Formulation and stability of biodegradable films made from cod gelatin and sunflower oil blends

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ABSTRACT

Sunflower oil was added to cod gelatin in proportions of 0, 0.3, 0.6, and 1% to improve the hydrophobic properties of the resulting films and decrease water vapor permeability and the soluble matter content. Mechanical and optical properties were evaluated to determine the influence of the added oil and the stability of the films during storage for one month. Adding oil increased film thickness, whiteness, and optical absorbance and decreased transparency; all these properties held stable over the storage period. Fourier transform infrared (FTIR) spectra revealed some lipid-protein interactions (hydrogen bonds, ester formation) and early oil oxidation. By the end of storage oxidation was well advanced and secondary oxidation products were present. Adding oil in any quantity decreased the puncture force but not the puncture deformation. Stability of these two mechanical properties decreased during storage in the films that contained ≥ 0.6 % added oil. Water vapor permeability did not decrease when oil was added and even increased with storage time, though adding oil yielded more insoluble films due to lipid-protein interactions.

Key words: cod gelatin, sunflower oil, edible film, stability, film properties

INTRODUCTION

Most films used to preserve foodstuffs are synthetic. Nevertheless, for environmental reasons, attention has lately been turned towards biodegradable and/or edible films. The materials used to make the films are ordinarily waste products from food processing, thereby enhancing processing sustainability. The biodegradable, edible nature of these films meant that they can be employed in both food and agricultural applications. The materials used are mainly carbohydrates, proteins, and lipids. Edible coatings have been used for many years, the oldest coatings being made from waxes, typically as coatings for cheese. However, in recent years carbohydrates and proteins have been extensively tested to develop biodegradable films having more and more versatile properties. Protein-based films offer better mechanical and barrier properties (Cuq, Gontard, Cuq & Guilbert, 1998) due both to the specific structure of the proteins and the ability of proteins to form stronger intermolecular covalent bonds than carbohydrates (Cuq, Aymard, Cuq, & Guilbert, 1995).

Studies characterizing film production using protein from fish gelatin are quite recent, all fish gelatins being observed to exhibit excellent film-forming properties (Avena-Bustillos, Olsen, Olson, Chiou, Yee, Bechtel & McHugh, 2006; Jongjareonrak, Benjakul, Visessanguan, Prodpran & Tanaka, 2006a,b; Gómez-Guillén, Ihl, Bifani, Silva & Montero, 2007). In general, fish gelatins have demonstrated suitable film-forming ability, yielding transparent, colorless, water-soluble and water vapor-permeable, and highly deformable films. For certain applications, the hydrophilic character of these types of film is a drawback, hence the interest in developing blends with other components with a view to decreasing their water vapor permeability and water solubility. Various mechanisms aimed at reducing water vapor permeability in polysaccharide or protein films have been tested, mainly by adding oils or

waxes to the emulsified film-forming solutions (Jongjareonrak et al., 2006b) or by making bilayer films (Debeaufort, Quezada-Gallo, Delporte & Voilley, 2000; Morillon, Debeaufort, Blond, Capelle & Voilley, 2002).

5 The advantageous or adverse alterations that take place on adding oil or wax to the film depend on the attributes of all the different components of the blend and hence on the interactions that can occur (Debeaufort et al., 2000; Jongjareonrak et al., 2006b, Morillon et al., 2002). Studies on gelatin films of this kind are quite recent, and the behavior and properties of the gelatins in the films differ according to their origin. Animal gelatin has more
10 crosslinking than fish gelatin, and gelatin from warm-water fish more crosslinking than gelatin from cold-water fish. These differing qualities are attributable to the differing amino acid compositions, especially with regard to hydroxyproline. The literature contains no reports on the cod-skin gelatin used here. Cod being a cold-water species, this gelatin has its own particularities, namely, low hydroxyproline concentrations and a low level of
15 crosslinking, and so forth.

In view of the nature of the components of the films, molecular changes and reorganization can be expected to take place over time. For these reasons, studies on the development of film-forming blends and the stability of the resulting films are of considerable interest, and
20 that is the object of the present experiment.

