EFFECTS OF GELATIN ORIGIN, BOVINE-HIDE AND TUNA-SKIN, ON THE
PROPERTIES OF COMPOUND GELATIN-CHITOSAN FILMS

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ABSTRACT

With the purpose to improve the physico-chemical performance of plain gelatin and chitosan films, compound gelatin-chitosan films were prepared. The effect of the gelatin origin (commercial bovine-hide gelatin and laboratory-made tuna-skin gelatin) on the physico-chemical properties of films was studied. The dynamic viscoelastic properties (elastic modulus $G'$, viscous modulus, $G''$ and phase angle) of the film forming solutions upon cooling and subsequent heating revealed that the interactions between gelatin and chitosan were stronger in the blends made with tuna-skin gelatin than in the blends made with bovine-hide gelatin. As a result, the fish gelatin-chitosan films were more water resistant (~18% water solubility for tuna vs 30% for bovine) and more deformable (~68%...
breaking deformation for tuna vs 11% for bovine) than the bovine gelatin-
chitosan films. The breaking strength of gelatin-chitosan films, whatever the
gelatin origin, was higher than that of plain gelatin films. Bovine gelatin-chitosan
films showed a significant lower water vapor permeability (WVP) than the
corresponding plain films, whereas tuna gelatin-chitosan ones were only
significantly less permeable than plain chitosan film. In spite of gelatin-chitosan
interactions, all the chitosan-containing films exhibited antimicrobial activity
against S. aureus, a relevant food poisoning. Mixing gelatin and chitosan may
be a means to improve the physico-chemical performance of gelatin and
chitosan plain films, especially when using fish gelatin, without altering the
antimicrobial properties.

Key words: bovine-hide gelatin, chitosan, fish gelatin, physico-chemical
properties, S. aureus, edible films
As a consequence of the problems associated with disposal of packaging plastics, there is a growing interest concerning the development of biodegradable materials. These newer materials can be obtained from several sources, which include proteins (collagen/gelatin, soya, whey, wheat, etc), polysaccharides (chitosan, starch, cellulose) and lipids (wax, fatty acids) (Gennadios, Hanna & Kurth, 1997; Tharanathan, 2003). Although the entire substitution of petrochemical polymers with bioplastics may not be possible due to the worse physicochemical properties of the latter, it is necessary to research on the improvement of such properties, as well as on the new applications, in order that bioplastics corner the market and, therefore, the traditional synthetic plastics are partially substituted.

Gelatin is a protein with a broad range of functional properties and applications, including film-forming ability. Bovine and porcine wastes are the most frequent sources to obtain gelatin of good quality. However other sources of gelatin are becoming increasingly relevant, such as fish bones and skins (Gomez-Guillen, Turnay, Fernandez-Diaz, Ulmo, Lizarbe & Montero, 2002). Whatever the species of origin, gelatin films fail in terms of mechanical properties and water resistance, which may limit its field of application. Several strategies have been used to improve the physical performance of gelatin films. These include chemical or enzymatic treatments (Cao, Fu & He, 2007; Chiou et al., 2008; de Carvalho & Grosso, 2004; Spanneberg, Osswald, Kolesov, Anton, Radusch & Glomb, 2010; Zhang et al., 2010)) and mixing with other polymers, as composite films may be designed to take advantages of pure components (Garcia, Pinotti, Martino & Zaritzky, 2004). For example mixing with apolar...
components such fatty acids or oils reduces water vapour transmission rate (Jongjareonrak, Benjakul, Visessanguan & Tanaka, 2006; Limpisophon, Tanaka & Osako, 2010; Perez-Mateos, Montero & Gomez-Guillen, 2009). Furthermore polymers can establish new bonds that may enhance the properties of the resulting materials (Denavi, Perez-Mateos, Anon, Montero, Mauri & Gomez-Guillen, 2009; Sionkowska, Wisniewski, Skopinska, Kennedy & Wess, 2004). Gelatin has been blended with casein (Chambi & Grosso, 2006), pectin (Liu, Liu, Fishman & Hicks, 2007), chitosan (Arvanitoyannis, Nakayama & Aiba, 1998; Kolodziejska & Piotrowska, 2007; Kolodziejska, Piotrowska, Bulge & Tylingo, 2006), starch (Arvanitoyannis et al., 1998) and soy protein (Denavi et al., 2009), achieving in general an improvement of its physical performance.

