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MICROBIOLOGICAL QUALITY OF CHICKEN BREAST MEAT AFTER APPLICATION OF THYME AND CARAWAY ESSENTIAL OILS

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ABSTRACT

The aim of the present study was to evaluate the effect of selected types of antimicrobial essential oils to the various groups of microorganisms during storage of chicken meat. The samples of chicken breast meat were used in the experiment. The number of lactobacilli, *Pseudomonas* spp., anaerobic plate count and *Enterobacteriaceae* after application of caraway and thyme essential oils (EO) at concentration 1% v/w in a combination with the ethylenediaminetetraacetate (EDTA) solution 1.5% w/w and vacuum packaging were evaluated. The samples were analyzed at 0, 4th, 8th, 12th and 16th day of storage of chicken meat at temperature 4 °C. Another aim was to determine the species of isolated microorganisms from samples of chicken meat by MALDI-TOF MS Biotyper (matrix assisted laser desorption ionization-time of flight mass spectrometry). The number of *Lactobalillus* spp. ranged from 1.35 log CFU.g^{-1} in all groups to 3.04 log CFU.g^{-1} on 0th day to 3.04 log CFU.g^{-1} on 4th day in control group stored in air. The *Pseudomonas* spp. was not found in all tested samples at the start of the experiment, the highest number of *Pseudomonas* spp. was in the control group on 16th day (2.68 log CFU.g^{-1}). Presence of *Pseudomonas* spp. were not found during storage in groups after treatment with caraway and thyme EO. The values of anaerobic plate count ranged from 2.81 log CFU.g^{-1} on 4th day in control group with vacuum packaging to 5.19 log CFU.g^{-1} on 16th day in control group in air condition. The *Enterobacteriaceae* was not found in all tested samples on 0th day and ranged to 4.46 log CFU.g^{-1} on 12th day in control group in air condition. From Lactobacillus spp., the most often identified species was *Lactobacillus paracasei*, from genus *Pseudomonas*, there were identified *Pseudomonas fluorescens* in two cases. From anaerobic plate count, there were isolated *Staphylococcus warneri* from control goup stored in air condition, *Kocuria rhizophila* from control group with vacuum packaging, *Staphylococcus warneri*, *Aeromonas salmonicida* and *Aeromonas popoffii* from control group treated with EDTA, *Staphylococcus hominis* and *Staphylococcus epidermidis* from group treated with caraway essential oil. From *Enterobacteriaceae*, the most bacteria were isolated from control group in air condition and from control group treated with EDTA.

Keywords: chicken meat; thyme; caraway; essential oils; microorganisms

INTRODUCTION

Meat hygiene is determined by different environmental factors, which could result in meat spoilage and food safety problems. The growth of bacteria is the main cause of the reduction of freshness for chilled meat **(Ercolini et al., 2009)**.

Consumers in the present time are demanding for minimally processed food products without chemical preservatives. Thus, the food industry has focused on the development of active packaging where active compound/s directly or indirectly interact with the packaged food products by avoiding the production of undesired compounds and restricting the growth of pathogens **(Jideani and Vogt, 2016)**.

Meat consumption is an important for human development and health maintenance, which is why safety of meat and meat products is of growing concern in modern society **(Cardoso Pereira and Vicente, 2013)**. A major issue related with meat consumption is the presence of pathogens, which can cause food-borne diseases **(Sofos, 2008)**. Raw meat is an ideal growth medium for many pathogens and spoilage bacteria. *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes, Escherichia coli, Staphylococcus aureus* are most common pathogens which cause a million episodes of illness every year **(Boskovic et al., 2013)**.

Poultry meat is a very popular food commodity due to its low production costs, low content of fat, high nutritional value and distinct flavor **(Patsias et al., 2008)**.

Poultry meat, namely parts containing skin, have a higher initial contamination rate than e. g. beef or pork and it thus is a fast perishable product, which deteriorates after 4 – 10 days post slaughter, even under cold conditions **(Meredith et al., 2014)**.

In recent years, attention has been focused on herbs and spices extracts, which have been used for centuries to improve the sensory characteristics and shelf-life of foods **(Fernandez-Gines et al., 2005)**.

