# Effect of *Thymus Serpyllum* and *Ocimum Basilicum* Essential Oils on the Shelf-Life of Chicken's Meat during Refrigerated Storage

**Miroslava Kačániová[1], Margarita Terentjeva[2], Henrieta Arpášová[3], Lukáš Hleba[1], Eva Ivanišová[1], Jana Petrová[1], Attila Kántor[1], Juraj Čuboň[1], Peter Haščík[1]**

[1]*Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia*
[2]*Latvia University of Agriculture, Faculty of Veterinary Medicine Institute of Food, Environmental Hygiene, K. Helmaņa iela 8, LV-3004, Jelgava, Latvia*
[3]*Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia*

**Abstract**
The aim of this study was to investigate the microbiological quality of chicken's thighs meat after application of *Thymus serpyllum* and *Ocimum basilicum* essential oils with combination of vacuum packaging and EDTA treatment. The microbiological quality of control samples without vacuum packaging, vacuum packed with and without EDTA treatment, vacuum packed and treated with EDTA and samples treated with the essential oils of basil and breckland thyme were followed for 16 days. Microbiological analyses were conducted with standard microbiological methods. For anaerobic plate count, PCA agar was inoculated and incubated for 2 days at 35°C anaerobically. *Pseudomonas* spp. count was determined on Pseudomonas Isolation agar after incubation at 48 h at 25°C. For lactic acid bacteria (LAB), MRS agar was inoculated and incubated for 48-78 h at 37°C microaerophilly. For *Enterobacteriaceae*, VRBG agar was inoculated and incubated at 37°C for 24 h. Anaerobic plate count ranged from 2.89 log CFU/g in all tested group on 0 day to 5.19 log CFU/g on 16 day in control group stored in air. LAB count ranged from 3.01 log CFU/g in all tested group on 0 day to 4.08 log CFU/g on 16 day in control group stored in air. *Enterobacteriacea* counts were from 1.33 log CFU/g on 0 day to 5.11 on 16 day in control group stored in air. *Pseudomonas* spp. were found only in control group stored in air on 12 and 16 day. The results of the present study suggest the possibility of application of *Thymus serpyllum* and *Ocimum basilicum* essential oils as natural food preservatives and potential sources of antimicrobial ingredients for food industry.

**Keywords**: chickens thighs, vacuum, EDTA, basil and breckland thyme essential oil

## 1. Introduction

Chicken meat is favored by consumers around the world because of the desirable nutritional qualities, such as a low fat content and a relatively high concentration of polyunsaturated fatty acids [1]. Fresh meat products are usually marketed at refrigerated temperatures (2+5°C) [2].

The microbiological safety and quality of poultry meat are equally important for producers, retailers and consumers then microbial contamination of raw and the processed product is important. Among the microorganisms two different groups are relevant: foodborne pathogens and organisms that are generally harmless to human health, but, being psychrotrophic, are able to multiply on the product during chill storage. Spoilage results mainly in loss of typical sensory characteristics of

* Corresponding author: Miroslava Kačániová, phone: +421376414494, e-mail: miroslava.kacaniova@gmail.com

product and limited shelf-life of the product. The product shelf-life determines both the number of spoilage organisms present initially, the temperature history of the product at all stages of production and subsequent storage and handling, as well as post-processing contamination Because of the impact of spoilage processes of shelf-life of the product, the possibility to delay development of spoilage microflora is necessary to ensure the good quality of foods. [3].

Throughout the history, the aerial parts and the volatile constituents of *Thymus* species have been highly recommended for different applications. They were commonly used as herbal teas, condiments, spices, and for various medicinal purposes [4]. Many ethnomedicinal properties are attributed to infusions and decoctions produced from the aerial parts of *Thymus* species. Infusions and decoctions contain essential oils (EOs) with exhibit tonic, carminative, digestive, antispasmodic, antimicrobial, antioxidant, antiviral, anti-inflammatory and expectorant activities, so as could be used for the treatment of cold [5-7]. *T. serpyllum* is a well-known as wild thyme and is native to Mediterranean Europe and North Africa where grows mainly at the higher altitudes. It is acknowledged for use in various traditional home remedies. This aromatic plant possess antiseptic, diaphoretic, analgesic, carminative, expectorant, diuretic, emmenagogue, and stimulant properties, and it has been also used in mouth washes and gargles, against cough and cold. Its EO contains various, powerful ingredients with proven disinfectant and immune stimulating features, capable to fight a range of infections. The oil relieves rheumatism, and is also used in hair loss treatments [8].

