

## **Biodegradable fish gelatin/chitosan composite films: homogeneous and bi-layer structures**

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### **ABSTRACT**

Fish gelatin and chitosan were used to develop composite biodegradable films using two strategies: (i) the mixture of both polymers to obtain homogeneous polymer matrixes and (ii) the formation of two layers. The films produced were characterized in terms of water absorption, solubility, water vapour permeability and mechanical properties.

Both strategies revealed to produce films with a high strain at break (60-70%), and a quite significant solubility in water and in acetic acid 1.7% (around 40%). Little differences were observed between homogeneous and bi-layer films. On the other hand, homogenous films revealed to have a lower water sorption capacity, compared to pure gelatin and bi-layer films. The results have shown that, under the conditions tested, there was no advantage on using the bi-layer technique.

A high affinity towards liquid water was observed in all films tested, even though a crosslinking agent (poly(ethylene glycol) diglycidyl ether) was added to the film formulations. This fact may be due to a low crosslinking degree, added to the hydrophilic nature of the crosslinking agent itself.

*Keywords: Films; fish gelatine, chitosan, bi-layer*

### **INTRODUCTION**

One of the current trends in the food industry consists on the substitution of chemical additives and packaging materials by natural compounds, especially in the area of food safety and preservation. In response to this consumer requirement, the innovative concept of active packaging appears to be an interesting strategy. Natural biopolymers and their composites (e.g. polysaccharides and proteins), have been applied on the development of biodegradable films for packaging purposes [1,2]. However, these films still present some limitations, needing further technological developments.

Fish gelatin is produced from fish skins that would otherwise be discarded or made into fish meal. Currently, large volumes of food-grade skins are mechanically separated when boneless fillets are produced. These skins can provide an economical source of raw material for the production of fish-skin gelatin for food and pharmaceutical uses. This protein has demonstrated the ability to produce edible films that may be used in food products [3]. Chitosan is a polysaccharide derived from chitin, the second most abundant biopolymer (after cellulose) found in the exoskeleton of crustaceans and fungal cell walls. It is a quite interesting material because it possesses antimicrobial properties in conjunction with its cationic character and its film-forming properties. The combination of chitosan and fish gelatin to produce films is worthy to be studied, since it combines the use of raw materials, in which one of them exhibits interesting properties beyond film forming capacity.

The aim of the present study was to develop and characterize composite bioactive films based on fish gelatin and chitosan. The films were prepared, either from aqueous mixtures of both polymers, or by casting a chitosan solution on a fish gelatin layer to form a bi-layer. The films obtained from both processes were compared in terms of transparency, mechanical and hygroscopic properties, water absorption capacity, solubility in aqueous solutions, water vapor permeability and colour.

### **MATERIALS & METHODS**

The materials used were chitosan (deacetylation degree of 75-85%, Sigma Aldrich), fish gelatin (Lapi Gelatine, S.A., Italy), acetic acid (Sigma Aldrich), glycerol (Sigma Aldrich) as plasticizer, poly(ethylene glycol) diglycidyl ether (EX810, Sigma Aldrich) as crosslinker agent and nisin (Nisaplin, Danisco) as antimicrobial.

Homogeneous films with different fish gelatin contents (25, 50, 75 and 100%, dry weight) were prepared by dissolving the required amount of both biopolymers in an acetic acid solution (1.7%). The final polymer

concentration was 5% (dry weight). Afterwards, glycerol (20% of polymers dry mass), the crosslinker (molar ratio crosslinker:polymer of 2:1) and nisin (50 UI/ml of solution), were added. The mixture was centrifuged to remove insoluble particles, and a specific amount was transferred to an acrylic flat plate and let to dry, at controlled temperature ( $T=22^{\circ}\text{C}$ ) and relative humidity ( $\text{RH}=50\%$ ), to form a film.

Bi-layer films were obtained preparing a first gelatin layer, by drying a film-forming solution composed by gelatin (5%), glycerol (20% of polymers dry mass), crosslinker (molar ratio crosslinker:polymer of 2:1) and nisin (50 UI/ml of solution). After that, a chitosan solution (1.5%) (containing the same concentration of the other components), was spread over the dried gelatin layer. The thickness of the gelatin layer was adjusted to vary from 25% to 100% of the overall thickness.

Films were characterized in terms of their mechanical properties (using a TA-XT2 Stable Mycro Systems texturometer), transparency, solubility, swelling and water permeability.

The rheological characterization of the filmogenic solutions (viscosity and viscoelastic properties) was carried out by performing steady-state and oscillatory tests using a controlled stress rheometer (Haake RS75).

