

EFFECT OF EDIBLE FILMS AND COATINGS CONTAINING
ANTIOXIDANT ON THE QUALITY OF RABBIT MEAT
DURING LOW TEMPERATURE STORAGE

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Abstract

Rabbit meat is very susceptible to spoilage due to its high moisture and protein content. Hence, the application of natural antioxidants is important to restrict the deterioration and maintain its high quality and shelf life. This study was designed to investigate the effect of edible films and coatings made of chitosan and containing rosemary extract on the pH, colour and lipid oxidation in rabbit meat during low temperature storage. Samples of the loin and hind leg derived from New Zealand rabbits were divided in five groups – control (C), F1 – wrapped into chitosan film; F2 – wrapped into chitosan + rosemary film; S1 – immersed into chitosan solution, S2 – immersed into chitosan + rosemary solution. The meat was stored at 4 °C for 7 days and then at –20 °C up to day 90. The values of pH increased overtime in the loin and leg, and were higher in the treated groups, especially F1 and F2. The higher values of pH in F1 and F2 corresponded to the decreased lightness (L*) in these groups in both loin and leg. The redness (a*) and yellowness (b*) remained unaffected by the treatment and storage in the loin. In the leg, we observed decreased a* in S1 and S2 after 7 days, and in F2, S1 and S2 after 90 days. The lowest value of b* in the leg was exhibited in F2 after frozen storage. Regardless of the treatment and storage period, TBARS remained lower in the meats with edible films and coatings, demonstrating their high potential to restrict oxidation.

Key words: rabbit meat, edible films, edible coatings, antioxidants, meat quality

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Introduction. Meat is an important component of the diet, however, the high levels of its consumption especially of red meats has been criticized lately. This is due to the increased saturated fats provided by the red meats and the associated risk of chronic diseases such as cardiovascular diseases, diabetes and certain types of cancer [1]. White meat (mainly derived from poultry) is considered a healthier option by many. Studies suggest that it may protect from all-cause mortality, stroke and cancer, although no clear evidence exists in regard to heart disease and diabetes [2]. Rabbit meat is one of the best white meats, being an excellent sustainable source of protein. In addition, it has low fat and cholesterol content, but high content of unsaturated fatty acids, B vitamins, potassium, phosphorus and magnesium [3]. In 2022, the global rabbit meat production was 756 438 t, with Asia being the main producer (64.3%), followed by Europe (19.5%) [4]. Inside the EU, Spain, Italy and the Czech Republic are the biggest rabbit meat consumers (57%) followed by France, Germany, Bulgaria, and Slovakia. The storage under low temperatures includes traditionally refrigeration and frozen storage and is the most common way to preserve fresh meat [5]. Although low temperatures can effectively inhibit the growth of microorganisms and the effect of enzymes thus preventing the meat spoilage, still oxidation processes occur and lead to changes in the sensory qualities of meat [6]. Edible films and coatings are structures from biological macromolecules used to form thin layers to protect the products from environmental damage and to increase their shelf life [7]. Although both terms are used interchangeably, films in general are preformed, thin layers, and are freestanding, whereas, coatings are formed directly on the food product [8]. The use of edible films and coatings is an option for meat, especially if enriched with natural antioxidants, to offer both the packaging and the necessary protection during storage with natural and biodegradable components. Chitosan edible films and coatings have drawn much attention due to their antioxidant activities in food systems [9]. The aim of this study was to investigate the effect of application of edible chitosan films and coatings containing rosemary extract on the quality attributes of rabbit meat during low temperature storage.

Material and methods. For the purposes of the experiment the edible films and coatings were made of chitosan and contained rosemary (*Rosmarinus officinalis*) extract.

Preparation of the edible films. Chitosan (at a concentration of 1.5% w/v) was dispersed in 1% acetic acid solution. The mixture was homogenized until the chitosan was completely dissolved and glycerol (0.8 g/g polymer) was added. To prepare the composite films ethanol extract of rosemary was added to the film forming solution at amount 2%. In the pure chitosan films, the rosemary extract was replaced by 70% ethanol. The film-forming solutions were then sonicated for 10 min to remove air bubbles and distributed in Teflon (non-stick) trays in amounts of 0.300 g/cm². The trays were dried at 47 °C in SPT-200 Vacuum Drier for 16 h. The films were then separated, cut into pieces and stored in a desiccator until analysis.

