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The water vapour permeability, mechanical properties and solubility of fish gelatin–chitosan films modified with transglutaminase or 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and plasticized with glycerol

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Abstract

The effect of glycerol on the mechanical and water barrier properties, as well as on the water solubility, of fish gelatin–chitosan films (4:1, w/w) cross-linked with TGase or EDC was determined. The addition of glycerol in concentrations up to 30% (of the substrate mass) to the fish gelatin–chitosan films modified with TGase or EDC did not change their solubility in buffers of pH 3 and 6 at 25 °C or during heating at 100 °C for 60 min. The chemical and enzymatic cross-linking of the components did not increase the water barrier properties of the films. WVP of the films modified with EDC and TGase was not affected by glycerol at concentrations up to 25% of the substrate mass. Tensile strength of the films decreased after modification of the components with TGase or EDC, respectively, by about 25% and 40%. The elongations of the enzymatically modified films containing 20% of glycerol and of chemically modified films containing 15% of glycerol were, respectively, about 8 and 13 times higher than those of unplasticized films; however, the tensile strengths of plasticized films were, respectively, 2.5 and 5 times lower.

Keywords: Fish gelatin–chitosan films; Crosslinking; Transglutaminase; 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide; Glycerol; Water vapour permeability (WVP); Mechanical properties

1. Introduction

So far non-biodegradable, synthetic materials are still those most often used as food packages. The very promising possibility of reducing wastes is to replace such materials with environmentally friendly packaging materials based on natural polymers, such as proteins and polysaccharides. Additionally, these biodegradable, edible films have abilities to carry food additives, such as antimicrobial agents, antioxidants, vitamins, flavours and colours (Appendini & Hotchkiss, 2002; Kester & Fennema, 1986; Krochta & De Mulder-Johnston, 1997). Furthermore, natural polymers (e.g. collagen and gelatin or chitosan or mixtures of these components) obtained from by-products of the food industry can be used for producing edible films. Chitosan is a valuable component of natural packaging films. It is generally obtained from natural chitin after its *N*-deacetylation by an alkaline treatment. Chitosan is a biodegradable and non-toxic polymer. Because of its biological activity, it can potentially serve also as a bactericidal agent in food packaging materials (Coma, Deschamps, & Martial-Gros, 2003; Coma et al., 2002; Tanabe, Okitsu, Tachibana, & Yamauchi, 2002; Zhai, Zhao, Yoshii, & Kume, 2004; Zheng & Zhu, 2003). The source of protein in the composite films could be gelatin obtained from fish offals.

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The properties of gelatins are largely influenced by the origin of raw material. The gelatin produced from skins of fish living in cold waters does not gel at room temperature (Norland, 1990). Until now, gelatin from skins of cold-adapted fish has not been applied as a component of biodegradable packages. However, films prepared from cod skin gelatin are completely soluble in water, even at room temperature (Piotrowska, Kołodziejska, Januszewska-Jóźwiak, & Wojtasz-Pająk, 2005). Therefore, such films are not suitable for coating or packaging of many food products. This problem arises when the packaging material should be resistant to solubilization in contact with acidic foods or during heating. Our previous experiments showed that the solubility of fish gelatin films and fish gelatin–chitosan films (4:1, w/w) could be limited by cross-linking of the components with transglutaminase (TGase) or with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (Kołodziejska, Piotrowska, Bulge, & Tylingo, 2006; Piotrowska, 2004). EDC, similarly to TGase, participates in forming amide bonds (Kuijpers et al., 2000; Wissink et al., 2001). Moreover, in the presence of EDC, apart from amide bonds involving the amine groups of chitosan or proteins, additional bonds are formed, involving the amine or carboxylic groups of protein and hydroxyl groups of chitosan (Chiou & Wu, 2004; Wang et al., 2003).

TGase has been used to crosslink some protein films (Mahmoud & Savello, 1993; Motoki, Aso, Seguro, & Nio, 1987), whereby the solubility of the films was decreased (de Carvalho & Grosso, 2004; Yildirim & Hettiarachchy, 1998). However, the results (regarding the effect of cross-linking), using TGase, on the water vapour permeability (WVP) and mechanical properties were inconsistent, probably due to different protein components of the films, various conditions of enzymatic reaction and preparation of films. Therefore, the first aim of our study was to determine the mechanical and water barrier properties of the fish gelatin–chitosan films modified with TGase. To compare results, parallel experiments were carried out using films, chemically modified with EDC.