MATERIALS AND METHODS

Raw materials. Cod (*Gadus morhua*) skin gelatin was obtained, basically by following the procedure reported in Gómez-Guillén and Montero (2001). A commercial brand of sunflower oil was purchased from a local store.

Film preparation. To prepare the film forming solution, first, gelatin powder (4%, w/v) was dissolved in distilled water by heating in a water bath at 60 °C for 2 h. Then, 0.75 % glycerol and 0.75 % sorbitol (w/v) were added as plasticizers. Different amounts of sunflower oil (0, 0.3, 0.6, and 1% w/v) were incorporated to the film forming solution and the mixture was homogenized in a blender (Osterizer, model 86750E, USA) at the maximum speed setting for 4 min. A few drops of food-grade silicone antifoaming agent (Dow Corning 1510 antifoam) were added to prevent foam formation. Amounts of 40 mL of the film-forming solution were poured onto plexiglass plates (11.5 x 11.5 cm²) and dried in an oven with air renewal and circulation (Binder, model FD 240, Tuttlingen, Germany) at 45 °C for 18-20 h. The dried films were conditioned in desiccators with saturated solutions of NaBr at room temperature at 58 % relative humidity for two days before analysis, and then half of the films were peeled from the casting surface for analysis as described below. For the stability study, the remaining films were stored in the desiccators at 58 % relative humidity and 22°C for a month, and then analyses were repeated. Film thickness was measured using a digital micrometer (Mitutoyo, model MDC-25M, Kanagawa, Japan), averaging nine different locations.

Color. Films were placed between two steel dishes with a hole 5.7cm in diameter. Film color readings were then taken using a tristimulus colorimeter (HunterLab model D25 A-9, Hunter Associates Laboratory Inc., Reston, VA, USA), applying the CIELab scale (C/2°) where L*

(black 0 to light 100), a^* (red 60 to green -60) and b^* (yellow 60 to blue -60) were used to measure lightness, redness, and yellowness. The whiteness index was defined as $100 - [100 - L^*]^2 + a^{*2} + b^{*2}]^{1/2}$. Results were expressed as the means of six samples.

5 **Light absorbance.** The light barrier properties of gelatin films were measured by exposing the films to light at wavelengths ranging from 200 to 700 nm, using a UV-1601 spectrophotometer (Model CPS-240, Shimadzu, Kyoto, Japan). The transparency of the films was calculated by the equation $T = \text{Abs}_{600}/x$, where Abs_{600} was absorbance at 600 nm and x was film thickness (mm).

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Fourier transform infrared spectroscopy (FTIR). Pieces of film 2 cm in diameter were sandwiched between two KBr disks. FTIR spectra were recorded from wavenumber 400 to 4000cm^{-1} in a Bruker IFS28 spectrometer (Bruker Banner Lane, Coventry, UK). Absorbance spectra were obtained with a spectrum of the KBr disk as background. In all, 50 interferograms
15 were compiled for each spectrum.

Mechanical properties. Films were fixed in a 5.6 cm-diameter cell and perforated to breaking point using a texturometer (mod. 4501, Instron Engineering Corp., Canton, MA, USA) with a round-ended stainless steel plunger ($\varnothing=3\text{mm}$). A 100-N load cell was used, and cross-head
20 speed was 60 mm/min. Puncture force [N] and deformation [mm] were determined by means of force-deformation curves. Puncture deformation (%) was calculated according to Sobral, Menegalli, Hubinger and Roques (2001). All determinations were carried out at least in quadruplicate.

Water vapor permeability measurements. Water vapor permeability was measured using the method described by Sobral et al. (2001). A round portion of film was cut out and mounted on a plastic cup (permeation area = 15.90 cm²) containing silica gel, and the cups were then placed in desiccators with distilled water on the bottom. Weights were taken every hour for six hours.

- 5 Water vapor permeability was calculated from the equation $WVP = \frac{w \cdot x \cdot t^{-1} \cdot A^{-1} \cdot \Delta P^{-1}}$, where w was weight gain (g), x was film thickness (mm), t was time of gain (h), A was permeation area, and ΔP was the change in the partial atmospheric vapor pressure with the silica gel and pure water (2642 Pa at 22 °C). Results were expressed as g.mm.h⁻¹.cm⁻².Pa⁻¹. All measurements were carried out in triplicate.

