Antioxidant effect of oregano essential oil during various storage meat
time of hybrid combination ross 308

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ABSTRACT
This study was conducted to evaluate the effect of Origanum vulgare L. Hirtum essential oil on the oxidation stability of raw chicken meat. Oregano essential oil was applied in a different way, on the one hand in a feed for broiler chickens (E1) and on the other hand on a surface of chicken thighs (E2). Broiler chickens were fed during the experimental period in the all groups with commercial feed mixtures except the experimental group of E1 (with the addition of 0.05% oregano essential oil, 50 g EO per 100 g of the feed mixture). In E2 was application of oregano essential oil (0.5%) on surface of thighs 1 mL per 60 g of meat realized. The oxidative stability of the chicken meat was investigated in the same way, 8th days after vacuum-packed and stored at temperature 4 °C and 6, 9 and 12 months after vacuum-packed and storage at -18 °C. The samples of the E1 consisted of breast and thigh muscles with skin (150 g) and of the E2 thigh muscle with skin (60 g). The impact of oregano essential oil was measured by content of fat and peroxide value (PV). Fat content in both experiments was not affected by storage time and EO addition. Content of chicken meat fat in E1 in control group ranged between \( M = 9.64 - 12.95 \, g.100 \, g^{-1} \) and in experimental group contained similar amount of fat mean from \( M = 9.94 - 12.24 \, g.100 \, g^{-1} \); E2: in control group \( M = 7.01 - 7.73 \, g.100 \, g^{-1} \) and in experimental group \( M = 6.15 - 8.03 \, g.100 \, g^{-1} \). Measured peroxide values confirm that oregano essential oil has effect on broiler chicken meat oxidative stability, if applied to feed, manifested statistically significant differences between control and experimental group. The mean of peroxide value in control group of E1 was \( M = 0.58 - 3.60 \, \mu \text{mol} \, \text{O}_2.\text{kg}^{-1} \) and in experimental group was \( M = 1.06 - 2.11 \, \mu \text{mol} \, \text{O}_2.\text{kg}^{-1} \). We found not statistically significant difference in peroxide values, if applied oregano essential oil to raw chicken meat. The results impact of oregano essential oil on chicken meat comparable to control group, but a tendency to improve oxidative stability was indicated.

Keywords: oregano essential oil; antioxidant; chicken meat storage; oxidative stability; peroxide value

INTRODUCTION
The relationship between the consumption of meat and health is multifaceted, and it needs to be analyzed in detail, with specific attention to the relevant differences that characterize the effects of the different meat types. A variable but moderate energy content, highly digestible proteins of good nutritional quality, unsaturated lipids, B-group vitamins and minerals make poultry meat a valuable food. Epidemiological studies performed across the world, in highly diverse populations with different food preferences and nutritional habits, provide solid information on the association between poultry consumption, within a balanced diet, and good health (Marangoni et al., 2015).

Meat and meat products are important sources of high-quality protein. Their acceptance of consumers depends mainly on the proportion of fat, that is responsible for its taste properties and structure (Lorenzo a Franco, 2012). However, despite this benefit, fat is the cause of deterioration in the nutritional and sensory quality of the meat due to its greater susceptibility to oxidative damage (Qi et al., 2015).

Angelovič et al. (2015) constate that the mechanisms of oxidative degradation can be autoxidation in presence of atmospheric oxygen.

The intake of lipids from chicken meat is variable dependent on the cut considered by consumers. Fats are mainly found in the skin and can, therefore, be easily removed during processing or consumption of meat. The lipid content varies from 1% in poor parts such as breasts to 17% in chicken wings with skin. The inclusion of skin can increase these values (Marangoni et al., 2015).

Muscular lipids are highly susceptible to oxidation due to a high degree of unsaturation. Oxidation leads to a deterioration in the taste, color, structure and nutritional value of the meat. Of great importance in the oxidation of...
Chicken meat are some ingredients (iron content, antioxidants) as well as external factors (feeding with fodder feed, stress, killing process, temperature, processing procedures, storage conditions, etc.) (Estévez et al., 2014).