One of the unfavourable properties of natural polymer films, especially protein films, apart from excessive water solubility and poor WVP, is the fragility of the material. For this reason, such films have to be plasticized. Hydrophilic plasticizers are most often used to improve flexibility of the films (Arvanitoyannis & Biliaderis, 1999; Arvanitoyannis, Nakayama, & Aiba, 1998a; Butler, Vergano, Testin, Bunn, & Wiles, 1996; Caner, Vergano, & Wiles, 1998; Cuq, Gontard, Cuq, & Guilbert, 1997; Sobral, Menegalli, Hubinger, & Roques, 2001; Yang & Paulson, 2000). Incorporation of plasticizers into natural polymer films eliminates the fragility of the films and improves their elongation. However, some of the ways that plasticized films can undergo deterioration include increase in solubility of films and water vapour permeability or decrease in tensile strength. Because the gelatin–chitosan films, like other natural polymer films, had to be plasticized, the effect of glycerol on mechanical properties as well as on the water solubility of the films at different temperatures and pH values was also studied. The possibility of using glycerol as a plasticizing agent of fish gelatin–chitosan films was estimated. The final goal of our study was to design biodegradable material with good mechanical and barrier properties, suitable for packages of many kinds of food products with different acidities and contents of moisture.

2. Materials and methods

2.1. Materials

Chitosan (deacetylation degree 73%) was obtained from krill chitin in the Sea Fisheries Institute in Gdynia according to Kołodziejska, Wojtasz-Pająk, Ogonowska, and Sikorski (2000). Fish gelatin was obtained from Baltic cod skins, as described by Kołodziejska, Kaczorowski, Piotrowska, and Sadowska (2004). EDC was purchased from Sigma Chemical Co. The preparation of commercial TGase (Ajinomoto Co's Transglutaminase Activa®-WM, Japan), containing 99% of maltodextrin and 1% of enzyme, was used for the enzymatic modification. Six grams of the dry preparation were mixed with 20 ml of cold water in an ice bath for 15 min and centrifuged at 6000*g* for 15 min at 4 °C. The protein content in the enzyme solution was determined according to Lowry, Rosebrough, Farr, and Randall (1951).

2.2. Enzymatic and chemical modifications

Fish gelatin–chitosan films (4:1, w/w) were obtained by mixing a 2% solution of chitosan (pH 5 adjusted with 0.5 M HCl) with a 25% solution of gelatin. The resulting mixture was occasionally stirred during 2 h of incubation at 50 °C and centrifuged at 2000*g* and 20 °C for 15 min.

To the fish gelatin–chitosan solutions, glycerol was added in the range 15–35% of the substrate mass, EDC to the final concentration of 30 mM or TGase at 0.2 mg/ml. The films were formed just after adding of TGase or EDC, as described below. The enzymatic and chemical reactions were occurring during that process.

2.3. Film formation

Film-forming solution was poured on a polyester surface. To obtain similar thickness of the samples, varying areas of film-forming surfaces were used. The films modified with EDC were dried at room temperature for 24–48 h at 35–45% relative humidity (RH). The films modified with TGase were dried in two stages: 12 h at 65–70% RH, followed by 24–48 h at 35–45% RH. The thickness of the films was measured at five random locations with a hand-held micrometer. The average thickness of the films ranged from 0.10 to 0.12 mm.

2.4. Solubility

About 50 mg of the dry samples were immersed in 20 ml of 0.2 M Mc'Ilvaine buffer (pH 3 or 6) and incubated for 24 h at 25 °C or 15 min or 60 min at 100 °C. The insoluble material was separated on a funnel with cotton wool. Nitrogen contents were determined in the insoluble residues of the films.

The solubility of the films was evaluated on the base of nitrogen that was dissolved in the buffer and was expressed as the percentage of nitrogen contained in the initial films.

The nitrogen content was determined according to AOAC methods (1990).

2.5. Water vapour permeability

WVP was determined according to the ASTM method E 96–95 (ASTM, 1995). The films were conditioned for 24 h at 25 °C and 50% RH before determination. Film samples were mounted on cups filled with water. The cups were placed, at 25 °C and 50% RH in a desiccator. The weight of the cups was measured at 1 h intervals during 9 h. Simple linear regression was used to estimate the slope of weight loss vs. time plot.

WVP was calculated from:

$$\mathrm{WVP} = (\mathrm{WVTR}L)/\Delta p$$

where water vapour transmission rate (WVTR) is the slope/film area ($g/m^2 \times h$), L is the film thickness (mm), and Δp is the partial water vapour pressure difference (kPa) between the two sides of the film.

