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CHANGES IN COLOUR AND MYOGLOBIN OXIDATION IN CHICKEN MEAT AS AFFECTED BY ANTIOXIDANTS DURING STORAGE

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Abstract

The study aimed to investigate the effect of natural antioxidants derived from plants on the changes of colour, pH and myoglobin oxidation of chicken meat during storage. Samples of breast and thighs were divided in three groups – control (C), and treated with infusion of wild basil (T1) or oregano (T2). The meat was stored at 4 °C for 7 days and then at −20 °C up to day 90. The pH, colour, oxymyoglobin (OMb) and metmyoglobin (MetMb) were measured at 0 d, 7 d and 90 d. The antioxidant treatment affected the colour parameters of the chicken meat, as the effect differed according to the muscles and duration of storage. Contrary to breast that exhibited initially decreased yellowness (b*), in T1, the antioxidant treated thigh meat had increased lightness (L*) at 7 d and 90 d, increased b* at 0 d and decreased redness (a*) at 7 d. Duration of storage affected both breast and thigh meat colour, more visible in T1 and T2. Considerable increase in b* was observed in breast, while in thigh meat storage was associated with higher L* and lower a*. Both breast and thigh of the treated groups showed decrease in pH, particularly in the initial stages of storage. The percentage of OMb and MetMb in meat was less affected by the antioxidants. Their effect was stronger in breast, with lower OMb in T1 at the 90th day of storage. Furthermore, this group exhibited dramatic

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decrease in the OMb in the course of storage. MetMb increased in C and T1 during the storage period. The changes in the myoglobin redox forms in thigh meat are mainly due to storage and are similar to those observed in breast.
Key words: chicken meat, antioxidants, colour, myoglobin forms

Introduction. Poultry meat production has been significantly increasing in recent years, with main producers being USA, China and Brazil. This growth is determined by the high consumers' demand for poultry meat, due to its lower price, easier processing and the awareness of the people about the health benefits of this kind of meat [1]. In addition, for rearing, chickens require 28 times less land and eleven times less water in comparison to cattle [2].

Among the most important factors affecting the decision of the consumers to purchase meat, is its colour. The latter provokes instant negative or positive psychological response [3], and is closely connected to the quality, safety and storage of meat [4,5]. Meat colour strongly depends on the content of heme pigments and especially myoglobin whose content in the red muscles might reach 70–95% of the total heme pigments [6]. Three types of heme pigments are responsible for the colour of poultry meat, namely myoglobin, hemoglobin and cytochrome C [7] and their contents vary among the muscles as well as the poultry species. Thigh muscles in chicken contain 5–10 times more pigments than breast [8]. Also, the myoglobin content in red poultry muscles vary between 2 and 4 mg/g, while in breast it is less than 0.5 mg/g [9,10], and thus the changes in meat colour are different for breast and thighs. In addition to the myoglobin content, the bright colour of the fresh meat depends on its redox forms.When exposed to oxygen, oxymyoglobin is formed on the meat surface accompanied by the development of a bright cherry red colour. When oxidized, oxymyoglobin turns to brown metmyoglobin that is associated with colour deterioration and negative perception in the consumers. The oxidation of myoglobin in meat might be facilitated by lipid oxidation as reported by several studies, however, the biochemical reactions directly responsible for myoglobin and lipid oxidation each generate products that can further accelerate oxidation in a reciprocal manner [11]. Restriction of oxidation has been successfully achieved through use of antioxidants. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been effectively used against lipid oxidation, however, their application in food industry has been limited since they are considered responsible for increasing the risk of cancer. A good alternative to the synthetic antioxidants are the natural ones, such as plant extracts derived from various herbs, spices, seeds and fruits. Hence, the aim of the study was to investigate the effect of natural antioxidants from plant extracts on the changes of the instrumental colour parameters and myoglobin redox forms during storage of chicken meat.

