Properties of Chitosan-Laminated Collagen Film

Nevena Krkić*, Vera Lazić, Ljiljana Petrović, Jasna Gvozdenović and Danijela Pejić

Faculty of Technology, University of Novi Sad, Bul. Cara Lazara 1, RS-21000 Novi Sad, Serbia

Summary

The objective of this study is to determine physical, mechanical and barrier properties of chitosan-laminated collagen film. Commercial collagen film, which is used for making collagen casings for dry fermented sausage production, was laminated with chitosan film layer in order to improve the collagen film barrier properties. Different volumes of oregano essential oil per 100 mL of filmogenic solution were added to chitosan film layer: 0, 0.2, 0.4, 0.6 and 0.8 mL to optimize water vapour barrier properties. Chitosan layer with 0.6 or 0.8 % of oregano essential oil lowered the water vapour transmission rate to $(1.85\pm0.10)\cdot10^{-6}$ and $(1.78\pm0.03)\cdot10^{-6}$ g/(m^2·s·Pa) respectively, compared to collagen film $((2.51\pm0.05)\cdot10^{-6}$ g/(m^2·s·Pa)). However, chitosan-laminated collagen film did not show improved mechanical properties compared to the collagen one. Tensile strength decreased from (54.0 ± 3.8) MPa of the uncoated collagen film to (36.3 ± 4.0) MPa when the film was laminated with 0.8 % oregano essential oil chitosan layer. Elongation at break values of laminated films did not differ from those of collagen film $((18.4\pm2.7)$ %). Oxygen barrier properties were considerably improved by lamination. Oxygen permeability of collagen film was $(1806.8\pm628.0)\cdot10^{-14}$ cm^3/(m·s·Pa) and values of laminated films were below $35\cdot10^{-14}$ cm^3/(m·s·Pa). Regarding film appearance and colour, lamination with chitosan reduced lightness (L) and yellowness ($+b$) of collagen film, while film redness ($+a$) increased. These changes were not visible to the naked eye.

Key words: collagen casings, chitosan film, physical and mechanical properties, oregano essential oil

Introduction

Chitosan is a natural biopolymer derived from deacetylation of chitin, a polysaccharide that is the major component of the shells of crustaceans (crabs, shrimps and crawfish) and of fungal cell wall (*1*). Chitosan films exhibit significant antimicrobial activity against yeasts, moulds and Gram-positive bacteria, and to a lesser extent against Gram-negative bacteria. Antioxidant activity has also been reported (*1–4*). Because of its good film-forming capacity, chitosan has been investigated for its application in food packaging as a natural active edible film. However, the high water sensitivity of chitosan films remains the main issue to overcome (*5*).

Collagen is a group of naturally occurring proteins. In nature it is found exclusively in animals. It is the main component of connective tissue and is the most abundant protein in mammals, making up about 25 to 35 % of the whole body protein content. Collagen, in the form of elongated fibrils, is mainly found in fibrous tissues such as tendon, ligament and skin (*6*).

Traditional collagen casings are mostly used for dry fermented sausage manufacturing. Regarding the manufacturing process, the desirable properties for traditional collagen casings are good penetration of flavours and aromas, permeability to water and steam, and resistance during filling and clipping. After the sausage production is finished, further water loss, penetration of aromas and oxygen becomes undesirable and it shortens the shelf-life of the product (*4*).

*Corresponding author; Phone: ++381 21 485 3713; Fax: ++381 21 450 413; E-mail: nevena.krkic@uns.ac.rs

Regarding the above mentioned, it might be useful to combine collagen films for casings with chitosan, which should be a good barrier for gasses and protect the surface from microbial growth. Besides, to lower the water vapour transmission of chitosan film, different amounts of oregano essential oil have been reported to be added to filmogenic solution (2,7,8). Thus, the objective of this study is to determine physical and mechanical properties of chitosan-laminated collagen film with the addition of different volumes of oregano essential oil to the chitosan layer.