The *Ocimum* genus, commonly known as basil, comprises 30– 160 annual and perennial herbs and shrubs, which are native species in tropical and subtropical regions of Asia, Africa, and Central and South America. Among several species of the *Ocimum genus, O. basilicum, O. tenuiflorum, O. canum, O. gratissimum* and *O. minimum* are cultivated for both culinary and medicinal purposes. For example O. *basilicum* is an important culinary herb and it contains a high proportion of phenolic derivatives including eugenol and linalool. It is extensively grown because of its adaptability to a range of distinct environmental conditions. *O. basilicum* has shown various disease-curing abilities, such as anti-ulcer, anti-microbial, anti-hyperlipidaemic, and anti-viral activities. The pharmacological and economic value of *Ocimum* spp. is mainly due to its aromatic compounds (methyl eugenol, eugenol and linalool) that have been reported to exert nematicidal activity and have been used as pro-oxidants, anti-oxidants, dental anaesthetics and disinfectants, as well as protecting agent against nicotine toxicity in murine peritoneal macrophages [9-11].

The purpose of this study was to investigate the effects of *Thymus serpyllum* and *Ocimum basilicum* essential oils combination with EDTA and vacuum packaging on the microbiological of chicken thighs.

## 2. Materials and methods

### Preparation of samples

For evaluation of the antimicrobial activity of essential oils, the chicken thigh with skin was taken. The chicken thigh fresh samples were prepared as follow: for air-packaging (AC, control samples) chicken thigh was packaged in polyethylene bags and stored aerobically in refrigerator at 4±0.5°C; for vacuum-packaged (VPC, control samples) chicken thigh was packaged in polyethylene bags and stored anaerobically in vacuum and refrigerator 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, packaged in polyethylene bags and stored anaerobically in vacuum in refrigerator at 4±0.5°C; for vacuum-packed samples with *O. basilicum* 0.20% v/w (OBO) chicken thigh was treated with basil oil for 1 min, packaged to polyethylene bags and stored anaerobically in vacuum in refrigerator at 4±0.5°C; for vacuum-packed samples with *T. serpyllum* 0.20% v/w, (TSO) chicken thigh was treated with breckland thyme oil for 1 min, packaged to polyethylene bags and stored anaerobically in vacuum, in refrigerator at 4±0.5°C.. For sample packaging, a vacuum packaging machine type VB-6 (RM Gastro, Czech Republic) was used, each sample were packed immediately after treatment. A stock solution of 500 mM concentration of EDTA was prepared by diluting 186.15g in 1 L distilled water (EDTA, $C_{10}H_{14}N_2O_8.Na_2.2H_2O$), 99.5% purity, analytical grade (Invitrogen, USA). A final

concentration of 50 mM EDTA, pH 8.0 solution was prepared from the stock solution. Basil and breckland thyme essential oil (Hanus, Nitra, Slovakia) was added to coat the chicken thigh surface of both sides with 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 and forceps and immediately transferred into a sterile stomacher bag containing 90 mL of 0.1% peptone water (pH 7.0). Samples were homogenized for 60 s in a peristaltic blender at room temperature. Sampling was carried out on 0, 4, 8, 12 and 16 days of experiment. Chicken thighs were stored in vacuum packaging at 4±0.5°C during the experiment. Microbiological analyses were conducted with standard microbiological methods. For anaerobic plate count (APC), Plate Count Agar (PCA, Oxoid, UK) was inoculated with 1 mL of sample suspension and incubated for 2 days at 35°C anaerobically. For *Pseudomonas* spp., an amount of 0.1 mL of chicken homogenates was spread onto the surface of the Pseudomonas Isolation agar (PIA, Oxoid, UK) and incubated for 48 h at 25°C. For LAB, a 1.0 mL sample were inoculated into Rogosa, Sharpe agar (MRS, Oxoid, UK) and incubated for 48-72 h at 37°C in an aerobic atmosphere supplemented with carbon dioxide (5% $CO_2$). For *Enterobacteriaceae*, a 1.0 mL sample was inoculated into 10 mL of molten (45°C) violet red bile glucose agar (VRBL, Oxoid, UK). Inoculated agars were incubated at 37°C for 24 h. All plates were examined for typical colony types and morphology characteristics associated with each medium applied for incubation. Only typical colonies were counted.