The antimicrobial activities of essential oils are correlated to the presence of their bioactive volatile components **(Mahmoud and Croteau, 2002)**. Chemically the essential oils contain terpene compounds (mono-, sesqui- and diterpenes), alcohols, acids, esters, epoxides, aldehydes, ketones, amines and sulfides. The components of essential oils are divided into two groups: terpene compounds and aroma compounds **(Bakkali et al., 2008)**.

The composition, structure as well as functional groups of the essential oils are very important in determining their antimicrobial activity. Compounds with phenolic groups are most effective against microbial population **(Dorman and Deans, 2000)**.

Carum carvi, which is also known as caraway, is one of the oldest spices cultivated in Europe. The dried ripe fruits (schizocarp) of *C. carvi* L. family *Apiaceae (Umbelliferae*) are extensively being used in folk medicine as a carminative, found to be effective against spasmodic gastrointestinal complaints, irritable stomach, indigestion, lack of appetite and dyspepsia in adults, and in relieving flatulent colic of infants **(Thippeswamy et al., 2013)**. Caraway has been used traditionally as a spice due to its pleasant flavor. Caraway seeds and crude extracts are used as a flavoring of bread (e.g., rye bread), cheese, sauerkraut, candies, meat products, pickled fruits, sauces, and chewing gums. Ground caraway seeds are used as a component of teas and herbal mixtures **(Johri, 2011)**. Caraway essential oils are usually volatile, odorous, and may contain up to 100 individual components that are composed mostly from monoterpenes, sequiterpenes, phenylpropanoids, and isothiocyanates. The oil possesses bactericidal, virucidal, fungicidal, analgesic, sedative, antiinflammatory, spasmolytic, and anesthetic activities **(Raal et al., 2003)**.

Thyme (*Thymus vulgaris* L.), belonging to the *Lamiaceae* family, is a well-known spice plant which possesses very good medicinal properties. The major components of *Thymus vulgaris* oil and extract are thymol, p-cymene, carvacrol, and γ-terpipene. They show very strong antibacterial, antifungal and antioxidant activities. Thyme essential oils retard food spoilage and increase the shelf-life of foods **(Mandal and DebMandal, 2016)**.

The aim of the present study was to monitor the microbiological quality of chicken breast after treatment by selected essential oils (caraway, thyme) soluted in ethylenediaminetetraacetate (EDTA) in combination with vacuum packaging stored at 4 °C.

MATERIAL AND METHODOLOGY

Preparation of samples

The aim of the present study was to monitor the effect of caraway (*Carum carvi*) and thyme (*Thymus vulgaris* L.) essential oils to micobiological quality of chicken meat during 16 days of storage. Samples of chicken breast muscles were used to experiment.

Microbiological analyzes were performed on 0^{th}, 4^{th}, 8^{th}, 12^{th} and 16^{th} day of storage at 4 ±0.5 °C.

The chicken breast samples were prepared as follow:

-control group (CG) – chicken breast samples were packaged to polyethylene bags and stored aerobically at 4 ±0.5°C;

-control group vacuum-packaged (C vacuum) – chicken breast samples were packaged to polyethylene bags and stored anaerobically at 4 ±0.5°C;

-control group vacuum-packaged with EDTA (C EDTA) – chicken breast samples were treated with EDTA solution, 1.5% w/w for 1 minute and samples were packaged to polyethylene bags and stored anaerobically at 4 ±0.5°C;

-vacuum-packaged samples with *Carum carvi* 1% v/w – chicken breast samples were treated with caraway essential oil (*Carum carvi)* (Hanus, Nitra, Slovakia) for 1 minute and samples were packaged to polyethylene bags and stored anaerobically at 4 ±0.5°C;

-vacuum-packaged samples with *Thymus vulgaris* L. 1% v/w – chicken breast samples were treated with thyme essential oil (*Thymus vulgaris* L.) (Hanus, nitra, Slovakia) for 1 minute and samples were packaged to polyethylene bags and stored anaerobically at 4 ±0.5°C;

Vacuum packing machine VB-6 (RM Gastro, Česká republika) was used to vacuum packaging of samples. Each sample was packed immediately after treatment with the EDTA solution (pH 8.0, 99.5% purity, Invitrogen, USA). The final concentration of EDTA solution used for the treatment of meat samples was 50 mM.

Microbiological analysis

The following groups of microorganisms were determined in samples of chicken breast:

-*Lactobacillus* spp.