Materials and Methods

Reagents

Highly viscous commercial chitosan powder from crab shells was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Collagen casings were bought from a local manufacturer Cotex Viscofan (Novi Sad, Serbia). Oregano essential oil (carvacol) was purchased from Aromara (Zagreb, Croatia), glacial acetic acid and Tween 20 were obtained from Superlab (Belgrade, Serbia).

Film preparation

Chitosan filmogenic solutions were prepared by dissolving chitosan powder in 1 % acetic acid to reach the mass concentration of 4 kg/m^3. The solution was stirred overnight on a magnetic stirrer to dissolve chitosan. Different volumes of oregano essential oil (OEO) per 100 mL of filmogenic solution were added: 0, 0.2, 0.4, 0.6 and 0.8 mL. Wetting agent Tween 20 was added to reach 50 % of OEO volume of the solution. Filmogenic solutions were coated on collagen film surface to make laminated films. A collagen film without coating was used as reference. All films were labelled according to the OEO content of the chitosan layer from A0 to A4, while K corresponded to the uncoated collagen film (Table 1).

Table 1. Film labelling scheme

	Film label					
	K	A0	A1	A2	A3	A4
γ(chitosan)/(kg/m^3)	0	4	4	4	4	4
φ(OEO)/%	0	0	0.2	0.4	0.6	0.8

In order to compare the obtained results, filmogenic solutions were also cast on Petri dishes to obtain chitosan films. Casting ratio was: 40 g of film-forming solution to 95 cm^2 of plate area.

Both chitosan and laminated collagen films were air dried (*t*=25 °C, relative humidity RH=65 %) and conditioned for at least two days (*t*=8 °C, RH=65 %) prior to analysis (2).

Mechanical properties

Film thickness was measured using a micrometer (Digico 1, Tesa Technology, Renens, Switzerland) with sensitivity of 0.001 mm. Average values of 15 measurements of thickness of each film were obtained.

Tensile strength and elongation at break of the films were measured on an Instron Universal Testing Instrument (Model No 4301, Instron Engineering Corp., Canton, MA, USA), according to ASTM standard method D882-01 (9). A rectangular film strip of 90 mm in length and 15 mm in width was used. The initial grip separation was set at 50 mm, and crosshead speed was set at 100 mm/min. The tensile strength and elongation at break of the strips were measured in a static mode. Tensile strength (MPa) was calculated by dividing the given peak load with the cross-sectional area of the film. Elongation at break was calculated as the percent of change by dividing the film elongation at the moment of rupture with the initial gage length of the specimen (50 mm) and multiplying by 100 (9). Tensile strength and elongation at break measurements for each type of film were repeated 15 times, from which an average was obtained.

Swelling property

Films were cut into pieces of 1×2 cm in size and weighed in air-dried conditions (*t*=25 °C, RH=65 %). They were then immersed in deionized water (25 °C) for 2 min. Wet samples were wiped with filter paper to remove excess liquid and weighed. The amount of adsorbed water was calculated as:

$$\text{Swelling}=(m_1-m_2)/m_2\cdot100 \qquad /1/$$

where m_1 and m_2 are the mass of the wet and the air-dried samples, respectively (10). Average values of 5 measurements of each film were reported in the paper.

Water vapour barrier properties

Water vapour barrier properties of films were determined gravimetrically according to the ASTM E 96-95 desiccant method (11). The method involves sealing a known open area of an impermeable container with the film to be tested. Anhydrous silica gel was used to maintain a 0 % atmosphere inside the cells. Distilled water was used to maintain 100 % RH outside the cells. Test cells were stored under controlled temperature ((23±2) °C) and weighed periodically until a constant rate of mass gain was reached. The obtained weighing values were used for the calculation of the amount of water vapour transferred through the film. The water vapour transmission rate [WVTR/(g/(m^2·s·Pa))] of the film was calculated as follows:

$$\text{WVTR}=\Delta m/(t\cdot A\cdot\Delta p) \qquad /2/$$

where Δm (in g) is the mass gain of the tested cells, t is the time between weighings (in this case 24 h), A is the area of exposed film (50 cm^2) and Δp is partial water vapour pressure (in Pa) difference between the two sides of the film. Under the conditions of determination, the Δp was 1753.55 Pa.

