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Antimicrobial effect of oregano-chitosan double coatings on *Listeria monocytogenes* in meat products

S Mojsova¹, L Angelovski¹, D Jankuloski¹, J Simonovska² and E Velickova²

¹ Food Institute, Faculty of Veterinary Medicine Skopje, Ss. Cyril and Methodius University in Skopje, Macedonia

² Department of Food and Biotechnology, Faculty of Technology and Metallurgy, Ss. Cyril and Methodius University in Skopje, Macedonia

E-mail: kostova.sandra@fvm.ukim.edu.mk

Abstract. The aim of this study was to investigate the effect of oregano and chitosan applied as edible coatings on the chemical and microbiological properties of different type of meat products. The main focus from the microbiological aspect was on *Listeria monocytogenes* as a pathogen that can survive for a long period on this type of product and, hence, present a risk for consumers. *In vitro* testing showed chemical quality after 7 and 14 days' storage of the three types of products was stable. Promising results were obtained for the microbial analysis, whereby large reductions in *L. monocytogenes* numbers after 7 and 14 days' storage at 4°C was measured in the coated products. The results indicated the chitosan and oregano combination can be an effective inhibitor of *L. monocytogenes* growth in chilled meat products.

1. Introduction

The food industry is focusing on finding modern methods and technologies to increase the shelf life of foods. The susceptibility of ready-to-eat meat products to spoilage is an economic problem for producers, and various methods have been applied to enhance the shelf life of these foods [1]. On the other hand, consumer demands for healthy meals that are free of chemical preservatives are greater now than in the past.

Biodegradable, edible chitosan films combined with plant extracts as double coatings have recently been widely used to enhance the qualitative and microbial stability of food [2], [3], [4]. Edible coatings are applied on the surface of the food in thin mono- or multi-layers. Direct incorporation of essential oils in foods such as meat products will result in the immediate reduction of the bacterial population but can alter the sensory characteristics of added food. This incorporation of essential oils in edible films may be particularly interesting, and some studies showed that oregano and garlic oil were, for example, effective in whey protein-based films against *Staphylococcus aureus*, *Salmonella* Enteritidis, *Listeria monocytogenes*, *Escherichia coli* and *Lactobacillus plantarum* [5].

This study was performed using oregano-chitosan double coating to improve the microbial stability of meat products against *L. monocytogenes*. The control and coated meat samples were stored in plastic containers at 4°C for two weeks. The quality was evaluated by determining the water and fat content and the microbial safety by following the numbers of *L. monocytogenes* as indicators of the potential antimicrobial effect of the added extracts as coatings.

2. Materials and methods

2.1. Materials

Chitosan was purchased from Sigma Aldrich (St. Louis, USA), while glycerol, Tween 80 and acetic acid were from Merck, Germany. All chemicals were of analytical grade. The alcohol oregano extract was obtained with supercritical extraction with CO₂ from dried oregano herb.



2.2. Preparation of the edible coatings

Chitosan solution (1%, w/v) was prepared by dissolving 1 g of chitosan in 100 mL of acetic acid (1% v/v) with continuous stirring at room temperature (20±2°C). After the complete dissolution of the chitosan, 0.2 g of glycerol and 0.2 g of Tween 80, were added and the solution was stirred for another 30 min.

The oregano extract was prepared by dissolving 1g of extract in 10 ml 97% ethanol.

2.3. Preparation of the meat samples

Meat products (smoked pork neck, ham and smoked beef tenderloin), commercially produced, were purchased at retail. The plastic packaging was removed and slices were cut. The double coating was applied by brushing the slices on their surface with the oregano extract and letting it dry for 20 min. Then, the second coating, chitosan, was applied, again by brushing and then drying for additional 20 min.

After the drying of the two coatings, *L. monocytogenes* ATCC 13932 was added to the meat products. The bacterial concentration was approximately 1.5x10⁶ cfu/ml and the coated slices were dipped for 30 seconds in the bacterial suspension. Four groups of meat product slices were prepared.

1. Meat products for chemical and sensory analysis without chitosan and oregano, no contamination with *L. monocytogenes*; A – smoked pork neck, B – ham, C – smoked beef tenderloin.
2. Meat products for chemical and sensory analysis with chitosan and oregano, no contamination with *L. monocytogenes*; CA – smoked pork neck, CB – ham, CC – smoked beef tenderloin.
3. Meat products for microbiological analysis without chitosan and oregano and contaminated with *L. monocytogenes*; AM – smoked pork neck, BM – ham, CM – smoked beef tenderloin.
4. Meat products for microbiological analysis with chitosan and oregano and contaminated with *L. monocytogenes*, CAM – smoked pork neck, CBM – ham, CCM – smoked beef tenderloin.