-*Pseudomonas* spp.

-Anaerobic plate count

-*Enterobacteriaceae*

Plate dilution method was used for the quantitative determination of the number of colony forming units (CFU) of each group of microorganisms. An amount of 5 g of the chicken breast was took using sterile scalpels and transferred into a sterile stomacher bag containing 45 mL of 0.1% physiological solution (pH 7.0) and homogenized for 60 seconds. Microbiological analyses were conducted with accordance to standard microbiological methods. De Man, Rogosa, Sharpe agar (MRS, Oxoid, UK) was used for isolation of *Lactobacillus* spp. Inoculated agar was incubated in a thermostat (CO_2 incubator ATP.Line CB, Binder GmbH, Tuttlingen, Germany) with 5% CO^2 in atmosphere at 37 °C for 48 – 72 hours. Pseudomonas Isolation Agar (PIA, Oxoid, UK) was used for isolation of *Pseudomonas* spp. Inoculated agar was incubated at 35 °C ±1 °C during 48 hours. Plate Count Agar (PCA, Oxoid, UK) was used to isolation of Anaerobic plate count. PCA agar was after inoculation incubated at 35 °C for 48 hours in anaerobic conditions. Violet Red Bile Glucose agar (VRBL, Oxoid, UK) was used to isolation of *Enterobacteriaceae*. Inoculated agar was incubated at 37 °C for 24 hours.

Identification of microorganisms by MALDI-TOF MS

MALDI-TOF MS (Bruker Daltonics, Germany) was used to identification of microorganisms from meat samples.

The matrix (HCCA) preparation:

The matrix solution used was a saturated solution of α-cyano-4-hydroxycinnamic acid (HCCA) (Bruker Daltonics, Germany) dissolved in 50% acetonitrile, 47.5% ultra-pure water, 2.5% trifluoroacetic acid. The solution was used as the organic solvent. Then 1 mL of organic solvent was prepared by addition of 500 µL of 100% acetonitrile, 475 µL of ultra-pure water and 25 µL of 100% tri-fluoro acetic acid, the mixture was mixed thoroughly. There were added 250 µL of organic solvent to HCCA and vortexed.

Isolated colonies were taken and suspended in 300 mL of distilled water and mixed thoroughly. There were added 900 µL of ethanol (99.8%). The mixture was centrifuged at 13 000 × g for 2 minutes. The supernatant was discarded, then the pellet was centrifuged again. Residual ethanol was removed and the pellet was allowed to dry at room temperature. There was added 50 µL of 70% formic acid and mixed thoroughly. Then, 50 µL of acetonitrile was added and solution was centrifuded at maximum speed for 2 minutes. Then 1 µL of the supernatant was spotted on a polished MALDI target plate (Bruker Daltonics, Germany). After drying 1 µL of the matrix solution was added to each spot and allowed to air dry and samples were processed in the MALDI-TOF MS.

RESULTS AND DISCUSSION

Naturally occurring antimicrobial compounds can be used as food preservatives. Essential oils and other extracts from herbs and spices, have shown antimicrobial activity against many pathogens and spoilage microorganisms **(Bakkali et al., 2008)**.

The number of *Lactobacillus* spp. in chicken breast ranged from 1.35 log CFU.g^{-1} on 0th day to 3.04 log CFU.g^{-1} on 4th day of storage in control group stored in air. The number of *Lactobacillus* spp. ranged from od 1.35 log CFU.g^{-1} on 0th day to 2.69 log CFU.g^{-1} on 12th day of storage in samples of control group treated with EDTA. The number of *Lactobacillus* spp. ranged from 1.35 log CFU.g^{-1} on 0th day to 3.00 log CFU.g^{-1} on 8th day of storage in the control group with vacuum packaging. In the group after treatment with caraway essential oil, the number of *Lactobacillus* spp. ranged from 1.35 log CFU.g^{-1} on 0th day to 2.63 log CFU.g^{-1} on 16th day and in the group after treatment with thyme essential oil, the number of *Lactobacillus* spp. ranged from 1.35 log CFU.g^{-1} on 0th day to 2.86 log CFU.g^{-1} on 8th day (figure 1).

