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# MICROBIOLOGICAL QUALITY OF CHICKEN THIGHS MEAT AFTER APPLICATION OF ESSENTIAL OILS COMBINATION, EDTA AND VACCUM PACKING

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## ABSTRACT

The aim of the present work to monitoring chicken the microbiological quality of vaccum packaged thighs after treatment by ethylenediaminetetraacetate (EDTA), anise (*Pimpinella anisum*), spearmint (*Mentha spicata* var. *crispa*), thyme (*Thymus vulgaris* L.) oregano (*Origanum vulgare* L.) essential oils and stored in at 4 ±0.5 °C for a period of 16 days. The following treatments of chicken thighs were used: air-packaged control samples, control vacuum-packaged samples, vacuum-packaging with EDTA solution 1.5% w/w, control samples, vacuum-packaging after treatment with *Pimpinella anisum, Mentha spicata* var. *crispa* essential oil at concentrations 0.2% v/w, vacuum-packaging after treatment with *Thymus vulgaris* L., *Origanum vulgare* L. essential oil at concentration 0.2% v/w. The quality assessment of all samples was done microbiologically and following microbiological parameters were detected: the anaerobic plate count, *Enterobacteraceae* counts, lactic acid bacteria and *Pseudomonas* spp. counts. The number of anaerobic plate count ranged from 3.69 log CFU.g$^{-1}$ in all tested group on 0 day to 5.68 log CFU.g$^{-1}$ on 16 day in control group stored in air condition. The number of lactic acid bacteria ranged from 2.00 log CFU.g$^{-1}$ in all tested group on 0 day to 4.82 log CFU.g$^{-1}$ on 16 day in group with oregano, thyme essential oils combination. *Enterobacteriacea* counts in chicken thighs was 0.68 log CFU.g$^{-1}$ on 0 day to 7.58 CFU.g$^{-1}$ on 16 day in air-packed meat samples. The *Pseudomonas* spp. was not found in all tested samples. Among the antimicrobial combination treatments examined in this work, the as application of vacuum packaging, EDTA and essential oils treatment was the most effective against the growth of *Enterobactericeae*, inhibitory effect on anaerobic plate count also was observed. The results of this present study suggest the possibility of application the *Pimpinella anisum*, *Mentha spicata* var. *crispa*, *Thymus vulgaris* L., *Origanum vulgare* L. essential oil of as natural food preservatives and potential sources of antimicrobial ingredients for food industry for chicken thighs meat treatment.

**Keywords**: meat; microorganisms; essential oils; vaccum; EDTA

## INTRODUCTION

Poultry meat is a very popular food commodity around the world due to its low cost of production, low fat content, high nutritional value, distinct flavor **(Barbut, 2001; Patsias et al., 2008)**. The diverse nutrient composition of meat makes it an ideal environment for the growth and prolifiration of meat spoilage micro-organisms, as well as food-borne pathogens **(Zhou et al., 2010)**. Therefore is essential to apply adequate preservation technologies to extend the shelf life of perishable meat products which is a major concern for the meat industries **(Wang et al., 2004)**.

Special attention in poultry meat production is paid to the fact that live animals are hosts to a large number of different microorganisms residing on their skin, feathers and in the alimentary tract. During the slaughter a majority of these microorganisms are eliminated, but subsequent contamination is possible at any stage of the production process including contamination from feather plucking and evisceration equipment, washing before storage, cooling or freezing. Microorganisms from the environment, equipment and operators' hands also can contribute to contamination of meat. During the processing the changes in the microflora of meat are reported from, in general, Gram-positive rods (micrococci) to Gram-negative bacteria including Enterobacteriaceae, *Pseudomonas* spp., which were isolated the most frequently. Industrial poultry slaughterhouses have a particular technological process, the individual stages of which are not in conformity with modern principles of hygienic meat production and processing. Factors, which alter the mcrobiological quality of poultry meat can occur during the all processing steps **(Kozačinski et al., 2006)**.