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Water solubility of the films. Solubility determinations were carried out in triplicate according to the method of Gontard, Guilbert & Cuq (1992). Three pieces of film 2 cm in diameter (about 0.25g total) were immersed in 10 mL of distilled water and the system slowly stirred at room temperature (22-25°C) for 24 hours. The samples were then filtered through Whatman no. 1

15 filter paper. The insoluble fraction was dried in a forced-air oven (105°C, 24 hours) to determine the water-soluble matter as a percentage of the initial weight before and after solubilization.

The protein content of the soluble fraction was also measured using a LECO FP-2000 nitrogen

20 determinator (Leco Corporation, St Joseph, MI, USA) calibrated with EDTA by the Dumas method following A.O.A.C. 992.15 (A.O.A.C. 2000). Results are the means of three determinations and have been expressed as percentage soluble protein (Nx5.4). The non-protein fraction was determined as the difference between the total soluble matter and the protein fraction.

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Statistical analysis. Data were subjected to analysis of variance using SPSS computer program (SPSS 14.0, SPSS INC., Chicago, Illinois, USA), paired comparisons were made using Bonferroni test or Tamhane test, with significance of difference set at $p \leq 0.05$, depending on variance homogeneity. Differences of means due to storage time were using a T test.

RESULTS AND DISCUSSION

The estimated thickness was 100 μm over an experimental range of between 95 and 119 μm . There was a tendency for thickness to increase with the amount of oil added, although only the film with 1 % oil differed significantly, suggesting conformational changes in the film matrix at high added oil concentrations. The films made with gelatin alone or with low oil concentrations were thus relatively denser. Thickness held steady over the storage period.

Optical properties

Visually, the cod-skin gelatin films were quite transparent, though the films turned whiter when sunflower oil was added due to the light-scattering effect of the emulsion that was formed by the composite gelatin-oil film blends. At the same time, film whiteness increased with the amount of oil added ($p \leq 0.05$) [Table 1]. The whiteness of the films held steady over the 30-day storage period ($p \leq 0.05$).

The film without any added oil was quite clear, with transparency values of nearly 100% (Table 1) [the lower the transparency index value ($A(600)/\text{thickness}$), the higher transparency]. Transparency decreased perceptibly on adding oil ($p \leq 0.05$), but only minimal changes were observed between the films with the different oil concentrations. Bertan,

Tanada-Palmu, Siani and Grosso (2005) also reported increased film opacity with increasing concentrations of hydrophobic substances. Transparency index values for all the samples were unchanged after 30 days of storage.

5 Spectroscopic scanning of the films at wavelengths in both the UV (200-400 nm) and visible (350-800nm) ranges (Figure 1) yielded light absorbance spectra with similar profiles for the different formulations, with a single peak at around 280 nm. Absorbance values increased with oil concentration. This could mean that, when applied to food systems, oil-containing films could be an excellent barrier preventing UV light-induced lipid oxidation. Our results are in agreement
10 with those of Jongjareonrak et al. (2006b), who reported reduced light transmission with fatty acid content in gelatin-fatty acid film blends. Again, after storage for 30 days, there were only slight changes in the light barrier properties, with the sole exception of the 1% added-oil film, which had an appreciably smaller maximum absorbance peak.

15 **FTIR**

In figure 2.A is shown the FTIR spectra from wavenumber 400 to 4000cm⁻¹ and in figure 2.B from 1400 to 1800 cm⁻¹.

Region between 2700 and 3700 cm⁻¹

The gelatin film exhibited a pronounced absorbance band between 3510 and 3550cm⁻¹, due
20 primarily to OH stretching vibrations. According to Yakimets, Wellner, Smith, Wilson, Farhat, & Mitchell (2005), increased absorbance in the 3000–3600 cm⁻¹ range signifies adsorbed water molecules in the gelatin film, possibly brought about by the presence in the film of relatively high amounts of glycerol and sorbitol as plasticizers. After 30 days of storage, new absorbance peaks appeared between 3300 and 3500cm⁻¹. This wavenumber
25 range was largely attributable to stretching vibrations of the amino groups on the gelatin

chains, which may have been more exposed to the molecular surface through conformational changes.