Zhang et al. (2016) studied the antimicrobial effect of rosemary (RO) and clove (CL) essential oils in chicken meat during storage against total viable counts (TVC), lactic acid bacteria (LAB) counts, *Enterobacteriaceae* counts and *Pseudomonas* spp. The antimicrobial and effects of two spice extracts and their combination on raw chicken meat during storage for 15 days at 4 °C were studied. Initial lactic acid bacteria (LAB) counts (p <0.05) were found to be 4.26 log CFU.g^{-1} for all meat samples. The values increased in C and PC samples and reached 6.20 and 5.96 log CFU.g^{-1} at the end of the storage period. LAB counts were found to be lower (p <0.05) in spice-treated samples relative to those measured for the control samples. During storage, chicken fillet samples treated with RO-CL showed a lower LAB count compared with the counts measured for the control and CL- and RO-treated samples. On day 15 of storage, LAB counts reached 5.47, 5.43 and 5.08 log CFU.g^{-1} for the RO, CL, and RO-CL samples, respectively.

LAB are the most resistant bacteria among gram-positive bacteria against the antimicrobial action of EOs **(Kostaki et al., 2009)**. **Holley and Patel (2005)** reported that the high tolerance of LAB toward the action of essential oils is attributed to their ability to generate ATP and to tolerate conditions of osmotic stress.

In the control group stored in air condition, the number of *Pseudomonas* spp. ranged from 0 log KTJ.g^{-1} 0th day to 2.68 log CFU.g^{-1} on 16th day. In the control group stored in vacuum packaging, the number of *Pseudomonas* spp. ranged 0 log CFU.g^{-1} on 0th and 4th day to 2.13 log CFU.g^{-1} on 12th day. In the control group after treatment with EDTA, the number of *Pseudomonas* spp. ranged from 0 log CFU.g^{-1} on 0th and 4th day to 2.13 log CFU.g^{-1} on 12th day of storage. Presence of *Pseudomonas* spp. were not

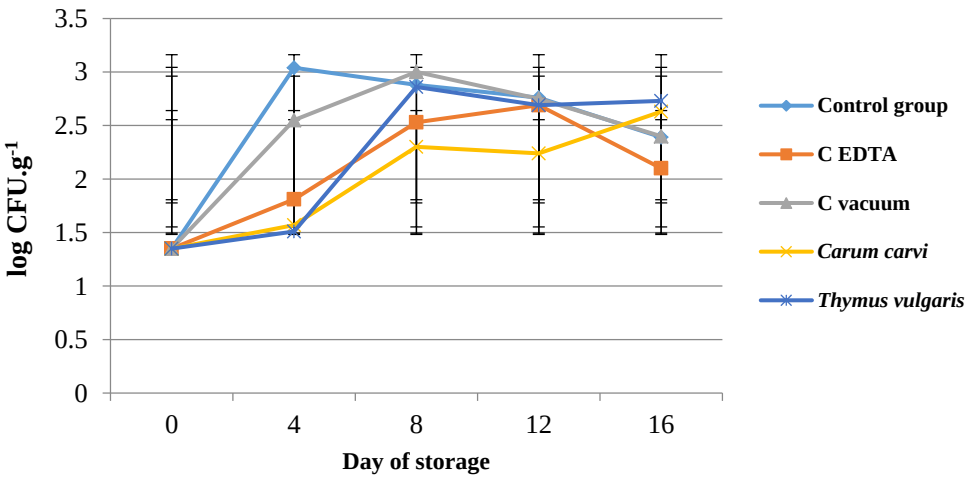

Figure 1 Number of *Lactobacillus* spp. in the chicken breast during storage.