Chitosan (poly b-(1,4)-N-acetyl-D-glucosamine) polymer is industrially produced by chemical deacetylation of the chitin found in arthropod exoskeletons. Chitosan is largely utilized not only due to its film forming capability but also because of its antimicrobial properties (Helander, Nurmiaho-Lassila, Ahvenainen, Rhoades & Roller, 2001; Jung, Youn, Lee, No, Ha & Prinyawiwatkul, 2010). For this reason, the use of chitosan as an edible coating or film to extend the shelf-life of foods and inhibit pathogens is of growing interest (Aider, 2010; Gomez-Estaca, Montero, Gimenez & Gomez-Guillen, 2007; Lopez-Caballero, Gomez-Guillen, Perez-Mateos & Montero, 2005). Specifically, chitosan has been found to be active against S. aureus (Fernandes et al., 2008). Some S. aureus strains are able to produce staphylococcal enterotoxins and are the causative agents of staphylococcal food poisonings (Le Loir, Baron & Gautier, 2003). The antimicrobial action of chitosan is supposed to be derived from the positive charge that amino groups present at
acidic pH (below 6.5), which lead to cellular membrane depolarization and microbial death. However the main drawback of chitosan under this condition is its intrinsic water solubility, which limits the utilisation as self-standing packaging material. Attempts to increase the water resistance of chitosan have been made including crosslinking with glutaraldehyde, glyoxal or epichlorohydrin (Suto & Ui, 1996; Tual, Espuche, Escoubes & Domard, 2000; Zheng, Du, Yu & Xiao, 2000). However, in a subsequent work (Tang, Du & Fan, 2003) it was confirmed that the antimicrobial capacity of chitosan films diminishes with an increase in the cross-linking. Mixing of chitosan with other biopolymers to obtain more insoluble matrices has been proved as an effective means to improve the water resistance of chitosan maintaining its antimicrobial properties (Fernández-Saiz et al., 2008). Chitosan has also been blended with other biopolymers such as methylcellulose and starch resulting in an improvement of its physico-chemical properties (Garcia et al., 2004; Garcia, Pinotti & Zaritzky, 2006).

According to Taravel & Domard (1995), the interactions between gelatin and chitosan are produced by both electrostatic and hydrogen bonding, with the blends taking on new physical properties and thus becoming suited to potential new applications (Sionkowska et al., 2004). Accordingly, combining these biopolymers seems to be a promising way to enhance the physical properties of the resulting materials (Huang, Onyeri, Siewe, Moshfeghian & Madihally, 2005; Mao, Zhao, Yin & Yao, 2003). There are some reports dealing with the physico-chemical properties of compound fish gelatin-chitosan films as well as its improvement by adding different crosslinkers (Arvanitoyannis et al., 1998; Kolodziejska et al., 2007; Kolodziejska et al., 2006). However there is no previous report on the effect of the gelatin origin on the film forming ability and
the physico-chemical properties of the gelatin-chitosan admixtures. The objective of the present work has thus been to study the physico-chemical properties of compound gelatin-chitosan films as function of gelatin origin: a commercial bovine-hide gelatin and a laboratory-obtained tuna-skin gelatin. The antimicrobial activity of the resulting films over *S. aureus* was also evaluated.

**MATERIALS AND METHODS**

**Materials**

Tuna-skin gelatin was extracted according to a patented method (Gómez-Guillén & Montero, 2001). A commercial type A bovine-hide gelatin (Bloom 200/220) was purchased from Sancho de Borja S.L. (Zaragoza, Spain). Chitosan from shrimp shells (85 % deacetylated; 141,000 Da) was purchased from Guinama (Valencia, Spain). Glycerol and sorbitol were from Panreac (Barcelona, Spain).

For the microbial analyses, brain heart infusion (BHI) broth was purchased from Oxoid (Basingstoke, UK) and bacteriological agar were from Scharlau (Barcelona, Spain). *Staphylococcus aureus* (CECT 240) was obtained from the Spanish Type Culture Collection (Valencia, Spain).

