Effect of lyophilized plant aqueous extracts on pH, colour and myoglobin in rabbit meat during refrigerated and frozen storage

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Abstract. The effect of the treatment with lyophilized antioxidant extracts of wild basil (*Clinopodium vulgare* L.) and basil (*Ocimum basilicum* L.) in rabbit meat on pH, colour and myoglobin changes during refrigerated and frozen storage was investigated. The experimental design included 3 groups of meat – one control and two treated with lyophilized plant extracts (in amount 1:100). The meat was derived from three carcass parts- loin, hind leg and fore leg The meat samples were stored at 4°C for 7 days, after that the storage proceeded for 90 days at -45°C. The study demonstrated different effect of the plant antioxidants on pH and colour, as well as the changes in the myoglobin and its redox forms that depended on the carcass part. The values of pH and decreased during frozen storage. The treatment with plant extracts and storage led to considerable discoloration of the meat from hind leg and fore leg, displayed by decrease of the redness, corresponding to lower percentage of OMb but higher of MetMb. The results suggest certain pro oxidative effect of the plant extracts in this study and require further research with different doses, storage times and temperatures in order to determine the optimal antioxidant application.

1 Introduction

During the last decade, there has been growing interest in developing different non-invasive ways to determine and ensure food quality and safety [1]. Increasing the demand for minimally processed and "fresher" foods, together with the globalization of production, creates major challenges in terms of food quality and safety. Furthermore, there is a growing interest in the use of natural ingredients in foods and beverages. Consumers increasingly prefer food products that contain natural ingredients and are concerned about the adverse health effects of the synthetic additives, especially some antioxidants. Oxidation of foods, including meat, has a variety of adverse effects – deterioration of colour, browning, development of rancid odour and taste. In addition, oxidation reduces nutritional value and poses significant health risks. In this regard, the use of extracts of spices and herbs as antioxidants to restrict oxidative processes is promising. Spices and herbs are rich sources of powerful antioxidants and are an alternative for controlling oxidation in meat products. The use of spices and herbs for flavouring, improving colour and aroma of food dates back to ancient times. For this reason, in addition to their efficiency as antioxidants, spices and herbs are classified as natural - a quality that appeals to consumers. Interest in their application in the food industry will continue to increase, as will scientific research and technology in this area. Finding better ways to derive active substances from plants, as well as designing more efficient combinations of natural antioxidant supplements in foods is a major challenge to food science and technology. The working hypothesis of our study is based on the possibility of stabilizing and improving the quality of raw meat and extending its shelf life by adding natural antioxidants. Based on an exhaustive literature search and own studies, basil (*Ocimum basilicum* L.) and wild basil (*Clinopodium vulgare* L.) were selected for the experiment. Basil essential oil has analgesic [2], anti-inflammatory [3, 4], antibacterial [5], hepatoprotective [6, 7] and immunomodulating properties [8, 9]. It is used as an additive to protect against oxidation, as an antimicrobial agent, or as an ingredient to influence the taste and aroma of food products [10, 11]. Wild basil, on the other hand, is used in traditional medicine in Bulgaria and is shown to have strong antibacterial action [12] . The plant is rich in phenolic acids and flavonoids, which are well known for their antioxidant potential. The strong antioxidant activity of aqueous and lyophilized extracts of wild basil was also

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confirmed by our previous research [13]. The use of natural antioxidants in the producing and processing of foods, including meat and meat products have drawn considerable interest due to the claims that the synthetic ones pose significant risks for the human health. In addition to the use of the antioxidants, the good quality and safety of the meat and meat product can be guaranteed by proper storage conditions. Freezing is one of the most important methods applied to achieve longer shelf life of foods. However, freezing duration and temperature can induce significant changes in the quality characteristics of meat. Although, freezing temperature of -18°C is widely recommended in food industry, according to Estévez [14], the optimum temperature for frozen storage is set to -40°C. It was shown that at this temperature, only a very small amount of water remains unfrozen, but it is chemically inactive. The aim of this study was to investigate the effect of lyophilized aqueous extracts of basil and wild basil on the pH, colour and myoglobin changes in rabbit meat during low temperature storage.

2 Material and methods

2.1 Plant extracts, treatment of the meat and storage

Plant extracts were derived from dry basil (*Ocimum basilicum* L.) and wild basil (*Clinopodium vulgare* L.), purchased from a drugstore. The aqueous extracts (infusions) were prepared as 10 g of the herbs were added to 150 ml boiling distilled water and kept for 60 minutes. The extracts were cooled, filtered, filled with distilled water to a total volume of 150 ml. They were further concentrated and freeze dried in lyophiliser TG-16.50 ("Hochvacuum", Germany).