Preparation of the coatings. Two percent (w/v) solution of chitosan in 1% acetic acid solution was prepared. Ethanolic extract of rosemary was added to the chitosan solution to obtain final concentration of 2%. The solution was homogenized at 800 rpm for 10 min. In the pure chitosan solutions, the rosemary extract was replaced by ethanol. The meat was immersed into the solutions for 10 s and then immersed into Na_2CO_3 solution prior storage.

Meat treatment. Rabbit loins ($n = 6$) and hind legs ($n = 6$) were obtained from New Zealand White rabbits (2.50–2.80 kg), raised in the Institute of Animal Science – Kostinbrod. The loins and legs were trimmed from visible fat and cut into portions ($5 \times 5 \times 1.5$ cm). The portions from each meat cut were divided into 5 groups – C (muscle without treatment), F1 – wrapped into chitosan film; F2 – wrapped into chitosan+rosemary film; S1 – immersed into chitosan solution, S2 – immersed into chitosan + rosemary solution. The samples from each group were individually packaged into bags and stored for 7 d (4°C) and 90 d (-20°C) until analysis.

pH and colour measurements. The measurement of pH was done using pH meter (Jenway 3300), with glass electrode, calibrated at pH 4.0 and 7.0. The colour values (L^* – lightness, a^* – redness and b^* – yellowness) were measured on the surface of the meat using colourimeter PCE-CSM 4 (PCE Instruments). Both pH and instrumental colour measurements were done on fresh meat and after 7 and 90 days of storage on three locations of the sample, as the results were averaged.

Oxidation of lipids. The oxidation of lipids was measured determining the amount of 2-thiobarbituric acid reactive substances (TBARS), expressed as mg malondialdehyde per kg of meat (mg MDA/kg meat) [10, 11]. All the analyses were performed in triplicate.

Statistical evaluation. The statistical processing of the data was done through ANOVA procedure of the JMP Pro 17 software package [12]. The effects of the antioxidant treatment and the storage were assessed. When significant effects were found, the difference between the means were evaluated through post hoc comparisons (Tukey HSD), at $P < 0.05$. The results were presented as Mean $\pm$ SD.

Results and discussion. The pH of the fresh loin was 5.81 (Table 1) and of the leg, 5.83 (Table 2).

These values were slightly higher than the ones reported in New Zealand rabbits [13], however, they fall within the range of the meat of commercial hybrids. When measured after refrigerated storage the values of pH showed significant differences among treatments in the loin ($P = 0.0008$) and leg ($P < 0.0001$). The control groups showed the lowest pH when compared to the treated meats during this period. After the frozen storage, we also observed considerable differences between the control and the treated samples in the loin ($P = 0.0097$) and the leg ($P < 0.0001$). Furthermore, F1 and F2 samples had considerably higher

T a b l e 1

Effect of the edible films and coatings on the pH and colour of rabbit loin

Trait	Treatment	Storage			P-value
		0 d	7 d	90 d	
pH	C	5.81 ± 0.16^{AB}	5.99 ± 0.08^{cA}	5.58 ± 0.06^{bB}	0.0103
	F1	5.81 ± 0.16^B	6.71 ± 0.24^{aA}	6.06 ± 0.02^{abB}	0.0015
	F2	5.81 ± 0.16^B	6.39 ± 0.09^{abA}	6.27 ± 0.28^{aAB}	0.0235
	S1	5.81 ± 0.16^{AB}	6.16 ± 0.12^{bcA}	5.78 ± 0.16^{abB}	0.0334
	S2	5.81 ± 0.16^B	6.28 ± 0.06^{bcA}	5.80 ± 0.26^{abB}	0.0262
	P value		0.0008	0.0097	
L*	C	53.78 ± 1.41	55.28 ± 0.20^{ab}	52.11 ± 2.67^{ab}	0.1651
	F1	53.78 ± 1.41^A	50.44 ± 2.18^{bcA}	45.94 ± 1.44^{bB}	0.0041
	F2	53.78 ± 1.41	49.44 ± 2.23^c	48.02 ± 3.48^{ab}	0.0706
	S1	53.78 ± 1.41	54.28 ± 2.13^{abc}	51.36 ± 3.60^{ab}	0.3847
	S2	53.78 ± 1.41^B	58.90 ± 2.50^{aA}	54.33 ± 2.27^{aB}	0.0471
	P-value		0.0012	0.0285	
a*	C	9.57 ± 0.66	10.25 ± 1.80	9.51 ± 1.33	0.7675
	F1	9.57 ± 0.66	13.44 ± 3.69	9.78 ± 1.32	0.1454
	F2	9.57 ± 0.66	13.62 ± 5.84	9.97 ± 3.27	0.4243
	S1	9.57 ± 0.66	13.57 ± 2.90	12.54 ± 3.10	0.2029
	S2	9.57 ± 0.66	10.84 ± 3.99	9.92 ± 2.60	0.8491
	P-value		0.7056	0.5770	
b*	C	5.80 ± 0.36	6.37 ± 0.35	6.74 ± 0.93	0.2410
	F1	5.80 ± 0.36^{AB}	8.87 ± 2.50^A	4.86 ± 0.73^B	0.0406
	F2	5.80 ± 0.36	9.04 ± 2.81	5.81 ± 1.82	0.1416
	S1	5.80 ± 0.36	7.47 ± 0.79	6.28 ± 1.13	0.1102
	S2	5.80 ± 0.36	7.46 ± 0.94	6.69 ± 1.16	0.1513
	P-value		0.3796	0.3563	