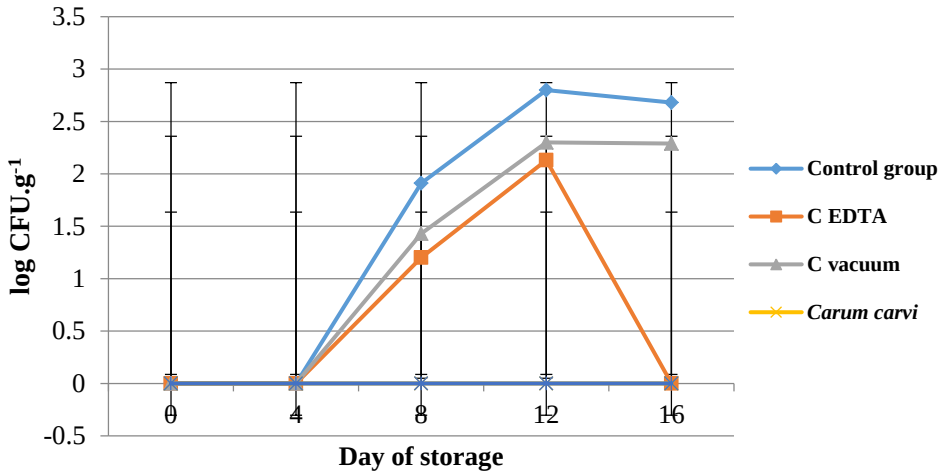

Figure 2 Number of *Pseudomonas* spp. in the chicken breast during storage.

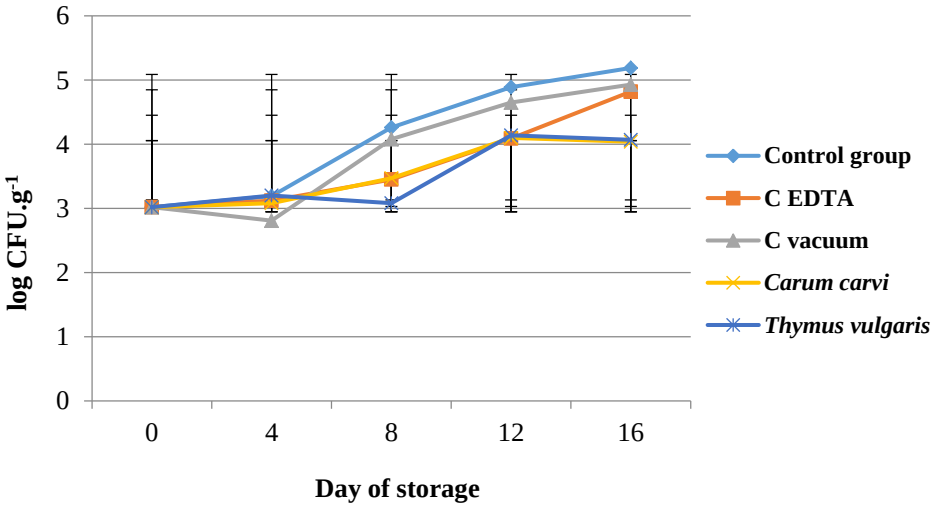

Figure 3 Anaerobic plate count values in the chicken breast during storage.

found during storage in groups after treatment with caraway and thyme essential oils (Figure 2).

Zhang et al. (2016) investiagated the antimicrobial activity of rosemary, clove and clove-rosemary essential oils in their study. Rosemary essential oil showed a low inhibition effect. But the clove and clove-rosemary essential oils showed significant inhibition effects on the *Pseudomonas*. The combination of essential oils was the most effective in reducing the population of pseudomonas from 5.6 (control samples) to 4.76 log CFU.g^{-1} after 15 days of storage (p <0.05).

Lv et al. (2011) evaluated the antimicrobial activity of selected plant essential oil combinations against four food-related microorganisms. Ten essential oils were tested against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Saccharomyces cerevisiae* using agar disk diffusion and broth dilution methods. The highest activity against tested bacteria was shown when testing the oregano essential oil. Basil and bergamot essential oils were active against the Gram-positive bacteria (*S. aureus* and *B. subtilis*), while perilla essential oil strongly inhibited the growth of yeast (*S. cerevisiae*).

The values of anaerobic plate count ranged from 3.02 log CFU.g^{-1} on 0th day to 5.19 log CFU.g^{-1} on 16th day in the control group stored in air condition. The values anaerobic plate count ranged from 3.02 log CFU.g^{-1} on 0th day to 4.82 log CFU.g^{-1} on 16th day in the control group in vacuum packaging and treated with EDTA. The values of anaerobic plate count ranged from 2.81 log CFU.g^{-1} on 4th day to 4.93 log CFU.g^{-1} on 16th day in the control group in vacuum packaging. The values of anaerobic plate count ranged from 3.02 log CFU.g^{-1} on 0th day to 4.04 log CFU.g^{-1} on 16th day in group after treatment with caraway essential oil. The values of anaerobic plate count ranged from od 3.02 log CFU.g^{-1} on 0th day to 4.14 log CFU.g^{-1} on 12th day in group treated with thyme essential oil (Figure 3).