The experiment was carried out with rabbit meat derived from three carcass parts – loin (n=6), hind leg (n=6) and foreleg (n=6), harvested from New Zealand rabbits supplied from the Institute of Animal Science-Kostinbrod, Bulgaria (Fig. 1). The animals were stunned, slaughtered and exsanguinated. After decapitation, the skin, viscera and paws from each carcass were removed. The carcasses were put in ice and transported to the laboratory. They were then kept into refrigerator at 4° C for 24 h. After that the loin, fore leg and hind leg were separated. The meat from each part was deboned and minced and divided into three groups – control, treated with wild basil (*Clinopodium vulgare* L.) and basil (*Ocimum basilicum* L.). The antioxidants were applied through mixing with the meat in amount 1 g of antioxidant per 100 g meat. Further, small patties from the minced meat of each part were formed for the control and two treated groups. The patties were kept in refrigerator for 7 days, and then the storage continued up to the 90th day at -45°C.

2.2 Measurement of pH and colour

The measurement of pH was done through pH meter (Jenway 3300), equipped with glass electrode, calibrated at pH 4.0 and 7.0. The instrumental colour parameters (L*-lightness, a*-redness and b*-yellowness) were measured on the surface of the patties using colorimeter PCE-CSM 4 (PCE Instruments). The pH and instrumental colour measurements were done on the fresh meat patties, then after 7 and 90 days of storage on three locations of the sample.

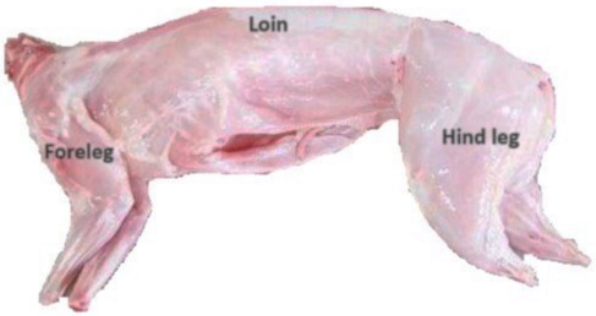

Fig. 1. Rabbit carcass parts, included in the study

2.3 Changes in myoglobin (redox forms)

The total myoglobin content (Mb) was determined as 2 g of the meat were taken from the surface and put in a tube with 20 ml 0.04M Na/K buffer and homogenized using Ultraturrax at 10 000 rpm for 20 s. The homogenates were kept in ice bath for 1 h and they were centrifuged at 10000 rpm for 30 min. The supernatant was filtered and the volume was brought up to 25 ml. Then the absorbance of the supernatant was read at 503, 525, 557 and 582nm. For determination of the % oxymyoglobin (OMb), metmyoglobin (MetMb) and deoxymyoglobin (DeoxyMb) the following equations were used [15]:

$$\% \text{ OMb} = (0.722R1 - 1.432R2 - 1.659R3 + 2.599) \times 100;$$
$$\%\text{MetMb} = (-0.159R1 - 0.085R2 + 1.262R3 - 0.520) \times 100;$$

$$\%\text{DeoxyMb} = (-0.543R1+1.594R2+0.552R3-1.329) \times 100, \text{ where}$$

R1=A582/A525;
R2=A557/A525;
R3=A503/A525

2.4 Statistical analysis

The statistical analysis of the data was performed through ANOVA procedure of the JMP v.7 software package [16]. The effects of the antioxidant treatment and the storage as well as their interaction were assessed. In case of significant effects, the difference between the means was evaluated through Tukey HSD at $P<0.05$.

3 Results and discussion

3.1 Measurements of pH and colour

The antioxidant treatment did not significantly affect the pH of the rabbit meat obtained from loin (Table 1), hind leg (Table 2) and fore leg (Table 3). In the patties made of loin (Table 1), however the values of pH tended to be lower in the group treated with wild basil ($P=0.0568$).