Naturally occurring antimicrobial compounds have good potential to be applied as food preservatives. Essential oils, other extracts from plants, herbs, spices, some of their constituents have shown antimicrobial activity against different food pathogens and spoilage microorganisms **(Bakkali et al., 2008; Burt, 2004; Holley and Patel, 2005)**. Plants, plants products have been claimed to have

health-promoting effects, which may be related to the antioxidant activity *in vivo* **(Ivanišová et al., 2013; Ivanišová et al., 2015a, b)**.

Anise (*Pimpinella anisum* L.), which belongs to the family *Apiaceae*, is an important spice, medicinal plant used for pharmaceutics, perfumery and food industry. The fruits as well as the essential oils are characterized by antispasmodic, antioxidant, antimicrobial, insecticidal and antifungal effects **(Gülcin et al., 2003; Özcan, Chalchat, 2006; Tepe et al., 2006; Tirapelli et al., 2007)**. Its fruits which are called aniseed contain around 1.5-5.0% of essential oil mainly composed of volatile phenylpropanoids like trans-anethole with around 90% **(Tabanca et al., 2005)**. In addition, the essential oil of the anise fruit also contains a small proportion of estragol, anisaldehyde, himachalene and cis-anethole **(Omidbaigi et al., 2003; Tabanca et al., 2006)**.

The genus *Mentha* of the family *Lamiaceae* comprises about 19 species, 13 natural hybrids, is widely distributed across the Europe, Africa, Asia, Australia and North America **(Kumar et al., 2011)**. *Mentha spicata* L., commonly known as spearmint, is a native of Africa, temperate Asia and Europe. It is an herbaceous, rhizomatous, perennial plant growing up to 40x130 cm in height. A literature review shows the antifungal effect of *M. spicata* EO (essential oil) against some food-poisoning fungi **(Sokovic et al., 2009)**, other storage insects **(Lee et al., 2002)**, but reports are lacking about this EO's ability to counter aflatoxin production.

Antimicrobial activity of thyme or oregano essential oil incorporated edible films have been evaluated by a number of researchers, however, limited data exist on the application of antimicrobial edible films incorporated with essential oils in real food systems **(Seydim and Sarikus, 2006; Chi et al., 2006; Oussalah et al., 2006; Du et al., 2008)**. Among *Lamiaceae* species, oregano (*Origanum vulgare* L.), thyme (*Thymus vulgaris* L.), wild thyme (*Thymus serpyllum* L.) have been studied widely for their antioxidant activity due to the high content of phenolic compounds **(Vichi et al., 2001; Zandi and Ahmadi, 2000)**.

The aim of this study was to investigate the effects of anise, spearmint, thyme, oregano essential oils and ethylenediaminetetraacetate in combination with vacuum packaging on the microbiological properties of chicken thighs.

## MATERIAL, METHODOLOGY

### *Preparation of samples*

To evaluate the antimicrobial activity of essential oils the chicken thigh with skin for each experimental group was taken. The chicken thigh fresh samples with were prepared as follow: for air-packaging (AC, control samples) chicken thigh fresh meat was packaged to polyethylene bags and stored aerobically at 4 ±0.5°C; for vacuum-packaged (VPC, control samples) chicken thigh fresh meat was packaged to polyethylene bags, stored anaerobically in vacuum at 4 ±0.5°C; for vacuum-packed samples with EDTA solution 1.5% w/w (VPEC, control samples) chicken thigh was treated with EDTA for 1 min, then packaged to polyethylene bags, stored anaerobically in vacuum at 4 ±0.5°C; for vacuum-packed samples treated with *Pimpinella anisum* + *Mentha spicata* var. *crispa* 0.20% v/w (VP+PAO+MSO) chicken thigh was treated with anise in combination with mint oil for 1 min, packaged to polyethylene bags, stored anaerobically in vacuum at 4 ±0.5°C; for vacuum-packed samples treated with *Thymus vulgaris* L. In combination with *Origanum vulgare* L. 0.20 % v/w (VP+TVO+OVO) chicken thigh was treated with essential oil for 1 min, packaged to polyethylene bags, stored anaerobically in vacuum at 4 ±0.5°C. For sample packaging, a vacuum packaging machine type VB-6 (RM Gastro, Czech Republic) was used. Each sample was packaged immediately after treatment. EDTA solution (pH 8.0, 99.5% purity, analytical grade, Invitrogen, USA) was prepared at final concentration of 50 mM and used in treatment of chicken thigs samples. Anise, spearmint, thyme and oregano essential oils (Hanus, Nitra, Slovakia) was added to coat the surface of chicken thigh on both sides of each sample using a micropipette. Final concentration of 0.2% v/w of EO was used for treatment.

