# Microbiological Quality of Chicken Thighs after Vacuum Packaging, EDTA, *Coriandri aetheroleum* and *Menthae spicata aetheroleum*

**Miroslava Kačániová[1], Jana Petrová[1], Margarita Terentjeva[2], Martin Mellen[3], Attila Kántor[1], Maciej Kluz[4], Lukáš Hleba[1], Peter Haščík[5], Juraj Čuboň[5]**

[1]*Slovak University of Agriculture,Faculty of Biotechnology and Food Sciences, Department of Microbiology, Tr. A. Hlinku 2, 949 76Nitra, Slovak Republic*
[2]*Latvia University of Agriculture,Faculty of Veterinary Medicine, Institute of Food and Environmental Hygiene, K. Helmaņa iela 8, LV-3004, Jelgava, Latvia*
[3]*Hydina Slovakia, s.r.o., Nová Ľubovňa 505, 065 11 Nová Ľubovňa, Slovakia*
[4]*University of Rzeszow, Department of Biotechnology and Microbiology, Rzeszow, Poland*
[5]*Slovak University of Agriculture, TFaculty of Biotechnology and Food Sciences, Department of Animal Products Evaluation Processing, r. A. Hlinku 2, 949 76 Nitra, Slovak Republic*

**Abstract**
The aim of the present work was monitoring chicken thighs microbiological quality after treatment by ethylenediaminetetraacetate (EDTA), coriander (*Coriandri aetheroleum*) and spearmint (*Menthae spicata aetheroleum*) essential oil, stored under vacuum packaging, at 4±0.5°C for a period of 16 days. The following treatments of chicken thighs were used: Air-packaging control samples, control vacuum-packaging samples, vacuum-packaging with EDTA solution 1.50% w/w, control samples, vacuum-packaging with *Menthae crispae aetheroleum* essential oil at concentrations 0.2% v/w and vacuum-packaging with *Coriandri aetheroleum* essential oil at concentration 0.2% v/w. The quality assessment of all samples was established by microbiological analysis. The microbiological parameters as the total viable count, *Enterobacteraceae* genera counts, lactic acid bacteria and *Pseudomonas* spp. were detected. The results of this present study suggest the possibility of application the essential oil of *Coriandri aetheroleum* and *Menthae spicata aetheroleum* as natural food preservatives and potential sources of antimicrobial ingredients for food industry.

**Keywords**: chicken thighs, vacuum. EDTA, microbiological quality, essential oils

## 1. Introduction

The microbiological quality of meat and meat products is very important with regards to public health significance. There are several reports on outbreaks of food borne illnesses because of consumption of meat [1-3]. The meat is potentially subjected to contamination from a variety of sources within and outside animal during the slaughter of animal and during its sale. In living animals, those surfaces in contact with the environment harbor a variety of microorganisms.

*Coriandrum sativum* L. (*Apiaceae*) has been cultivated for thousands of years in India, China and Egypt and today is native to the Mediterranean region and cultivated in many temperate countries. Coriander is the dried nearly ripe fruit of the *Coriandrum sativum* L. plant. The coriander leaves and seeds are used as seasoning agent in liqueurs, teas, meat products and pickles. The oil isolated from dried seeds is important ingredient in modern perfumery. Numerous papers [4-25] describe the chemical composition of the

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

oil from coriander leaves and seeds using different oil isolation methods (extraction, steam and hydrodistillation, supercritical fluid extraction with $CO_2$, headspace method). By distillation 0.1 - 5.2 % of essential oil can be recovered from coriander seeds, which contains mainly linalool. Saturated and unsaturated aliphatic aldehydes C8 - C14 are the main components (nearly 90 %) of coriander leaf oil [6-7,18,23]. The principial constituent of the coriander oil is linalool [6,11,13- 16,23-25] or linalool and geranyl acetate 20 or *trans*-2-decenal [23]. By the standard of European Pharmacopoeia [25] (EP) coriander (*Coriandri fructus*) should contain not less than 3 ml/kg of essential oil. Coriander oil (*Coriandri aetheroleum*) contain by EP 65 - 78 % of linalool, also the ranges of several other constituents are stated.

The genus *Mentha,* belonging to the family Lamiaceae, consists of more than 30 species. The plants of this family are a rich source of polyphenols and hence could possess strong antioxidant properties [26]. *Mentha spicata* L. is characterized by its volatile oil of economical importance. It is widely cultivated in many places around the world for the production of essential oil [27-29]. Traditionally, *M. spicata* has been utilized in the foods as a flavoring agent and as an herbal medicine in folk remedies [30]. Recently, *M. spicata* has become a subject of scientific interest in view of other potential uses of its essential oil and extracts, for the most part, as antimicrobial and antioxidant agents [31].