Table 1. pH and instrumental colour measurements in loin according to the antioxidant treatment (A) and storage time (S)

A	S	pH	L*	a*	b*
contr.	0 d	6.19	60.14	13.44	11.10
contr.	7 d	6.07	60.26	13.30	11.46
contr.	90 d	5.90	62.09	11.64	11.52
w. basil	0 d	6.10	60.34	11.81	12.42
w. basil	7 d	5.94	57.38	9.75	14.00
w. basil	90 d	5.92	58.17	11.20	14.33
basil	0 d	6.11	59.82	13.06	17.66
basil	7 d	6.14	56.30	9.95	14.94
basil	90 d	6.00	58.01	10.36	12.09
RMSE		0.08	3.84	1.99	2.62
A	contr.	6.06	60.83	12.79	11.36^{b}
	w. basil	5.99	58.63	10.92	13.58^{ab}
	basil	6.08	58.04	11.12	14.90^{a}
S	0 d	6.14^{a}	60.10	12.77	13.73
	7 d	6.05^{a}	57.98	11.00	13.47
	90 d	5.94^{b}	59.42	11.07	12.65
Sig. (P)	A	0.0568	0.2912	0.1186	0.0321
	S	0.0004	0.5010	0.1298	0.6658
	A*S	0.1744	0.8624	0.5735	0.1884

* Means connected with different letters differ significantly, P<0.05

On the other hand, in loin patties, storage induced significant decrease in pH (P<0.0004), that reached minimal values on 90th day.

Table 2. pH and colour measurements in the hind leg according to the antioxidant treatment (A) and storage time (S)

A	S	pH	L*	a*	b*
contr.	0 d	6.20	66.36	12.97	10.53
contr.	7 d	6.01	61.62	10.70	13.08
contr.	90 d	5.85	57.44	13.63	11.04
w. basil	0 d	6.10	66.14	11.87	10.51
w. basil	7 d	6.28	60.20	10.10	13.48
w. basil	90 d	5.90	56.00	11.04	13.78
basil	0 d	6.16	60.17	13.39	15.93
basil	7 d	5.98	62.40	9.98	16.06
basil	90 d	6.02	58.15	11.28	15.10
RMSE		0.21	2.58	1.14	2.40
A	contr.	6.02	61.81	12.43^{a}	11.55^{b}
	w. basil	6.09	60.78	11.01^{b}	12.59^{b}
	basil	6.05	60.24	11.55^{ab}	15.70^{a}
S	0 d	6.15	64.22^{a}	12.75^{a}	12.32
	7 d	6.09	61.41^{b}	10.26^{b}	14.21
	90 d	5.93	60.78^{b}	11.98^{a}	13.31
Sig. (P)	A	0.7806	0.4411	0.0495	0.0048
	S	0.0889	<0.0001	0.0007	0.2747
	A*S	0.3746	0.0506	0.2647	0.6005

* Means connected with different letters differ significantly, P<0.05

In the patties made of the fore leg (Table 3), significant interaction between the antioxidant treatment and storage in regard to pH values was observed (P=0.0328). It was also noted, that pH decreased in the frozen samples from the control group and those treated with wild basil, more substantially in the former.

The application of the plant extracts was associated with significant changes in the meat colour that were different according to the carcass part. The antioxidant treatment led to significant increase in the yellowness (b*) in the patties made of loin (0.0321), hind leg (0.0048) and fore leg (P=0.0413). This effect was particularly pronounced in the meat treated with basil extract, when compared to the control samples on the other hand, the redness of the meat (a*) decreased in hind leg (P=0.0495) and fore leg (P=0.0021) meat samples after plant extract treatment. The storage affected the redness as well, showing decrease in the values of this parameter on the 7th day of storage in both hind leg (P=0.0007) and fore leg patties (P=0.0004), regardless of the antioxidant treatment Furthermore, in hind leg patties, storage affected significantly the lightness (L*) (P<0.0001), as on 7th and 90th day the values of this coordinate were significantly lower in comparison to the fresh meat.

pH and colour have crucial importance for the quality of meat. The values of pH determine the possibilities for further processing and storage, whereas the colour is directly linked to the consumers' perception and their willingness to purchase meat [17]. So far, the studies on the effect of antioxidants in meat storage and processing report contradictory results in regard to pH. In line with our observations, Mancini et al. [18] did not find any particular changes in pH in rabbit burgers due to antioxidant treatment. Moawad et al. [19], reported considerably lower pH values in rabbit meat treated with extracts from mulberry and olive leaves. Both studies showed significant increase in pH in the course of refrigerated storage, that we failed to observe. The lower

Table 3. pH and instrumental colour measurements in fore leg according to the antioxidant treatment (A) and storage time (S)

