CHITOSAN AND LYSOZYME AS COMPONENTS OF BIOCOMPOSITE EDIBLE COATINGS

A. Zimoch[1], A. Jarmoluk[1] and K. Marycz[2]

[1]Department of Animal Products Technology and Quality Management, University of Environmental and Life Sciences, Wroclaw, Poland

[2]Department of Animal Hygiene and Animal Welfare, University of Environmental and Life Sciences, Wroclaw, Poland

Abstract - Requirements for protective coatings for meat and meat products are multidirectional and related to: lack of negative environmental impact, mixing of the individual coating components, antioxidant and antimicrobial activity. In addition, the coating should be characterized by good mechanical and barrier properties against oxygen and moisture and should not change the natural appearance of the product. Coatings as an integral part of food product have to be safe for consumers and justify the high costs of their production. The aim of the study was to develop a preservation coating with good thermo-mechanical properties and compact structure for application on chilled fresh meat. Biocomposites produced with hydroxypropyl methylcellulose (HPMC), chitosan and lysozyme were investigated for phase angle (DMA), tensile strength, microstructure (SEM) and antimicrobial activity on fresh meat. The smaller the size of tan δ the greater the hydrolysis of HPMC and chitosan by lysozyme or lower concentration of both polymers. The ability of chitosan to increase mechanical properties was confirmed. Scanning electron microscopy revealed a problem with structure integrity when chitosan was added to the coatings, but the addition of lysozyme improved their morphology. The total reduction in bacterial numbers by about 2.5 log CFU/g was obtained for meat dipped in biocomposite containing HPMC 0.5%, chitosan 2% and lysozyme 1% after four wk of storage.

Key Words – bioactive film, meat preservation

I. INTRODUCTION

Chitosan coatings are characterized by moderate water permeability, and may be used to prevent drying of food, and can extend freshness [1]. In addition, chitosan has the ability to reduce lipid oxidation and discoloration, while maintaining the quality of fresh foods with high water activity during storage at low temperatures. Protective films prepared with chitosan may reduce the absorption of oil from fried breaded products [2]. Biocomposites of chitosan with pectin are characterized by reduced solubility compared to individual polymers, and in combination with HPMC, chitosan forms a compact, flexible films with high tensile strength [3]. Yin produced a transparent and brittle coating with chitosan and hydroxypropyl methylcellulose [4].

Lysozyme is a known muramidase discovered as a bacteriostatic agent by Alexander Fleming in 1922. Production of protective coatings with lysozyme and chitosan expands the range of its effects on bacteria such as: *Escherichia coli* and *Streptococcus faecalis* [5]. The enzyme mixture with chitooliogomers shows a strong inhibition effect bacteria growth such as *Escherichia coli*, *Pseudomonas fluorescens*, *Bacillus cereus* and *Staphylococcus aureus* in minced meat that is stored at refrigeration temperatures [6].

The objectives of this study were to develop composite biodegradable coatings for meat and meat products with HPMC, chitosan (CH) and lysozyme and to characterize the microstructure of the biocomposites by SEM, and evaluate thermo-mechanical and antimicrobial properties.

II. MATERIALS AND METHODS

Supplies used included: Longissimus ("Dworeccy", Poland), HPMC (Walocel HM 100 PA 2208 FG, Dow Wolff Cellulosics) and CH (low molecular weight, DD: 75-85%, low viscosity ranged between 20 and 200 cps, Aldrich), DL - lactic acid (85%, Sigma), glycerol 99% (Aldrich), lysozyme (Ovopol, Poland).

HPMC was dissolved in distilled water by stirring at 400 rpm. A solution of CH was prepared in diluted lactic acid. The stock solution of lysozyme was prepared by dissolving in distilled water. The

solutions were mixed in the appropriate proportions of final concentrations as shown in Table 1. Samples of biocomposites were then cast on previously leveled Teflon-coated glass plates and dried at 25°C and 60% RH for 48 h.

Table 1 Experimental design

Variants	Variability factors		Constant components		
	Chitosan [%]	Lysozyme [%]	HPMC [%]	Glycerol [%]	Lactic acid [%]
1.		0			
2.	0	0.5			
3.		1			
4.		0		25 (of the	
5.	1	0.5	0.5	total polymer	2
6.		1		weight)	
7.		0			
8.	2	0.5			
9.		1			

Dynamic Mechanical Analysis was performed in TRITEC 2000 DMA from Triton Technology. Strips of experimental films (5 mm length and 7 mm width) were heated from -80°C to 70°C at 2°C/min and the frequency of 1 Hz. tan δ (phase angle) was recorded.