The aim of the present work was monitoring chicken thighs microbiological quality after treatment by ethylenediaminetetraacetate (EDTA), coriander (*Coriandri aetheroleum*) and spearmint (*Menthae spicata aetheroleum*) essential oil, stored under vacuum packaging, at 4±0.5°C for a period of 16 days.

## 2. Materials and methods

### Preparation of samples

To evaluate the antimicrobial activity of essential oils the chicken thigh with skin of 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 in refrigerator; for vacuum-packaged (VPC, control samples) chicken thigh fresh meat was packaged to polyethylene bags and stored anaerobically in vacuum and in refrigerator; for vacuum-packed samples with EDTA solution 1.5% w/w (VPEC, control samples) chicken thigh fresh meat was treated with EDTA for 1 min and packaged to polyethylene bags and stored anaerobically in vacuum and in refrigerator; for vacuum-packed samples with *Coriandri aetheroleum* 0.20% v/w (VP+CEO) chicken thigh fresh meat was treated with coriander oil for 1 min and packaged to polyethylene bags and stored anaerobically in vacuum and in refrigerator; for vacuum-packed samples with *Menthae spicata aetheroleum* 0.20 % v/w, (VP+MEO) chicken thigh fresh meat was treated with spearmint oil for 1 min and packaged to polyethylene bags and stored anaerobically in vacuum and in refrigerator (4±0.5°C). For sample packaging a vacuum packaging machine type VB-6 (RM Gastro, Czech Republic) was used and 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 solution was prepared from the stock solution. The pH of the solution was adjusted to 8.0 with the addition of the appropriate quantity of NaOH solution. Coriander and mint essential oil (Hanus, Nitra, Slovakia) was added to coat chicken thigh surface (both sides) of each sample using a micropipette. Final concentration of 0.2% v/w of EO was used for treatment.

### Microbiological analysis

Approximately 10 g (10 $cm^2$ ) of the chicken thigh was sampled using sterile scalpels and forceps, 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 was carried out after certain time intervals: 0, 4, 8, 12 and 16 days. Chicken thigh were stored under vacuum packaging, at 4±0.5°C during experiment. Microbiological analyses were conducted by using standard microbiological methods. Total viable counts (TVC) were determined using Plate Count Agar (PCA, Oxoid, UK), after incubation for 2 days at 37°C. For *Pseudomonas* spp. enumerations, 0.1 mL from 1:10 prepared serial dilutions (0.1% physiological solution) of chicken

homogenates was spread onto the surface of solid media. *Pseudomonas* was determined on Pseudomonas Isolation agar (PIA, Oxoid, UK) after incubation at 48 h at 25°C. For lactic acid bacteria enumeration, a 1.0 mL sample were inoculated into Rogosa and Sharpe agar (MRS, Oxoid, UK) after incubation 48-78 h at 37°C in an aerobic atmosphere supplemented with carbon dioxide (5% $CO_2$). For members of the family *Enterobacteriaceae*, a 1.0 mL sample was inoculated into 10 mL of molten (45°C) violet red bile glucose agar (VRBL, Oxoid, UK). After setting, a 10 mL overlay of molten medium was added and samples incubated at 37°C for 24 h. The large colonies with purple haloes were counted. All plates were examined for typical colony types and morphology characteristics associated with each medium applied for incubation.

## 3. Results and discussion

Total viable count (TVC) values for the tested groups of chicken thigh are showed in Fig. 1. The initial TVC value of chicken thigh was 3.36 log cfu/g on 0 day.

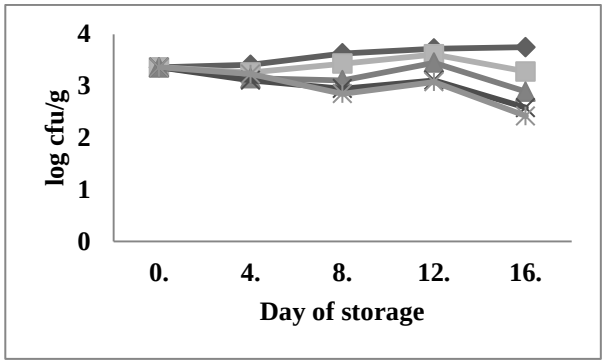


**Figure 1.** Changes (log cfu/g) in population of total viable count in chicken thigh stored in air (AC, ♦); stored under vacuum (VPC, ■); stored under vacuum packaging with EDTA (VPEC, ▲); stored under vacuum packaging with *Coriandri aetheroleum* 0.2% essential oil (VP+CEO, ×); stored under vacuum packaging with *Menthae spicata aetheroleum* 0.2% essential oil (VP+MEO, ●).