### Microbiological analysis

An amount of 10 g (10 $cm^2$) of the chicken thigh was sampled using sterile scalpels, forceps and immediately transferred into a sterile stomacher bag containing 90 mL of 0.1% peptone water (pH 7.0) and homogenized for 60 s in a Stomacher at room temperature. Sampling and microbiological testing was carried out after certain time intervals: 0, 4, 8, 12, 16 days of experiment. Chicken thighs were stored in vacuum packaging at 4 ±0.5 °C. Microbiological analyses were conducted with accordance to standard microbiological methods. Anaerobic plate count (APC) was determined on Plate Count Agar (PCA, Oxoid, UK) after incubation for 48 h at 35 °C in anaerobic conditions. For *Pseudomonas* spp., 0.1 mL from prepared chicken meat suspension was spread onto the Pseudomonas Isolation agar (PIA, Oxoid, UK). After inoculation PIA was incubated for 48 h at 25 °C. For lactic acid bacteria enumeration, a 1.0 mL of sample was inoculated onto Rogosa, Sharpe agar (MRS, Oxoid, UK), Inoculated agar was incubated for 48-78 h at 37 °C in an aerobic atmosphere supplemented with carbon dioxide (5% $CO_2$). For *Enterobacteriaceae* counts, a 1.0 mL of sample was transferred into 10 mL of molten (45 °C) Violet Red Bile Glucose agar (VRBL, Oxoid, UK). After setting, a 10 mL molten medium was added to cover the suspension. Inoculated VRBL agars were incubated at 37 °C for 24 h. All plates were examined for typical colony appearance and morphology characteristics associated with each medium applied for cultivation of microorganisms.

## RESULTS, DISCUSSION

Essential oils have not only antibacterial properties, but their application in meat can affect some meat characteristics as well. Based on antibacterial properties of EOs, type of affected pathogen, some essential oils are better than others for application in meat industry. Concentration of essential oils, which should be added to meat in order to prevent the oxidation, proliferation of foodborne pathogens, or to extend shelf-life by inhibition of background microflora, is usually higher than one used

in *in vitro* conditions because of interaction with meat components **(Bošković et al., 2013)**.

Anaerobic plate count (AC) values for the tested groups of chicken thigh are showed in Figure 1. The initial anaerobic plate count value of chicken thigh was 3.69 log CFU.g$^{-1}$ on 0 day and the number of microorganisms increases to 5.68 log CFU.g$^{-1}$ on 16 day in control group stored in air condition. In control group stored in vacuum packaging the AC counts were from 3.69 log CFU.g$^{-1}$ on 0 day to 5.12 log CFU.g$^{-1}$ on 16 day of experiment. In control group stored in vacuum packaging and EDTA treated the AC ranged from 3.69 log CFU.g$^{-1}$ on 0 day to 4.78 log CFU.g$^{-1}$ on 16 day. In the group after treatment with anise and spearmint essential oils combination, AC ranged from 3.69 log CFU.g$^{-1}$ on 0 day to 4.56 log CFU.g$^{-1}$ on 16 day. In group after treatment with thyme and oregano essential oils combination, the AC ranged from 3.69 log CFU.g$^{-1}$ on 0 day to 4.45 log CFU.g$^{-1}$ on 16 day. The lowest number on APC on 16 days was found in the group treated with oregano and thyme essential oil combination (4.45 log CFU.g$^{-1}$).