Material and methods. Plant extracts, treatment and storage of the meat. Plant extracts were derived from dry wild basil (*Clinopodium vulgare* L.) and

oregano (*Origanum 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 min. The infusions were cooled, filtered, filled with distilled water to form a total volume of 150 ml and stored at 4 °C until their use on meat. The experiment was carried out with chicken breasts ($n = 18$) and thighs ($n = 18$) harvested from male chickens of "Salmon" cross, developed in the Institute of Animal Science – Kostinbrod. The meat was deboned and divided into three groups – control (C), treated with wild basil (T1), and treated with oregano (T2). Each of the groups contained six breasts and six thighs. The breast and thigh meat were soaked into the infusions for 20 h at 4 °C. The samples were kept in refrigerator for 7 days, and then the storage continued up to the 90th day at −20 °C.

pH and colour measurements. The measurement of pH was done through 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.

Myoglobin redox forms. Two grams of the meat taken from the surface were put in a tube with 20 ml 0.04 M Na/K buffer and homogenized using Ultra-turrax at 10 000 rpm for 20 s. The homogenates were kept in ice bath for 1 h and they were centrifuged at 10 000 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 582 nm. For determination of the % OMb and MetMb the following equations were used [12]:

$$\% \text{ OMb} = (0.722\text{R}_1 - 1.432\text{R}_2 - 1.659\text{R}_3 + 2.599) \times 100,$$
$$\% \text{ MetMb} = (-0.159\text{R}_1 - 0.085\text{R}_2 + 1.262\text{R}_3 - 0.520) \times 100,$$

where $\text{R}_1 = \text{A}^{582}/\text{A}^{525}$; $\text{R}_2 = \text{A}^{557}/\text{A}^{525}$; $\text{R}_3 = \text{A}^{503}/\text{A}^{525}$.

Statistical evaluation. The statistical processing of the data was done through ANOVA procedure of the JMP v.7 software package. 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±SD.

Results and discussion. *pH and colour.* Antioxidant treatment affected significantly the yellowness of the breast ($P = 0.0441$) as shown in Table 1. Differences were found between the breasts of C and T1 on day 0, as the meat of T1 showed considerable decrease in b*. On the other hand, b* values were significantly influenced by the storage period in T1 ($P < 0.0001$), as on day 90 we found the highest values of this coordinate.

Table 1

Effect of antioxidant treatment and storage on the colour and pH in chicken breasts

Indicator	Treatment	Storage			Sig.
		0 days	7 days	90 days	
L*	C	58.94 ± 4.14	55.73 ± 4.63	55.39 ± 3.78	$P = 0.5530$
	T1	54.47 ± 3.77	56.31 ± 2.75	56.49 ± 1.60	$P = 0.6492$
	T2	56.00 ± 3.30	60.16 ± 4.33	53.02 ± 2.36	$P = 0.1081$
	Sig.	$P = 0.3904$	$P = 0.3934$	$P = 0.3473$	
a*	C	10.36 ± 3.36	8.08 ± 1.64	8.58 ± 1.79	$P = 0.5099$
	T1	7.16 ± 2.13	7.52 ± 1.58	6.80 ± 1.91	$P = 0.8972$
	T2	7.67 ± 0.70	8.01 ± 1.35	6.88 ± 0.64	$P = 0.3904$
	Sig.	$P = 0.2716$	$P = 0.8887$	$P = 0.3506$	
b*	C	4.91 ± 1.02^{a}	7.08 ± 1.53	8.24 ± 1.46	$P = 0.0594$
	T1	3.20 ± 0.39^{bB}	4.30 ± 0.74^{B}	9.05 ± 0.64^{A}	$P < 0.0001$
	T2	3.97 ± 0.18^{ab}	7.74 ± 3.44	8.12 ± 2.03	$P = 0.1275$
	Sig.	$P = 0.0441$	$P = 0.2122$	$P = 0.7203$	
a*/b*	C	2.19 ± 0.86	1.20 ± 0.42	1.05 ± 0.25	$P = 0.0996$
	T1	2.21 ± 0.43^{A}	1.74 ± 0.07^{A}	0.74 ± 0.16^{B}	$P = 0.0015$
	T2	1.94 ± 0.20^{A}	1.17 ± 0.45^{AB}	0.88 ± 0.21^{B}	$P = 0.0151$
	Sig.	$P = 0.8166$	$P = 0.1715$	$P = 0.2813$	
pH	C	6.18 ± 0.06^{aB}	6.43 ± 0.01^{aA}	6.31 ± 0.07^{AB}	$P = 0.0038$
	T1	5.83 ± 0.04^{bB}	6.34 ± 0.02^{bA}	6.28 ± 0.03^{A}	$P < 0.0001$
	T2	5.81 ± 0.09^{bB}	6.28 ± 0.02^{cA}	6.29 ± 0.06^{A}	$P = 0.0001$
	Sig.	$P = 0.0007$	$P = 0.0001$	$P = 0.7723$	