Water vapour permeability [WVP/(g/(m·s·Pa))] was calculated as:

$$\text{WVP}=\text{WVTR}\cdot d \qquad /3/$$

where d was the film thickness (in µm).

Oxygen permeability

Oxygen permeability was measured using the Lyssy method, according to DIN 53 380 standard on an oxygen permeability tester (Lyssy GPM-200, Systech Instruments, Oxfordshire, United Kingdom) with an appropriate gas chromatograph (GC-320, Gasukuro Kogyo, Tokyo, Japan) and an integrator (HP 3396, Hewlett-Packard, Palo Alto, CA, USA).

Colour measurement

Colour values of films were measured with a chromometer (CR-400 Chroma Meter, Minolta Camera Co., Osaka, Japan) using D-65 lighting, a 2° standard observer angle and an 8-mm aperture in the measuring head. Film specimens were placed on the surface of a white standard plate and Hunter colour parameters were determined as *L* (lightness, 0=black, 100=white), *a* (–*a*=greenness, +*a*=redness), and *b* (–*b*=blueness, +*b*=yellowness). Colour measurement of the film was replicated five times and average values were calculated. Before each series of measurements, the instrument was calibrated using a white ceramic tile.

Statistical analysis

Statistical analysis was carried out using ORIGIN v. 7.0 (*12*). Measurements were done at least in five replicates. All data were presented as mean values with their standard deviation indicated (mean±S.D.). One--way ANOVA and Bonferoni tests, using $p \leq 0.05$ level of significance, were applied to compare the mean values of the results.

Results and Discussion

Chitosan films made from film-forming solution without OEO were transparent and colourless. Incorporation of the emulsifier and OEO resulted in milky and opaque films (Fig. 1a). Among the chitosan-laminated collagen films, no visible differences in appearance, transparency and colour were detectable to the naked eye (Fig. 1b).

Mechanical properties

Lamination of collagen film with chitosan affected the thickness in most cases (Fig. 2). Lamination with chitosan film without OEO (A0) did not affect the thickness of collagen film significantly, but with the addition of OEO, thickness values increased from (68.5±2.9) µm of collagen film and (72.1±2.3) µm of A0 laminated collagen film to (102.6±4.4) µm of A4 laminated collagen film. Similar behaviour was recorded for chitosan films, where thickness increased from (38.5±0.5) µm of A0 chitosan film to (92.9±2.6) µm of A4 chitosan film. Increase in thickness followed the increase in OEO content. Standard deviation of thickness values for each film was less than 10 %.
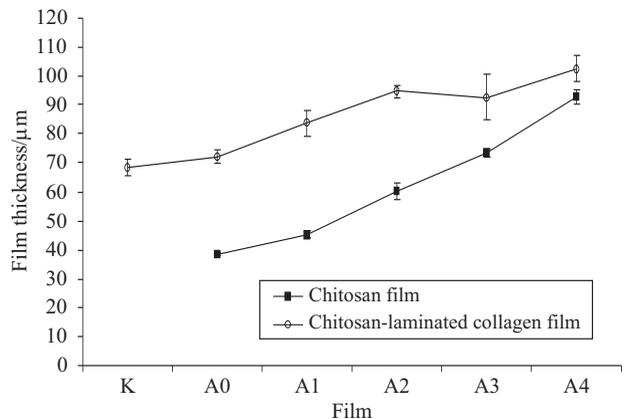
Fig. 2. Thickness of chitosan and chitosan-laminated collagen films. Bars represent the mean values±S.D. of 15 independent measurements. For film labels see Table 1

Regarding chitosan films, the increase of the added oil led to significant ($p<0.05$) decrease in film strength (Fig. 3). The film without oil exhibited a tensile strength of (44.5±2.6) MPa. The addition of oil decreased chitosan film strength, and the tensile strength of the film with 0.8 % oil was minimal (13.5±0.9) MPa. When the oil con-