In study of **Radha Krishnan et al., (2014)**, *Enterobacteriaceae*, a psychrotrophic facultative anaerobic bacterial group, formed a substantial part of the chicken meat microbial flora and reached the final counts of 4.68, 3.76 for samples from the initial count of 3.32 log10 CFU.g$^{-1}$. For other samples, final counts were obtained as 4.59, 4.41, 3.91, 4.26, 4.51, 4.01, 4.11, 3.84 log10 CFU.g$^{-1}$ for, samples respectively. **Radha Krishnan et al., (2014)** confirmed that the bacterial counts obtained from spice treated samples were lower than those from the control samples. It is important to point out, that the samples treated with combination of different spice extracts showed lower counts in comparison with the samples treated with extracts of individual spices.

The results of **Kačániová et al., (2015)** study suggest the possibility of using the essential oil of *Pimpinella anisum* L. And *Mentha piperita* as natural food preservatives and potential source of antimicrobial ingredients for meat. Among the treatments of antimicrobial combination examined in this work, the application of vacuum packaging, EDTA and essential oils treatment were the most effective against the growth of lactic acid bacteria, *Enterobactericeae.* Inhbitory effect on total viable count also was observed. Based on microbiological analyses, treatments with *Pimpinella anisum* L. and *Mentha piperita* essential oils resulted in shelf-life extension in comparison with the control samples. The similar results were found in our study in group with combination of anise, spearmint essential oils were used.

The primary objective of chilling poultry is to reduce microbial growth to a level that will maximize both food safety and shelf life **(Popelka et al., 2014)**. Hovewer, psychrotrophic nature of lactic acid bacteria enhancing their survival and multiplying on meat and supporting the spoilage of products. Lactic acid bacteria (LAB) values for the tested groups of chicken thigh are showed in Figure 2. The initial TVC value of chicken thigh was 2.00 log CFU.g$^{-1}$ on 0 day. The number of lactic acid bacteria ranged from 2.00 log CFU.g$^{-1}$ in all tested group on 0 day to 4.82 log CFU.g$^{-1}$ on 16 day in group treated with oregano and thyme essential oils combination.

In control group stored in air condition, the number of LAB ranged from 2.00 log CFU.g$^{-1}$ on 0 day to 3.98 log CFU.g$^{-1}$ on 16 day. In control group stored in vacuum packaging LAB counts ranged from 2.00 log CFU.g$^{-1}$ on 0 day to 4.12 log CFU.g$^{-1}$ on 16 day. In control group stored in vacuum packaging after EDTA treatment, LAB ranged from 2.00 log CFU.g$^{-1}$ on 0 day to 4.23 log CFU.g$^{-1}$ on 16 day.