Adding 0.3% and 0.6% sunflower oil to the gelatin film formulation markedly increased the number of bands appearing between 3200 and 3500 cm^{-1} . This could be a direct consequence of the presence of long-chain fatty acids, which would increase the number of CH groups in the films. Various mechanisms, all taking place at the same time, could account for this high number of new peaks.

On the one hand, the stretching vibration of the glyceride ester carbonyl produces a maximum absorbance peak at around 3470 cm^{-1} as reported by Guillen, Ruiz and Cabo (2004) for salmon lipids (3468–3469 cm^{-1} for edible oils). In addition, a decrease in the wavenumber position and broadening of the OH-stretching vibration band could be indicative of a hydrogen bonding interaction between two materials in a composite film (Xie, Zhou, & Qian, 2006). In this connection, as noted earlier by Djagny, Wang, & Xu (2001), the added oil may induce esterification reactions between the hydroxyl functional groups on the gelatin and the fatty acids in the sunflower oil, giving rise to new peaks characteristic of the fatty acid carbon chains attached to the gelatin molecules. Moreover, the carboxyl groups on the fatty acids are able to interact with the glycerol and sorbitol molecules added to the film as plasticizers.

Furthermore, according to Guillen et al. (2004), new bands in the vicinity of 3287 and 3530 cm^{-1} may also arise from the generation, through a primary oxidation step, of functional groups capable of forming hydrogen bonds, such as hydroperoxides, alcohols, and derivatives, thereby increasing the number of absorbance peaks in this spectral range. In this connection, Hayati, Man, Tan, & Aini (2005) reported that the OH stretching band of

hydroperoxides in oxidized soybean oil emulsions resulted in a distinctly visible band at 3417 cm^{-1} . Thus, in our composite gelatin-oil films the presence of numerous absorbance peaks in this frequency interval may be indicative of early oil oxidation in the film, most likely occurring during the drying step.

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In contrast to the film without oil, the infrared spectra of the films containing 0.3% and 0.6% oil showed a high number of peaks in the frequency range between 2800 and 3200 cm^{-1} , which were more intense and more numerous in the film with 0.6% added oil. A band at 3009 cm^{-1} has been associated with the stretching vibration of *cis* olefinic CH double bond in
10 sunflower oil (Vlachos, Skopelitis, Psaroudaki, Konstantinidou, Chatzilazarou, & Tegou, 2006). A similar frequency band has also been reported in lipids from other sources, i.e., at 3012 cm^{-1} in salmon (Guillen et al., 2004) and at 3006–3007 cm^{-1} in pork (Guillen & Cabo, 2004).

15 The film containing 1% oil exhibited a noticeably different infrared spectrum in this frequency range compared with the films with lower proportions of added oil, most of the peaks being recorded at wavenumbers above 3600 cm^{-1} . The mechanism is unknown but could be the result of excess oil in the film hindering infrared absorbance in this frequency range.

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As already observed in the gelatin-only films, after storage for 30 days the oil-containing films displayed a pronounced peak at 3500–3550 cm^{-1} , in large measure attributable to OH stretching vibrations. In addition, a sharp decrease in peak number and intensity was also observable at 2800–3500 cm^{-1} . This may be ascribable to advanced oxidation of the oil in the
25 film. Thus, the expected hydroperoxide-related peaks may be decreased by conversion of this

component into such secondary oxidation products as aldehydes, ketones, alcohols, etc. Furthermore, the lower absorbance intensity at around 3009–3012 cm^{-1} could indicate a decrease in the degree of lipid unsaturation caused by a reduction in the number of *cis* CH double bonds. The films with 0.3% and 0.6% added oil displayed an appreciable increase in absorbance at frequencies ranging from 2700 and 2800 cm^{-1} . This effect could be ascribed to CH stretching of the aldehyde groups. This agrees with Mirghani, Man, Jinap, Baharin, & Bakar (2002), who reported several bands in the 2832 and 2695–2699 cm^{-1} frequency regions in a palm olein system as a result of the accumulation of malonaldehyde and other secondary oxidation products. In addition, according to Guillen et al. (2004) hydroperoxides may also be converted into alcohols and hydroperoxide derivatives, which are recorded at frequencies above 3500 cm^{-1} .