**Preparation of the film-forming solutions and films**

Seven different formulations were prepared: The batches were: B (bovine-hide gelatin), T (tuna-skin gelatin), Ch (chitosan), B-Ch 0.75% (bovine-hide gelatin plus 0.75% chitosan), T-Ch 0.75% (tuna-skin gelatin plus 0.75% chitosan), B-Ch 1.5% (bovine-hide gelatin plus 1.5% chitosan), T-Ch 1.5% (tuna-skin gelatin
plus 1.5% chitosan). The gelatin film-forming solutions (solutions B and T) were prepared at a concentration of 4 g gelatin/100 ml of distilled water. The chitosan film-forming solution (solution Ch) was prepared by dissolving chitosan in a proportion of 3 g/100 mL in 0.15 M acetic acid. The gelatin-chitosan film-forming solutions (solutions B-Ch 0.75%, B-Ch 1.5%, T-Ch 0.75%, T-Ch 1.5%) were prepared by mixing a 4% bovine-hide or tuna-skin gelatin solution with a 1.5% or 3% solution of chitosan in 0.15 M acetic acid, in a proportion of 1:1 (v/v), to obtain a film-forming solution containing 2% gelatin and 0.75% or 1.5% chitosan, respectively. A mixture of glycerol and sorbitol was added to the film-forming solutions as previously described (Thomazine, Carvalho & Sobral, 2005) at a concentration of 0.15 g each per gram of the total polymeric agent (gelatin and/or chitosan). Plasticizing molecules of this kind reduce inter-chain interactions, improving film flexibility and, consequently, film handling.

The film-forming solutions, which had a pH of 4.6 ± 0.3 in all cases, were warmed and stirred at 45 ºC to obtain a good blend. The films were made by casting an amount of 40 mL onto plexiglass plates (12 x 12 cm) and drying at 45 ºC in a forced-air oven for 15 h to yield a uniform thickness of 100 μm ± 11 in all cases except the 0.75% chitosan formulations, which was 80 μm ± 7 thick.

Prior to the determinations, the films were conditioned at 22 ºC over a saturated solution of NaBr (58% RH) in desiccators for 3 d.

Physical characterization of the film-forming solutions

Dynamic viscoelastic analysis of the film-forming solutions was carried out on a Bohlin CSR-10 rheometer/rotary viscometer (Bohlin Instruments Ltd.,
Gloucestershire, UK) using a cone-plate geometry (cone angle = 4º, gap = 0.15 mm). Cooling and heating from 40 to 6 ºC and back to 40 ºC took place at a scan rate of 1 ºC/min, a frequency of 1 Hz, and a target strain of 0.2 mm. The elastic modulus (G'; Pa), viscous modulus (G’’; Pa) and phase angle (º) were determined as functions of temperature. Two determinations were performed for each sample, with an experimental error of less than 6% in all cases.

The film-forming solutions were poured into glasses 2.3 cm in diameter and 3.6 cm in height and left to mature in a refrigerator at 2 ºC for 16-18 h. Gel strength at 9±1 ºC was determined on an Instron model 4501 Universal Testing Machine (Instron Co., Canton, MA, USA) with a 100 N load cell, a crosshead speed of 1 mm/s, and a flat-faced cylindrical plunger 1.27 cm in diameter. The maximum force (g) reading was taken when the plunger had penetrated 4 mm into the gelatin gels.

**Physico-chemical characterization of the films**

**Mechanical properties**

A puncture test was performed to determine the maximum strength and deformation of the films at the breaking point. The films were placed in a cell 5.6 cm in diameter and perforated to the breaking point using an Instron model 4501 Universal Testing Machine (Instron Co., Canton, MA, USA) with a round-ended stainless-steel plunger 3 mm in diameter at a crosshead speed of 1 mm/s and a 100 N load-cell. Breaking strength was expressed in N and breaking deformation in percent as previously described (Sobral, Menegalli, Hubinger & Roques, 2001). All determinations were the means of at least five measurements.
Thermal properties