Tensile strength of the dried samples was determined with Deben Microtest table for Dynamic Materials Testing (M Test 200 SEM). The broken coatings were placed on carbon discs and sprayed with a layer of gold within 150 s with a sputter coater (EDWARDS type SCAN COAT 6).

Internal microstructure of the test coatings was visualized using Scanning Electron Microscopy (Zeiss EVO 15 LS with SE1 detector) in the accelerating voltage = 20 kV EHT.

Fresh meat covered by biocomposite coatings were vacuum packaged and stored for 1, 2, 3 and 4 wk at 4°C. Experiment was replicated 3 times. To determine the total number of bacteria, meat was minced and homogenized in sterile bags. Serial dilutions were prepared and inoculated using a pour plate technique to a medium containing: tryptone, yeast extract, glucose and agar. After 72 h incubation at 30°C (PN-A-82055-6), colonies were counted. The results were converted to log CFU/g.

Response surface methodology (RSM) was used to investigate the simultaneous effect of the two experimental factors of chitosan and lysozyme at three levels. Statistical analysis was performed using the Statistica software (version 7.1, Statsoft, Inc.). Significance of differences was defined at $p \leq 0.05$.

III. RESULTS AND DISCUSSION

Glass-rubber transition temperatures of experimental biocomposites were observed as a result of dynamic mechanical analyses. Fig. 1 shows temperature dependence of the tan δ of the selected coatings based on HPMC. Glass transition is observed as a peak of tan δ when energy is given to the measurement system [7]. HPMC has a peak at 25 - 30°C and is designateded as α relaxation. Higher concentrations of CH in experimental biocomposites caused a shift in the temperature of α relaxation in higher region, from 30°C for coating with 1% CH to 55°C for film with 2% CH. Peaks observed at temperatures close to 0°C or below correspond to β relaxation (Fig. 1). Phase angle changes are often used to characterize α and β relaxation at a certain oscillation frequency [8]. This analysis is 1000 times more sensitive than differential scanning calorimetry – DSC [9]. Polysaccharides concentration is reflected in the size of phase angle peaks [9]. When lysozyme was added to the coating composition, a smaller glass transition peak was noted as a result of HPMC and CH hydrolysis (Fig. 1).

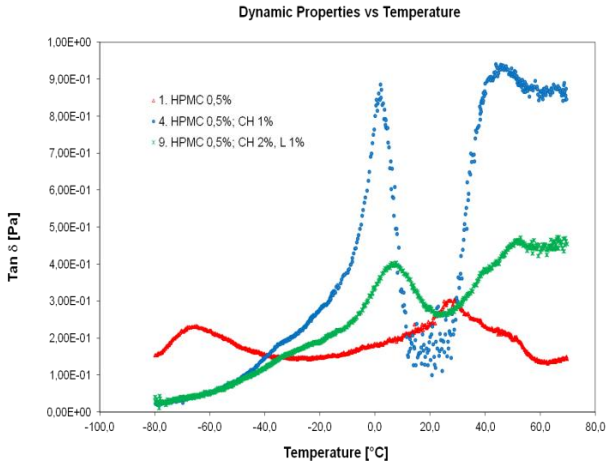

Figure 1. Relationship of tan δ on chitosan and lysozyme as a function of temperature

Fig. 2. shows the dependence of tensile strength on concentration of CH and lysozyme. Addition of CH to the coatings resulted in a statistically significant increase in tensile strength. Keratin films produced with CH also increased flexibility, as noted by Tanabe [10]. Simultaneously, a significance influence of lysozyme concentration in the film was not observed, but Park showed that the addition of lysozyme significantly weakened the structure of CH films and their integrity, which finally decreased their tensile strength [5].