Ghabraie et al. (2016) studied the antibacterial activity of 32 essential oils against four pathogenic bacteria (*Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella Typhimurium*) and one spoilage bacterium (*Pseudomonas aeruginosa*). In solid phase, red thyme, red bergamot, ajowan, summer savory, chinese cinnamon and cinnamon bark had higher inhibitory zone (20 – 40 mm) against five tested bacteria compared with

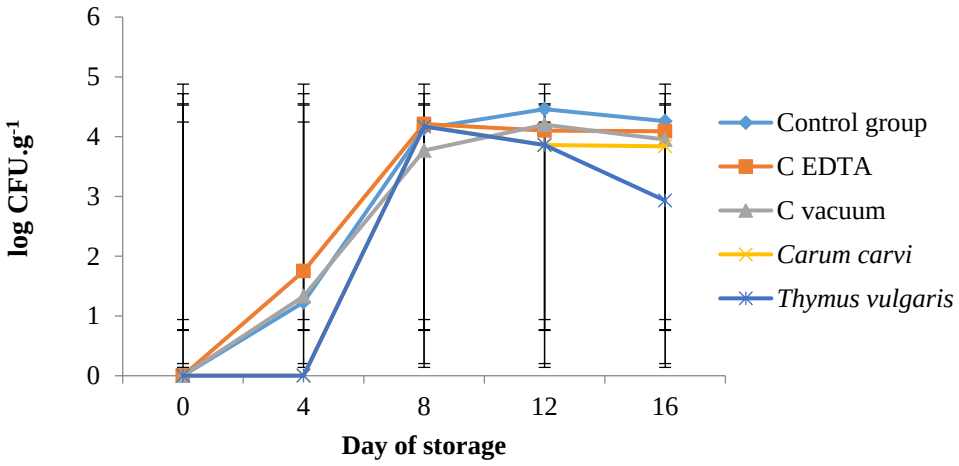

Figure 4 Number of *Enterobacteriaceae* in chicken breast during storage.

other essential oils. Red thyme, red bergamot, ajowan, summer savory inhibited *S. aureus* by more than 60 mm. In vapour phase, at 30 µL of tested essential oils, chinese cinnamon and red bergamot were the only essential oils that inhibited all bacteria with the inhibition zone from 20 to more than 60 mm depending on target bacteria. In liquid phase, chinese cinnamon showed the best antibacterial activity among all essential oils and it inhibited *S. aureus* and *E. coli* at low minimum inhibitory concentration of 470 ppm.

In the control group stored in air condition, the number of *Enterobacteriaceae* genera ranged from 0 log CFU.g^{-1} on 0th day to 4.46 log CFU.g^{-1} on 12th day. In the control group treated with EDTA, the number of *Enterobacteriaceae* genera ranged from 0 log CFU.g^{-1} on 0th day to 4.21 log CFU.g^{-1} on 8th day. In the control group in vacuum packaging, the number of *Enterobacteriaceae* genera ranged from 0 log CFU.g^{-1} on 0th day to 4.20 log CFU.g^{-1} on 12th day. In the group treated with caraway essential oil, the number of *Enterobacteriaceae* genera ranged from 0 log CFU.g^{-1} on 0th day to 4.18 log CFU.g^{-1} on 8th day. In the goup treated with thyme essential oil, the number of *Enterobacteriaceae* genera ranged from 0 log CFU.g^{-1} on 0th day to 4.17 log CFU.g^{-1} on 8th day (figure 4).

Boskovic et al. (2015) investigated the antibacterial activity of thyme and oregano essential oil against food-borne bacteria. They reported antibacterial activity of these essential oils against *Salmonella* Enteritidis, *Salmonella* Thyphimurium, *Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus.*

Essential oils possess the strongest antibacterial properties when contain a high percentage of phenolic compounds. Oregano essential oil contains a high concentration of phenols, which showed a higher antimicrobial activity compared to thyme essential oil **(Burt, 2004)**.

Antimicrobial mechanism of the two major constituents carvacrol and thymol, is based on their ability to disintegrate the outer membrane of Gram-negative bacteria, releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to ATP **(Lambert et al., 2001)**.