## RESULTS & DISCUSSION

Since gelatin is able to form gel systems, a first study regarding the sol-gel transition of the film-forming solutions, was carried out. The sol-gel transition temperatures, obtained from oscillatory temperature sweeps, are presented in Table 1.

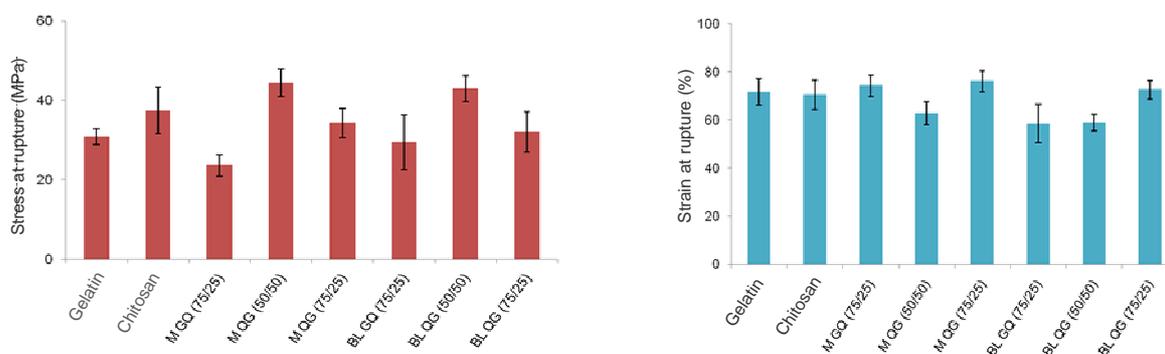
**Table 1. Sol-gel transition temperatures and  $\eta_0$  of film formulations**

Solution	T <sub>gel</sub> (°C)	$\eta_0$ (Pa s)
Gelatin	14.6	0.013
M GQ (75/25)	13.1	0.071
M GQ (50/50)	11.4	0.112
M GQ (25/75)	5.8	0.112

It can be seen that the sol-gel transition temperature decreases as the chitosan content increases. The presence of chitosan molecules is probably making more difficult the interaction between gelatin chains and the establishment of the gel networks. We can conclude that, during the drying step at  $T=22^{\circ}\text{C}$ , the film solutions are well above T<sub>gel</sub>. Thus, no gel is present when films are formed. From steady shear measurements, the viscosity of such solutions was also determined. They present a Newtonian behavior, with an increasing viscosity ( $\eta_0$ ) with increasing chitosan content (Table 1).

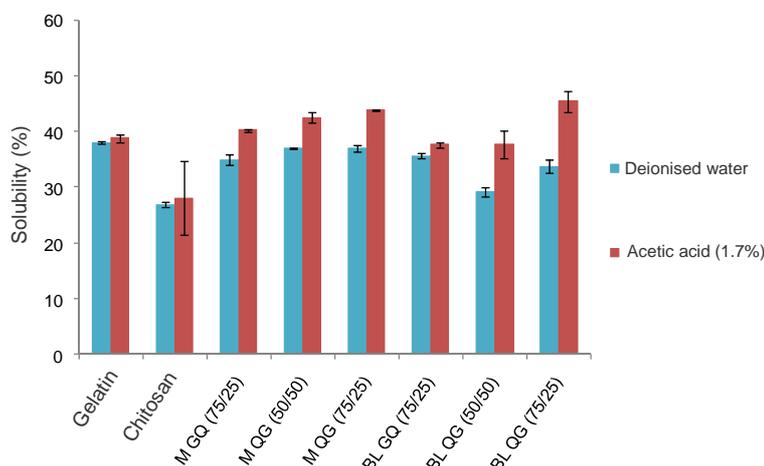
The films obtained were flexible when handled. Color parameters (of CIElab colour space) of colored paper sheets did not change significantly when covered by the films prepared. The application of either homogeneous, or bi-layer films, induced color differences below 9, indicating quite transparent films.

Tensile tests revealed a high strain at break (from 60-70%) and a stress at break from 30 to 45 Mpa (Figs 1A and 1B). However, no significant differences were detected between pure gelatin, bi-layer and homogeneous mixtures films.