The infrared spectrum of the 1% oil-containing film on day 2 of storage exhibited a distinct absorbance band at frequencies around 2800 cm^{-1} , which, as noted earlier, might be explained by the accumulation of aldehydes subsequent to possible lipid oxidation in the film during drying. In contrast to the findings for the 0.3% and 0.6% oil-containing films, no such accumulation was perceptible after 30 days, suggesting that they later underwent degradation. As will be discussed below, this coincided with increased absorbance intensities in other spectral regions associated with other functional groups involved in lipid oxidation.

Region between 1400 and 1800 cm^{-1}

The gelatin film prepared without any added oil was characterized by the presence of an absorbance band at frequencies between 1700 and 1640 cm^{-1} , i.e., the amide I region of collagen and gelatin, with a principal peak at 1687 cm^{-1} . An amide I component at around

1690 cm^{-1} has been reported in bovine gelatin (Paschalis, Verdelis, Doty, Boskey, Mendelsohn, & Yamauchi, 2001) and also in fish skin gelatin (Muyonga, Cole, & Duodu, 2004) and has been attributed to helices of aggregated collagen-like peptides. There was a second, faint peak appearing in this same spectral region at a wavenumber of 1656 cm^{-1} . This frequency position has been ascribed to the presence of random coils in commercial mammalian gelatin (Prystupa & Donald, 1996) and in Nile perch skin gelatin (Muyonga et al., 2004). Several workers have reported a band at 1660 cm^{-1} related to the presence of triple helical structures in collagen or gelatin (Prystupa & Donald, 1996; Muyonga et al., 2004) and also in mammalian gelatin films (Tsunoda, Sugiura, Sonoyama, Yajima, Ishii, Taniyama & Itoh 2001; Yakimets et al., 2005). However, no such band was clearly detected for the cod gelatin films used in the present study, suggesting an absence of triple helices and a prevalence of random coils and helices of aggregated gelatin chains.

An increase in the number and intensity of the peaks occurring in this frequency range was observable after storage for 30 days. Besides a more intense peak at 1687 cm^{-1} , new peaks and shoulders could be seen between 1703 and 1734 cm^{-1} , attributable to increased intermolecular associations in the form of C=O bonds due to film aging. Similarly, Xu & Ruckenstein (1993) found a peak at 1735 cm^{-1} in the spectra of crosslinked gelatins. These researchers reported that after a few days gelatin gels no longer dissolved in water or melted at 50 C, suggesting that chemical crosslinking had occurred.

In the films with the added sunflower oil, a peak was visible at around 1700 cm^{-1} , and according to (Djagny et al., 2001), it could correspond to carbonyl groups in the ester bonds formed between the fatty acids and the gelatin chains. These authors reported that an absorbance peak for the carbonyl groups in the ester bonds between a pig skin gelatin and

lauric acid occurred at 1700cm^{-1} . However, a peak at 1703 cm^{-1} was also recorded in the film without oil after 30 days of storage, attributed to crosslinking of the gelatin chains. Analysis of the spectra of the oil-containing films after storage for one month revealed an appreciable increase in the intensity of the peak at 1702 cm^{-1} , especially in the films with 0.3% and 0.6% added oil. This suggests a higher degree of gelatin crosslinking in the films with added oil, probably induced by esterification reactions with the fatty acids.

Addition of the sunflower oil also led to the appearance of a band at 1744 cm^{-1} in the film containing 0.3% oil and at 1749 cm^{-1} in the film containing 0.6%. A band at approximately 1746 cm^{-1} has been ascribed to the stretching vibration of the ester carbonyl functional group of the triglycerides in edible oils (Vlachos et al., 2006) and also in lipids in pork adipose tissue (Guillen & Cabo, 2004) and farmed salmon (Guillen et al., 2004). According to these researchers, this band may undergo important changes because of oxidation. After 30 days' storage, the band at around 1746 cm^{-1} appeared as a trace shoulder in the films with 0.3% and 0.6% oil, and a peak shift towards lower frequency values ($\approx 1733\text{ cm}^{-1}$) was observed. This change was attributed to the formation and accumulation in the films of saturated aldehydes or ketones as secondary oxidation products, which have been reported to appear at 1728 cm^{-1} in lipids having diverse origins (Guillen et al., 2004; Vlachos et al., 2006). The film that contained 1% oil also exhibited distinct increases in absorbance at 1739 and 1750 cm^{-1} . The former increase was attributable to the accumulation of secondary oxidation products, as was observed in the films with the lower amounts of lipids. The peak at 1750 cm^{-1} , in contrast, could simply be ascribable to triglyceride ester accumulation, as reported by Vlachos et al. (2006).