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 ($P < 0.05$)

Redness index (a/b) changed significantly during storage in T1 ($P = 0.0015$) and T2 ($P = 0.0151$), as in both groups it showed the lowest values on the 90th day of storage. Similar trend was found in the breast of the control group ($P = 0.0996$).

Unlike colour, the values of pH in breast meat were affected to a greater extent by the antioxidant treatment. Significant effect of the plant infusions was observed on days 0 ($P = 0.0007$) and 7 ($P = 0.0001$) of the sample storage. At the initial stages of the storage, pH showed the highest value in C when compared to T1 and T2. However, on the 7th day, we observed gradual increase of the pH values but again they were the highest in the control breasts. In addition, differences between both treated groups were found, T2, showing lower pH in comparison to T1. The duration of the storage influenced significantly the changes in pH in C ($P = 0.0038$), T1 ($P < 0.0001$) and T2 ($P = 0.0001$). C group showed increased pH values on the 7th day of storage, following decrease on the 90th day. In T1 and T2 we observed similar patterns, as the lowest pH values were measured on day 0 and after increasing until day 7 the changes in the values remained negligible until the end of the storage.

Table 2

Effect of antioxidant treatment and storage on the colour and pH in chicken thighs

Indicator	Treatment	Storage			Sig.
		0 days	7 days	90 days	
L*	C	43.22 ± 3.25	44.51 ± 2.43^{b}	42.27 ± 4.33^{b}	$P = 0.7371$
	T1	46.65 ± 1.93^{B}	54.17 ± 3.07^{aA}	50.38 ± 2.52^{aAB}	$P = 0.0312$
	T2	45.39 ± 3.00^{C}	55.93 ± 0.73^{aA}	50.66 ± 0.62^{aB}	$P = 0.0012$
	Sig.	$P = 0.3724$	$P = 0.0018$	$P = 0.0203$	
a*	C	19.30 ± 0.90	19.01 ± 1.94^{a}	15.60 ± 3.14	$P = 0.1517$
	T1	19.75 ± 0.98^{A}	14.96 ± 1.29^{bB}	11.52 ± 0.45^{C}	$P = 0.0001$
	T2	18.83 ± 1.79^{A}	15.30 ± 1.38^{abAB}	13.19 ± 1.64^{B}	$P = 0.0143$
	Sig.	$P = 0.7011$	$P = 0.0347$	$P = 0.1262$	
b*	C	6.46 ± 0.72^{b}	7.79 ± 1.93	9.30 ± 1.47	$P = 0.1344$
	T1	8.26 ± 0.83^{a}	9.65 ± 0.54	10.43 ± 1.52	$P = 0.1076$
	T2	7.91 ± 0.46^{ab}	11.83 ± 2.13	9.59 ± 1.73	$P = 0.0637$
	Sig.	$P = 0.0394$	$P = 0.0694$	$P = 0.6798$	
a*/b*	C	3.01 ± 0.33^{A}	2.50 ± 0.40^{aA}	1.68 ± 0.23^{aB}	$P = 0.0070$
	T1	2.40 ± 0.22^{A}	1.56 ± 0.20^{bB}	1.12 ± 0.19^{bB}	$P = 0.0007$
	T2	2.39 ± 0.25^{A}	1.34 ± 0.39^{bB}	1.39 ± 0.18^{abB}	$P = 0.0071$
	Sig.	$P = 0.0573$	$P = 0.0132$	$P = 0.0399$	
pH	C	6.39 ± 0.07^{aB}	6.62 ± 0.01^{A}	6.52 ± 0.08^{AB}	$P = 0.0081$
	T1	5.96 ± 0.04^{bB}	6.51 ± 0.14^{A}	6.39 ± 0.06^{A}	$P = 0.0008$
	T2	6.05 ± 0.14^{bB}	6.65 ± 0.11^{A}	6.51 ± 0.11^{A}	$P = 0.0022$
	Sig.	$P = 0.0031$	$P = 0.2602$	$P = 0.2084$	