A	S	pH	L*	a*	b*
contr.	0 d	6.24^{a}	62.18	18.16	11.76
contr.	7 d	6.23^{a}	54.38	15.84	13.19
contr.	90 d	5.83^{b}	52.36	17.68	14.86
w. basil	0 d	6.14^{ab}	59.54	16.33	12.97
w. basil	7 d	6.14^{ab}	61.13	12.48	15.76
w. basil	90 d	5.92^{ab}	55.09	14.33	14.08
basil	0 d	6.16^{ab}	56.27	17.01	15.41
basil	7 d	6.04^{ab}	54.53	10.94	16.82
basil	90 d	6.12^{ab}	58.77	14.41	13.96
RMSE		0.12	5.127	1.74	1.63
A	contr.	6.10	56.31	17.23^{a}	13.27^{b}
	w. basil	6.07	58.59	14.38^{b}	14.27^{ab}
	basil	6.11	56.52	14.12^{b}	15.40^{a}
S	0 d	6.18	59.33	17.17^{a}	13.38
	7 d	6.14	56.68	13.09^{b}	15.26
	90 d	5.96	55.41	15.47^{a}	14.30
Sig. (P)	A	0.7495	0.5906	0.0021	0.0413
	S	0.0022	0.2784	0.0004	0.0754
	A*S	0.0328	0.2017	0.4696	0.1189

* Means connected with different letters differ significantly, P<0.05

pH values in this study were found in the meat during frozen storage. Similar to us, Leygonie et al. [20] observed decrease in pH in frozen ostrich meat, assuming that lower pH values might be attributed to formation of exudate in thawing containing minerals and protein components and that increase the concentration of H^+ and hence the acidity of the meat. According to Lan et al. [21], the formation of free fatty acids during storage might as well help increase acidity. We may suggest that such processes are additionally favoured by mincing the meat as in our case.

In contrast with our results, Al Jumai et al. [22], reported increase of L* and a * in rabbit meat, treated with plant antioxidants. On the other hand, the authors observed higher values of b* in the antioxidant treated meat as well as decrease of a* during storage that coincides with our findings. Decrease of L* and a* as well as increase in b* coordinates in samples of rabbit meat treated with antioxidants was observed also by Mancini et al. [18]. In rabbit meat, Wang et al. [23] showed strong negative correlation between yellowness and TBARS formation, indicating that increased b*values are associated with higher degree of lipid oxidation. Since the higher values of b* in this study are observed in the samples treated with antioxidants, it could be suggested that there is certain pro-oxidative effect of the plant extract, probably due to the dose applied. This, however, warrant further research.

During storage, we found decrease of the lightness and redness of the meat regardless of the antioxidants. Such changes in the colour of rabbit meat were reported and might be attributed to the changes in the myoglobin and formation of its redox forms [23].

3.2 Changes in myoglobin and its redox forms

Myoglobin content in the rabbit meat was affected in a different way according to the carcass part. In loin patties (Fig. 2) the antioxidant treatment and storage interacted significantly (P=0.0172), showing decrease in the Mb percentage in the control and wild basil treated group in the course of storage. On the other hand, the group treated with basil showed increase in the Mb after 7th and 90th day of storage. The content of total Mb tended to be higher in the patties from hind legs treated with basil (Fig.3), while in the fore leg samples (Fig. 4) there was significant decrease of the Mb during storage (P=0.0310).

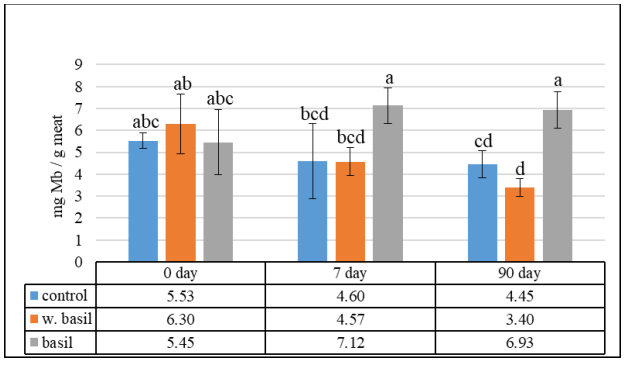

Fig. 2 Content of Mb in loin according to the antioxidant treatment and storage (means connected with different letters differ significantly, P<0.05)

