Study of the effect of treatment with aqueous extracts by oregano and wild basil on raw poultry meat

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Abstract. The effect of treatment with aqueous antioxidant extracts of oregano (*Origanum vulgare L.*) and wild basil (*Clinopodium vulgare L.*) in refrigerated storage of raw poultry meat was studied. Physicochemical analyzes of meat samples were performed - total protein, ash, fat, dry matter, cooking loss and pH value in dynamics. The content of malondialdehyde (MDA) and the protein profile were determined. The microorganisms' growth rate in meat during storage at 4°C for a period of 14 d was monitored. After the 7th d, the total number of mesophilic microorganisms in the meat samples increased to 7.00 log cfu/g, which is indicative of decay. A significant increase in pH value was observed after 14 d of storage, but there were no significant changes in total protein content and protein profile. In all meat samples, the amounts of MDA on days 7 and 14 were significantly below the thresholds indicated in the literature. Experimental groups treated with extracts showed lower values for MDA content compared to the control, which is an indication of certain inhibition of lipid oxidation processes in meat.

1 Introduction

Meat and meat products are a major food group rich in important nutrients. Chicken meat is considered one of the greatest sources of affordable quality protein because it is significantly cheaper compared to, for example, beef. The price of one kg of beef is equivalent to the price of 3 - 4 kg of chicken [1]. Raw chicken meat is a product with a limited expiry date, as the main reasons for the short shelf life in refrigerated storage are microbial growth and lipid oxidation. Oxidation of lipids leads to changes in the quality parameters of the meat such as color, smell and taste, as well as to the accumulation of secondary products, which have an adverse effect on the health of the consumer. Antioxidants are used in the meat processing industry to prevent these changes. They are substances that, in low concentrations, slow down the oxidation of lipids and proteins in meat products. The use of antioxidants in food is controlled by the relevant country's regulatory authorities or international standards.

Although there are many compounds exhibiting antioxidant properties, few of them are approved for use in foods [2]. Accumulating data on the toxic and/or carcinogenic effects of synthetic antioxidants, as well as consumer preferences for natural and healthy products, is the reason for the great interest of researchers and the meat-processing industry in alternative solutions to minimize oxidative rancidity and increase the shelf life of meat [3, 4]. Some vitamins (ascorbic acid, α-tocopherol and their derivatives) are currently used as natural agents for reducing lipid oxidation in food products. In chicken meat, they inhibit lipid peroxidation and metmyoglobin formation, stabilizing meat color during storage [5].

Research has been conducted to show that many fruits such as plums, grapes, cranberries, pomegranates, citrus fruits, carobs, etc. have an antioxidant effect on meat products [4].

Rich sources of natural antioxidants are a number of herbs (rosemary, oregano, sage, basil, etc.) and spices (cinnamon, cloves, nutmeg, ginger, black pepper, garlic) [3, 6].

Wojdyło et al. investigated 32 species of herbs and spices belonging to different botanical families and found that a large number of them, especially from the *Lamiaceae* family, exhibited significant antioxidant activity and could be used as natural antioxidant supplements [7].

The aim of the study was to investigate the possibilities of extending the shelf life of raw chicken meat by treatment with aqueous extracts of oregano and wild basil and to examine the effect of treatment on the meat's physicochemical properties, microbiological status and TBARS content during storage at 4°C.

2 Material and methods

2.1. Materials

The studies were conducted with chicken breast meat, from a local producer. The samples, 50 g each, were divided into three groups - control (CG) and two experimental groups, treated (marinated) with aqueous

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extracts of wild basil (TGCv) and oregano (TGOv), respectively. Treatment with plant extracts of the experimental groups was carried out for 20 hours at a temperature of 4°C. All the chemicals and reagents used were analytical grade. Trichloroacetic acid (TCA), ethylenediaminetetraacetic acid (EDTA), propyl gallate and gallic acid were purchased from Merck. DPPH (1,1diphenyl-2-picrylhydrazyl radical), Trolox (6-hydroxy2,5,7,8-tetramethylchroman-2-carboxylic acid), 2-thiobarbituric acid (TBA) and 1,1,3,3-tetraethoxypropane (TEP) were from Sigma-Aldrich. All the chemicals, reagents for SDS-PAGE were purchased from SERVA, protein markers - from Sigma-Aldrich.

2.2. Preparation of aqueous extracts

For preparation of aqueous extracts (infusions), aerial parts of wild basil (*Clinopodium vulgare L.*) and oregano (*Origanum vulgare L.*), purchased from a herbal pharmacy in the city of Sofia, were used. 150 ml of boiling distilled water was added to 10 g of the plant material with a subsequent stay for 60 min. The infusions were filtered, topped up with distilled water to obtain a total volume of 150 ml and cooled to a temperature of 4˚C.