All of the prepared meat product slices were stored at 4°C and were analysed on days 7 and 14 of storage.

2.4. Quality control of meat samples

Water and moisture contents of the coated and uncoated meat slices were analysed using reference methods. For the total fat content, analysis was according to ISO1443:1973 and moisture content was according to ISO 1442:1997.

2.5. Microbiological analysis of meat samples

Detection and enumeration of *L. monocytogenes* was performed according to ISO 11290:2017. For the isolation of *L. monocytogenes*, approximately 25 g of each sample was homogenised with 225 mL of half-strength Fraser broth in a stomacher for 2 minutes. This homogenate was then incubated at 30 °C for 24 h. An aliquot of 1 mL was transferred to tubes containing Fraser broth supplemented with Fraser selective supplement and incubated at 37 °C for 48 h. The cultures were streaked onto plates containing the *Listeria* agar Ottaviani & Agosti and incubated at 37 °C for 24 h. Afterwards, 3-5 suspect colonies were selected for confirmation. The confirmation of *L. monocytogenes* colonies after isolation on suitable media was based on several methods, including Gram staining and measurement of haemolytic activity on sheep blood agar, the carbohydrate utilisation pattern, the catalase reaction and tumbling motility.

2.6 Sensory analysis of the meat products

A six member trained panel evaluated the meat product slices according to standard protocol (white light illumination, room temperature of 23±2°C, relative humidity of 50%). Ten gram samples were coded randomly and were served in white plastic plates along with a glass of water for neutralisation

of the taste. The sensory panel evaluated the attributes of colour, odour and general appearance using a 9 point intensity scale [6].

2.7 Statistical analysis

All data were analysed statistically ($n=6$ and $p=0.05$ or 5% significance level) by one way analysis of variance homogeneity test and Duncan's Multiple Range Test (DMRT) using SPSS-16.0 software package for standard methods [7].

3. Results and discussion

3.1 Chemical analysis

The chemical parameters, water and fat content, are given in Table 1. These parameters are the most variable during storage, so were chosen as indicators of the products' stability. The main characteristic of the edible coatings was anticipated to be their antimicrobial effect against *L. monocytogenes*. However, chemical analyses proved the double coating also exhibited good barrier properties, since there was no significant change in the water and fat content of the coated meat slices, meaning the coatings prevented the meat products (CA, CB and CC) from drying which could later cause auto-oxidation of lipids and loss of sensorial attributes. The uncoated meat products (A, B and C) showed loss of water during storage, proving they were more susceptible to drying than their coated counterparts.

Table 1. Chemical analysis of the meat product slices

Meat product group	Water content (%)		Fat content (%)	
	7 day	14 day	7 day	14 day
CA	66.5±1.5 ^a	65.1±2.1 ^a	2.5±0.5 ^a	2.9±0.4 ^a
A	68.5±2.0 ^a	65.4±0.9 ^b	3.0±0.7 ^a	3.2±0.2 ^a
CB	71.6±1.9 ^a	72.3±1.4 ^a	2.4±0.4 ^a	2.6±0.3 ^a
B	73.2±2.2 ^a	70.0±0.6 ^b	2.5±0.3 ^a	2.4±0.1 ^a
CC	53.1±1.8 ^a	58.2±2.9 ^a	2.5±0.3 ^a	2.4±0.2 ^a
C	60.4±2.8 ^a	54.5±2.8 ^b	2.6±0.6 ^a	2.9±0.3 ^a

Mean values and standard deviation, ($n = 3$); Different small letters within a row for each analysis indicate significant differences due to storage time within the same sample; Meat product slices with double coating, CA – smoked pork neck, CB – ham, CC – smoked beef tenderloin; meat product slices without coating, A – smoked pork neck, B – ham, C – smoked beef tenderloin.

3.2 Microbiological analysis

Our investigation showed that *L. monocytogenes* remained viable on the meat products for the examined 14-day period (Table 2). That will surely influence the expected shelf-life of such products, and if they do contain this pathogen, could affect consumers' health.

Contact of *L. monocytogenes* with chitosan-oregano double coating resulted in decreased bacterial populations (Table 2 and Figures 1-3). Populations of *L. monocytogenes* ATCC 13932 inoculated on the meat products in the presence of chitosan-oregano double coating decreased from 6 log₁₀ cfu/g to undetectable levels after 14 days at 4°C in two types of meat products (smoked pork neck and smoked beef tenderloin) while in the third meat product group (ham), a large decrease in the microbial load was measured. The mechanism of this antimicrobial activity can be explained in various ways. Some authors have suggested that the interaction between positively-charged chitosan molecules and negatively-charged microbial surfaces results in the disruption of cell membranes, leakage of intracellular constituents, and ultimately, microbial cell death [8]. Concerning the meat products without coating, microbiological analysis showed stable populations of the inoculated pathogen.