Fig. 1. Photographs showing the effect of the addition of different volumes of oregano essential oil (OEO): a) samples of chitosan films, b) samples of collagen film and chitosan-laminated collagen films. For film labels see Table 1
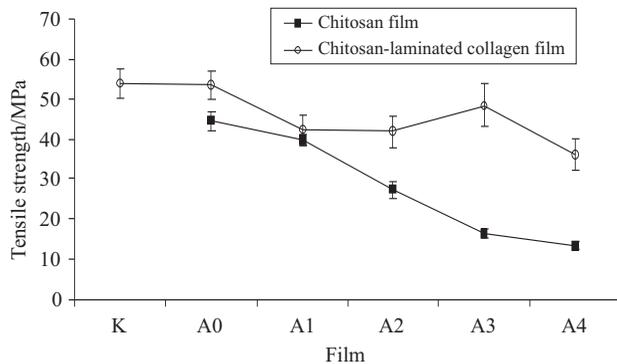
Fig. 3. Tensile strength of chitosan and chitosan-laminated collagen films. Bars represent the mean values±S.D. of 15 independent measurements. For film labels see Table 1

tent increases, the greater molecular contact between chitosan and oil compounds may weaken the polymer chain aggregation forces, making the matrix more open, which provokes a loss of film cohesion and mechanical resistance (*13*). Shuang (*2*) obtained comparable results for 1 % chitosan film with the addition of 1, 2, 3 or 4 % OEO. Similar effect was observed for laminated collagen films, although less pronounced. Tensile strength decreased from (54.0±3.8) MPa of collagen film (K) to (36.3±4.0) MPa of A4 laminated collagen film.

The addition of oil did not significantly (p>0.05) affect elongation at break values of chitosan films (Fig. 4). Elongation at break of the chitosan film without the added oil was (9.9±1.1) % and of the film with 0.8 % oil it was (7.9±1.1) %. With the addition of oil and the decrease of

were poor compared to the mentioned plastic films, which have elongation at break values above 200 % (*14*).

Swelling property

For chitosan film without oil (A0), it was not possible to determine the swelling degree, because the film partly dissolved in water. The addition of OEO affected the hydrophilic character of pure chitosan film and lowered film swelling in water from (2355.3±278.4) % of A1 chitosan film to (708.8±91.9) % of A4 chitosan film, as shown in Fig. 5. These results were expected because the essential oil is highly hydrophobic, and the increase in hydrophobic ingredients in the film matrix reduced water absorption. Similar results were obtained by Shuang (*2*) for water absorption of 1 % medium molecular mass chitosan film with 1, 2, 3 or 4 % OEO.

For laminated collagen films, swelling degree was around 200 % (Fig. 5). The addition of oregano essential oil did not have significant (p>0.05) effect. The swelling degree of uncoated collagen film (K) of (168.6±13.2) % was increased slightly by lamination with chitosan, and this increase was due to the swelling of the chitosan layer, but the effect was not significant (p>0.05). This might be because chitosan layer is much thinner than the collagen one, and thus the effect of its swelling was not significant. A0 laminated collagen film contains a layer of chitosan that is highly hydrophilic. Unlike A0 chitosan film, which dissolved in water, A0 laminated collagen film was protected on one side by the collagen and did not dissolve, but it swelled.

Fig. 4. Elongation at break of chitosan and chitosan-laminated collagen films. Bars represent the mean values±S.D. of 15 independent measurements. For film labels see Table 1
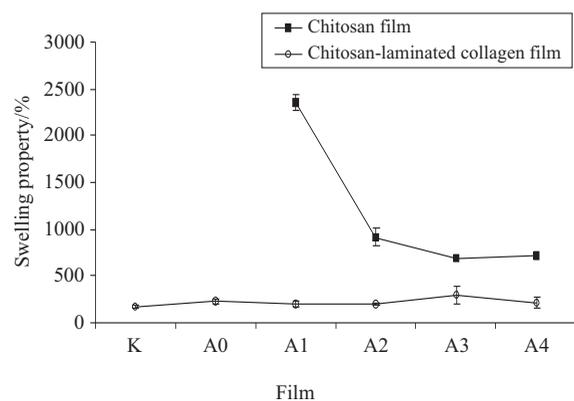
Fig. 5. Swelling property of chitosan and chitosan-laminated collagen films. Bars represent the mean values±S.D. of 5 independent measurements. For film labels see Table 1