Region between 2000 and 2200 cm⁻¹

Although the newly formed gelatin films exhibited practically no absorbance in this spectral range, the films without oil underwent an appreciable increase in band intensity after 30 days. This was attributed to the formation of gelatin crosslinking C≡C and C≡N type bonds as a consequence of film aging. In the films with the added oil crosslinking of this kind was not in evidence, probably because the oil hindered interactions between the peptide chains.

Mechanical properties

Puncture force and deformation values at rupture tended to decrease significantly ($p \leq 0.05$) with the amount of oil in the films (Figure 3). Bertan et al. (2005) reported a decrease in the tensile strength of films made from bovine hide gelatin with the addition of hydrophobic substances, though the percentage elongation increased. Jongjareonrak et al. (2006b) also found a decrease in tensile strength in a fish gelatin film with the addition of fatty acids, though tensile strength increased with the concentration of sucrose fatty acid esters.

In the present study the puncture force of the cod-skin gelatin films without added oil was lower than the values reported by Gómez-Guillén et al. (2007) for tuna gelatin films (≈ 6 N) and by Sobral & Habitante (2001) for pigskin gelatin films (14 N) prepared using comparable amounts of plasticizer. However, the puncture deformation, and hence film flexibility, was much higher than in the above-mentioned tuna ($\approx 14\%$) and pigskin ($\approx 3.5\%$) gelatin films. These differences could be explained by the origin of the gelatin and the plasticizers used. In this respect, glycerol is well known to increase film flexibility (Cuq et al., 1995; Gennadios, Weller, Hanna, & Froning, 1996). Here, however, this was most likely not the cause, since the amount of glycerol used was lower as compared to the study on tuna gelatin films. Thus, the

most likely explanation was chemical and structural differences among the gelatins. Gómez-Guillén, Turnay, Fernandez-Díaz, Ulmo, Lizarbe and Montero (2002) discussed the rheological characteristics (viscoelasticity and gel strength) and chemical/structural properties (amino acid composition, molecular weight distribution, and triplex helix formation) of gelatins from the skin of different species as factors responsible for differences in behavior. Thus, cod-skin gelatin had a lower concentration of alanine and imido acid along with a small degree of proline hydroxylation, whereas cod and hake gelatin presented a low α_1/α_2 ratio (~ 1) compared with gelatins from flatfish species (sole and megrim).

After 30 days' storage, the puncture force values decreased significantly, even in the film without any added oil. Film deformation also fell sharply in the films containing the highest amounts of oil (0.6% and 1.0%).

Water vapor permeability

The water vapor permeability of the cod-skin gelatin film without added oil was $4.87 \times 10^{-8} \text{ g} \cdot \text{mm} \cdot \text{h}^{-1} \cdot \text{cm}^{-2} \cdot \text{Pa}^{-1}$ (Table 1). This film was about two times more permeable to water than a tuna gelatin film (Gómez-Guillén et al., 2007). Using pig skin gelatin, Sobral and Habitante (2001) reported a linear increase in film WVP from 1.8 to $3.2 \times 10^{-8} \text{ g} \cdot \text{mm} \cdot \text{h}^{-1} \cdot \text{cm}^{-2} \cdot \text{Pa}^{-1}$, between 15 and 65 g sorbitol / 100g of gelatin. Similar behavior has recently been reported for fish gelatin films (Jongjareonrak et al., 2006a), and it was pointed out that the differences observed between gelatin films from two species (*Priacanthus macracanthus* and *Lutjanus vitta*) could depend on differences in the protein composition, emulsifying protein composition, and emulsifying properties of the gelatins, while hydrophobicity (lower WVP) increased with the addition of long-chain fatty acids.