Table 2. *L. monocytogenes* numbers on sliced meat products with and without coating during 14 days' storage at 4°C

Meat product group	<i>Listeria monocytogenes</i> log cfu/g	
	7 days	14 days
CAM	3.36	0
AM	5.32	4.70
CBM	3.83	1.47
BM	5.66	5.08
CCM	2.96	0
CM	5.53	4.23

Meat product slices with coating, CAM – smoked pork neck, CBM – ham, CCM – smoked beef tenderloin; meat product slices without coating, AM – smoked pork neck, BM – ham, CM – smoked beef tenderloin.

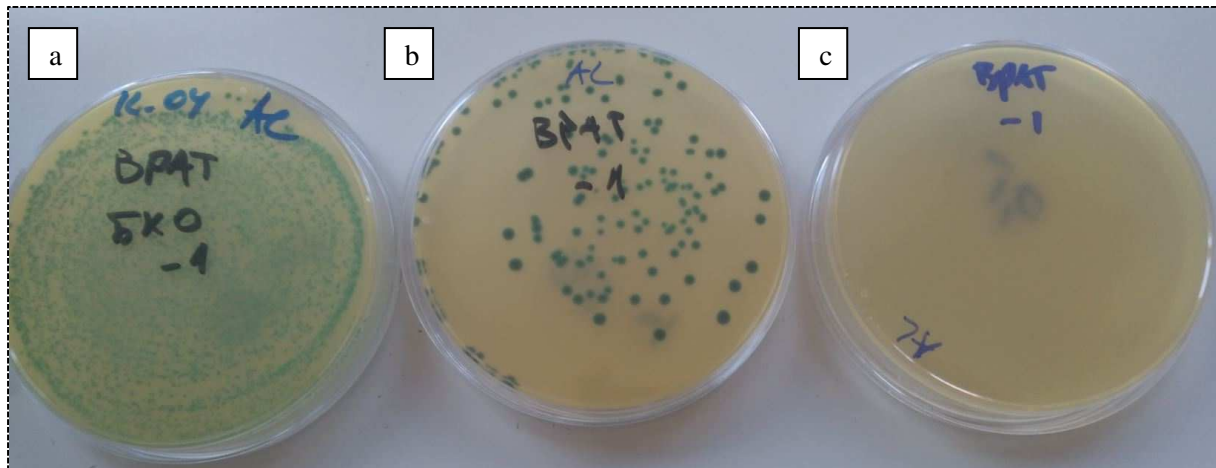


Figure 1. Presumptive *Listeria* derived during storage from AM – smoked pork neck, no coating, and; CAM – smoked pork neck with coating. AM after 7 days, b) CAM after 7 days and c) CAM after 14 days

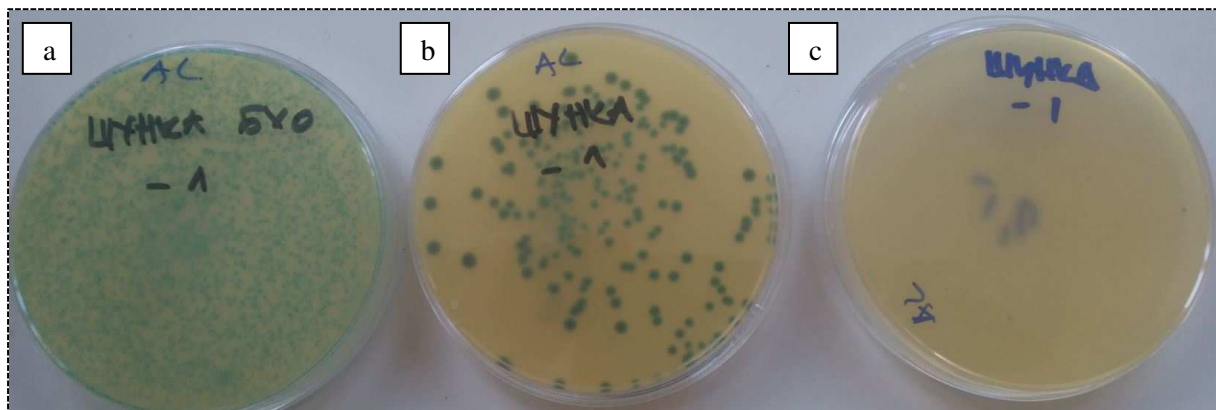


Figure 2. Presumptive *Listeria* derived during storage from BM – ham no coating, and; CBM – ham with coating. a) BM after 7 days, b) CBM after 7 days and c) CBM after 14 days