Means with different uppercase superscripts in a row are significantly different ($P < 0.05$); means with different lowercase superscripts in a column are significantly different ($P < 0.05$)

pH than S1 and S2. This might indicate presence of proteolytic bacteria on the films that might occur during the process of their preparation. In contrast to our results, EL BAYOMI et al. [14] demonstrated significant decrease in rabbit meat dipped in rosemary essential oil, chitosan or combination of both, thus suggesting antimicrobial activity of these coatings. In this experiment we did not observe such effect. Despite the increase of the pH in the antioxidant treated meat, its values were similar to those reported in other studies [15]. In line with our results, increased pH in rabbit meat with alginate coating containing clove essential oil during storage were reported [16]. The authors suggest this might be attributed to the higher pH of the alginate or higher microbial count on the rabbit carcasses. During the first 7 days of the storage under refrigerated conditions, the pH showed

T a b l e 2

Effect of the edible films and coatings on the pH and colour of rabbit leg

Trait	Treatment	Storage			<i>P</i> -value
		0 d	7 d	90 d	
pH	C	5.83 ± 0.11^B	6.06 ± 0.09^{cA}	5.70 ± 0.03^{bB}	0.0062
	F1	5.83 ± 0.11^C	6.58 ± 0.10^{aA}	6.08 ± 0.08^{aB}	0.0002
	F2	5.83 ± 0.11^B	6.29 ± 0.07^{bA}	6.18 ± 0.16^{aA}	0.0076
	S1	5.83 ± 0.11^B	6.12 ± 0.01^{bcA}	5.62 ± 0.05^{bC}	0.0004
	S2	5.83 ± 0.11^A	6.26 ± 0.06^{bB}	5.78 ± 0.04^{bB}	0.0004
	<i>P</i> -value		< 0.0001	< 0.0001	
L*	C	51.32 ± 2.43	52.31 ± 2.26^{ab}	54.83 ± 2.04^a	0.2234
	F1	51.32 ± 2.43^A	48.93 ± 3.77^{bAB}	42.67 ± 2.06^{bB}	0.0242
	F2	51.32 ± 2.43	49.61 ± 3.40^{ab}	52.28 ± 1.90^a	0.4982
	S1	51.32 ± 2.43	56.87 ± 2.34^a	56.89 ± 3.63^a	0.0870
	S2	51.32 ± 2.43	53.13 ± 1.10^{ab}	51.45 ± 3.54^a	0.6490
	<i>P</i> -value		0.0343	0.0008	
a*	C	13.88 ± 1.98	14.29 ± 2.72^{ab}	11.41 ± 2.57^{ab}	0.3601
	F1	13.88 ± 1.98	15.59 ± 1.36^{ab}	17.70 ± 1.35^a	0.0678
	F2	13.88 ± 1.98^{AB}	16.93 ± 0.83^{aA}	9.90 ± 2.34^{bB}	0.0096
	S1	13.88 ± 1.98	11.92 ± 1.84^b	8.94 ± 2.29^b	0.0655
	S2	13.88 ± 1.98	12.51 ± 0.15^b	10.54 ± 3.05^b	0.2270
	<i>P</i> -value		0.0190	0.0082	
b*	C	6.05 ± 2.99	8.51 ± 1.39	7.34 ± 1.96^{ab}	0.4466
	F1	6.05 ± 2.99	8.29 ± 1.30	8.63 ± 1.37^a	0.3142
	F2	6.05 ± 2.99^{AB}	9.09 ± 1.43^A	3.99 ± 0.46^{bB}	0.0472
	S1	6.05 ± 2.99	6.45 ± 3.11	5.55 ± 1.63^{ab}	0.9179
	S2	6.05 ± 2.99	7.09 ± 0.56	5.40 ± 1.52^{ab}	0.5962
	<i>P</i> -value		0.3983	0.0239	

Means with different uppercase superscripts in a row are significantly different ($P < 0.05$); means with different lowercase superscripts in a column are significantly different ($P < 0.05$)

considerable increase regardless of the treatment in the loin (Table 1) and in the leg (Table 2).