Lactic acid bacteria (LAB) values for the tested groups of chicken thigh are showed in Fig. 2. The initial TVC value of chicken thigh was 2.98 log cfu/g on 0 day.

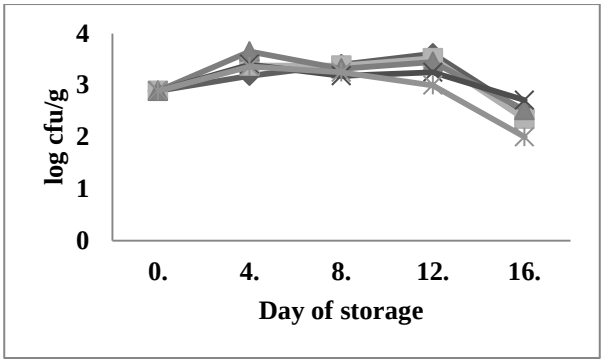


**Figure 2.** Changes (log cfu/g) in population of lactic acid bacteria in chicken thigh stored in air (AC, ♦); stored under vacuum (VPC, ■); stored under vacuum packaging with EDTA (VPEC, ▲); stored under vacuum packaging with *Coriandri aetheroleum* 0.2% essential oil (VP+CEO, ×); stored under vacuum packaging with *Menthae spicata aetheroleum* 0.2% essential oil (VP+MEO, ●).

*Enterobacteriaceae* genera values for the tested groups of chicken thigh are showed in Fig. 3. The initial TVC value of chicken thigh was 0 log cfu/g on 0 day. Presence of this bacteria were found on all groups at 16 day.

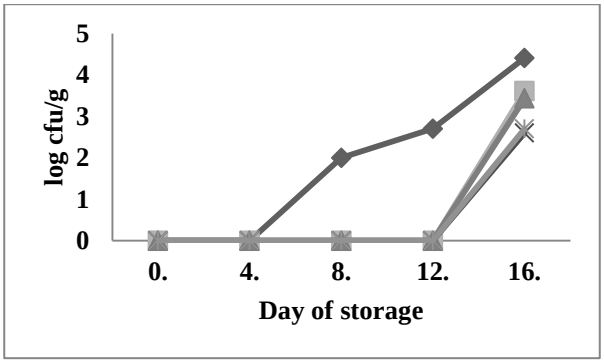


**Figure 3.** Changes (log cfu/g) in population of *Enterobacteriaceae* genera in chicken thigh stored in air (AC, ♦); stored under vacuum (VPC, ■); stored under vacuum packaging with EDTA (VPEC, ▲); stored under vacuum packaging with *Coriandri aetheroleum* 0.2% essential oil (VP+CEO, ×); stored under vacuum packaging with *Menthae spicata aetheroleum* 0.2% essential oil (VP+MEO, ●).

The presence of *Pseudomonas* spp. bacteria in this study were not found in all tested groups.

Of the antimicrobial combination treatments examined in the study of Nizimani et al. [32]., the use of treatments, EDTA–lysozyme–rosemary oil

(VP+EL+R) and EDTA–lysozyme–oregano oil VP+EL+O) were the most effective against the growth of Gram-negative, Gram-positive bacteria, and to a lesser extent on yeasts. The presence of rosemary oil (0.2% v/w) in cooked VP+EL+R and VP+EL+O samples produced a distinct but acceptable pleasant odor and taste, well received by the panellists. The application of oregano oil in cooked chicken samples was not as pleasant as compared to that of rosemary oil. Based on both microbiological (TVC data) and sensory (taste attribute) analyses, treatments VP+EL+R and VP+EL+O produced a shelf-life extension of 7–8 days, as compared to the control samples.

The results of Hasapidou and Savvaidis [33] study indicate that the shelf-life of fresh chicken liver stored under refrigeration, can be extended, by either packaging the product under MAP (M treatment) or with EDTA (ME treatment) and additionally by adding oregano essential oil (MER1, MER2 treatments). The product under these treatments (M, ME, MER1 and MER2) maintains its freshness and quality (sensorial) characteristics. However an important parameter, when evaluating the potential use of EOs such oregano, thyme etc. as preservative in foods, needs to be taken into consideration in terms of the sensorial acceptability of the treated with the EOs product.