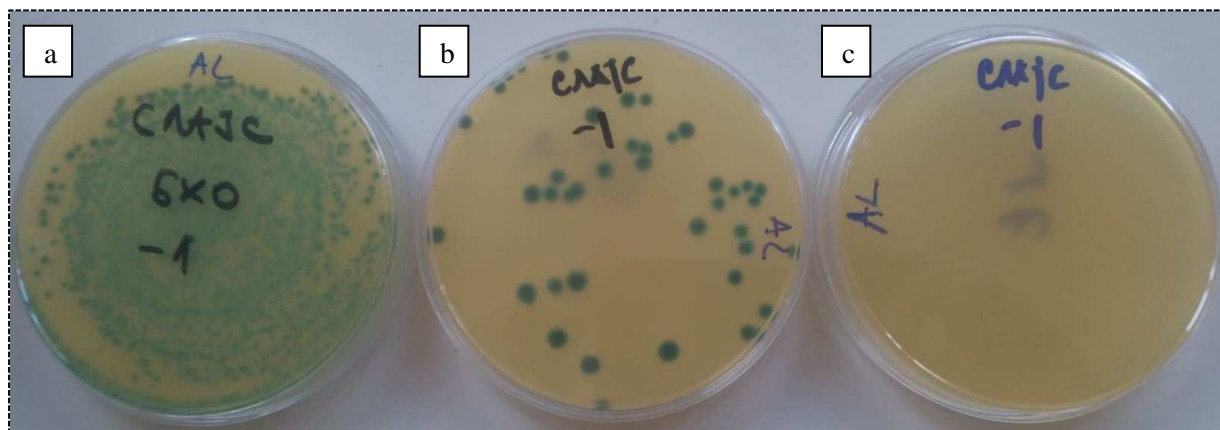


Figure 3. Presumptive *Listeria* derived during storage from CM – smoked beef tenderloin, no coating, and; CCM – smoked beef tenderloin with coating. a) CM after 7 days, b) CCM after 7 days and c) CCM after 14 days

3.3 Sensory analysis

The trained panellists evaluated the meat products during storage in terms of colour, odour and general appearance (Table 3). As expected, the coated meat product slices had slightly lower scores compared to their corresponding controls, but were still very well graded, with more than 75% of panellists finding them acceptable. At the beginning, the acceptability difference was the most noticeable, being around 12% difference between control and coated meat products, and was most noticeable for ham. After the first week, the score difference was 9-10% between control and coated meat products, while after the second week of storage, this sensory difference was even smaller and ranged around 6-7%. These results confirm that the edible coating, besides its beneficial effect on the products' stability, also, had good sensorial characteristics, since oregano is among the most common herbs, and consumers usually accept its flavour without problem.

Table 3. Sensory analysis of the coated and uncoated meat slices

Meat product group	Colour		Odour		General appearance	
	7 days	14 days	7 days	14 days	7 days	14 days
CA	7.4 ± 0.42 ^a	7.0 ± 0.52 ^b	8.3 ± 0.82 ^a	7.6 ± 0.56 ^b	8.0 ± 0.67 ^a	7.6 ± 0.51 ^b
A	8.1 ± 0.31 ^a	7.5 ± 0.52 ^b	8.5 ± 0.84 ^a	7.9 ± 0.69 ^b	8.4 ± 0.51 ^a	7.9 ± 0.42 ^b
CB	8.0 ± 0.70 ^a	7.7 ± 0.48 ^b	8.4 ± 0.51 ^a	7.6 ± 0.69 ^b	8.0 ± 0.47 ^a	7.9 ± 0.31 ^b
B	8.7 ± 0.67 ^a	8.6 ± 0.69 ^b	8.4 ± 0.84 ^a	7.9 ± 0.31 ^b	8.6 ± 0.51 ^a	8.3 ± 0.48 ^b
CC	7.9 ± 0.52 ^a	7.3 ± 0.82 ^b	8.2 ± 0.42 ^a	7.6 ± 0.51 ^b	8.5 ± 0.70 ^a	8.0 ± 0.47 ^b
C	8.3 ± 0.31 ^a	8.0 ± 0.48 ^b	8.5 ± 0.84 ^a	8.3 ± 0.67 ^b	8.8 ± 0.42 ^a	8.6 ± 0.51 ^b

Mean values and standard deviation, ($n = 6$); Different small letters within a row indicate significant differences due to storage time within the same sample.

Meat product slices with double coating, CA – smoked pork neck, CB – ham, CC – smoked beef tenderloin; meat product slices without coating, A – smoked pork neck, B – ham, C – smoked beef tenderloin.

4. Conclusion

Oregano-chitosan double coatings can improve the microbial safety of meat products during refrigerated storage and increase the shelf life while maintaining the quality parameters. However, there is still a requirement to improve and standardise the coating procedures according to food industry needs (reduced costs and increased shelf life) and to meet consumer demands without altering the sensory characteristics of the meat products.

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