Calorimetric analysis was performed using a model TA-Q1000 differential scanning calorimeter (TA Instruments, New Castle, DE, USA) previously calibrated by running high purity indium (melting point 156.4 °C; enthalpy of melting 28.44 W/g). Sample amounts on the order of 10 mg ± 0.002 weighed out using a Sartorious model ME235S electronic balance (Goettingen, Germany) were tightly encapsulated in aluminum pans and scanned under dry nitrogen purge (50 mL/min). Freshly conditioned films were rapidly cooled to 0 °C and scanned between 0 and 90 °C, at a rate of 10 °C/min. Glass transition temperatures, Tg (°C), were determined on first heating scans consistently with the other physical determinations carried out on the same original (after conditioning at 58% RH) material. Tg was estimated as the midpoint of the line drawn between the temperature at the intersection of the initial tangent with the tangent through the inflection point of the trace and the temperature of the intersection of the tangent through the inflection point with the final tangent. Tg data were the mean values of at least three replications per film sample.

Water solubility

Film portions measuring 4 cm² were placed in aluminum capsules with 15 mL of distilled water and shaken gently at 22 °C for 15 h. The solution was then filtered through Whatman no. 1 filter paper to recover the remaining undissolved film, which was desiccated at 105 °C for 24 h. Film water solubility was calculated using the equation $FS (%) = ((W_o-W_f)/W_o) \cdot 100$, where $W_o$ was the initial weight of the film expressed as dry matter and $W_f$ was the weight of the
undissolved desiccated film residue. All determinations were carried out in triplicate.

Water vapour permeability

Water vapour permeability (WVP) was determined by a gravimetric method. Films were attached over the openings of cells (permeation area = 15.9 cm$^2$) containing silica gel, and the cells were placed in desiccators with distilled water at 22 ºC. The cells were weighed daily for 7 d. Water vapour permeability was calculated using the equation $WVP = w \cdot x \cdot t^{-1} \cdot A^{-1} \cdot \Delta P^{-1}$, where $w$ was weight gain (g), $x$ film thickness (mm), $t$ elapsed time (h) for the weight gain, and $\Delta P$ the partial vapour pressure difference between the dry atmosphere and pure water (2642 Pa at 22 ºC). Results have been expressed as g·mm·h$^{-1}$·cm$^{-2}$·Pa$^{-1}$. All determinations were carried out in duplicate.

Microbial analysis

For microbial analysis, prior to film casting, the pH of all the film-forming solutions was adjusted to 6. This pH was considered suitable to enable any potential in vitro antibacterial effects of the film-forming solutions and/or films to be attributed to the chitosan rather than to the pH. The antimicrobial activity of both the film-forming solutions and the films was determined against S. aureus. The bacteria were stored at -80 ºC in BHI broth with 25 % glycerol until use. Strain was grown in BHI (Oxoid) broth at 37 ºC overnight to a final bacterial concentration of $10^7$-$10^8$ cfu/mL. Spread plates of brain heart agar were prepared. Sterile filter paper disks immersed in the film-forming solutions or
pieces of the films themselves were placed on the plate surfaces and incubated at 37 °C for 24 h. The appearance of a clear area below or around the film or filter paper disks was deemed to be positive for antimicrobial activity.

Statistical analysis

Statistical tests were performed using the SPSS® computer program (SPSS Statistical Software, Inc., Chicago, IL, USA). One-way analysis of variance was carried out. Differences between pairs of means were compared using a Tukey test. The level of significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Film-forming solutions

The changes in the viscoelastic properties of the film-forming solutions during cooling and subsequent heating have been plotted in Figure 1. Solution B had a higher elastic modulus ($G'$) value and had thermal transition (gelling and melting) points at higher temperatures than solution T. These findings are indicative both of bovine-hide gelatin's greater capacity to refold into triple-helix chains as it cools and its higher thermostability. These findings were fully expected and wholly consistent with the different origins of the gelatins, as reported previously (Joly-Duhamel, Hellio, Ajdari & Djabourov, 2002; Joly-Duhamel, Hellio & Djabourov, 2002). Bovine-hide gelatin has a higher imino acid (Pro+Hyp) content than tuna-skin gelatin (210 residues in bovine gelatin vs. 185 residues in tuna gelatin) (Gomez-Estaca, Montero, Fernandez-Martin &
Gomez-Guillen, 2009), and this is well known to be related to enhanced physical properties and higher thermostability of the resulting gelatin gels (Ledward, 1986; Norland, 1990).