This indicates protein hydrolysis due to the ageing of the meat and release of different amino acids. Considerable increase in pH during refrigeration was reported in rabbit muscles stored for 18 days [17]. Further, in the frozen meat, pH displayed significant decrease and its values were similar to those of the fresh meat. Such changes in pH during storage were reported in beef [18].

The instrumental colour parameters of the meat were affected to a different extent by the antioxidant treatment. In loin, L* showed significant differences among the groups after 7 days ($P = 0.0012$) and 90 days ($P = 0.0285$). At both stages of storage, the highest L* was observed in S2, while F1 and F2 had the

lowest values of this parameter. In the course of storage, there was significant decrease in L^* in the loins of S2 ($P = 0.0471$) and F1 ($P = 0.0041$) that reached minimum after 90 days of frozen storage. We did not observe any significant effect of the antioxidants or storage on a^* and b^* of the loin. Similar to loin, in the hind leg, L^* was affected by the treatment. After 7 days of storage, the darkest colour was observed in the F1 and F2 meat ($P = 0.0343$), however, after 90 days of frozen storage, the lowest L^* was observed in F1 ($P = 0.0008$). During the storage, the lightness of F1 showed gradual decrease ($P = 0.0242$). In contrast to the loin, the application of edible films and coatings had significant effect on a^* and b^* of the leg. S1 and S2 showed lower values of a^* when compared to the other groups both after 7 days ($P = 0.0190$) and after 90 days ($P = 0.0082$). Furthermore, after 90 days of storage F2 also showed low a^* values. With regard to b^* , significant effect of the treatment was observed only in the meat after 90 days of frozen storage ($P = 0.0239$), where the lowest values were measured in F2, while the highest were registered in F1. The storage of the meat affected significantly only F2, where considerable decrease in a^* ($P = 0.0096$) and b^* ($P = 0.0472$) was observed. The colour of the meat is one of its most important characteristics for the consumers. Regardless of the storage stage, in this study we observed considerable decrease in the lightness of the meat in the groups wrapped with either chitosan or chitosane + rosemary films. Such decrease was not observed in beef packaged in chitosan films [19], however, in beef with active edible coatings such increase of the darkness was documented [20]. Contrary to our results, increase of L^* was reported in rabbit meat with alginate coating on day 5 and day 7 during refrigerated storage [16]. Our results indicated that the effect of the treatment on the instrumental colour parameters is stronger in the hind leg meat and leads to considerable decrease in the redness of the S1 and S2 samples.

Lipid oxidative changes were presented through the content of TBARS measured in the loin (Fig. 1) and the leg (Fig. 2).

There was significant effect of the films and coatings treatment on the TBARS after 7 days of refrigerated storage in the loin ($P = 0.0095$), showing lower values in the meat from the groups either wrapped in film or dipped into the solution. The same trend was observed in the loin after frozen storage, however, the differences among groups were not significant. In the hind leg significant decrease in the TBARS due to the application of the films and coatings was displayed after frozen storage ($P = 0.0015$), nevertheless similar trend was also shown in the groups after 7 days of refrigerated storage. The results indicate the positive effect of the antioxidant treatment for restriction and prevention of the oxidative processes in the meat. Our results are consistent with those of El Bayomi et al. [14], who reported substantial decrease in TBARS in rabbit meat treated with antioxidants during refrigerated storage.