In the oil-containing films, WVP values dropped significantly with the amount of sunflower oil added. Adding lipids can increase the hydrophobicity of the film matrix depending on the kind and amount of lipid (Bertan et al., 2005). A tendency of the WVP to decrease has also been reported in fish gelatin films containing fatty acids and sucrose fatty acid esters (Jongjareonrak et al., 2006b). However after 30 days of storage, this tendency is lost (e.g., permeability increases), though there were no significant differences between the film formulations. This could be the result of a certain degree of film matrix disruption after storage, which would agree with the declining mechanical property values.

10 **Film solubility**

The water solubility of gelatin films is very high around 88 % (Table 2), and adding oil at >0.6% lowered the solubility ($p \leq 0.05$). The higher the oil concentration in the film, the lower the protein fraction in the soluble matter ($p \leq 0.05$), probably because interactions between the protein and the oil in the film contributes to protein insolubilization. Non-protein soluble matter increased with film oil concentration ($p \leq 0.05$), so protein–oil interactions appear to be conducive to the release of plasticizer. Considerably lower solubility (around 30%) has been reported in films based on gelatin of bovine origin (Bertan et al., 2005), this high difference largely attributable to structural differences and differences in amino acid composition. Bertan et al. (2005) also observed that adding hydrophobic substances brought about a significant increase in the soluble matter in the films at all concentration ranges tested as compared to gelatin/triacetin (15%) films.

After 30 days' storage water solubility increased in the gelatin only films and decreased in the gelatin/oil film blends ($p \leq 0.05$) because of higher interactions between components, specially, in the formulation with higher amount of oil (1%) which the non-protein matter proportion

decreased with storage time ($p \leq 0.05$), perhaps because oil-protein interactions are more stable than plasticizer-protein interactions.

Around 77.50% of the protein was soluble in the gelatin only films, a proportion that fell by more than 20% with the addition of 1% oil to the films. This behavior was constant during the 30-day storage period ($p \leq 0.05$), as already mentioned, suggesting that protein interactions in the matrix was stable.

To summarize, the structure of cod-skin gelatin in the film matrix exhibited a prevalence of helices of aggregated collagen-like peptides along with a certain proportion of random coils. The absence of a triple helix structure is largely a direct consequence of the lower hydroxyproline content reported in cold-water fish collagen, resulting in poorer rheological properties of the gelatin compared with those of gelatins from warm-blooded animals. Following the addition of sunflower oil, FTIR spectra revealed lipid-protein interactions that could be responsible for the poorer rheological properties and for the lower water vapor permeability and water solubility of the composite gelatin-oil films. Gelatin crosslinking took place during a 30-day storage period and appeared to be heightened by lipid-protein interactions in the sunflower oil-containing films. Moreover, lipids underwent appreciable oxidation in the gelatin only films, and in the case of the formulation with the highest proportion of oil was distinctly discernible from the earliest stage of storage. Both gelatin crosslinking and lipid oxidation may disrupt the film matrix during aging, leading to film insolubilization and a certain reduction in mechanical and water barrier properties. The light barrier properties of the films were enhanced by adding the oil and remained stable over the storage period. These light barrier properties, together with the lower solubility, were the main advantages of the composite cod-skin gelatin/sunflower oil films. Thus, according to the

results, adding sunflower oil alters the properties of cod gelatin, yielding a more insoluble film, although permeability was not diminished. After storage for 30 days, the optical properties, altered mechanical properties and water vapor permeability held stable, but the insolubility remains.

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Acknowledgements

This research was supported by the Spanish Ministry of Education and Science under project AGL2005-02380/ALI and project CYTED XI.20.