Of the antimicrobial combination treatments examined in the study of Pavelkova et al. [34], the use of treatments, EDTA, oregano oil ad thymus oil were the most effective against the growth of Gram-negative bacteria and to a lesser extent on total viable count and lactobacilli. Based on microbiological analyses, treatments oregano oil and thymus oil produced a shelf-life extension of 8e9 days, as compared to the control samples. The ability of vacuum packaging to inhibit spoilage organisms is well documented, but many pathogenic organisms are less affected. Therefore, the combined effect of essential oils as oregano and thymus and vacuum packaging on the safety of the meat could be investigated.

To our knowledge, the effect of all three antibacterial hurdles investigated in this study has not yet been reported to date in the bibliography and therefore any comparison of our data to the results of other workers is not feasible.

## 4. Conclusions

The results of this present study suggest the possibility of using the essential oil of coriander and spearmint as natural food preservatives and potential source of antimicrobial ingredients for meat. Of the antimicrobial combination treatments examined in the work, the use of storage condition as vacuum packaging, EDTA, and essential oils were the most effective against the growth of lactic acid bacteria and *Enterobactericeae* family and to a less extent on total viable count. Based on microbiological analyses, treatments with coriander and spearmint essential oils resulted in shelf-life extension, as compared to the control samples. Therefore, the combined effect of essential oil and vacuum packaging on the safety of the meat could be investigated.

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## References

1. Lunden, J.M., Autio, T.J. Sjoberg A.M., Korkeala, H.J., Persistent and nonpersistent *Listeria monocytogenes* contamination in meat and poultry processing plants, Journal of Food Protection, 2003, 66, 2062-2069

2. Prakash, B., Krishnappa, G., Muniyappa L. and Kumar, B.S., Epidemiological characterization of avian Salmonella enterica serovar infections in India, International Journal of Poultry Science, 2005, 4 (6), 388-395

3. Bhandare, S. G., Sherikar, A. T., Paturkar, A. M., Waskar, V. S., Zende R. J., A comparison of microbial contamination of sheep/goat carcasses in a modern Indian abattoir and traditional meat shops, Food Control, 2007, 18, 854-868