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 ($P < 0.05$)

In contrast to the breast, the antioxidant treatment had considerable effect on the values of the three colour coordinates in thighs (Table 2). Both T1 and T2 thighs had increased L* values on days 7 and 90 when compared to C ($P < 0.05$). Furthermore, the lightness was also affected and increased by the duration of storage in both T1 ($P = 0.0312$) and T2 ($P = 0.0012$). The redness decreased in the experimental groups on the 7th day ($P = 0.0347$), and also was lower in T1 ($P = 0.0001$) and T2 ($P = 0.0143$) in the course of storage. The antioxidants increased the yellowness b* of the thighs at the beginning of the storage in the fresh meat ($P = 0.0394$). The redness index was lower in T1 and T2 during the refrigerated ($P = 0.0132$) and frozen storage ($P = 0.0399$), when compared to the control group. The values of this parameter changed during the storage period in all examined groups. The thighs of C group showed considerable decrease of this ratio on day 90, while in both antioxidant treated groups, the decrease of this parameter starts earlier on day 7.

The antioxidants decrease the pH values in T1 and T2 ($P = 0.0031$) on day 0, however, in the later stages of storage they did not exert any effect on this trait in thighs. In the course of storage, after the 7th day, the pH values in all three groups increased significantly.

As seen from the results in Table 2, the effect of the antioxidant components of the plant extracts differed according to the type of meat and duration of storage. Plant extracts have been widely applied in meat processing, however, the effects on the colour parameters that have been reported remain contradictory. Combination of rosemary and cloves extract had positive effect on the colour characteristics of raw chicken meat, stored for 15 days [13]. In contrast to our results, the authors reported increased values of L* during storage in breast, as well as increased redness and yellowness in this meat treated with the antioxidants. In vacuum packed beef and pork, the effect of mixture of aqueous extracts of grape seed, rosemary and oregano was associated with increased L*, and decreased b*, while no changes were detected in a* after four months of frozen storage [14]. This partially agrees with our results where increased L* was observed in thighs of the treated groups. On the other hand, the decreased redness of the meat during storage that we observed in thighs could be associated with oxidative changes in myoglobin.

Myoglobin redox forms. The antioxidant treatment of the breast meat had significant effect on the percentage of OMb on day 0 ($P = 0.0003$) and day 90 ($P = 0.0176$) of the storage (Table 3). The highest percentage of OMb was found in the T1 fresh meat, while at the end of the storage, the freezing conditions led to lowest values of this parameter in the group. The percentage of MetMb was also influenced by the antioxidants, again on day 0 ($P < 0.0001$) and day 90 ($P = 0.0061$). The highest MetMb content was found in T2 initially, while on day 90, this group showed the lowest MetMb percentage. During the storage, the percentage of OMb decreased in group C ($P = 0.0012$) and T1 ($P < 0.0001$). On the other hand, these groups exhibited considerable increase in MetMb percentage after the 7th day of storage.

T a b l e 3

Effect of antioxidant treatment and storage on the myoglobin redox forms in chicken breasts

Indicator	Treatment	Storage			Sig.
		0 days	7 days	90 days	
OMb	C	24.31 ± 4.49^{bA}	9.19 ± 2.25^{B}	7.38 ± 2.25^{abB}	$P = 0.0012$
	T1	67.01 ± 11.76^{aA}	7.87 ± 2.22^{B}	4.53 ± 1.51^{bB}	$P < 0.0001$
	T2	14.29 ± 1.36^{b}	10.30 ± 1.02	11.53 ± 2.39^{a}	$P = 0.0679$
	Sig.	$P = 0.0003$	$P = 0.3631$	$P = 0.0176$	
MetMb	C	56.25 ± 2.08^{bB}	67.93 ± 2.93^{A}	73.56 ± 2.36^{aA}	$P = 0.0004$
	T1	39.34 ± 4.52^{cB}	69.00 ± 0.56^{A}	71.58 ± 0.22^{aA}	$P < 0.0001$
	T2	68.75 ± 1.13^{a}	67.48 ± 0.79	66.53 ± 1.77^{b}	$P = 0.1913$
	Sig.	$P < 0.0001$	$P = 0.5903$	$P = 0.0061$	