tensile strength, elongation at break was expected to increase (*2,13*). This probably did not happen because of low OEO concentrations (below 1 %). Recorded values of elongation at break of laminated collagen films did not differ from K film (18.4±2.7) %, except for A3 film, which showed slightly higher elongation at break value, (22.4±3.8) % (Fig. 4).

Laminated collagen films showed tensile strength that could be compared to widely used plastic films, such as LDPE (23.6 MPa) and HDPE (47.4 MPa). The obtained results for elongation at break of laminated collagen films

Water vapour barrier properties

Chitosan film is hydrophilic and it acts like a semipermeable barrier to moisture, so its contribution to water vapour barrier properties of collagen film is limited. The addition of OEO increased the hydrophobic character of the films and this is the reason why A3 and A4 laminated collagen films showed lower water vapour transmission rate, $(1.8±0.1)·10^{-6}$ and $(1.78±0.03)·10^{-6}$ g/(m²·s·Pa) respectively, compared to collagen (K) film, $(2.51±0.05)·10^{-6}$ g/(m²·s·Pa) (p≤0.05; Fig. 6). These results can be compared to the results of Shuang (*2*), who showed that
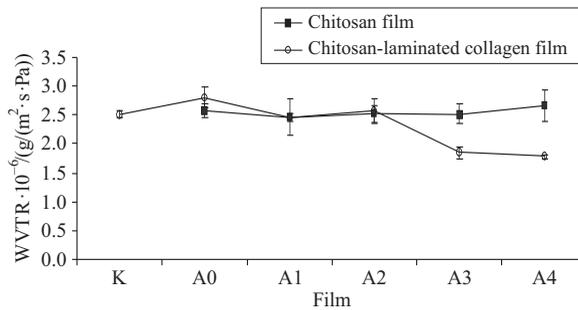
Fig. 6. Water vapour transmission rate (WVTR) of chitosan and chitosan-laminated collagen films. Bars represent the mean values ±S.D. of 5 independent measurements. For film labels see Table 1

chitosan film water vapour permeability could be lowered with the addition of OEO.

Water vapour permeability of chitosan film ($(99.0\pm9.3)\cdot10^{-12}$ g/(m·s·Pa)) increased with the addition of OEO (Fig. 7), following the increase of thickness values. Water vapour permeability of A4 chitosan film was $(248.1\pm25.1)\cdot10^{-12}$ g/(m·s·Pa). Similarly, water vapour permeability of laminated collagen films increased at first, starting from $(171.2\pm3.4)\cdot10^{-12}$ g/(m·s·Pa) for K film to $(242.7\pm20.2)\cdot10^{-12}$ g/(m·s·Pa) for A2 film and then decreased for A3 ($(171.0\pm9.3)\cdot10^{-12}$ g/(m·s·Pa)), and A4 laminated films $(182.4\pm3.6)\cdot10^{-12}$ g/(m·s·Pa) because of the hydrophobic effect of OEO.
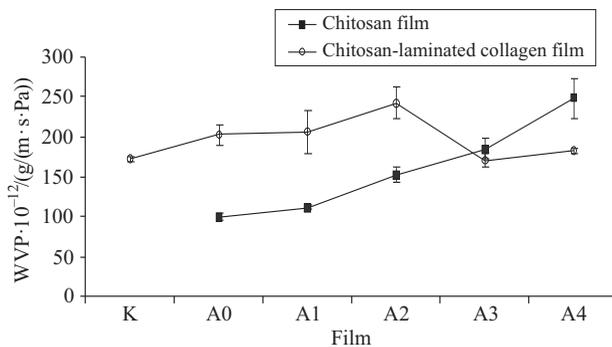
Fig. 7. Water vapour permeability (WVP) of chitosan and chitosan-laminated collagen films. Bars represent the mean values ±S.D. of 5 independent measurements. For film labels see Table 1