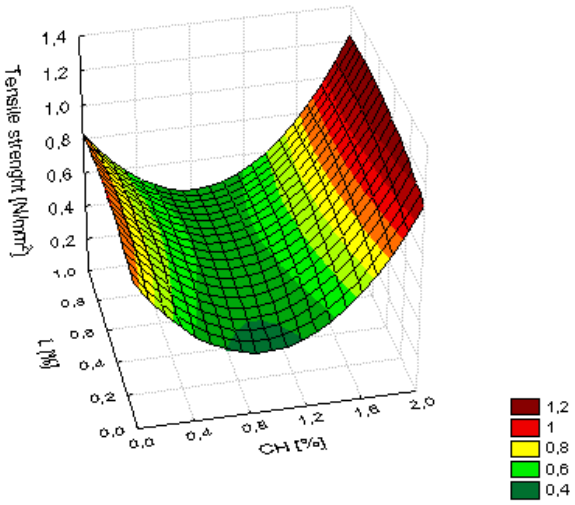

Figure 2. Changing tensile strength value as a function of changing concentrations of lysozyme and chitosan.

The cross – section microstructure of the biocomposite coatings was studied by scanning electron microscopy. Fig. 3 shows homogenous morphology and continuous structure of hydroxypropyl methylcellulose, while a rough structure with pores and cracks of matrix can be seen in Fig. 4. It may confirm the partial immiscibility of the polysaccharides, because interaction at the molecular level of two polymers can be seen in dynamic mechanical analysis as a sharp single peak [11], which was not observed in our study. However, it has to be noted that the cross-section structure of biocomposites between HPMC, chitosan and lysozyme (Fig. 5) is more homogenous than the structure of the biocomposite without added enzyme (Fig. 4). A compact structure without any pores in the CH – lysozyme blend was observed by Park, which shows the good miscibility of these two polymers [5]. Similar improvements of coating morphology among HPMC, CH and lysozyme can been seen in Fig. 5.

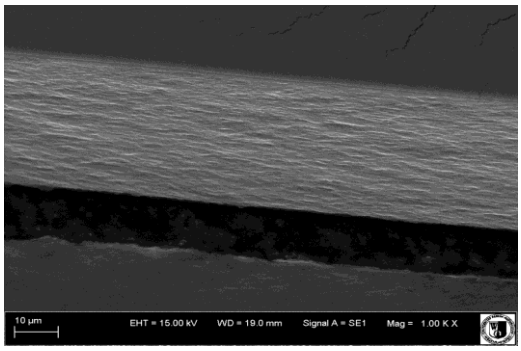

Figure 3. Scanning electron micrographs of a cross section of a HPMC coating (variant 1)

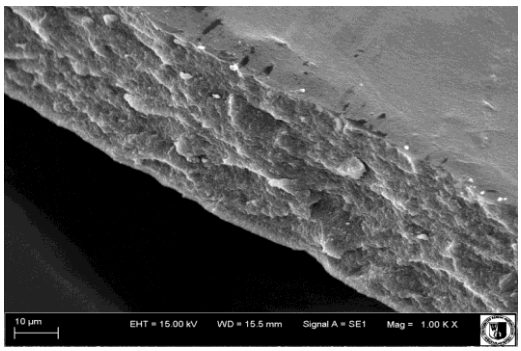

Figure 4. Scanning electron micrographs of a cross section of a HPMC and chitosan coating (variant 4)

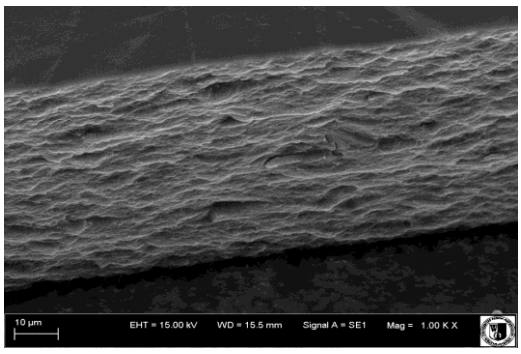

Figure 5. Scanning electron micrographs of a cross section of a HPMC, chitosan and lysozyme coating (variant 9)

After 4 weeks of storage, the total number of bacteria was reduced on meat covered with experimental coatings from around 8 log CFU/g for the control to 5.6 log CFU/g for variant 4 and 9

(Fig. 6). Inhibition of bacteria growth were also noted for meat samples covered with a coating produced with HPMC and lactic acid (variant 1) the beginning of storage, which may be related to the presence of lactic acid. Higher concentrations of CH in the biocomposite resulted in a greater reduction in bacteria growth. Similar results were obtained by several authors [12, 13]. Sagoo recorded a reduction in the number of bacteria about 3 log CFU/g in minced meat dipped in 0.3 and 0.6% CH solution [12]. Rao also obtained similar results for inhibition of the growth of bacteria in minced meat prepared with chitooligosaccharide and lysozyme [13].