Adding chitosan modified the viscoelastic properties of both gelatins. The maximum G' values were considerably lower than the values for solutions B and T, and the thermal transition points also occurred at lower temperatures. This is indicative of a pronounced loss of the gelatin's ability to refold into triple-helix chains in the presence of chitosan. A decrease in percentage renaturation of food grade gelatin by incorporation of chitosan has been previously reported (Arvanitoyannis et al., 1998). Adding chitosan at the higher concentration led to a substantial increase in the viscous modulus (G'') during cooling. Furthermore, phase angle values at both concentrations were higher than the values for the corresponding gelatin solutions at temperatures below 10 °C. Both these effects also indicate that chitosan interferes with protein network formation as a result of gelatin-chitosan interactions. These effects were considerably more marked for the tuna-skin gelatin, especially at the higher chitosan concentration, as indicated by the higher increase in G'' and the not so steep slope of the phase angle during cooling and subsequent heating. Thus, the chitosan interacted more with the tuna-skin gelatin than with the bovine-hide gelatin. Interactions between chitosan and collagen have previously been described (Sionkowska et al., 2004) and were also reflected by an increase in the viscosity values. In the present experiment the collagen had previously been denatured to obtain soluble gelatin, presumably heightening this interaction. The interactions between gelatin and chitosan are produced by both electrostatic and hydrogen bonding (Taravel & Domard, 1995). The former are a consequence of the
different charges of gelatin, an anionic biopolymer, and the cationic chitosan. The latter occur extensively between the \(-\text{COOH}, -\text{NH}_2, \text{and} -\text{OH}\) groups on the amino acids in the gelatin and the \(-\text{OH} \text{and} -\text{NH}_2\) groups on the chitosan, and are particularly notable at low temperatures. Regarding the contribution of electrostatic interactions, it should be noted that both gelatins are type A, i.e., they had been subjected to an acidic pre-treatment, therefore both are presumed to have similar isoelectric points, being net positively charged at pH 6. The most likely explanation for such a different interaction could be the differing imino acid content (Pro+Hyp), which was considerably lower in the tuna-skin gelatin. The abundance of pyrrolidine iminoacid content (Pro, and especially Hyp), is well known to be directly implicated in the gelling mechanism promoting the formation of junction zones of triple collagen-like helices (Ledward, 1986). In this connection, the lower iminoacid content in the tuna gelatin would lead to a less extensive self-aggregation of the gelatin chains, which might have contributed to an increment of the gelatin-chitosan interactions.

The gel strength determinations (Figure 2) carried out on the cold-matured film-forming solutions revealed a decrease in the mechanical properties of the gelatin gels when chitosan was added, which is ascribed not only to a dilution effect of the gelatin in the film-forming solution but also to the gelatin-chitosan interactions. The effect was more pronounced at the higher concentration of added chitosan, indicating that chitosan not only interfered with nucleation point formation but also with triple-helix chain growth during cold maturation. No differences were found between both types of gelatin.
Films

Warm-water fish gelatins have been reported to have quite similar properties to mammalian gelatins (Gilsenan & Ross-Murphy, 2000), and the tuna-skin gelatin films showed Tg values that were very slightly lower (within experimental error) than the bovine-hide gelatin films (Figure 3a, curves B and T; Table 1), in agreement with previous reports (Gomez-Estaca et al., 2009). Films made from various chitosans (different degrees of deacetylation, OH and amino groups, crystallinity, etc.) in the presence of various amounts of different hydrophilic plasticizers have been reported to exhibit very high (>>100ºC) glass transition temperatures (Suyatma, Tighzert & Copinet, 2005). The chitosan films tested here, however, had a rather low glass transition temperature, even lower than the values for the two gelatins (Table 1); as the ratio between the polymer and the glycerol plus sorbitol equiproportional mixture remained constant in all cases, the considerable higher water content in Ch (~22%) was presumably a main responsible. The value seemed to be consistent with a phase diagram previously published (Lazaridou & Biliaderis, 2002) for a system comprising 70 % chitosan and 30 % sorbitol at different moisture contents.

