., 2018).

The results are in accordance with those reported by Andonegi et al. (2020), who observed that the contact angle with water increased as the amount of chitosan in a mixture with collagen decreased. The addition of hydrophilic groups promotes an increase in hydrophilicity in collagen films, as mentioned in the study carried out by Perumal *et al.* (2018), where the collagen films had a CA of 90°, and this decreased with the addition of a sulfated polysaccharide, going from 90° to 63.8°.

3.2.5 Mechanical properties

The results obtained from the tests in which the mechanical properties of the films were evaluated are found in Table 4 , where significant differences can be seen in the formulations in terms of the percentage of elongation at break; it is observed that the PCL presented the largest of these being 1067 ± 222.6 (Figure 5A), which could be related to greater flexibility and stretching capacity before rupture. Unlike PLA, which obtained the lowest elongation percentage of all, being 4.39 ± 0.40 is why it is considered a rigid plastic because it has low flexibility and fragility and stands out for small elongations ranging from 0.5 to 5% (Méndez-Bautista, 2010).

However, PLA has similar mechanical properties or is in the same range as polymers made from petroleum derivatives, except for the low elongation percentages that differentiate it from them. Regarding the resistance due to the tension exerted on the samples, PLA achieved the highest result, 30.76 MPa, followed by PCL with a value of 21.17 MPa. Some studies relate that the thickness of the films may influence these data. The study by Arredondo (2017) reported that the thickest film presented the most significant resistance to breaking and tension. The greater the thickness, the greater the force required to break the film. On the other hand, the elastic modulus shows that PLA is the significantly stiffest material. Likewise, as the amount of collagen increases in relation to chitosan, the films become less rigid and less strong and acquire greater deformation capacity. The collagen shows the polymer matrix's plasticization capacity, as seen in the curves in Figure 5B.

Table 4. Mean values and standard deviation of thickness, tensile strength (TS), Elastic Modulus (EM) and elongation at break point (E) in mechanical tests applied to monolayer films.

Formulations	Thickness (μm)	TS (MPa)	EM (MPa)	E (%)
F1	130 ± 20^{c}	19 ± 4^{bc}	5.3 ± 0.4^{b}	21 ± 6^{b}
F2	90 ± 20^{d}	13 ± 3^{c}	4.20 ± 0.03^{c}	25 ± 6b
F3	120 ± 10^{c}	11 ± 2^{c}	0.56 ± 0.08^{e}	30 ± 4b
PCL	260 ± 10^{a}	22 ± 5^{b}	1.74 ± 0.11^{d}	1067 ± 122^{a}
PLA	160 ± 10^{b}	31 ± 3^{a}	11.0 ± 0.9^{a}	4.4 ± 0.4^{c}

Different superscript letters between the columns indicate significant differences ($p < 0.05$) between the formulations.

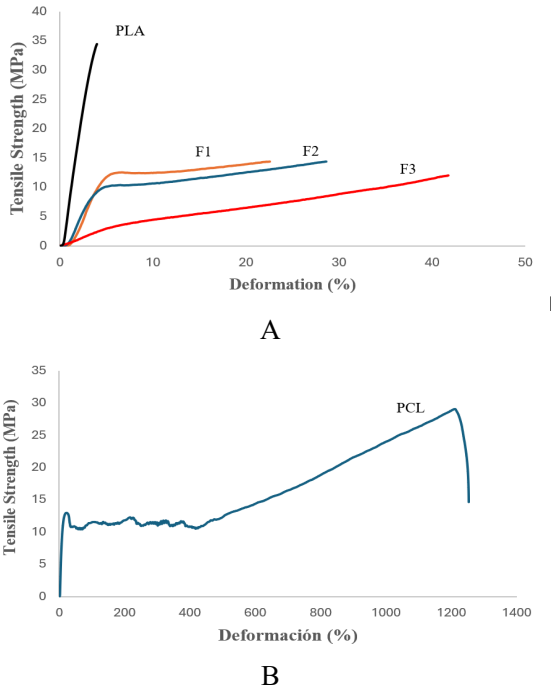

Figure 5. Tensile properties of studies formulations: A) PLA, F1, F2 and F3; B) PCL.