2.6. Mechanical properties

Tensile strength (TS) and elongation at break (E) were determined according to ASTM method D 882-00 (ASTM, 2001) with a model 5543 Instron Universal Testing Machine (Instron Co., Canton, MA, USA). Initial grip separation and cross-head speed were set at 50 mm and 25 mm/min, respectively. TS was calculated by dividing the maximum load by the initial cross-sectional area of the sample and expressed in MPa. E was calculated as a ratio of the elongation at the point of sample rupture to the initial length of a sample as a percentage. Strips of film samples (2.5 by 10 cm) were conditioned for 24 h at 25 °C and 50% RH before determination of TS and E.

3. Results and discussion

3.1. Effect of glycerol on the solubility of gelatin–chitosan films modified enzymatically and chemically

The solubilities of unmodified fish gelatin–chitosan films at 25 °C in buffers of pH 6 and 3, were 65% and 96%, respectively (Table 1a). After modification with TGase, the solubility of the films decreased to 26% at pH 6 and 33% at pH 3 (Table 1a). Moreover, enzymatic treatment limited solubility of films at 100 °C and pH 6, from 92–98% (for not cross-linked samples) to 30–35% (Table 1a and b). The films cross-linked with TGase were solubilized completely during heating for 1 h at 100 °C and pH 3. However, solubility of films after 15 min of heating at 100 °C amounted only to 38% (Table 1c). This could indicate that crosslinking of components was not enough to limit solubility of films during prolonged heating at pH 3. The EDC modified films were less soluble in buffers, especially at pH 3 and 100 °C than were films modified with TGase (Table 1). The results suggest that chemical cross-linking of components was more effective than enzymatic modifications.

Table 1
Effects of glycerol on the solubility of fish gelatin–chitosan films modified with TGase at a concentration of 0.2 mg/ml and EDC at a concentration of 30 mM

Glycerol concentration (% of the substrate mass)	Solubility (%)[A]			
	Films modified with TGase		Films modified with EDC	
	pH 3	pH 6	pH 3	pH 6
(a) 25 °C, 24 h				
0[B]	96[a]	65[a]	96[a]	65[a]
0	33[b]	26[bc]	15[b]	22[b]
20	35[b]	25[b]	15[b]	23[b]
25	35[b]	28[c]	17[b]	21[b]
30	33[b]	26[bc]	14[b]	21[b]
(b) 100 °C, 1 h				
0[B]	97[a]	98[a]	97[a]	98[a]
0	94[a]	35[b]	38[a]	40[b]
20	91[b]	37[bd]	37[a]	41[b]
25	90[b]	41[c]	36[a]	39[b]
30	90[b]	39[cd]	38[a]	41[b]
(c) 100 °C, 15 min				
0[B]	96[a]	92[a]	96[a]	92[a]
0	38[b]	30[b]	16[a]	26[b]
20	38[b]	30[b]	24[b]	31[cd]
25	36[b]	27[c]	22[b]	33[c]
30	38[b]	30[b]	23[b]	30[d]

[A] Values for a particular column followed by different letters differ significantly ($P < 0.05$).
[B] Unmodified films (Kołodziejska et al., 2006).

It was shown that the addition of glycerol, in concentrations up to 30%, did not increase the solubility of films modified with TGase in buffers of pH 3 and 6 at 25 °C and during heating at 100 °C for 15 and 60 min (Table 1). Although there were sometimes statistically significant differences in the solubility, a regular character of such changes was not shown. As the value of solubility of modified films is a reflection of components cross-linking, such results show that the presence of glycerol does not interfere with the enzymatic reaction.

In the case of chemical modification, there was only a significant increase in the solubility of films containing 20% of glycerol heated for 15 min at 100 °C (Table 1c). However, the higher concentration of the plasticizer did not cause further changes in the solubility of the films.

Cuq et al. (1997) showed that solubility in water of the films prepared from fish muscle proteins plasticized with glycerol, sorbitol or saccharose was higher than that of films without plasticizers. However, according to these authors, the loss of film mass was caused by solubilization of glycerol, not by proteins. Sothornvit and Krochta (2000) also reported that glycerol did not affect the solubility of whey protein films. The authors also reported that greater effects on the solubility were exerted by pH and temperature rather than by the plasticizer. On the other hand, Mahmoud and Savello (1993) observed the increase in the solubility of protein films. This can be explained as a result of decrease of protein interactions in the polymer network.