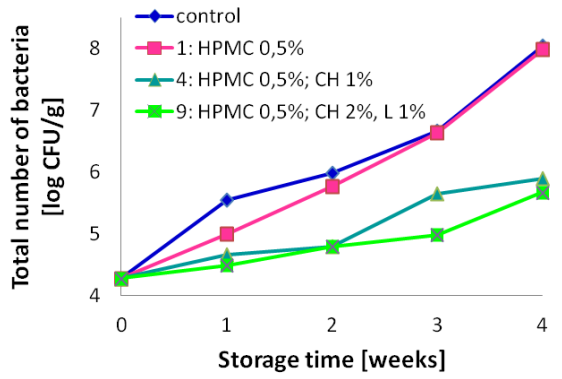

Figure 6. Total number of bacteria in minced meat with coatings (■▲) and without coatings (♦)

IV. CONCLUSION

Addition of lysozyme can improve the morphology of experimental coatings, but at the same time can lower their mechanical properties. Increasing concentration of chitosan in films is reflected in increasing values of tan δ and tensile strength as was expected. It is possible to improve the microbiological quality of fresh meat using chitosan and lysozyme in production of biocomposite coatings.

ACKNOWLEDGEMENTS

Authors thank for co-funding of the European Union through the European Regional Development Fund under the Operational Programme Innovative Economy, 2007-2013, the project "Innovative technologies for the production of biopreparates based on the new generation eggs (OVOCURA)"

REFERENCES

1. Guilbert S., Gontard N., Gorris L. G. M. (1996). Prolongation of shelf life of perishable food products using biodegradable films and coatings. Labensmittel-Wissenschaft und Technologie 29: 10-17.
2. Guilbert S. (2000). Edible films and coatings and biodegradable packaging. Bulletin of the IDF 346: 10-16.
3. Fell J.T, Ofori-Kwakye K. (2003). Leaching of pectin from mixed films containing pectin, chitosan and HPMC intended for biphasic drug delivery. International Journal of Pharmaceutics 250: 251-257.
4. Yin J., Luo K., Chen X. Khutoryanskiy V.V. (2006). Miscibility studies of the blends of chitosan with some cellulose ethers. Carbohydrate Polymers 63: 238-244.
5. Park S. – I., Daeschel M. A., Zhao Y. (2004). Functional properties of antimicrobial lysozyme-chitosan composite films. Journal of Food Science 8: 215-221.
6. Rao M. S., Chander R., Sharma A. (2008). Synergistic effect of chitooligosaccharides and lysozyme for meat preservation. Food Science and Technology 41: 1995-2001.
7. Manley T. R. (1989). Thermal analysis of polymers. Pure & Applied Chemistry 61: 1353-1360.
8. Lai Y. C., Sung P.H., Chien J. T. (2000). Evaluation of compatibility of rice starch and pectins by Tg and sub-Tg endotherms and the effect of compatibility on gel viscosity and water loss. Cereal Chemistry 77: 544-550.
9. Wetton R. E. (1986). Dynamic mechanical thermal analysis of polymers and related systems. In Dawkins JV, editor. Developments in polymer characterization (pp 179-222). New York: Elsevier Applied Science.
10. Tanabe T., Okitsu N., Tachibana A., Yamauchi K. (2002). Preparation and characterization of keratin – chitosan composite film. Biomaterials 23: 817-825.
11. Wu Y. B., Yu S. H., Mi F. L., Wu C. W., Shyu S. S., Peng C. K., Chao A. C. (2004). Preparation and characterization on mechanical and antibacterial properties of chitsosan/cellulose blends. Carbohydrate Polymers 57: 435–440.
12. Sagoo S., Board R., Roller S. (2002). Chitosan inhibits growth of spoilage microorganisms in chilled pork products. Food Microbiology 19: 175-182.
13. Rao M.S., Chander R., Sharma A. (2008). Synergistic effect of chitooligosaccharides and lysozyme for meat preservation. Food Science and Technology 41: 1995-2001.