The differences, however, were observed between the control and treated groups, and no such were reported between the groups with the different coat-

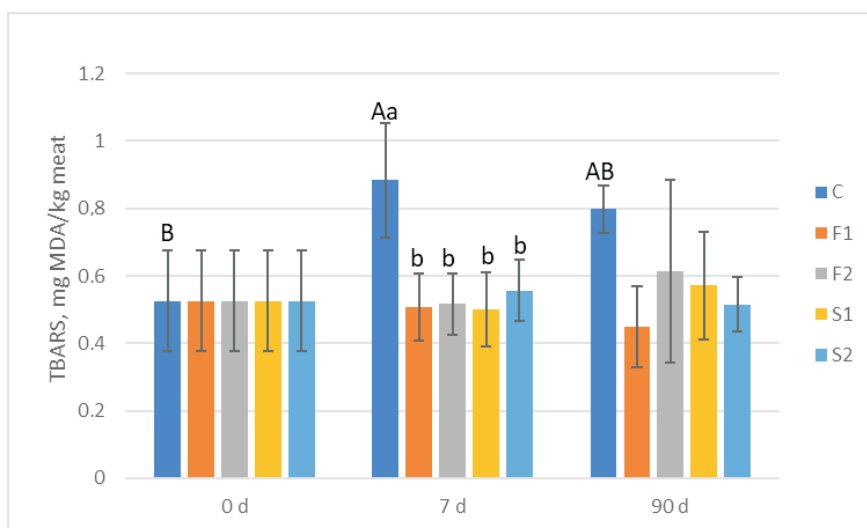


Fig. 1. Effect of the edible films and coatings on the TBARS content in rabbit loin during storage. Significant effect of treatment (7 d, $P = 0.0095$); significant effect of storage (C, $P = 0.0414$). For the same group the different uppercase letters mean significant difference during storage ($P < 0.05$). For a storage interval, the different lowercase letters mean significant differences among groups ($P < 0.05$)

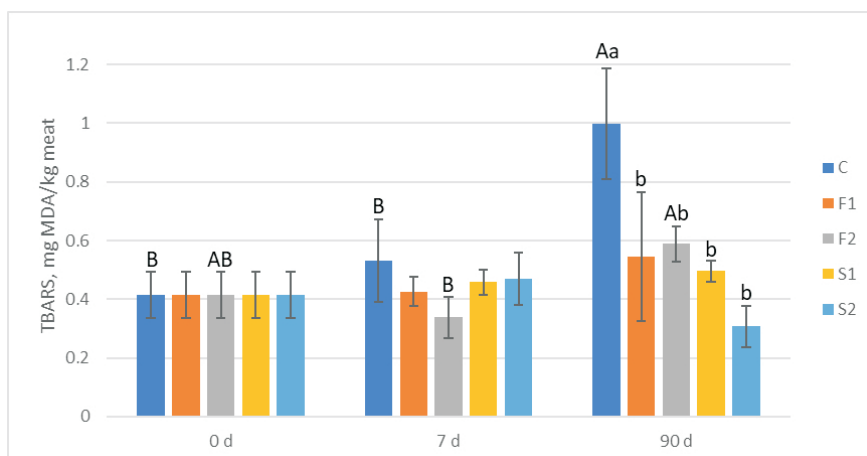


Fig. 2. Effect of the edible films and coatings on the TBARS content in rabbit leg during storage. Significant effect of treatment (90 d, $P = 0.0015$); significant effect of storage (C, $P = 0.0057$ and F2, $P = 0.0129$). For the same group the different uppercase letters mean significant difference during storage ($P < 0.05$). For a storage interval, the different lowercase letters mean significant differences among groups ($P < 0.05$)

ings. Decreased lipid oxidation was also observed in rabbit patties dipped in lemongrass or Black seed oils as antioxidants [15]. During the storage period significant differences between the stages were observed only in the control group of

the loin ($P = 0.0414$) with marked increase of TBARS after 7 d and 90 d. Similar to the loin, the storage induced significant increase in the secondary products of lipid oxidation in the control group ($P = 0.0057$) and F2 ($P = 0.0129$) of the hind leg.

Conclusions. The study presented new data and insight concerning the application of the edible films and coatings aiming to restrict the process of deterioration and maintain the high quality of rabbit meat. The results showed that the values of pH increased overtime in the loin and leg, and were higher in the treated groups, especially in F1 and F2, but still remained within the acceptable for rabbit meat. The higher pH values of F1 and F2 meat corresponded to the lower L^* in these groups in both loin and leg. The a^* and b^* coordinates remained unaffected by the treatment and storage in the loin. In the hind leg, we observed decreased a^* in S1 and S2 after 7 days, and in F2, S1 and S2 after 90 days. The lowest value of b^* in the leg was observed in F2 after frozen storage. Regardless of the treatment and storage period, TBARS remained lower in the treated meats. This indicates the significant potential of the edible films and coatings to prevent oxidation.

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