The main factors influencing lipid oxidation in meat are oxygen, which reacts with unsaturated lipids to form lipid peroxides. The resulting lipid peroxides lead to the formation of various chemical compounds such as alcohols, aldehydes and ketones (Domínguez et al., 2014), which are responsible for the unpleasant taste and odor and thus also for the reduction of the sensory and nutritional quality of the meat.

Lipid oxidation is possible to prevent through adding synthetic and natural antioxidants to animal feed or to processes meat and meat products. Synthetic antioxidants have been confirmed for their toxicological and carcinogenic effects. Natural antioxidants may be found in any plant part and most natural antioxidants are phenolic compounds, and the most important are the tocopherols, flavonoids, and phenolic acids (Kumar et al., 2015).

The genus Origanum belongs to the family of Labiatae and includes many species that are commonly found as wild plants in the Mediterranean areas. Thirty-eight (38) Origanum species are recognized in the world. Due to the variability in chemical and aroma characteristics is used Origanum in cosmetic industries, as a culinary herb, flavoring substances of food products, alcoholic beverages and perfumery for their spicy fragrance (Pirigharnaei et al., 2011).

Essential oils (EO) are volatile, natural compounds formed by aromatic plants as secondary metabolites, known for their antiseptic, bactericidal, vircidal and fungidical, and medicinal properties and their fragrance, they are used in preservation of foods and as antimicrobial, analgesic, sedative, anti-inflammatory, spasmylytic and locally anesthetic remedies (Bakkali et al., 2008).

Dominant components of oregano EO are composed of carvacrol and thymol, followed by γ-terpinene, p-cymene, linalool, terpinen-4-ol and sabine hydrate. O. vulgare ssp. hirtum, contained a high amount of EO. The content of EO as high as 8% with carvacrol as dominant component (95%) was reported for this subspecies (Kumar et al., 2015).

Freezing is one of the several methods of preserving meat and protecting its quality until it reaches the consumer (Xia et al., 2012).

The main objectives of this preservation method are to inhibit microbial growth, delay metabolic activities and oxidative damages. It allows maintaining almost all product characteristics of products and to stock them for long periods (He et al., 2013). Despite being one of the least aggressive preservation methods, freezing still produces modifications in foods.

Bennett et al. (2014) report that oxidation can be initiated by the formation of lipid peroxides, which can be detected by measuring the peroxide value, that quantifies the levels of peroxides and hydroperoxides formed in the initial phase of lipid oxidation. The determination of the fat peroxide value is used to determine the degree of fat loss. The low peroxide fat value usually indicates that the fat has not become rancid and will have good stability.

Fresh fats have a peroxide value of 1 – 2, whereas rancid fats have a peroxide value of 15 – 20. Rancidity is caused by oxidation and hydrolysis (Sharma, Giriprasad and Goswani, 2013).

**Scientific hypothesis**

The aim of this paper is to study the effect of oregano essential oil supplementation on chicken meat quality and lipid oxidation stability during various storage time of meat.

**MATERIAL AND METHODOLOGY**

**Biological material**

Two experiments were carried out. The difference between the 1st (E1) and 2nd (E2) experiment was in the way of application of oregano essential oil (EO). The application of oregano essential oil was performed in the E1 in the feed and in the E2 on the surface of the chicken thighs. The oxidative stability of the chicken meat was investigated in the same way in both experiments, after vacuum-packed 8 days after vacuum-packed and storage at 4 °C and 6, 9 and 12 months after vacuum-packed and storage at -18 °C.