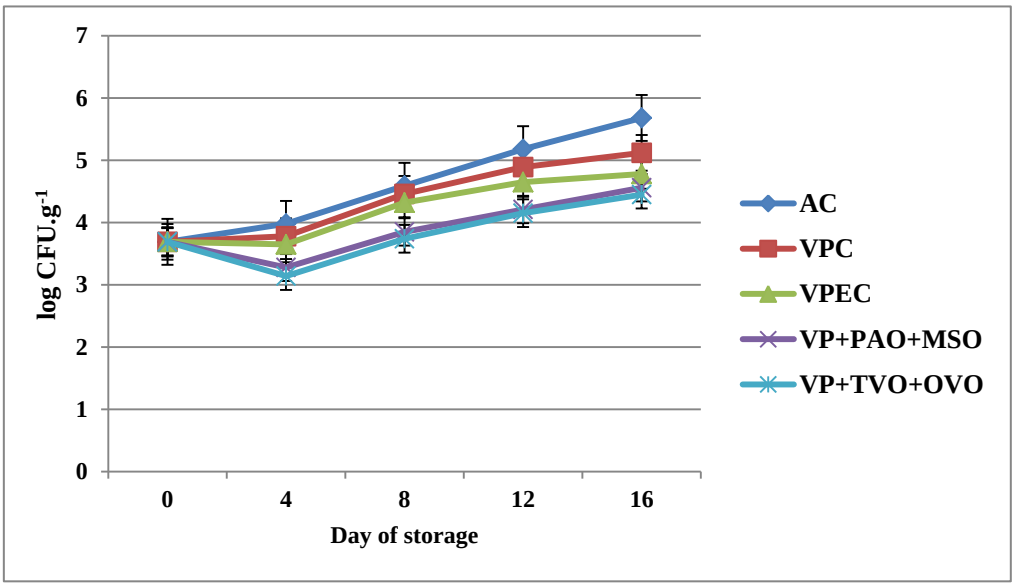


**Figure 1** Changes (log CFU.g$^{-1}$) in population of anaerobic plate count in chicken thigh stored in air (AC); stored in vacuum (VPC); stored in vacuum packaging with EDTA (VPEC); stored in vacuum packaging after treatment with *Pimpinella anisum* + *Mentha spicata* var. *crispa* 0.20% v/w combination (VP+PAO+MSO); stored in vacuum packaging after treatment with *Thymus vulgaris* L. + *Origanum vulgare* L. 0.20 % v/w, combination (VP+TVO+OVO).

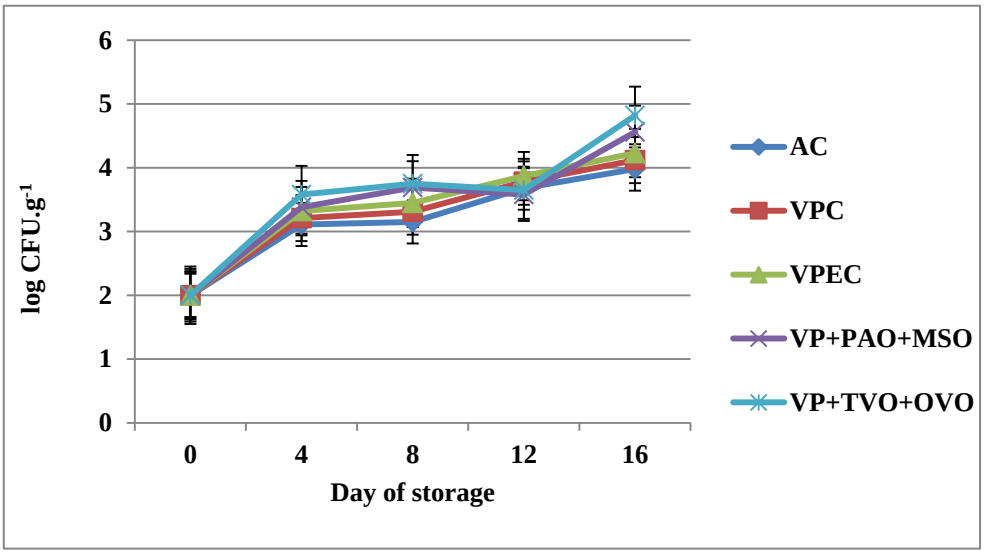


**Figure 2** Changes (log CFU.g$^{-1}$) of lactic acid bacteria counts in chicken thigh stored in air (AC); stored in vacuum (VPC); stored in vacuum packaging with EDTA (VPEC); stored in vacuum packaging after treatment with *Pimpinella anisum* + *Mentha spicata* var. *crispa* 0.20% v/w combination (VP+PAO+MSO); stored in vacuum packaging after treatment with *Thymus vulgaris* L. + *Origanum vulgare* L. 0.20 % v/w, combination (VP+TVO+OVO).