As discussed above, chitosan has been reported to interact molecularly with collagen by forming a wide range of blends where new hydrogen bonding networks appear, the triple helical structure of the collagen being replacing as the chitosan level increases (Sionkowska et al., 2004). In other work, the formation of gelatin-chitosan polyelectrolyte complexes has been shown to give rise to a decrease of the crystallinity of the system (Yin, Yao, Cheng & Ma, 1999). Other factors affecting the properties of gelatin-chitosan films are the evaporation temperature and the presence of plasticizers (water and polyols).
(Arvanitoyannis et al., 1998). Furthermore, the interactions between gelatin and chitosan are supposed to be dependent on the physical and chemical properties of the gelatin, which vary considerably with gelatin origin. As a general rule, mammalian gelatins afford better physical properties and thermostability than fish gelatins, and there are, moreover, appreciable differences among gelatins from different fish species (Gomez-Guillen et al., 2002). The main factors determining the physical properties of a gelatin and hence gelatin quality are the amino acid composition and the molecular weight distribution (Gomez-Guillen et al., 2002). Mammal’s gelatins are typically richer in imino acids whereas the average molecular weight highly depends on the extraction procedures. Both these factors will presumably affect the interactions between the gelatin and chitosan. Thus, the physico-chemical properties, and ultimately the potential applications of the resulting materials will all be closely related to the properties of the polymer admixture employed.

In the present work, the glass transition temperatures of compound films were higher than those for the single components, indicating some level of interaction/association. At each of the two chitosan levels, the Tg values for both series of compound gelatin-chitosan blends were quite close and at the lower Ch concentration were nearly 6 °C higher than for the gelatins alone (Table 1). Augmenting the chitosan component resulted in a further increase of around 3 °C in the Tg. Higher water contents may produce higher plasticization effects in the blended films by an increase in the macromolecules mobility. However, it appeared clearly overcame by the increase matrix density effect caused by the strong associations between gelatins and chitosan, with the final result of glass transition processes occurring at higher temperatures in the
blends than in the single films. Initially, the polyols mixture is highly hygroscopic so that water may plasticize both the low-molecular-weight mixture and the high-molecular-weight components. The water-alone contribution to the plasticization of the different polymeric blends seemed hardly difficult to being separated from the whole plasticizer system. Additionally the humidity values for the B-Ch and T-Ch films were quite similar; small differences in water content could be basically modulated by the glycerol plus sorbitol plus water entire system, so that small variations in water are expected not to play an essentially differential role in the plasticization process and Tg values of the complex films. Therefore, the glass transition behaviour of the B- and T-type compounded films with Ch reasonably showed a paralleled evolution.