Comparable values were shown in the work of Ojagh *et al.* (*15*), where water vapour permeability of chitosan film was $(225\pm7)\cdot10^{-12}$ g/(m·s·Pa). In their work, however, with the addition of 0.8 and 2 % of essential cinnamon oil, water vapour permeability decreased to $(123\pm4)\cdot10^{-12}$ and $(100.3\pm6.7)\cdot10^{-12}$ g/(m·s·Pa), respectively. This might be because film thickness increased slightly with the addition of oil and did not influence water vapour permeability values to such an extent, as it was the case in this work.

If water vapour permeability is compared to plastic films like LDPE or OPP, which have shown water vapour permeability of $20\cdot10^{-12}$ and $6\cdot10^{-12}$ g/(m·s·Pa) respectively (*16*), it can be seen that this is the property that should be further optimized.

Oxygen permeability

Chitosan films are good barrier to gases. Results in literature vary depending on the chitosan origin, method of film preparation and testing conditions. Di Pierro *et al.* (*17*) determined oxygen permeability of chitosan film in 24 h to be approx. 25 (cm^3·µm)/(m^2·kPa) or $28.94\cdot10^{-14}$ cm^3/(m·s·Pa) for 2.5 % chitosan films. Olabarrieta (*18*) presented values of 1 % chitosan film oxygen permeability between $0.38\cdot10^{-14}$ and 3.82 cm^3/(m·s·Pa). Oxygen permeability of chitosan film is in the range of commercial plastic film permeability, such as polyvinylidene chloride (PVdC) ((0.4–$5.8)\cdot10^{-14}$ cm^3/(m·s·Pa)) or ethylene vinyl alcohol (EVOH) ((0.12–$13.89)\cdot10^{-14}$ cm^3/(m·s·Pa)) (*18*,*19*). Oxygen permeability of chitosan film is shown in Fig. 8. The permeability of the chitosan film without added oil was $(4.9\pm1.0)\cdot10^{-14}$ cm^3 cm^3/(m·s·Pa). The addition of the essential oil to the chitosan film did not affect this property significantly (p>0.05).
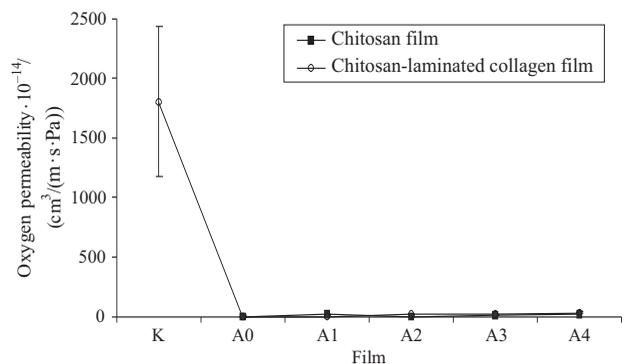
Fig. 8. Oxygen permeability of chitosan and chitosan-laminated collagen films. Bars represent the mean values±S.D. of 5 independent measurements. For film labels see Table 1

Uncoated film (K) showed very high oxygen permeability of $(1806.8\pm628.0)\cdot10^{-14}$ cm^3/(m·s·Pa), as expected (Fig. 8). High standard deviation value is probably a result of manufacturing process of commercial collagen casings. After lamination with chitosan layer, the permeability of the film decreased to values under $35\cdot10^{-14}$ cm^3/(m·s·Pa). Barrier films used commercially for packaging in vacuum and modified atmosphere are expected to show such results for oxygen permeability (*18–22*). For example, PA/PE films show oxygen permeability of $5\cdot10^{-14}$ to $20\cdot10^{-14}$ cm^3/(m·s·Pa).