2.3. Determination of total phenolic compounds

The content of total phenolic substances (TPC) in the extracts was determined by spectrophotometric method with Folin-Ciocalteu reagent and expressed as gallic acid equivalents - GAE mg/ml [13].

Antioxidant activity of the extracts was evaluated by DPPH method [14, 15], with a slight modification: 0.3 ml of a solution of 0.2 mM DPPH in methanol was mix with 1.2 ml of methanol and 0.5 ml of the corresponding dilution of the extract in 80% methanol. The samples were incubated for 60 min in the dark at room temperature and the decrease in absorbance at 517 nm was recorded. In the control, the sample solution is replaced by 0.5 ml of 80% methanol. The standard curve was prepared from a standard solution of Trolox at concentration from 1.25 to 7.5 µg/ml, and the antioxidant activity was expressed as Trolox equivalents - TE µg/ml.

2.4. Analyzes of control and experimental chicken meat samples

2.4.1. Physicochemical analysis of meat samples

The moisture content was measured with Sartorius Thermo Control YTC 01L balances.

Total protein content - by Kjeldahl method according to ISO 937:1978 [16].

Total fat – by extraction with hexane in a "Soxtec 2005" apparatus.

Total ash – by mineralization of the sample in a muffle furnace, according to ISO 936:1998 [17].

2.4.2. pH determination

The pH was measured potentiometrically with a pH-meter (Jenway 3300), pre-calibrated at pH 4.0 and 7.0. The measurements were performed on fresh meat and after storage at 4°C for 7 and 14 in three points of the sample, and the obtained results were averaged.

2.4.3. Cooking loss

Samples of approximately 15.0 g were taken from control (CG) and experimental (TGCv and TGOv) chicken meat groups, distributed in separate petri dishes and placed for 6, 12 or 21 min in the middle part of an oven preheated to 200°C. Then they were left to cool to room temperature in a desiccator. All samples were weighed on an analytical balance before and after cooking.

The percentage of cooking loss is calculated by eq. (1):

$$Cooking\ loss\ (\%)\ \frac{raw\ weight - cooked\ weight}{raw\ weigh} \times 100 \quad (1)$$

2.4.4. Protein electrophoresis (SDS-PAGE)

Electrophoretic analysis was performed according to Laemmli [18], with slight modification. Samples of 5 g each of chicken meat (CG, TGCv and TGOv) were homogenized with 45 ml of 5% SDS solution, then heated in a water bath (85°C) for 30 min and centrifuged at 8000 rpm for 10 min. A half ml of the supernatant was mixed with 0.5 ml of sample buffer (0.2 M Tris-HCl, pH 6.80, 2% SDS, 16% glycerol, 10 mM DDT, and 0.01% bromophenol blue). The electrophoresis was carried out at concentrations of stacking gel – 6% and separating gel – 10% and a constant current - 30 mA, using an OmniPAGE WAVE Electrophoresis System (Cleaver Scientifics). The gel was stained with 0.1% COOMASSIE® Brilliant Blue G-250 (30 - 40 min), then destained for 4 h. The distances from the start to each band are measured and the Rf values of the protein fractions are determined.

2.4.5. Microbiological analysis

The total number of aerobic mesophilic microorganisms was determined according to EN ISO 4833-1:2013 in the control and experimental chicken meat samples [19]. The dynamics of the growth of microorganisms for the entire period of refrigerated storage of the meat was monitored.

2.4.6. Content of TBARS

To determine the content of thiobarbituric acid reactive substances in meat samples, a spectrophotometric method was applied [20, 21]. Briefly: portions of 15 g of ground (chopped) meat were placed in beakers and mixed with 30 ml of 7.5% trichloroacetic acid solution with added 0.1% EDTA and 0.1% propyl gallate. The mixture was homogenized and filtered through filter paper. The filtrate was centrifuged at 6000 rpm at 4°C for 10 min (Beckman

Coulter). The supernatant was collected and used in the assay. Five ml of the supernatant was placed in a capped tube, mixed with 5 ml of 20 mM thiobarbituric acid (TBA) solution, homogenized and placed in a boiling water bath for 60 min. After cooling, the absorbance of the resulting pink-coloured complex was measured at 530 nm. TEP (1,1,3,3-tetraethoxypropane) was used as malondialdehyde (MDA) standard without prior hydrolysis, and for the construction of the standard curve the corresponding concentration of malondialdehyde was from 0.072 to 0.548 µg/ml. Results are presented as mg MDA/kg meat.