The results reported by Slimane and Sadok (2018) suggest that the addition of collagen increased the mechanical deformation of the films. The authors observed a significant increase when adding collagen, going from 4.25% in pure chitosan films to 4.49% and 5.67% for the composite films. Li *et al*. (2014) reported data in terms of TS for films composed of collagen and cellulose nanocrystal (CNC), where the addition of this filler increases the TS more significant than that of pure collagen (from 16Mpa to 22 MPa). Another study by Liu et al. (2021) showed that adding vegetable-tanned collagen fibers (VCF) of less than 25% by weight showed no significant effects for TS and E. Adding more than 25% collagen promotes a decrease in TS from 19.7 to 1.9 MPa.

3.2.6 Structural properties

3.2.6.1 Micrographs

In the surface optical micrographs, homogeneous and smooth surfaces are observed for PLA and PCL, with some deformations that correlate with the molds used for their production. In the cases of films F1 to F3, particles dispersed in a continuous medium are observed. The dispersed reddish particles probably correspond to collagen in the middle of a continuous chitosan matrix. It is observed that the number of dispersed particles increases from F1 to F3—Figure 6.

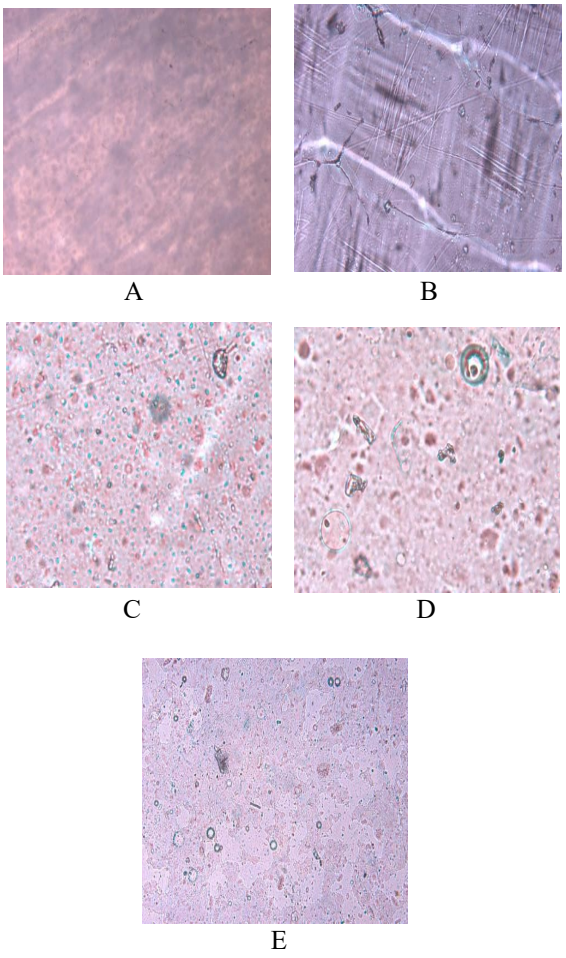

Figure 6. Monolayer film micrographs: A) PLA; B) PCL; C) F1; D) F2; E) F3, at 10x magnification.

The results obtained by Andonegi *et al*. (2020) through a scanning electron microscopy (SEM) analysis showed that the addition of chitosan promoted the visualization of dispersed collagen fibers and a rough surface; these changes were mainly noted in the films with the addition of chitosan with a high molecular weight, they also highlight that the collagen fibers were morphologically modified with a decrease in the diameter of the fibers due to the addition of chitosan.

The study conducted by Wenhang *et al*. (2016) observed that collagen films with the addition of microcrystalline cellulose (MC) showed rougher surfaces compared to pure collagen films, which showed a smooth and homogeneous surface.

Conclusion

Tilapia skin collagen was used to make films, yielding 36.5%. The analysis showed that the collagen samples had a maximum absorbance between 220 and 230 nm. The physicochemical properties of the films varied depending on the composition and number of

layers. Films with chitosan had higher solubility and lower barrier against water vapor. Films with PLA or PCL had lower water vapor permeability. The contact angle indicated an interaction between water and the collagen and chitosan films, while it was greater than 90° in the PLA and PCL films, indicating less interaction. PCL had the highest deformation capacity, and collagen showed a plasticizing effect on the chitosan matrix. Overall, the developed films are promising for food packaging applications.

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