3.2. The WVP of gelatin–chitosan films modified enzymatically and chemically

As could be suspected, unmodified fish gelatin–chitosan films were not good barriers for water (Table 2). The WVP coefficient, 2.42 $g \times mm/kPa \times h \times m^2$, was also higher than that reported by Arvanitoyannis, Nakayama, and Aiba (1998b) for mammal gelatin–chitosan films. The discrepancy in these data may be caused by different properties of fish and mammal gelatins, differences in molecular mass and deacetylation degree of chitosans, as well as, by different conditions used during preparation of the films.

The water barrier properties of fish gelatin–chitosan films were not improved by cross-linking of the components with TGase and EDC (Table 2). Yildirim and Hettiarachchy (1998) showed that modification, with TGase, of whey protein isolate, soybean globulin 11S, or mixtures of these components, even increased the WVP of films by 55–64%. On the other hand, de Carvalho and Grosso (2004) found that the WVP coefficient of TGase crosslinked gelatin films was about 40% lower than that of unmodified films.

As a rule, incorporation of hydrophilic plasticizers into protein and polysaccharide films increases the WVP. This effect of plasticizers results from the increase in the free volume between polymer chains due to decreasing attractive intermolecular forces that make the polymer network less dense and then more permeable (Banker, Gore, & Swabrick, 1966; Cuq et al., 1997).

Arvanitoyannis et al. (1998b) showed that WVP of chitosan–pigskin gelatin films (1:1) in the presence of glycerol at 19% of the substrate mass was much higher than that of unplasticized films. However, glycerol at concentrations up to 20% of the substrate mass did not affect the WVP of fish gelatin–chitosan films modified with TGase (Table 3). The WVP was 35% higher only when the concentration of glycerol amounted to 30%. In the case of films modified with EDC, WVP was not affected by glycerol in the whole tested range of concentration (Table 3). These results may suggest that chemical cross-linking makes the polymer network denser than that of the films modified with TGase because, apart from the amide bonds, other linkages may also be formed.

3.3. The mechanical properties of gelatin–chitosan films modified enzymatically and chemically

The mechanical properties of fish gelatin–chitosan films (4:1) are presented in Table 4. The TS of unmodified films was about 46 MPa. Pigskin gelatin–chitosan films (1:1) showed TS values three times higher (Arvanitoyannis et al., 1998b). This difference may result from different properties of the substrates and procedures for preparing

Table 2
The WVP of fish gelatin, chitosan and fish gelatin–chitosan films

Components of films	WVP[A] ($g \times mm/kPa \times h \times m^2$)
Chitosan	2.31[a]
Fish gelatine	2.54[a]
Fish gelatin–chitosan	2.42[a]
Fish gelatin–chitosan modified with EDC (30 mM)	2.53[a]
Fish gelatin–chitosan modified with TGase (0.2 mg/ml)	2.40[a]
LDPE[B]	0.003

[A] The values followed by the same letter do not differ significantly ($P > 0.05$).
[B] Krochta and De Mulder-Johnston (1997).

Table 3
Effects of glycerol on the WVP of fish gelatin–chitosan films

Glycerol concentration (% of the substrate mass)	WVP[A] ($g \times mm/kPa \times h \times m^2$)	
	Films modified with TGase in concentration of 0.2 mg/ml	Films modified with EDC in concentration of 30 mM
0	2.40[a]	2.53[a]
20	2.45[a]	2.50[a]
25	2.45[a]	2.45[a]
30	3.26[b]	2.56[a]

[A] Values for a particular column followed by different letters differ significantly ($P < 0.05$).

Table 4
Effects of glycerol on tensile strength and elongation of fish gelatin–chitosan films

Glycerol concentration (% of the substrate mass)	Films modified with TGase[A] (0.2 mg/ml)		Films modified with EDC[A] (30 mM)	
	TS (MPa)	E (%)	TS (MPa)	E (%)
0[B]	46.3[a]	1.9[a]	46.3[a]	1.9[a]
0	35.3[b]	2.4[a]	28.8[b]	8.8[b]
15	15.1[c]	3.5[a]	5.7[c]	112.4[c]
20	13.4[c]	20.0[b]	4.3[c]	115.9[c]
25	4.1[d]	167.6[c]	3.7[c]	117.9[c]
30	1.8[d]	420.1[d]	2.9[c]	14.2[c]