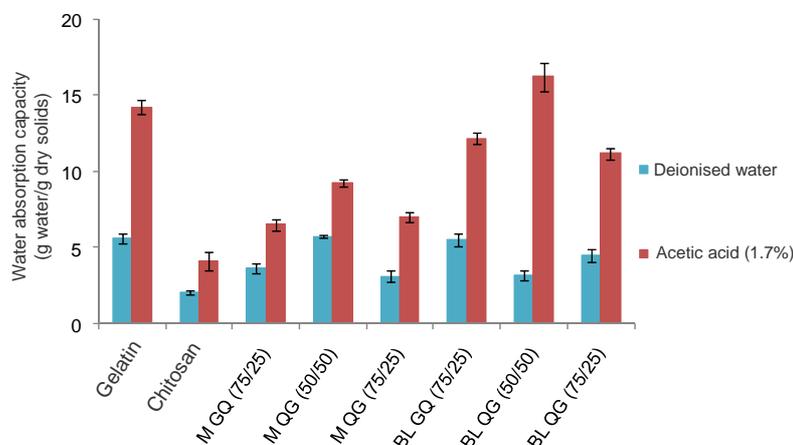
**Figure 1:** Tensile tests of mixture (M) and bi-layer (BL) gelatine/chitosan films: (A) stress at break, (B) strain at break.

The solubility of both types of films prepared was studied immersing film samples in deionised water and in an acetic acid solution (1.7%). It was observed a high solubility in both aqueous media (around 40% of samples dry mass was dissolved). However, no significant differences were observed, either by varying the gelatin content in homogeneous films, or the thickness of the gelatin layer in bi-layer films (Figure 2).



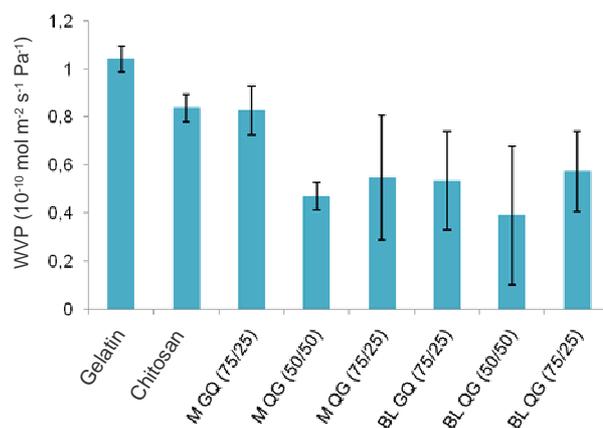
**Figure 2:** Solubility of mixture (M) and bi-layer (BL) gelatine/chitosan films in deionised water and acetic acid 1.7%.

The water absorption capacity was also studied in deionised water and acidic conditions (acetic acid 1.7%). The results are shown in Figure 3. A quite high water adsorption was observed, ranging from 2.5 up to 16.0 (g water/g dry solids), which is in line with the rather significant solubility in aqueous media observed. However, the water absorption was much higher at low pH (5.0-16.0 g water/g dry solids) than in deionised water (2.5-7.0 g water/g dry solids). This fact is probably due to the higher solubility of chitosan and gelatin (I.P.  $\approx$  7) in acidic media. Furthermore, although small differences were detected on the water absorption capacity in deionised water with the introduction of chitosan, the same did not happen in acetic acid 1.7%. In this case, the water absorption capacity of pure gelatin films was similar to that of bi-layers (13-16 g water/g dry solids), but much higher than the value observed for homogeneous chitosan/gelatin films (6-9 g water/g dry solids).



**Figure 3:** Water absorption capacity of mixture (M) and bi-layer (BL) gelatine/chitosan films.

Regarding the water vapour permeability, the value observed for bi-layer films and homogeneous gelatin/chitosan films, was slightly lower than the one registered for pure gelatin films (Figure 4). The barrier properties to water vapour were somewhat improved, with the introduction of chitosan.



**Figure 4:** Water vapour permeability (WVP) of mixture (M) and bi-layer (BL) gelatine/chitosan films.

The rather high affinity towards water of the films studied may be related to a low extension of the crosslinking reaction. In addition, the unreacted crosslinker molecules were not removed, remaining as part of the polymeric matrix. As so, they may have functioned as plasticizer and as alternative water binding sites, due to their hydrophilic character.

## CONCLUSION

With the additives tested (glycerol as plasticizer and poly(ethylene glycol) diglycidyl ether as crosslinker), the films obtained from the two methodologies revealed to have similar properties. The results indicate that, in this case, there was no advantage on using the bilayer technique. Future work will be focused on improving crosslinking reactions, either by changing the reaction conditions, or by choosing a less hydrophilic crosslinking agent. The main goal is to obtain films with improved resistance to liquid water.

## REFERENCES

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