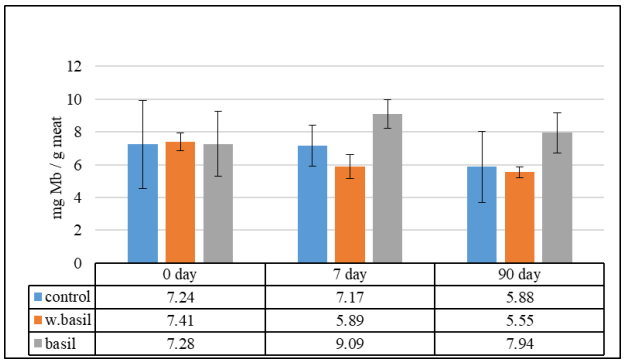

Fig. 3. Content of Mb in hind leg according to the antioxidant treatment and storage (means connected with different letters differ significantly, P<0.05)

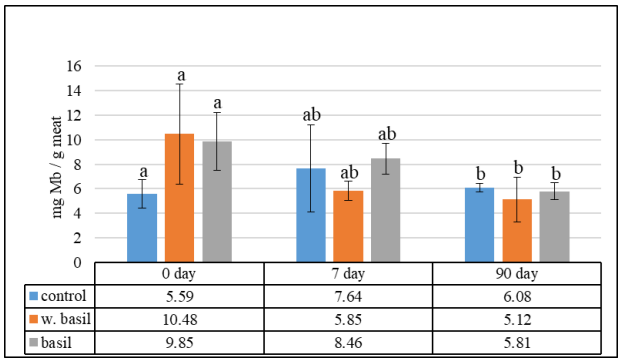

Fig. 4. Content of Mb in foreleg according to the antioxidant treatment and storage (means connected with different letters differ significantly, P<0.05)

Table 4. Proportion of the myoglobin redox forms in loin according to the antioxidant treatment (A) and storage time (S)

A	S	DeoxyMb (%)	OMb (%)	MetMb (%)
contr.	0 d	20.41	20.02	59.43
contr.	7 d	23.00	16.92	65.80
contr.	90 d	16.01	26.19	56.88
w. basil	0 d	22.09	14.65	63.44
w. basil	7 d	21.00	13.37	65.80
w. basil	90 d	20.27	14.17	65.60
basil	0 d	21.77	10.03	68.91
basil	7 d	25.07	2.87	72.80
basil	90 d	20.91	13.66	65.58
RMSE		4.03	7.21	4.3106
A	contr.	19.81	21.04^{a}	60.70^{b}
	w. basil	21.12	14.06^{ab}	64.93^{ab}
	basil	22.58	8.85^{b}	69.10^{a}
S	0 d	21.43	14.90	63.93^{ab}
	7 d	23.02	11.05	68.14^{a}
	90 d	19.07	14.06	62.67^{b}
Sig. (P)	A	0.3643	0.0077	0.0025
	S	0.1398	0.1518	0.0374
	A*S	0.6821	0.7551	0.4773

* Means connected with different letters differ significantly, P<0.05

In the loin patties, the antioxidant treatment was associated with significant decrease of the percentage of OMb (P=0.077), especially in the group treated with basil. Furthermore, the percentage of MetMb increased in the

antioxidant treated groups, again more pronounced in the basil group (P=0.025). Regardless of the antioxidants, this trait was also affected by the storage period (P=0.0374), with highest values measured on the 7th day of storage.

Table 5. Proportion of the myoglobin redox forms in hind leg according to the antioxidant treatment (A) and storage time (S)

A	S	DeoxyMb (%)	OMb (%)	MetMb (%)
contr.	0 d	19.53	20.93^{a}	59.32^{d}
contr.	7 d	24.07	9.10^{cd}	67.24^{ab}
contr.	90 d	21.85	15.93^{abc}	62.28^{cd}
w. basil	0 d	20.89	17.02^{ab}	62.24^{cd}
w. basil	7 d	22.94	10.02^{bcd}	67.32^{ab}
w. basil	90 d	23.89	5.33^{d}	71.38^{a}
basil	0 d	19.59	15.70^{abc}	64.84^{bc}
basil	7 d	22.90	7.79^{d}	69.66^{a}
basil	90 d	22.41	10.28^{bcd}	67.56^{ab}
RMSE		1.58	2.49	1.47
A	contr.	21.82	15.32	62.95
	w. basil	22.58	10.79	66.98
	basil	21.63	11.26	67.35
S	0 d	20.02^{b}	17.88	62.13
	7 d	23.30^{a}	8.97	68.07
	90 d	22.72^{a}	10.51	67.08
Sig. (P)	A	0.4255	0.0019	<0.0001
	S	0.0007	<0.0001	<0.0001
	A*S	0.5209	0.0115	0.0005