The E1 was conducted in poultry farm, in conditions of the welfare principles application whit deep litter breeding system. The conditions responded protection requirements for broilers chickens Council Directive 2007/43/EC. Microclimatic conditions (light, temperature, humidity and air exchange) were uniform for both groups in accordance with recommendations for the meat broiler chickens ROSS 308. Broiler chickens (40 one-day-old broiler chickens) were divided in the E1 into 2 groups, control (CG) and experimental – EG (n = 20). Broiler chickens were fed during the experimental period in the control group with commercial feed mixtures without oregano essential oil (EO) and in the experimental group with similar diets as in the control group but with the addition of 0.05% EO, in amount of 50 g of EO per 100 g of the feed mixture. Broiler chickens consumed of feed mixtures ad libitum.

The experimental period lasted 38 days and tree feed mixtures were used: starter feed mixture, for chickens to 18 days of age (feed from plate feeders and water from the hat drinkers located on the floor); grower mixture, from 19 to 31 day of age (feed from the tube feeders and drank water from bucket drinkers till end of the experiment) and finisher mixture, from 32 to 38 day of age. Broiler chickens were transported to the chemical laboratory of Department of Food Hygiene and Safety, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture Nitra and killed after ending experiment.

The breast and thigh muscles with skin were deboned and used for analyses (8th days after slaughter and storage at 4 °C and after 6, 9 and 12 months of storage at -18 °C of chicken meat).

In the E2 were broilers chickens killed on slaughterhouse and selected thighs randomly, after slaughtering. Chicken thighs were obtained from broiler chickens that were farmed and fed with commercial feed mixtures. Chicken thighs were deboned and used for analyses in the 8th day (measurement 30 minutes after application 0.5% of EO on the surface of thighs) and after 6, 9 and 12 months. In both measurements were thighs divided to two groups: control group (samples without treatment), experimental group (samples with application of EO on surface of thigh). Each
Laboratory testing

Sample preparation

The samples consisted of: E1: chicken muscles (breast and thigh) and skin; E2: thigh muscle and skin. Samples were deboned and collected on the day of slaughter vacuum-packed and stored at 4 °C for 8 days and at -18 °C for further analysis after 6, 9 and 12 months of storage. In the E2 were samples of experimental group with EO treated (1 ml of oregano essential oil applied on surface of thigh muscle 60 g). Time of EO treat was 30 minutes, amount 1 ml per 60 g of meat. Chemical analysis (fat content, peroxide value) of the meat samples were carried out after homogenization (according to the method AOAC 983.18), drying of the samples under the prescribed conditions at 105 °C to constant weight. The dried samples were then milled into powder using Grindomix GM 200 grinder and used to obtain fat by extraction (non-polar solvent) in a Soxhlet extractor and expressed as g per 100 g of fresh tissue.

Chemicals analyses

Oregano essential oil (Origanum vulgare sup. Hirtum) was purchased from Calendula a.s., Nová Lúbovňa, Slovensko with a total antioxidant activity of EO 93.85% and carvacrol content 57%.

Determination of fat content: was used an official method to determine fat content AOAC 991.36. Crude fat content is determined by extracting the fat from sample using a chemical solvent (petroleum) under the extraction method. Lipid extraction was carried out using a device Det-gras N, model 4002842.

Fat content calculation (g.100 g⁻¹):

\[
\text{Crude fat} = \frac{w_2 - w_0}{w_1} \times 100
\]

w2 – is weight of the extraction thimble with extracted fat
w0 – is weight of the empty extraction thimble
w1 - is weight of the sample

Determination of peroxide value (PV): was used an official method to determine peroxide value [IFRA (2011)] with minor modifications. Peroxide value test measures amount of iodine released from potassium iodide next estimated using a standard sodium-thiosulphate solution. Peroxide value is stated by millimoles of active oxygen per kilogram of lipids mmol O₂.kg⁻¹. Peroxide value was carried out using a chemical: chloroform, acetic acid, potassium iodide (freshly prepared saturated solution dark stored), sodium thiosulphate (0.01 mol.L⁻¹), starch.