**Statistical analysis**

Figures were done with EXCEL MS. Data for the mean from each replication was calculated and all data were log transformed. Statistical analysis were done with STATGRAPHICS 5 software (UMEX GmbH Dresden, Germany).

## 3. Results and discussion

The growing concern about food safety has led to the development of natural antimicrobials to control food borne pathogens and spoilage bacteria in food. Spices are one of the most commonly used natural antimicrobial agents in foods and have been used traditionally for thousands of years by many cultures for preserving foods and as food additives to enhance aroma and flavor [12]. The antimicrobial properties of some spices and their components have been documented [13-16]. The previous studies confirmed that garlic, onion, cinnamon, cloves, thyme, sage, and other spices inhibit the growth of both Gram-positive and Gram-negative food borne pathogens, as well as spoilage bacteria, yeast, and molds [12, 17].

Lactic acid bacteria (LAB) counts for the tested groups of chicken thighs are showed in Figure 1. The initial LAB value of chicken thighs was 3.01 log CFU/g on 0 day. The number of LAB ranged from 3.01 log CFU/g 0 day to 4.08 log CFU/g on 16 day in control group stored in air. In control groups stored in vacuum packaging and vacuum packaging and EDTA treatment LAB counts achieved, respectively, 3.80 and 3.30 log CFU/g on 16 day. In OBO and TSO groups LAB counts were 3.46 log CFU/g and 3.01 log CFU/g on 16 day of experiment.

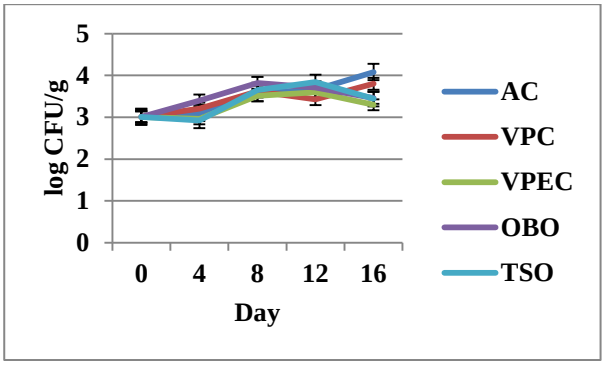


**Figure 1.** Changes (log cfu/g) in population of lactic acid bacteria in chicken thighs stored in air (AC); stored under vacuum (VPC); stored under vacuum packaging with EDTA (VPEC); stored under vacuum packaging with *Ocimum basilicum* 0.20% v/w (OBO); stored under vacuum packaging with *Thymus serpyllum* 0.20% v/w (TSO)

In the study of Petrová et al. (2016), LAB varied in similar values [18]. Unfortunately, some work has shown that spices stimulate the growth and acid production of LAB [19]. In a study by Nes and Skjelkvale [20], seasoned dry sausages were inoculated with three strains of *Lactobacillus plantarum*. By understanding and manipulating

additive or synergistic relationships among essential oils and other food additives, it is possible that reduced concentrations may be necessary for food preservation, resulting in fewer negative sensory changes occurring in food production. L AB spoilage in meats is a relevant problem as they are facultative anaerobes that can grow and continue to spoil foods under chilled conditions [21-22].

Anaerobic plate count (APC) for the chicken thighs are showed in Figure 2.

The number of anaerobic plate count ranged from 2.89 log CFU/g in all tested group on 0 day to 5.19 log CFU/g on 16 day in control group stored in air. In control group stored in vacuum packaging and vacuum packaging with EDTA, the APC counts were 4.41 log CFU/g and 4.12 log CFU/g on 16 day of experiment. APC counts reached 3.85 log CFU/g and 3.78 log CFU/g in samples treated with basil and breckland thyme essential oils on 16 days of experiment.

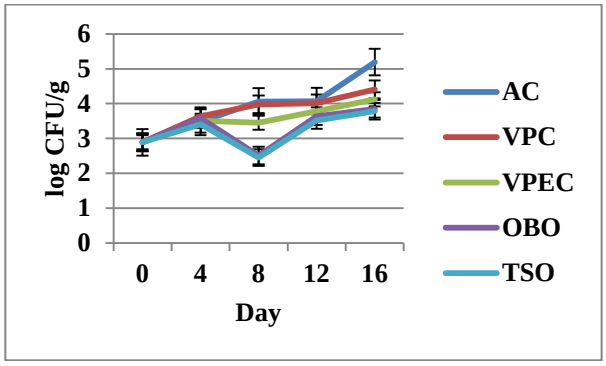


**Figure 2.** Changes (log cfu/g) in anaerobic plate count in chicken thighs stored in air (AC); stored under vacuum (VPC); stored under vacuum packaging with EDTA (VPEC); stored under vacuum packaging with *Ocimum basilicum* 0.20% v/w (OBO); stored under vacuum packaging with *Thymus serpyllum* 0.20% v/w (TSO)