[A] Values for a particular column followed by different letters differ significantly ($P < 0.05$).
[B] Unmodified films.

films, including thickness of films. It was reported that the mechanical properties of chitosan or composite films containing chitosan depend on the molecular mass of the polymer, the pH of the film-forming solution (Chen & Hwa, 1996) and on the deacetylation degree of the chitosan (Trung, Thein-Han, Qui, Ng, & Stevens, 2006). These properties are also affected by the kind of acid used to dissolve chitosan (Bégin & Van Calsteren, 1999), drying conditions of films (Srinivasa, Ramesh, Kumar, & Tharanathan, 2004) and contents of water (Lazaridou & Biliaderis, 2002). In the case of composite films, the ratio of the components may be important in creating the properties of such films. The concentration of fish gelatin in the studied composite films was four times higher than that of chitosan.

The TS of fish gelatin–chitosan films decreased after modification of the components with TGase and EDC, respectively, by about 25% and 40% (Table 4). Cross-linking of components usually increases the TS of the films. TGase-modified films of whey protein isolate, soybean 11 S globulin, films prepared from mixture of these components or pectin–soyflour films were stronger than the unmodified films (Mariniello et al., 2003; Yildirim & Hettiarachchy, 1998). On the other hand, de Carvalho and Grosso (2004) showed that enzymatic modification of gelatin with TGase had no effect on the TS of films. It appears that these variations in the TS values may be the result of different degrees of cross-linking of the polymers. Excessive cross-linking of fish gelatin worsened the properties of the gels (Kołodziejska et al., 2004). This effect could occur in the case of fish gelatin–chitosan films modified with TGase and EDC. Nevertheless, the TS was still in the moderate range of 10–100 MPa and was higher than that of LDPE films (Krochta & De Mulder-Johnston, 1997).

Enzymatic modification did not change the elongation of films, but crosslinking of the components with EDC increased this property from about 2% to 9% (Table 4). Glycerol, at a concentration of 15%, did not improve the flexibility of films modified enzymatically. Elongation of films containing glycerol in higher concentration, 20% of the substrate mass, was about 8 times greater than that of unplasticized samples; however, the TS was 2.5 times lower (Table 4). According to the values given by Krochta and De Mulder-Johnston (1997), the TS and elongation of the films were in the moderate range. A decrease of the TS with a simultaneous increase of elongation is a characteristic property of other polysaccharide or protein films plasticized with different hydrophilic compounds (Arvanitoyannis & Biliaderis, 1999; Arvanitoyannis et al., 1998a, 1998b; Tanaka, Iwata, Sanguandeekul, & Handa, 2001). Arvanitoyannis et al. (1998b) reported that in the case of non-crosslinked pigskin gelatin–chitosan films plasticized by glycerol at concentrations of 19% of the substrate mass, the TS was 1.5 times lower and elongation 6 times higher than those of the unplasticized films. Plasticized fish gelatin–chitosan films containing 25% and 30% glycerol (with reference to the substrate mass) had very great elongations, 167% and 420%, respectively (Table 4). However, this was accompanied by a drastic decrease of the TS (Table 4). Fish gelatin–chitosan films modified with EDC reached elongations of about 110% in the presence of glycerol at concentration of 15%, but the TS was five times lower than that of the unplasticized films. Glycerol, at this concentration, did not increase the elongation of films modified with TGase (Table 4). The elongation of chemically modified films was not affected by further increasing of glycerol concentration (Table 4).

4. Conclusions

The modifications of fish gelatin and chitosan with TGase allow reduction of the enhanced solubility of prepared films, even in such drastic conditions as acidic pH and high temperature. These properties widen the practical applications of modified films as packaging material. However, cross-linking of components with commercial preparations of TGase, differently from modification with EDC, increases the fragility of films. Therefore, especially, such films have to be plasticized. Fortunately, the plasticization of enzymatically-modified films does not increase the solubility of the polymers in aqueous medium, or at elevated temperature in acidic medium. Similarly, WVP of the crosslinked films is not affected by glycerol in relatively high concentration. On the other hand, glycerol at concentrations above 20% in modified films, drastically decreases their TS. Nevertheless, TS and elongation of films modified with TGase and plasticized by glycerol at a concentration of 20% of the substrate mass led to moderate mechanical properties of such materials. However, it is still necessary to select a plasticizer or mixtures of these substances, which allow better elongation of films, especially at low relative humidity, but without drastic decrease of TS.

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