Peroxide value calculation (mmol O₂.kg⁻¹):

\[
P_V = \frac{(V_1 - V_0) \times c \times 1000 \times T}{W}
\]

V1 – consumption of 0.01 mol.L⁻¹ sodium thiosulphate solution in the main test
V0 – consumption of 0.01 mol.L⁻¹ sodium thiosulphate solution in the blank test
\(c\) – molar concentration of the sodium thiosulphate solution
T – titre of the sodium thiosulphate solution
W – weighed portion of fat in grams

Statistic analysis

The statistical analysis was performed by the program SAS, version 9.1. The results of measurements were analysed using ANOVA for the repeated measure and Student t test for independent samples if the normality was met. If the normality wasn’t met, Friedman test and Mann-Whitney test were used. Significance was established at \(p < 0.05\).

RESULTS AND DISCUSSION

Fat content

The fat content of chicken meat after the application of EO to the feed and measured in raw chicken muscle homogenates depending on its storage, is reported in Table 2. In E1 mean content of chicken meat fat in control group ranged between \(M = 9.64\) g.100 g⁻¹ in 9th month and \(M = 12.95\) g.100 g⁻¹ in 12th months. The time has not a statistically significant effect on content of chicken meat fat in control group (\(F(1.94, 36.864) = 2.075, p > 0.05\)).
Chicken meat from experimental group contained similar amount of fat mean from \( M = 9.94 \) g.100 g\(^{-1}\) in 6\(^{th}\) month to \( M = 12.24 \) in 12\(^{th}\) months. We found out in experimental group too, that the time has not a statistically significant effect on content of chicken meat fat \((F(3, 57) = 1.749, p >0.05)\).

The difference between control group and experimental group of oregano essential oil feeding broiler chickens was not significant in measurements of fat content of chicken meat \(8\) days \((t(38) = 0.2, p >0.05)\), \(6\) months \((t(38) = 1.23, p >0.05)\), \(9\) months \((t(38) = -1.036, p >0.05)\), and \(12\) months \((t(38) = 0.735, p >0.05)\).

In E2 the fat content has been balanced during whole storage period \((F(1, 5) = 0.41, p >0.05)\). Fat content of chicken tight in experimental group ranged between mean \( M = 5.19 \) g.100 g\(^{-1}\) in 6\(^{th}\) month and \( M = 7.85 \) g.100 g\(^{-1}\) at 8\(^{th}\) day storage period. In experimental control group was not found a statistically significant effect of time on fat content \((F(3, 33) = 2.226, p >0.05)\).

In comparison of control group and experimental group we didn’t found a statistically significant difference in individual measurements \(8\) days \((t(22) = -0.877, p >0.05)\), \(6\) months \((t(22) = 1.219, p >0.05)\), \(9\) months \((t(22) = -0.032, p >0.05)\), and \(12\) months \((t(22) = 0.924, p >0.05)\).

These results correspond to the results of Liptaiová et al. (2010), which reported average fat content of chicken meat of broilers chickens fytodietives feeding with concentration 0.1% 9.9 g.100 g\(^{-1}\), 0.05% 9.5 g.100 g\(^{-1}\), 0.025% 10.45 g.100 g\(^{-1}\). The fat content of the chicken meat in the control group \((without fytodietives)\), was 9.8 g.100 g\(^{-1}\). Differences in the fat content of chicken meat between the groups were not statistically significant \((p >0.05)\).

Giannenas et al. (2016) in a 42-day-long fattening experiment with 5% oregano essential oil in feed mixtures \((300 \text{ g.ton}^{-1})\) of broiler chickens Ross 308 hybrid combination and 5% oregano essential oil and 0.5% virgin oil in feed mixtures \((500 \text{ g.ton}^{-1})\) indicated fat content in chicken breasts without skin of 5.4 g.100 g\(^{-1}\) in both groups compared to the control group of 5.5 g.100 g\(^{-1}\). Fat content in chicken thighs without skin 8.2 g.100 g\(^{-1}\) in both experimental groups and 8.4 g.100 g\(^{-1}\) in the control group without a statistically significant difference between the groups \((p >0.05)\).