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 ($P<0.05$)

Table 4

Effect of antioxidant treatment and storage on the myoglobin redox forms in chicken thighs

Indicator	Treatment	Storage			Sig.
		0 days	7 days	90 days	
OMb	C	23.81 ± 1.51^{bA}	15.68 ± 2.84^{B}	17.52 ± 0.46^{B}	$P = 0.0043$
	T1	57.79 ± 5.43^{aA}	14.46 ± 2.74^{B}	14.39 ± 3.52^{B}	$P < 0.0001$
	T2	19.84 ± 3.78^{b}	16.28 ± 1.99	19.14 ± 3.65	$P = 0.4159$
	Sig.	$P < 0.0001$	$P = 0.6906$	$P = 0.2132$	
MetMb	C	55.28 ± 0.30^{aB}	62.50 ± 1.97^{A}	65.03 ± 1.69^{A}	$P = 0.0005$
	T1	41.36 ± 4.17^{bB}	62.88 ± 1.86^{A}	63.40 ± 2.44^{A}	$P = 0.0002$
	T2	60.47 ± 2.85^{a}	58.78 ± 4.54	60.25 ± 2.23	$P = 0.8045$
	Sig.	$P = 0.0005$	$P = 0.2672$	$P = 0.0834$	

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 ($P < 0.05$)

Significant effect of the antioxidant treatment on the percentage of OMb and MetMb in thighs was observed only in the fresh meat on day 0. As seen in Table 4, the highest OMb percentage was present in T1. This group had also the lowest percentage of MetMb. Similar to breast, the effect of the duration of storage on these two parameters was found significant in C and T1 groups. In both groups, the values of OMb % decreased and MetMb increased considerably after day 7 of the storage. Usually, the application of the antioxidants on meat to restrict oxidative processes is also associated with restricted oxidation of meat pigments.

It was reported that plant extracts from rosemary, sage, mustard, clove, fenugreek and majorana restricted the oxidation of myoglobin to metmyoglobin in beef [15]. Also lyophilized black mulberry water extract was shown to reduce metmyoglobin formation in meat during storage, however, the effect was different and depended on the concentration of the antioxidant [16]. The results of this study concerning the oxidation of the main meat pigment and the formation of the forms that influence the colour showed inconsistent effect of the antioxidants. Furthermore, the effect differed among the plant species, and the type of the muscle studied, and changed during the storage of the meat. Based on our data we might suggest that some plant antioxidants might act as pro-oxidants and enhance the oxidation of myoglobin.

Conclusions. The results of our study showed that antioxidants derived from plant extracts affected the colour of the chicken meat, however, this effect depended on the type of the muscles and the duration of the storage. The changes in the colour were more pronounced in thighs, as the groups treated with antioxidants had lighter meat during the refrigerated and frozen storage, however, less red when refrigerated. The duration of storage affected both breast and thigh meat colour, more visible in groups with antioxidant treatment. Considerable in-

crease in b* was observed in breast, while in thigh meat storage was associated with higher L* and lower a*. The percentage of OMb and MetMb in meat remained less affected by the antioxidants. Their influence was stronger in breast, as wild basil treatment was associated with considerable decrease in the OMb in the course of storage. MetMb increased in C and T1 during the storage period. The changes in the myoglobin redox forms in thigh meat were mainly due to storage, identical to those observed in breast. So far, these results demonstrate the potential of the antioxidants contained in wild basil and oregano toward changes in the colour of the chicken meat during storage. Additional research with different doses of these plant extracts are necessary in the future, so that optimal conditions for producing meat with excellent colour are achieved.

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