In the study of Kluz et al. [23], the anaerobic plate count ranged from 2.77 log CFU/g in all tested group on 0 day to 5.45 log CFU/g on 16 day in control group stored in air. In control group stored vacuum packaged, stored vacuum packaged after EDTA treatment, after treatment with caraway and anise essential oils APC were 5.25 log CFU/g , 5.21 log CFU/g , 4.20 log CFU/g and 4.15 log CFU/g on 16 day of experiment. In this study caraway and anise essential oils had positive influence to number of all bacteria.

Results on counts of *Enterobacteriaceae* are shown in Figure 3. *Enterobacteriaceae* counts were from 1.33 log CFU/g to 5.11 log CFU/g in chicken thigh samples. In control group stored in vacuum packaging and vacuum packaging with EDTA, the *Enterobacteriaceae* counts were 5.01 log CFU/g and 4.12 log CFU/g on 16 day of experiment. *Enterobacteriaceae* counts reached 3.56 log CFU/g and 3.22 log CFU/g in samples treated with basil and breckland thyme essential oils on 16 days of experiment.

In the study of Kačániová et al. [24] the number of *Enterobacteriaceae* family ranged from 0.68 log CFU/g in all tested groups on 0 day to 7.58 log CFU/g on 16 day in control group stored in air. In control group stored in vacuum packaging and in vacuum packaging after EDTA treatment *Enterobacteriaceae* counts reached 7.25 log CFU/g and 7.20 log CFU/g on 16 day of experiment. In the groups of chicken thigh treated with anise and spearmint essential oils combination *Enterobacteriaceae* counts were 6.52 log CFU/g and 6.12 log CFU/g on 16 day.

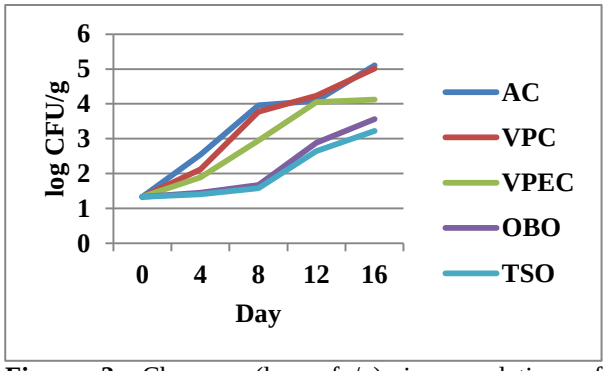


**Figure 3.** Changes (log cfu/g) in population of Enterobacteriaceae in chicken thighs stored in air (AC); stored under vacuum (VPC); stored under vacuum packaging with EDTA (VPEC); stored under vacuum packaging with *Ocimum basilicum* 0.20% v/w (OBO); stored under vacuum packaging with *Thymus serpyllum* 0.20% v/w (TSO)

*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. [25] who reported that both MAP and oregano oil had a strong effect on the reduction of *Enterobacteriaceae* counts. On day 9 of storage, the use of oregano oil at its lower concentration of 0.1% had practically no effect on *Enterobacteriaceae* counts while the higher concentration of 1% gave a reduction of more than

6 log CFU/g. On the same day, the *Enterobacteriaceae* counts were reduced by 1.5 log CFU/g (MAP 1), 1.8 log CFU/g (MAP 1, oregano oil 0.1%), more than 6 log CFU/g (MAP 1, oregano oil 1%).

The presence of *Pseudomonas* spp. bacteria in this study were found only in control group on 12 and 16 day of experiment.

## 4. Conclusions

Basil and breckland thyme essential oils exhibited good antimicrobial properties against anaerobic bacteria, lactic acid bacteria and *Enterobacteriacea* at 0.2% concentration. Meat is highly subjected to microbial deterioration, which ultimately affect the safety and quality characteristics if the meat have been not properly handled and preserved. Several plant-derived EOs can be effectively used in meat as natural alternatives to synthetic food additives, particularly as effective antimicrobial agents.

## Acknowledgements

This work was supported by grant VEGA 1/0611/14 and APVV-0304-12.

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