Comparable fat content introduced in their study by broiler chickens Ross 308 hybrid combination Čuboň et al. (2013), where measured in thigh muscle with skin 12.2 – 13.2 g.100 g\(^{-1}\), Milićević et al. (2014) measured in thigh muscle with skin 5.19 – 9.85 g.100 g\(^{-1}\).
Fat peroxide value during storage, mmol O₂·kg⁻¹.

<table>
<thead>
<tr>
<th>Group</th>
<th>Storage period</th>
<th>M ±SD</th>
<th>cᵢ(%)</th>
<th>M ±SD</th>
<th>cᵢ(%)</th>
<th>M ±SD</th>
<th>cᵢ(%)</th>
<th>M ±SD</th>
<th>cᵢ(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>8 days</td>
<td>0.58 ±0.34</td>
<td>58.62</td>
<td>1.39 ±0.54</td>
<td>38.85</td>
<td>2.40 ±1.87</td>
<td>77.92</td>
<td>3.60 ±1.70</td>
<td>47.22</td>
</tr>
<tr>
<td>EG</td>
<td>8 days</td>
<td>1.06 ±0.74</td>
<td>69.81</td>
<td>1.22 ±1.06</td>
<td>86.89</td>
<td>2.02 ±0.81</td>
<td>40.10</td>
<td>2.11 ±1.02</td>
<td>48.34</td>
</tr>
<tr>
<td>CG</td>
<td>6 months</td>
<td>1.76 ±0.42</td>
<td>23.86</td>
<td>1.56 ±0.53</td>
<td>33.97</td>
<td>2.22 ±2.03</td>
<td>91.44</td>
<td>3.40 ±3.84</td>
<td>112.94</td>
</tr>
<tr>
<td>EG</td>
<td>6 months</td>
<td>1.62 ±0.50</td>
<td>30.86</td>
<td>1.47 ±0.58</td>
<td>39.46</td>
<td>2.28 ±0.59</td>
<td>25.88</td>
<td>2.50 ±1.16</td>
<td>46.4</td>
</tr>
<tr>
<td>CG</td>
<td>9 months</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>EG</td>
<td>9 months</td>
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<tr>
<td>CG</td>
<td>12 months</td>
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<td>EG</td>
<td>12 months</td>
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</table>

**Legend:** CG – control group without oregano essential oil, EG – experimental group with oregano essential oil, $M$ – mean, $SD$ – standard deviation, $cᵢ$ – coefficient of variation.

**Figure 2** Fat peroxide value during storage, mmol O₂·kg⁻¹.

**Peroxide value**

The oxidative stability parameter of the peroxide value after the application of EO to the feed and measured in raw chicken muscle homogenates depending on its storage is reported in Table 3.

The mean of peroxide value in control group of E1 was $M = 0.58$ μmol O₂·kg⁻¹ at 8th day of storage time and increased during whole storage period up to $M = 3.60$ μmol O₂·kg⁻¹ in 12th months (Figure 2). There is a statistically significant effect of time on content of peroxide value in control group ($F(1.823, 34.634) = 19.357, p <0.05$).

Peroxide value in experimental group with EO was $M = 1.06$ μmol O₂·kg⁻¹ at 8th day of storage time and after 12th months $M = 2.11$ μmol O₂·kg⁻¹. There is a statistically significant difference between peroxide values in experimental group measured in various period ($χ^2(3) = 21.211, p <0.05$).

The difference between control group and experimental group with oregano essential oil feeding broiler chickens was statistically significant in first and last measurements (8 days ($U = 101, p <0.05$), 12 months ($U = 90.5, p <0.05$)). In first measurement was peroxide value significantly higher in experimental group ($Mdn = 0.85$) than in control group ($Mdn = 0.57$). In the last measurement it was inside out. Peroxide value was significantly higher in control group ($Mdn = 3.66$) than in experimental group ($Mdn = 1.98$).