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Film (% oil)	Whiteness		Transparency		Water permeability ($\times 10^{-8}$)	
	Before	After	Before	After	Before	After
0	18.74 $\pm$ 0.27 ^{a/x}	16.95 $\pm$ 0.43 ^{a/x}	0.30 $\pm$ 0.11 ^{a/x}	0.22 $\pm$ 0.02 ^{a/x}	4.31 $\pm$ 0.87 ^{a/x}	4.24 $\pm$ 0.72 ^{a/x}
0.3	25.25 $\pm$ 0.64 ^{b/x}	23.94 $\pm$ 1.22 ^{b/x}	5.12 $\pm$ 0.52 ^{b/x}	4.52 $\pm$ 0.76 ^{b/x}	3.70 $\pm$ 0.70 ^{a/x}	6.68 $\pm$ 0.74 ^{a/y}
0.6	27.70 $\pm$ 0.46 ^{c/x}	27.24 $\pm$ 0.88 ^{c/x}	7.41 $\pm$ 0.61 ^{bd/x}	7.17 $\pm$ 0.88 ^{c/x}	3.10 $\pm$ 0.48 ^{a/x}	5.79 $\pm$ 0.44 ^{a/y}
1	31.67 $\pm$ 1.09 ^{d/x}	28.69 $\pm$ 0.96 ^{c/x}	9.51 $\pm$ 0.75 ^{d/x}	8.00 $\pm$ 0.41 ^{c/x}	3.24 $\pm$ 1.16 ^{a/x}	6.11 $\pm$ 0.72 ^{a/y}

Table 1. Changes in whiteness, transparency and water permeability for cod-skin gelatin-based films with differing concentrations of added sunflower oil before and after storage. The different letters (a,b,c) indicate significant differences ($p \leq 0.05$) between the before and after samples of each film formulation, and the different letters (x,y) indicate significant differences ($p \leq 0.05$) between batches on each sampling date.

Film (% oil)	Total soluble matter (%)		Non-protein fraction (%)		Protein fraction (%)		Ps/Pt %	
	Before	After	Before	After	Before	After	Before	After
0	87.66 ± 0.46 ^{a/x}	93.75 ± 0.42 ^{a/x}	28.92 ± 2.91 ^{a/x}	34.46 ± 1.41 ^{a/x}	58.74 ± 2.91 ^{a/x}	59.29 ± 1.41 ^{a/x}	77.50 ± 3.85 ^{a/x}	78.80 ± 9.49 ^{a/x}
0.3	87.84 ± 1.54 ^{a/x}	85.08 ± 0.82 ^{a/y}	35.52 ± 0.56 ^{ab/x}	32.77 ± 2.36 ^{a/x}	52.32 ± 0.56 ^{ab/x}	52.31 ± 2.36 ^{b/x}	69.43 ± 7.33 ^{ab/x}	69.10 ± 8.61 ^{ab/x}
0.6	85.73 ± 0.76 ^{a/x}	78.78 ± 1.11 ^{a/x}	35.33 ± 1.07 ^{ab/x}	30.35 ± 1.53 ^{ab/x}	50.40 ± 1.07 ^{b/x}	48.43 ± 1.53 ^{b/x}	66.56 ± 4.89 ^{ab/x}	63.59 ± 3.22 ^{ab/x}
1	80.26 ± 0.07 ^{b/x}	66.39 ± 2.97 ^{a/y}	39.60 ± 3.64 ^{b/x}	26.03 ± 1.37 ^{b/y}	40.66 ± 3.64 ^{c/x}	40.36 ± 1.37 ^{c/x}	53.20 ± 7.16 ^{b/x}	52.73 ± 4.74 ^{b/x}

Table 2. Changes in total soluble matter (protein and non-protein fractions) and in the soluble protein (Ps) to total protein (Pt) ratio for cod-skin gelatin-based films with differing concentrations of added sunflower oil before and after storage. Meaning of letters is the same as those in table 1.

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Figure 1. Absorbance of cod-skin gelatin-based films with differing concentrations of added sunflower oil (0 %: circle, 0.3 % triangle, 0.6 % diamond, 1 % square) at wavelengths ranging from 200 to 700 nm before (blank bar) and after (solid bar) storage.

5 **Figure 2.** FTIR spectra of cod-skin gelatin-based films with differing concentrations of added sunflower oil between wavenumbers: (A) 4000 and 400 cm^{-1} and (B) 1800 and 1300 cm^{-1} before and after storage.

10 **Figure 3.** Puncture force (A) and puncture deformation (B) of cod-skin gelatin-based films with differing concentrations of added sunflower oil before (blank bar) and after (solid bar) storage. Error bars represent the standard deviation of six samples.