Colour

Lamination with chitosan slightly reduced collagen film lightness (*L*) from 72.2±0.3 of K film to 67.7±0.5 of A4 laminated one, reduced yellowness (+*b*) from 20.1±0.4 to 18.0±0.8 and increased redness (+*a*) from 0.1±0.1 to 1.2±0.3 (Fig. 9). Colour changes caused by lamination were not visible to the naked eye.

In chitosan films, the addition of oil increased film redness (+*a*) and consequently decreased film greenness (–*a*) from –0.3±0.3 of A0 chitosan film to 3.9±0.3 of A4 chitosan film (Fig. 9). The addition of oil increased yellowness (+*b*) from 4.9±0.5 in A0 chitosan film to 19.0±2.3 in A4 chitosan film, and decreased lightness (*L*) from
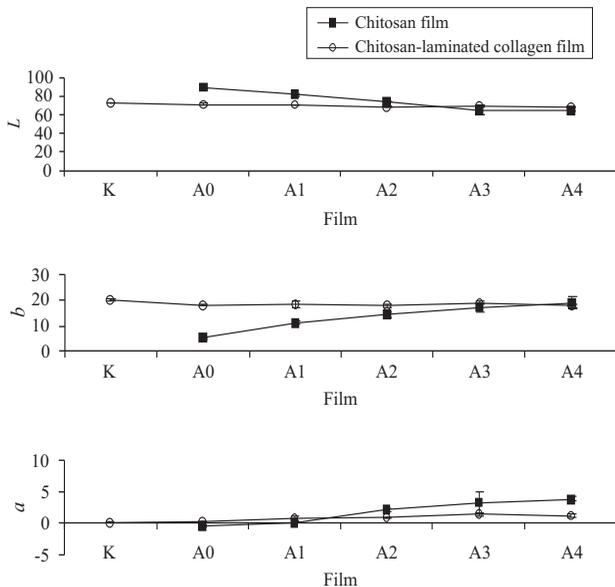
Fig. 9. Film colour parameters (*L*, *a* and *b* values) of chitosan and chitosan-laminated collagen films. Bars represent the mean values±S.D. of 5 independent measurements. For film labels see Table 1

88.9±1.1 in A0 chitosan film to 64.1±1.3 in A4 chitosan film. Films became more opaque, milky, whitish and yellowish, observed by the naked eye (Fig. 1). For pure chitosan films, the obtained colour results are similar to those presented by Li *et al.* (*23*) for 1 % low molecular mass chitosan film, and by Oguzlu and Tihminlioglu (*24*) for 3 % chitosan film.

Conclusion

Chitosan film proved to have good filmogenic property, and its commercial use in food packaging depends on its performance. Tensile strength of all chitosan-laminated collagen films was higher or comparable to those of widely used plastic films such as LDPE and HDPE. Laminated films showed considerably lower oxygen permeability compared to pure collagen casings. The addition of OEO to the chitosan layer had a positive effect by decreasing the water vapour transmission rate of the laminated collagen film, which makes it suitable for possible application in sausage preservation. Lamination with chitosan changed the colour of collagen film, but it was not visible to the naked eye, so it should not affect the typical colour. Laminated collagen film, which combines good tensile strength of the collagen film with good oxygen barrier property of the chitosan film, could find possible application in food industry. Our further work will focus on the use of chitosan lamination for dry fermented sausage protection.

References

1. N. Kandasamy, Development and characterization of anti-microbial edible films from crawfish chitosan, *PhD Thesis*, The Graduate School, Louisiana State University, Baton Rouge, LA, USA (2005).

2. C. Shuang, Development and characterization of antimicrobial food coatings based on chitosan and essential oils, *MSc Thesis*, The University of Tennessee, Knoxville, TN, USA (2004).

3. P.K. Dutta, S. Tripathi, G.K. Mehrotra, J. Dutta, Perspectives for chitosan based antimicrobial films in food applications, *Food Chem. 114* (2009) 1173–1182.

4. R.D. Joerger, Antimicrobial films for food applications: A quantitative analysis of their effectiveness, *Packag. Technol. Sci. 20* (2007) 231–273.