2.5. Statistical analysis

Analyzes of aqueous extracts were performed with three replicates. Data are presented as mean ± standard deviation (SD). Analyzes of variant chicken meat samples were performed with 5 replicates. Statistical evaluation of the results was performed with the Excel 2016 software package. A one-way analysis of variance was performed to determine the effect of meat treatment.

3 Results and discussion

The obtained water extracts from the plant material are transparent liquids with a light brown (wild basil) to red brown (oregano) color and a light pleasant aroma specific to the respective plant. The results of total phenolic content (TPC) and antioxidant activity assays are given in Table 1.

Table 1. TPC and antioxidant activity of aqueous extracts of *C. vulgare* and *O. vulgare*. Results are presented as means ± SD

Plant extract	TPC, mg GAE/ml	Antioxidant activity, µg TE /ml
Water extract of *C. vulgare*	0.849±0.014	274.05±8.70
Water extract of *O. vulgare*	4.781±0.125	768.10±4.95

Both extracts showed significant antioxidant activity. In the aqueous extract of oregano, the antioxidant activity is 2.8 times higher compared to the extract of wild basil, respectively: 768.10 µg TE/ml and 274.05 µg TE/ml. These results show a positive correlation with the data obtained for the content of total phenols in the extracts. Table 2 presents the results for moisture content, total protein, ash and fat of the control (CG) and treated meat samples (TGCv and TGOv) on the first day of storage at 4°C. No statistically significant differences were observed in the results of the physico-chemical analysis of the control and experimental groups of chicken meat, on the first day of cold storage. For all samples, the content of total protein, fat and ash is close to the values quoted in the literature [22, 23]. Cooking loss after heating with hot air in a convection oven increases with increasing heating time (Fig. 1).

Table 2. Physicochemical analysis of control and experimental groups of chicken meat on the first day of storage at 4°C. The results are presented as mean values ± SD

Chicken meat	Moisture, %	Protein, %	Fat, %	Ash, %
CG (control)	74.96 ±0.86	20.15±0.71	0.90±0.12	1.54±0.16
TGCv	75.08±1.05	19.94±0.53	0.88±0.09	1.49±0.08
TGOv	75.42 ±0.63	19.89±0.64	0.91±0.11	1.51±0.17

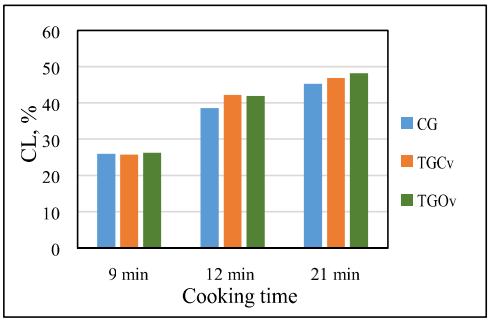

Fig. 1. Cooking loss of chicken meat samples - control and experimental groups at three different heating times

In the control group, cooking loss values increased from 25.90% (9 min) to 45.28% (21 min). Under identical test conditions, the results obtained in a study by Shaarani et al. [24] are slightly lower, respectively - 23% and 45%. A slight increase in cooking loss was observed in the experimental groups (TGCv and TGOv), but the differences were not statistically significant (P > 0.05).

Table 3. Effect of the treatment with aqueous extracts of *C. vulgare* and *O. vulgare* (TGCv and TGOv) on pH, protein content, TBARS-values and total viable count of chicken meat during refrigeration storage

Treatment	Storage days		
	0	7	14
	pH		
CG (control)	6.11±0.05	6.70±0.25	7.13±0.06
TGCv	6.08±0.02	6.10±0.06	6.67±0.08
TGOv	6.12±0.07	5.90±0.13	6.63±0.03
	Protein, %		
CG (control)	20.15±0.71	20.11±1.10	19.94±1.23
TGCv	19.94±0.53	20.03±0.54	19.93±0.85
TGOv	19.89±0.64	19.92±0.78	19.99±1,02
	TBARS values, mg MDA/kg		
CG (control)	0.027±0.005	0.164±0.019	0.273±0.017
TGCv	0.020±0.005	0.131±0.027	0.191±0.007
TGOv	0.018±0.002	0.127±0.021	0.183±0.031
	Total viable count, log10 CFU/g		
CG (control)	3.70±0.14	6.78±0.15	9.36±0.34
TGCv	3.61±0.18	6.67±0.16	9.24±0.21
TGOv	3.58±0.23	6.42±0.18	9.15±0.28

Table 3 presents the results for pH, total protein, TBARS-value and microbial status of the control and experimental groups of chicken meat after storage at 4°C for a period of up to 14 d.