All these findings were consistent with the thermal scan data obtained for the corresponding gels by rheometry, as shown in Figure 1 (bottom, curves a and b). At both Ch levels hysteresis of the sol-gel-sol transitions increased in the film-forming solutions of both gelatin-chitosan blends relative to those in the corresponding gelatins. Adding the larger amount of chitosan increased the complexity of the gel-sol and sol-gel profiles of both systems, particularly T-Ch1.5, which could be interpreted as a certain level of phase separation in the systems. Gelatin-rich phases (more frequent in the T systems than in the B systems) have higher gel-sol and sol-gel transition temperatures and, conversely, chitosan-rich phases have lower gel-sol and sol-gel transition temperatures. Although this gelling behaviour is not necessarily transferred to the respective films, DSC did not reveal any phase separation in the Ch1.5 systems.
Table 1 lists the glass transition temperature, mechanical properties (breaking strength and breaking deformation), solubility, and water vapour permeability of the different films. Breaking strength and water solubility values were similar for both gelatin films (B and T), a result attributable to the presence of very high-molecular-weight aggregates (Gomez-Estaca et al., 2009). In comparison, the chitosan film (Ch) was stronger than either of the gelatin films (B and T), although it was also more soluble in water. Perhaps the most interesting feature was that the breaking deformation value for film T was ~10 times higher than the values for films B and Ch. Deformation differences among gelatin films have been previously attributed to different imino acid contents, giving bovine-hide gelatin a higher degree of molecular rigidity (Gomez-Estaca et al., 2009). The water vapour permeability values showed all the films to be quite permeable, entirely consistent with films prepared from biopolymers plasticized with polyols. This has been reported to be also a result of increases in the free volume between polymer chains caused by a reduction in intermolecular attractive forces, making the polymer network less dense and thus more permeable (Cuq, Gontard, Cuq & Guilbert, 1997). However, some differences among the formulations were found. Thus, the Ch film was more permeable to water vapour than the B and T films. Gelatin origin was also a factor, T being less permeable than B, again attributable to both the amino acid profile and the molecular weight distribution (Avena-Bustillos et al., 2006; Gomez-Estaca et al., 2009). Comparisons among different authors are somewhat difficult because of the different film manufacture and measurement procedures. There is a study in which the WVP of compound fish gelatine-chitosan films as well as that of the pure components were studied (Kolodziejska et al., 2007). In this work neither
the gelatine and the chitosan pure films nor the compound ones were significantly different. In other study (Arvanitoyannis et al., 1998) mammal gelatine and chitosan were blended at a ratio 1:1, plasticized with different amounts and type of plasticizers and dried at different temperatures, resulting in WVPs ranging from $1.1 \times 10^{-11}$ g m/m² s Pa, which are three orders of magnitude lower than ours. In this case the single components were not analyzed.

On the whole, the physico-chemical properties of the compound gelatin-chitosan films were more appropriated for practical purposes than the properties of the films made from a single biopolymer only (films B, T, and Ch). As a general rule, the compound films had breaking strengths similar to the values for the Ch films ($p \leq 0.05$), the strongest films, and significantly higher ($p \leq 0.05$) than the values for the gelatin films. Breaking deformation either stayed the same (B-Ch blends) or decreased (T-Ch blends) significantly ($p \leq 0.05$) compared with the respective gelatin films. In spite of the decrease of breaking deformation in T-Ch films compared to T ones, these mixtures showed a higher breaking deformation than B, Ch and B-Ch films. Solubility of the compound films was significantly lower ($p \leq 0.05$) compared with that of chitosan films whatever the gelatin employed. Furthermore, in tuna-skin gelatin-chitosan mixtures the solubility was significantly ($p \leq 0.05$) lower than that of the tuna-skin gelatin. Water vapour permeability of the compound films decreased significantly ($p \leq 0.05$) compared with the single biopolymer component films Ch and B, and was similar to the values for T film, the one with the lower WVP among plain films, in all cases. All the alterations in the physico-chemical properties of the films can be attributed to the gelatin-chitosan interactions.
previously reported by different researchers (Sionkowska et al., 2004; Taravel et al., 1995; Yin et al., 1999) and confirmed in this study by the results for the viscoelastic properties and gel strength of the film-forming solutions. The interactions were observed to be stronger in the case of the tuna-skin gelatin, hence the alterations in the physical and chemical properties upon blending with chitosan were most discernible for the T-Ch formulations, especially regarding the film breaking deformation and water solubility.

It was also evaluated the possible loss of antibacterial activity of chitosan upon mixing with the bovine or the tuna gelatins. For this purpose, *S. aureus* was selected as a model of gram-positive microorganism that is also a relevant food poisoning microorganism. As expected, neither the B and T gelatin film-forming solutions nor the B and T gelatin films employed as controls exhibited any antibacterial effect (Figure 4). On the other hand, all the chitosan-containing formulations displayed distinctly discernible antimicrobial effects against *S. aureus*. For purposes of economy, only the photographs for the T films have been included here. So, the good inhibitory effects of the chitosan against *S. aureus* were maintained in spite of the gelatin-chitosan interactions observed. Other authors (Fernandez-Saiz, Lagaron, Hernandez-Munoz & Ocio, 2008) also found a gliadin-chitosan blend to effectively reduce the growth of *S. aureus*.

In conclusion, mixing gelatin and chitosan may be a means to improve the physico-chemical performance of gelatin and chitosan plain films, especially when using fish gelatin, without altering the antimicrobial properties.