Peroxide value of the samples in E2 evaluated in the control group ranged between $M = 1.56$ μmol O₂·kg⁻¹ and $M = 3.40$ μmol O₂·kg⁻¹. We didn’t found a statistically significant difference between peroxide values measured in various time ($χ^2(3) = 1.4, p >0.05$) in control group. In experimental group we observed increased peroxide value too and there was not found a statistically significant difference between peroxide values measured in various time ($χ^2(3) = 5.034, p >0.05$).

In comparison between control group and experimental group with EO, we found not a statistically significant difference in individual measurements (8 days ($U = 17.5, p >0.05$), 6 months ($U = 14.5, p >0.05$), 9 months ($U = 12.5, p >0.05$), 12 months ($U = 12.5, p >0.05$)).

Sing et al. (2014) noticed significantly higher peroxide value in control group without fytoadditives during storage at 4 °C after 7 and 9 days in raw chicken meat unlike our study, where was peroxide value of E2 after 8th days similar in both groups. In our experiments, the peroxide value was comparable to 8th day and after 6th months of storage, indicated oxidative damage to chicken meat, well below the peroxide limit reported Min and Ellefson (2010), which indicates, that a low peroxide value can represent either the beginning or advanced oxidation and can be distinguished based on the PV over time or by
measuring secondary oxidation products. A high quality, fresh fat has a PV of zero, on the other hand very poor-quality fats resulting in a PV ≥20 mmol.kg⁻¹. Peroxide value of chicken meat increased during the whole period of storage, but its value was not so high as to cause that meat to be damaged. In 12 months were in both experiments’ numbers of peroxide value higher in control group. The increase could be caused due to the faster rate of formation of new hydroperoxides than reduction of hydroperoxides into secondary oxidation products that signify effect of supplementation oregano essential oil in experimental groups.

Results of Dashti et al. (2015) showed that thyme essential oil was effective in preventing oxidative spoilage. No difference of peroxide value was observed between the treated samples and control during the first month of chicken nuggets storage but differences among samples can be observed from 2nd month to the 6th month of the storage time. Control samples showed higher oxidation rate than treated samples containing different concentrations of essential oil (0.1%, 0.2%, 0.05%) throughout the storage. In 6th months, peroxide value for all three concentrations was significantly different from samples without thyme essential oil. His research demonstrated the strongest effect of 0.2% essential oil that is lower concentration like in our experiment without significant difference in peroxide value of chicken thighs.

Dzomba et al. (2014) confirmed in their study with different parts of the chosen plants improves meat oxidative stability and provides better protection as synthetic antioxidant. The profile of the untreated meat was above all other profiles. Treating meat by mixing with 50 mg of Cleome gynandra leaf extract gave more promising results with a peroxide value that was below 50 mmol O₂.kg⁻¹ even after 26 days. It depicted the slowest rate of formation of peroxides as compared to synthetic antioxidant BHT.

Our results do not consist with authors, who reported that the peroxide value of mechanically deboned chicken meat treated with a polyphenol extract increases and thereafter decreases with storage time. They represented, that decomposition of hydroperoxides into secondary products increases at a higher rate as lipid oxidation progresses, as compared with the formation of new hydroperoxides, resulting in decreased peroxide value (Teets and Were, 2008, Soyer et al., 2010, Hwang et al., 2013).

CONCLUSION

Results of our experiments indicate that the oregano essential oil and storage time not influenced the fat content in chicken meat but manifested an impact on the oxidative stability of chicken meat its application to feed. This effect was statistically significant compared with control group. We found not statistically significant difference in peroxide values, if applied oregano essential oil to raw chicken meat. The results impact of oregano essential oil on chicken meat comparable to control group, but a tendency to improve oxidative stability was indicated. We recommend the use of 0.05% oregano essential oil for broiler chickens due to its antioxidant properties in amount 50 g of oregano essential oil per 100 g of the feed mixture. Oregano essential oil appears to be alternative to synthetic additives in broiler nutrition.

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