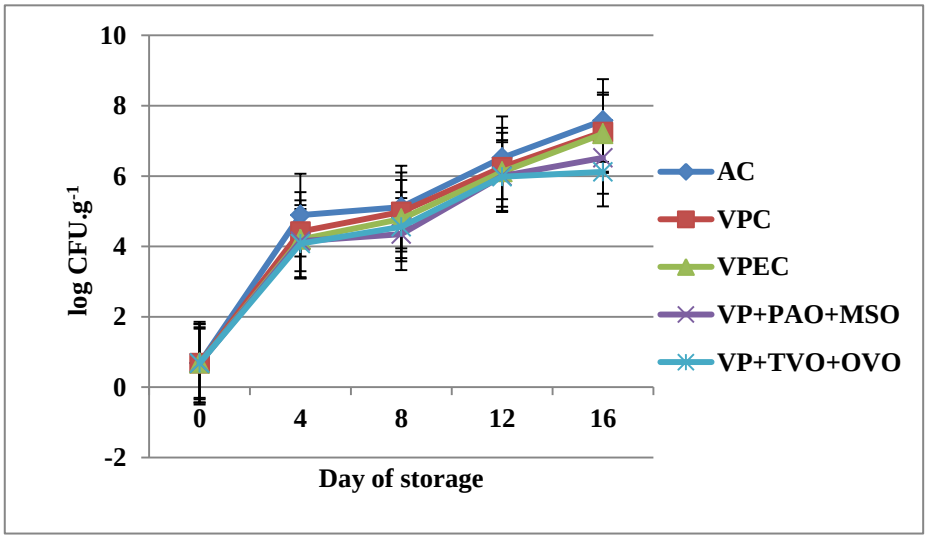


**Figure 3** Changes (log CFU.g$^{-1}$) in population of *Enterobacteriaceae* in chicken thigh stored in air (AC); stored in vacuum (VPC); stored in vacuum packaging with EDTA (VPEC); stored in vacuum packaging after treatment with *Pimpinella anisum* + *Mentha spicata* var. *crispa* 0.20% v/w combination (VP+PAO+MSO); stored in vacuum packaging after treatment with *Thymus vulgaris* L. + *Origanum vulgare* L. 0.20 % v/w combination (VP+TVO+OVO).

In the group after treatment with anise and spearmint essential oils combination, number of LAB ranged from 2.00 log CFU.g$^{-1}$ on 0 day to 4.56 log CFU.g$^{-1}$ on 16 day. In the group after treatment with oregano and thyme essential oils combination ranged from 2.00 log CFU.g$^{-1}$ on 0 day to 4.82 log CFU.g$^{-1}$ on 16 day.

LAB behaves as facultative anaerobes and able to grow under high concentrations of $CO_2$. Thus they constitute a substantial part of the natural microflora of VP meats. LAB are recognized as the important competitors to other spoilage related microbial groups under VP/MAP conditions **(Castellano et al., 2004; Doulgeraki et al., 2011; Zhang et al., 2009)**. Particularly, *Lactobacillus* spp., *Carnobacterium* spp., *Leuconostoc* spp. are associated to the spoilage of refrigerated raw meat **(Nychas, Skandamis, 2005)**. More species of lactobacilli can be found during the storage under the vacuum at 4°C including *Lb. algidus* beyond *Lb. sakei*. The results of **Ntzimani et al. (2010)** indicate that LAB was an important part of the precooked chicken microflora, irrespective of the packaging conditions, the antimicrobial treatment combination. The latter observations could probably help to explain their rapid growth between days 0, 2 of storage. This is also in agreement with LAB growth in beef stored under MAP at 5°C **(Skandamis and Nychas, 2001)**.