5. V.C. Souza, M.L.Monte, L.A.A. Pinto, Preparation of biopolymer film from chitosan modified with lipid fraction, *Int. J. Food Sci. Technol. 46* (2011) 1856–1862.

6. Y.C. Wu, S.P. Chi: *Handbook of Fermented Meat and Poultry*, Blackwell Publishing, Ames, IA, USA (2007).

7. U. Siripatrawan, S. Noipha, Active film from chitosan incorporating green tea extract for shelf life extension of pork sausages, *Food Hydrocolloids, 27* (2012) 102–108.

8. U. Siripatrawan, B.R. Harte, Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract, *Food Hydrocolloids, 24* (2010) 770–775.

9. Standard Test Method for Tensile Properties of Thin Plastic Sheeting. In: *Annual Book of ASTM Standards, No. D882--01*, American Society for Testing Materials, Philadelphia, PA, USA (2001).

10. A. Bigi, S. Panzavolta, K. Rubini, Relationship between triple-helix content and mechanical properties of gelatin films, *Biomaterials, 25* (2004) 5675–5680.

11. Standard Test Methods for Water Vapor Transmission of Materials. In: *Annual Book of ASTM Standards, No. E96-95*, American Society for Testing and Materials, Philadelphia, PA, USA (1994) 65–70.

12. ORIGIN Data Analysis and Graphing Software, v. 7.0, OriginLab Corporation, Northampton, MA, USA (*http://www.originlab.com*).

13. J. Bonilla, L. Atarés, M. Vargas, A. Chiralt, Effect of essential oils and homogenization conditions on properties of chitosan-based films, *Food Hydrocolloid*s, *26* (2012) 9–16.

14. S. Zivanovic, J. Li, P.M. Davidson, K. Kit, Physical, mechanical and antibacterial properties of chitosan/PEO blend films, *Biomacromolecules, 8* (2007) 1505–1510.

15. S.M. Ojagh, M. Rezaei, S.H. Razavi, S.M.H. Hosseini, Development and evaluation of a novel biodegradable film made from chitosan and cinnamon essential oil with low affinity toward water, *Food Chem. 122* (2010) 161–166.

16. R. Kulchan, W. Boonsupthip, P. Suppakul, Shelf life prediction of packaged cassava-flour-based baked product by using empirical models and activation energy for water vapor permeability of polyolefin films, *J. Food Eng. 100* (2010) 461–467.

17. P. Di Pierro, B. Chico, R. Villalonga, L. Mariniello, A.E. Damiao, P. Masi, R. Porta, Chitosan-whey protein edible films produced in the absence or presence of transglutaminase: Analysis of their mechanical and barrier properties, *Biomacromolecules, 7* (2006) 744–749.

18. I. Olabarrieta, Strategies to improve the aging, barrier and mechanical properties of chitosan, whey and wheat gluten protein films, *PhD Thesis*, KTH Fibre and Polymer Technology, Stockholm, Sweden (2005).

19. G. Robertson: *Food Packaging: Principles and Practice*, CRC Press, Taylor & Francis, Boca Raton, FL, USA (2005).

20. C. Weber, Biobased packaging materials for the food industry: Status and perspectives, *The Report of Food Biopack Project*, The Royal Veterinary and Agricultural University, Frederiksberg, Denmark (2005).

21. I. Cilla, L. Martinez, J.A. Beltrán, P. Roncalés, Dry-cured ham quality and acceptability as affected by the preservation system used for retail sale, *Meat Sci. 73* (2006) 581–589.

22. P.N. Skandamis, G.J.E. Nychas, Preservation of fresh meat with active and modified atmosphere packaging conditions, *Int. J. Food Microbiol. 79* (2002) 35–45.

23. J. Li, S. Zivanovic, P.M. Davidson, K. Kit, Characterization and comparison of chitosan/PVP and chitosan/PEO blend films, *Carbohydr. Polym. 79* (2010) 786–791.

24. H. Oguzlu, F. Tihminlioglu, Preparation and barrier properties of chitosan-layered silicate nanocomposite films, *Macromol. Symp. 298* (2010) 91–98.