Oregano and thyme essential oils showed great inhibitory activity against observed microorganisms, but higher concentration of these were needed to achieve bactericidal effects. Although oregano and thyme essential oils exhibited strong antibacterial activity, further researches are needed in order to determinate their antibacterial effects on pathogens in meat model media **(Ultee et al., 2002)**.

Kluz et al. (2016) reported, that caraway and anise essential oils exhibited good antimicrobial properties against anaerobic bacteria, lactic acid bacteria and *Enterobacteriacea* at 0.2% concentration.

Mass spectrometry has been used for bacterial identification since 1975 **(Anhalt and Fenselau, 1975)**, but a significant progress was marked by the introduction of MALDI-TOF MS **(Hillenkamp et al., 1991)**. In the present time, MALDI-TOF MS represents the most frequently used MS technique for a rapid and specific identification of bacteria. The MALDI-TOF technique is a soft ionization method allowing desorption of peptides and proteins from whole cells of cultured microorganisms. Due to the fact that bacterial cells have a high content of proteins and these proteins directly represent genetic information of the organism, the profiles of proteins are useful for identification of bacteria. Over the last years, MALDI-TOF MS technology (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) has proved to be a rapid method for the accurate identification of different microorganisms **(Patel, 2015)**.

From genus *Lactobacillus*, there were identified *Lactobacillus paracasei in three cases, Lactobacillus reuteri and Lactobacillus salivarius* in two cases. From genus *Pseudomonas*, there were identified *Pseudomonas fluorescens* in two cases, *Pseudomonas azotoformans, Pseudomonas taetrolens, Pseudomonas synxantha, Pseudomonas orientalis, Pseudomonas fragi* and *Pseudomonas veronii* in one case. Species of *Pseudomonas* spp. were isolated from control goup stored in air condition, control group treated with EDTA and control group in vacuum packaging (table 1).

Table 1 Isolated microorganisms of *Lactobacillus* spp. And *Pseudomonas* spp. in samples of chicken breast.

Samples	***Lactobacillus* spp.**	***Pseudomonas* spp.**
CG	*Lactobacillus reuteri, Lactobacillus paracasei*	*Pseudomonas fluorescens, Pseudomonas azotoformans,*
CG-V	*Lactobacillus reuteri Lactobacillus salivarius*	*Pseudomonas taetrolens, Pseudomonas fluorescens, Pseudomonas synxantha, Pseudomonas orientalis,*
CG-KEDTA	*Lactobacillus salivarius*	*Pseudomonas fragi, Pseudomonas veronii,*
Carum carvi	*Lactobacillus paracasei*	
***Thymus vulgaris* L.**	*Lactobacillus paracasei*	

Note: CG – control group stored in air condition, CG-V - control group with vacuum packaging, CG-KEDTA - control group treated with EDTA, *Carum carvi* - group treated with caraway essential oil, *Thymus vulgaris* L. - group treated with thyme essential oil.

Table 2 Isolated microorganisms of anaerobic plate count and *Enterobacteriaceae* in samples of chicken breast.

Samples	Anaerobic plate count	***Enterobacteriaceae***
CG	*Staphylococcus pasteuri*	*Serratia fonticola, Yersinia pseudotuberculosis, Buttiauxella noackiae, Buttiauxella agrestis,*
CG-V	*Kocuria rhizophila*	*Hafnia alvei, Citrobacter freundii, Escherichia coli*
CG-EDTA	*Staphylococcus warneri, Aeromonas salmonicida, Aeromonas popoffii,*	*Serratia fonticola, Rahnella aquatilis, Serratia liquefaciens, Buttiauxella noackiae*
Carum carvi	*Staphylococcus hominis, Staphylococcus epidermidis,*	*Serratia liquefaciens,*
***Thymus vulgaris* L.**		*Citrobacter freundii, Buttiauxella noackiae, Buttiauxella gaviniae*

Note: CG – control group stored in air condition, CG-V - control group with vacuum packaging, CG-KEDTA - control group treated with EDTA, *Carum carvi* - group treated with caraway essential oil, *Thymus vulgaris* L. - group treated with thyme essential oil.