4. Heath, H.B., Source Book of Flavors. AVI Publishing Company, Westport, Connecticut. 1981

5. Tashinen, J., Nykänen, L., Volatile constituents obtained by the extraction with alcohol-water mixture

and by steam distillation of coriander fruit, Acta Chemica Scandidavica, 1975, B29, 425-429
6. Lawrence, B.M., Recent progress in essential oils, Perfumer & Flavorist, 1977, 2, 53-55
7. Mc Leod, A.J., Islam, R., Volatile flavour components of coriander leaf, Journal of Sciences Food Agricultural, 1977, 27, 721-725
8. Chialva, F., Gabri, G., Liddle, P.A.P., Ulian, F., Qualitative evaluation of aromatic herbs by direct headspace GC analysis, Journal of high resolution chromatography & chromatography communications, 1982, 5(4), 181-188
9. Lamparsky, D., Klimes, I., Heterocyclic trace components in essential oil of coriander, Perfumer & Flavorist. 1988, 13(5), 17-25
10. Kerrola, K., Kallio, H., Volatile components and odor characteristics of carbon dioxide extracts of coriander (*Coriandrum sativum* L.) fruits, Journal of Agricultural Food Chemistry, 1993, 41(5), 785-790
11. Anitescu, G., Doneanu, C., Radulescu, V., Isolation of *Coriander* oil: comparison between steam distillation and supercritical $CO_2$ extraction, Flavour fragrance journal, 1997, 12(3), 173-176
12. Jeliazkova, E.A., Craker, L.E., Zheljazkov, V.D., γ-Irradiation of seeds and productivity of coriander, *Coriandrum sativum* L., Journal of Herbs, Spices and Medicinal Plants, 1997, 5, 73-79
13. Baratta, M.T., Dorman, H.J., Deans, S.G., Biondi, D.M., Ruberto, G. Chemical composition, antimicrobial and antioxidative activity of laurel, sage, rosemary, oregano and coriander essential oils, Journal of Essential Oil Research, 1998, 10(6), 618-627
14. Illés, V., Daood, H.G., Perneczki, S., Szokonya, L., Then, M., Extraction of coriander seed oil by CO2 and propane at super- and subcritical conditions. Journal of Supercritical Fluids, 2000, 17(2), 177-186
15. Smallfield, B.M., van Klink, J.W., Perry, N.B., Dodds, K.G., Coriander spice oil: effects of fruit crushing and distillation time on yield and composition. Journal of Agricultural Food Chemistry, 2001, 49(1), 118-123
16. Mazza, G., Minor volatile constituents of essential oil and extracts of coriander (*Coriandrum sativum* L.) fruits, Scientes de Aliments, 2002, 22(5), 617-627
17. Figueiredo, R.O., Nakagawa, J., Marques, M.O.M., Composition of coriander essential oil from Brazil. Future for Medicinal and Aromatic Plants 2004, 629, 135-137
18. Raal, A., Arak, E., Orav, A., Koriandriviljade eeterliku õli keemiline koostis ja vastavus Euroopa farmakopöa nõuetele, Agraarteadus, 2004, 15(4): 234-239.
19. Arak, E., Orav, A., Raal, A., Composition of the essential oil of *Coriandrum sativum* L. seeds from various countries. European Journal of Pharmacology Sciences, 2007, 32S, S22
20. Msaada, K., Hosni, K., Ben Taarit, M., Chahed, T., Kchouk, M.E., Marzouk, B., Changes on essential oil composition of coriander (*Coriandrum sativum* L.) fruits, Food Chemistry, 2007, 102(4), 1131-1134
21. Grosso, C., Ferraro, V., Figueiredo, A.C., Barroso, J.G., Coelho, J.A., Palavra, A.M., Supercritical carbon dioxide extraction of volatile oil from Italian coriander seeds. Food Chemiistry, 2008, 111(1), 197-203
22. Matasyoh, J.C., Maiyo, Z.C., Ngure, R.M., Chepkorir, R. Chemical composition and antimicrobial activity of the essential oil of *Coriandrum sativum*. Food Chemistry, 2009, 113(2), 526-529
23. Msaada, M., Ben Taarit, M., Hosni, K., Hammami, M., Marzouk, B. Regional and maturational effects on essential oils yields and composition of coriander (*Coriandrum sativum* L.) fruits, Sciences of Horticulture – Amsterdam, 2009, 122(1), 116-124
24. Msaada, K., Hosni, K., Ben Taarit, M., Ouchikh, O., Marzouk, B., Variations in essential oil composition during maturation of coriander (*Coriandrum sativum* L.) fruits, Journal of Food Chemistry, 2009, 33(5), 603-612
25. Neffati, M., Marzouk, B. (2010). Salinity impact on growth, essential oil content and composition of coriander (*Coriandrum sativum* L.) stems and leaves. Journal of Essential Oil Research, 2010, 22(1), 29-34
26. Mata, A.T., Proenca, C. Ferreira, A.R. Serralheiro, M.L.M. Nogueira, M., Araujo, E.M., Antioxidant and antiacetylcholinesterase activities of five plants used as Portuguese food species, Food Chemistry, 2007, 103, 778-786
27. Gulluce, M. Sahin, F. Sokmen, M. Ozer, H. Daferera, D. Sokmen, A. Polissiou, M. Adiguzel, A. Ozkan, H., Antimicrobial and antioxidant properties of the essential oils and methanol extract from Mentha longifolia L. ssp. Longifolia, Food Chemistry, 2007, 103, 1449-1456
28. Iscan, G., Kirimer, C. Kurkcuoglu, M., Baser, H.C., Demirci, F., Antimicrobial screening of Mentha piperita essential oils, Journal of Agricultural Food Chemistry, 2002, 50, 3943-3946
29. Pandey, A.K., Rai M.K., Acharya, A., Chemical composition and antimycotic activity of the essential oils of corn mint (Mentha arvensis) and lemon grass (Cymbopogon flexuosus) against human pathogenic Fungi, Pharmacological Biology, 2003, 41, 421-425
30. Lawrence, B.M., The composition of commercially important mints. In: Mint The Genus Mentha. Edits., B.M. Lawrence, pp. 280. CRC Press, Taylor and Francis Group, NY, 2007
31. Asekun, O.T., Grierson, D.S., Afolayan, A.J., Effect of drying methods on the quality and quantity of the essential oil of Mentha longifolia L. subsp. capensis. Food Chemistry, 2007, 101, 995-998
32. Hasapidou, A., Savvaidis, I.N. The effects of modified atmosphere packaging, EDTA and oregano oil

on the quality of chicken liver meat, Food Research International, 2011, 44, 2751-2756
33. Athina, G., Ntzimani, G., Giatrakou, V.I., Savvaidis, N.I., Combined natural antimicrobial treatments (EDTA, lysozyme, rosemary and oregano oil) on semi cooked coated chicken meat stored in vacuum packages at 4 °C: Microbiological and sensory evaluation. Innovative Food Science and Emerging Technologies, 2010, 11, 187-196
34. Pavelková, A., Kačániová, M., Horská, E., Rovná, K., Hleba, L., Petrová, J. The effect of vacuum packaging, EDTA, oregano and thyme oils on the microbiological quality of chicken's breast, Anaerobe, 2014, 29, 128-133.