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cross-linking on thermal, mechanical, barrier, and biodegradation properties. 

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**Figure captions**

Figure 1. Dynamic viscoelastic properties of the film-forming solutions. B (bovine-hide gelatin), T (tuna-skin gelatin), Ch (chitosan), B-Ch 0.75% (bovine-hide gelatin plus 0.75% chitosan), T-Ch 0.75% (tuna-skin gelatin plus 0.75% chitosan), B-Ch 1.5% (bovine-hide gelatin plus 1.5% chitosan), T-Ch 1.5% (tuna-skin gelatin plus 1.5% chitosan).

Figure 2. Gel strength of the film-forming solutions after cold maturation at 2 °C. Batch designations as in Figure 1.
Figure 3. Glass transition behaviour of one (a) and two (b) component films. (a) One-component films: Bovine-hide gelatin, curve B; tuna-skin gelatin, curve T; chitosan, curve Ch. (b) Two-component films: curves B-Ch 0.75%, B-Ch 1.5%, T-Ch 0.75%, T-Ch 1.5%.

Figure 4. Antimicrobial activity of the tuna-skin gelatin (T) and the tuna-skin gelatin plus 1.5% chitosan (T-Ch 1.5%) film-forming solutions (FFS) and films against *S. aureus*. Arrows indicate the inhibition areas.
Table 1. Physico-chemical properties (glass transition temperature, breaking strength and breaking deformation by puncture test, solubility, and water vapour permeability) of the film batches [B (bovine-hide gelatin), T (tuna-skin gelatin), Ch (chitosan), B-Ch 0.75% (bovine-hide gelatin plus 0.75 % chitosan), T-Ch 0.75% (tuna-skin gelatin plus 0.75 % chitosan), B-Ch 1.5% (bovine-hide gelatin plus 1.5 % chitosan), T-Ch 1.5% (tuna-skin gelatin plus 1.5 % chitosan)].

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<td><strong>Tg</strong> (ºC)</td>
<td>41.6±0.6a</td>
<td>41.0±0.6a</td>
<td>34.6±0.7b</td>
<td>46.9±0.8c</td>
<td>49.7±0.9d</td>
<td>47.4±0.9c</td>
<td>49.6±09d</td>
</tr>
<tr>
<td><strong>Breaking strength</strong> (N)</td>
<td>10.7 ± 2.2a</td>
<td>8.5 ± 1.6a</td>
<td>23.0 ± 2.9b</td>
<td>18.4 ± 3.6b</td>
<td>18.8 ± 3.3b</td>
<td>13.2 ± 2.2a</td>
<td>20.0 ± 3.5b</td>
</tr>
<tr>
<td><strong>Breaking deformation (%)</strong></td>
<td>14.1 ± 5.0a</td>
<td>154 ± 36b</td>
<td>19.2 ± 4.7a</td>
<td>11.0 ± 3.1a</td>
<td>19.9 ± 4.3a</td>
<td>68 ± 6c</td>
<td>40 ± 9a</td>
</tr>
<tr>
<td><strong>Solubility (%)</strong></td>
<td>34.3 ± 0.6ab</td>
<td>39.9 ± 1.3b</td>
<td>82 ± 11c</td>
<td>29.4 ± 2.0ab</td>
<td>33.1 ± 1.8ab</td>
<td>17.5 ± 1.6a</td>
<td>19.0 ± 4.4a</td>
</tr>
<tr>
<td><strong>WVP</strong> (10⁻⁸·g·mm·h⁻¹·cm⁻²·Pa⁻¹)</td>
<td>2.20 ± 0.11a</td>
<td>1.65 ± 0.4b</td>
<td>2.43 ± 0.26c</td>
<td>1.91 ± 0.06b</td>
<td>1.44 ± 0.23b</td>
<td>1.75 ± 0.37b</td>
<td>1.98 ± 0.13ab</td>
</tr>
</tbody>
</table>

Different letters (a, b, c) in the same row indicate significant differences (p ≤ 0.05).

*Tg* = Glass transition temperature

WVP = Water vapour permeability
Figure 1
Figure 2
Figure 3