Jääskeläinen et al. (2016) investigated the influence of packaging (under vacuum and in high oxygen atmosphere) on the development of microbial communities and metabolic activities at 6 °C. At the beginning of storage, the microbial community mostly consisted of *Carnobacterium* and *Lactobacillus*. After two weeks of storage, *Lactococcus* and *Lactobacillus* were the dominant genera under vacuum and *Leuconostoc* in high oxygen meat packages. This indicates that oxygen favoured the genus *Leuconostoc* comprising only heterofermentative species and hence potential producers of undesirable compounds. *Leuconostoc gelidum*, *Lactococcus piscium*, *Lactobacillus sakei* and *Lactobacillus algidus* were the most common species of bacteria.

Pseudomonas spp., especially *P. fragi*, play a significant role in the spoilage of meat **(Lebert et al., 1998)**. In several previous studies, *P. fragi* has been detected in almost all samples during the storage of beef in modified-atmosphere packaging (MAP) and vacuum packaging **(Pennacchia et al., 2011)**.

In the study of **Ercolini et al. (2011)**, the changes in microbial loads, microbial diversity, and metabolite release in meat during storage in air, modified-atmosphere packaging (MAP), vacuum packaging, and active vacuum packaging were evaluated. Their Results showed that Brochothrix thermosphacta dominated during the early stages of storage in air and MAP, while Pseudomonas spp. took over during further storage in air. Many different bacteria, several of which are usually associated with soil rather than meat, were identified in vacuum packaging and active vacuum packaging; however, lactic acid bacteria (LAB) dominated during the late phases of storage, and *Carnobacterium divergens* was the most frequent microorganism in active vacuum packaging.

From anaerobic plate count, there were isolated *Staphylococcus warneri* from control goup stored in air condition, *Kocuria rhizophila* from control group with vacuum packaging, *Staphylococcus warneri, Aeromonas salmonicida* and *Aeromonas popoffii* from control group treated with EDTA, *Staphylococcus hominis* and *Staphylococcus epidermidis* from group treated with caraway essential oil.

From *Enterobacteriaceae*, there were isolated *Buttiauxella noackiae* from control group stored in air condition, control group treated with EDTA and group treated with thyme essential oil. *Serratia fonticola* was isolated in two cases, from control group stored in air condition and from control group treated with EDTA. *Serratia liquefaciens* was isolated from control group treated with EDTA and group treated with caraway essential oil. *Yersinia pseudotuberculosis* and *Buttiauxella agrestis* were isolated from control group stored in air condition, *Hafnia alvei* and *Escherichia coli* were isolated from control group with vacuum packaging, *Rahnella aquatilis* was isolated from control group treated with EDTA, *Citrobacter freundii* and *Buttiauxella gaviniae* were isolated from group treated with thyme essential oil (Table 2).

Several authors detected many members of the *Enterobacteriaceae* on raw beef, lamb, pork, and poultry products. The genera *Serratia*, *Enterobacter*, *Pantoea*, *Klebsiella*, *Proteus* and *Hafnia*, often contribute to meat spoilage. Considering to their meat spoilage potential, the most important Enterobacteriaceae are the species *Serrata liquefaciens*, *Hafnia alvei* and *Enterobacter (Pantoea) agglomerans* **(Samelis, 2006)**. Among the *Enterobacteriaceae*, *Serratia* spp. is the most often found genus in meat. *Serratia grimesii* and *Serratia proteamaculans* occur in meat stored in air, MAP and in meat stored in vacuum packaging; although *S. grimesii* is often found at later stages of storage **(Pennacchia et al., 2011)**.

Doulgeraki et al. (2011) reported, that *Citrobacter freundii* was isolated from minced beef stored aerobically, while *Hafnia alvei* and *Proteus vulgaris* were isolated from meat storage under MAP. Storage conditions affected the *Enterobacteriaceae* community; modified atmosphere packaging increased both species and strain diversity.

CONCLUSION

The results of the present study show, that caraway and thyme essential oils can be use as natural food preservatives and they are also good source of antimicrobial ingredients for meat. Shelf-life of packaged fresh meat is very short in view of the fact, that its composition is the ideal environment for the growth and reproduction of spoilage and pathogenic microorganisms. High protein content and water activity promotes microbial spoilage of meat. The basic prerequisite for maintaining the quality and safety of meat is the prevention of microbial contamination.

Nowadays, the synthetic preservatives are often used to extend the shelf-life of meat. However consumers are interested in the natural substances limiting the growth of microorganisms in food. Essential oils and extracts can be used in meat and meat products as natural conservants.

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