At the beginning of the storage period (0 d), the pH values were not significantly different between the control and experimental groups. On the seventh day, the pH was significantly higher ($P \leq 0.05$) in the control group (CG) compared to the treated samples (TGCv and TGOv) and the trend was maintained until the 14th d when the pH of the CG was 7.13 (Table 3). The increase in pH values after the 7th d of storage may be due to the accumulation of ammonia and degradation products from amino acids released during protein degradation, caused by bacteria. In a study by Katiyo et al. [25] it was found that the pH of chicken meat increased to 7.08 on day 10 and reached 7.28 after 14 d of cold storage. The authors analyzed the microbial status of the meat and reported that on day 14 the total number of microorganisms was 9.13 log cfu/g, which is close to our result of 9.36 log cfu/g.

Analysis of microbiological test data showed a slight decrease of 0.36 log units (7 d) and 0.21 log units (14 d) in experimental group TGOv, which is not statistically significant. According to literature, oregano extracts have a significant antibacterial effect. The weak inhibitory effect in our study is most likely due to the lower concentration of the extract and the way the meat samples were treated. Martins et al. [26] reported different antibacterial activity of oregano depending on the type of extract and method of preparation. On the other hand, on the 7th and 14th d of storage, a significant ($p \leq 0.05$) decrease in TBARS values was observed in the experimental groups, which is indicative of the antioxidant effect of the extracts. A study by Zhang et al. [27] also found a significant reduction in TBARS in samples of raw chicken breast meat fillets treated with rosemary and clove extracts. The secondary products of lipid oxidation, mainly malondialdehyde, are determined with the TBARS analysis. MDA is the most commonly used marker when studying lipid peroxidation in foods. There is still no legislative limit on the concentration of MDA in meat samples, but MDA above 0.5 mg/kg indicates some oxidation and values above 1.0 mg/kg are considered unacceptable levels in several studies as they cause negative changes in the taste and aroma of the meat [28]. The mechanism of action of natural antioxidants is believed to be related to breaking the oxidation chain reaction by hydrogen release from phenolic groups to peroxide radicals and forming stable compounds [27]. In this sense, a high content of polyphenolic compounds determines the strong antioxidant effect of the extracts from *C. vulgare* and *O. vulgare*. For the entire period of refrigeration storage, no significant changes were found in the quantitative content of total protein in all groups. This result was confirmed by SDS-PAGE (Fig. 2). Multiple protein fractions with different electrophoretic mobilities were detected in all samples. An intense band for molecular weight of about 44 kDa, corresponding to actin is observed. Under these electrophoresis conditions, the other predominant protein in muscle tissue, myosin, remains at the start. No differences were found in the number and intensity of protein fractions for the entire storage period.

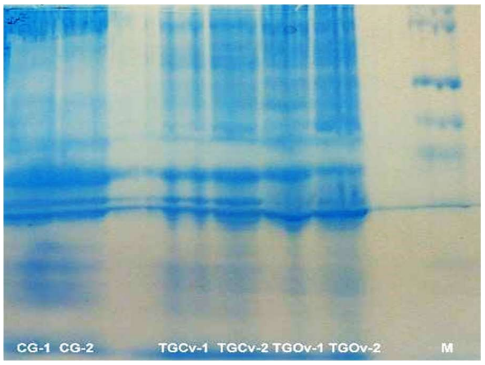

Fig. 2. SDS-PAGE of chicken meat after storage at 4°C: CG-1 TGCv-1 and TGOv-1 (0 d); CG-2, TGCv-2 and TGOv-2 (14 d), M-Sigma Marker high range

4 Conclusion

The results demonstrate the efficacy of aqueous extracts of wild basil and oregano in inhibiting lipid oxidation in chicken meat. A significant reduction in malondialdehyde content, compared to the control, was found when stored at 4°C for 14 d, without affecting the content of total protein, ash, fat and dry matter. In the experimental groups, there was a slight increase in cooking loss and a significantly lower pH-value on 7 d and 14 d compared to the control group. After the 7 d, the total number of mesophilic microorganisms in the meat samples increased to 7.00 log cfu/g, which is considered the upper limit of acceptability for fresh meat. Under the conditions of the experiment, the treatment with extracts of oregano and wild basil did not significantly affect the development of microorganisms in the meat samples. Further experiments are needed to optimize the concentration of the extracts and the treatment method to improve the quality characteristics of raw chicken meat and possibly extend the shelf life under refrigeration storage.

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