*Enterobacteriaceae* counts of the tested groups of chicken thigh are showed in Figure 3. The initial *Enterobacteriacea* genera value of chicken thigh was 0.68 log CFU.g$^{-1}$ on 0 day. Presences of these bacteria were found on all groups at 16 day. The number of *Enterobacteriaceae* genera ranged from 0.68 log CFU.g$^{-1}$ in all tested groups of samples on 0 day to 7.58 log CFU.g$^{-1}$ on 16 day in control group stored in air condition. In control group stored in air condition the number of *Enterobacteriaceae* genera ranged from 0.68 log CFU.g$^{-1}$ on 0 day to 7.58 log CFU.g$^{-1}$ on 16 day. In control group stored in vacuum packaging, *Enterobacteriaceae* counts ranged from 0.68 log CFU.g$^{-1}$ on 0 day to 7.25 log CFU.g$^{-1}$ on 16 day. In control group stored in vacuum packaging after EDTA treatment, *Enterobacteriaceae* counts ranged from 0.68 CFU.g$^{-1}$ on 0 day to 7.20 log CFU.g$^{-1}$ on 16 day. In the group of chicken thigh treated with anise and spearmint essential oils combination *Enterobacteriaceae* counts ranged from 0.68 log CFU.g$^{-1}$ on 0 day to 6.52 log CFU.g$^{-1}$ on 16 day. In the group of chicken thigh treated with oregano and thyme essential oils combination, *Enterobacteriaceae* counts ranged from 0.68 log CFU.g$^{-1}$ on 0 day to 6.12 log CFU.g$^{-1}$ on 16 day.

*Enterobacteriaceae* grew under vacuum packaging conditions at a slower rate than under aerobic packaging. This is in agreement with the results of **Chouliara et al., (2007)**, who reported that both MAP, oregano oil had a strong effect in the reduction of *Enterobacteriaceae* counts. On day 9 of storage, the use of oregano oil at its lower concentration (0.1%), had practically no effect on *Enterobacteriaceae* counts while the higher concentration (1%) gave a reduction of more than 6 log CFU.g$^{-1}$. On the same day, the *Enterobacteriaceae* counts were reduced by 1.5 log CFU.g$^{-1}$ (MAP 1), 1.8 log CFU.g$^{-1}$ (MAP 1, oregano oil 0.1%), more than 6 log CFU.g$^{-1}$ (MAP 1, oregano oil 1%), 3.4 log CFU.g$^{-1}$ (MAP2), 4.3 log CFU.g$^{-1}$g (MAP 2, oregano oil 0.1%), more than 6 log CFU.g$^{-1}$ (MAP 2, oregano oil 1%).

Growth of the *Enterobacteriaceae* was completely inhibited after thyme essential oil treatment was applied and final counts (ca. 4.0 log CFU.g$^{-1}$) were reduced (ca. 3 log cycle) significantly ($p$ <0.05) at the end of the storage period (day 12) in Giatrakou et al. (2010) study. The explanation of this was the antibacterial effects of the essential oils applied the study and this is in agreement with the results of the present study. Thymol essential oil treatment also produced the lower bacterial counts as compared to the control samples during the storage that is in agreement with our results.

Pseudomonas spp. were not isolated in the present study from all samples grou were tested. It is now well established that *Pseudomomas* spp. may form a significant part of the spoilage microflora of chicken meat stored under refrigeration **(Jay et al., 2005)**.

Among the treatments used for improving the shelf-life of products examined in the study of **Pavelkova et al., 2014**, the application of EDTA, oregano oil and thymus oil were the most effective against the growth of Gram-negative bacteria. Inhibitory effect on total viable count and LAB also was identified. Based on microbiological analyses, treatments with oregano and thymus oil combination produced a shelf-life extension of 8-9 days in comparison to the control samples. The ability of vacuum packaging to inhibit a growth of spoilage organisms is well documented, but many pathogenic organisms are less affected in this process. Therefore, the combined effect of essential oils as oregano and thymus including vacuum packaging on the safety of the meat could be investigated.

## CONCLUSION

The results of the present study suggest the possibility of using the essential oil of anise, spearmint, thymol, oregano as natural food preservatives and potential source of antimicrobial ingredients for meat. Among the combinations of treatments, which may pose antimicrobial activity and examined in the present work, the use of modified storage condition as vacuum packaging, treatment with EDTA and essential oils were the most effective against the growth of lactic acid bacteria, *Enterobactericeae* family. Also the growth of anaerobic microorganisms were inhibited. Based on microbiological analyses, the treatment with anise, spearmint, thyme, oregano essential oils resulted in shelf-life extension as compared to the control samples. The combined effect of four essential oils, EDTA, vacuum packaging can significantly contribute the shelf-life and safety of the chicken thigh.

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