* Means connected with different letters differ significantly, P<0.05

Table 6. Proportion of the myoglobin redox forms in fore leg according to the antioxidant treatment (A) and storage time (S)

A	S	DeoxyMb (%)	OMb (%)	MetMb (%)
contr.	0 d	11.15	38.72^{a}	49.98^{b}
contr.	7 d	21.45	15.33^{b}	63.46^{a}
contr.	90 d	19.14	17.94^{b}	62.76^{a}
w. basil	0 d	20.45	16.96^{b}	62.65^{a}
w. basil	7 d	21.26	16.45^{b}	62.51^{a}
w. basil	90 d	22.55	9.88^{b}	67.71^{a}
basil	0 d	19.38	18.54^{b}	62.04^{a}
basil	7 d	20.40	12.22^{b}	67.29^{a}
basil	90 d	22.06	8.73^{b}	69.50^{a}
RMSE		3.57	5.70	3.33
A	contr.	17.25	24.00	58.73
	w. basil	21.42	14.43	64.29
	basil	20.61	13.16	66.28
S	0 d	16.99	24.74	58.22
	7 d	21.04	14.67	64.42
	90 d	21.25	12.18	66.66
Sig. (P)	A	0.0536	0.0012	0.0004
	S	0.0351	0.0004	<0.0001
	A*S	0.1827	0.0295	0.0371

* Means connected with different letters differ significantly, P<0.05

In the samples of the hind leg and fore leg, we observed significant interaction between the antioxidant treatment and the storage period in regard to both OMb and MetMb. The percentage of DeoxyMb was affected by the storage in both hind leg (P=0.0007) and fore leg patties (P=0.0351). Generally, in the course of storage the percentage of DeoxyMb increased as in the hind leg samples this was more prominent on the 7th day of storage. In addition, in the samples of therefore leg, the content of DeoxyMb tended to increase in the antioxidant treated groups (P=0.0536).

Myoglobin is heme sarcoplasmic protein, responsible for the meat colour and specific for the different species [24]. In rabbit and chicken meat, the myoglobin content is low (<5mg/g) [25]. In our study the content of myoglobin varied according to the carcass part with lowest levels in the loin (5.37 mg/g vs.7.07 mg/g for the hind leg and 7.20 mg/g for the fore leg). This can be explained with the different type of muscles that are located in these particular parts. It is known that generally m. *Longissimus dorsi* that is located in loin contains less pigments when compared to the red muscles such as m. *Supraspinatus* in fore leg and m. *Biceps femoris* in hind leg [26, 27]. The present study demonstrated that the proportion of DeoxyMb was not affected by the antioxidants in all three carcass parts, but increased during storage in the hind leg and fore leg patties. The proportion of OMb and MetMb in the loin showed inverse relationship and were affected by the antioxidants, showing strongest effect in the basil group. On the other hand, the storage, especially in refrigerator up to 7 days in the loin was associated with highest degree of formation of MetMb, suggesting oxidative processes at this temperature. It should be noted that despite the antioxidant compounds, the groups treated with basil and wild basil showed the highest degree of discoloration. Despite the significant interaction between the antioxidant treatment and the storage time in regard to the proportion of OMb and MetMb in hind leg and foreleg samples, there was decrease in the OMb in the treated groups while the MetMb proportion increased. This corresponded with the loss of the redness in these groups. The observed discoloration of the meat in the groups treated with antioxidants from all three carcass parts might suggest a certain pro oxidative effect of the compounds in the dose applied in this study. In this regard, further research is warranted to determine the exact dose of the plant extracts so that the colour of the meat is stabilized. In our study demonstrated effects of the storage time and low temperatures associated with decreased OMb and increased MetMb in the rabbit meat, corresponding to lower a* values. Similar results indicating strong effect of the storage temperature and duration on the loss of redness and the changes of the proportions in MetMb were reported in rabbit meat [23, 28].

4 Conclusions

The study showed that application of plant antioxidants in rabbit meat had different effect on the quality characteristics in terms of pH and colour, as well as the changes in the myoglobin and its redox forms according to the carcass part. The values of pH were s affected by the storage, showing significant decrease in the frozen samples. Both antioxidant and storage led to decrease of

the redness in the meat from hind leg and foreleg, corresponding to lower percentage of OMb but higher of MetMb. The results suggest certain pro oxidative effect of the plant extracts leading to discoloration of the meat and require further research with different doses, storage times and temperatures in order to determine the most efficient antioxidant application in the rabbit meat.

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