

THE ANTIMICROBIAL EFFECT OF THYME AND ROSEMARY ESSENTIAL OILS AGAINST *LISTERIA MONOCYTOGENES* IN SOUS VIDE TURKEY MEAT DURING STORAGE

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**ABSTRACT**

The research was aimed to study the impact of sous vide thermal treatment on the microbiological quality of fresh turkey breast meat after treatment with thyme and rosemary EOs and the survival of *Listeria monocytogenes* on the turkey meat samples. The samples were vacuum-packed and cooked at 55 °C, 60 °C, and 65 °C for 5, 15, 30 and, 60 min. There was an amount of 5 g (5 ±0.2 g) of the sample placed in PA/PE film bags and inoculated with 100 µL of *L. monocytogenes* inoculum. The sample was incubated at 37 °C for 18 h after bag sealing. The samples were tested on the 1<sup>st</sup> and 3<sup>rd</sup> days of experiments. The microbiological quality of fresh turkey breast meat was assessed by the detection of total microbial counts and meat microbiota was identified by mass spectrometry using MALDI-TOF MS Biotyper (Bruker Daltonics, Germany). Microbial counts differed significantly depending on temperature and time and the microbial counts ranged from 2.21 log cfu.g<sup>-1</sup> to 8.26 log cfu.g<sup>-1</sup> on the 1<sup>st</sup> and 3<sup>rd</sup> day of the experiment. The study shows that the sous vide method with essential oils combination is an effective method and it can be used to protect the microbiota of turkey meat and *L. monocytogenes* survival, however, the quality of raw material is crucial.

**Keywords:** bacteria; MALDI-TOF MS Biotyper; turkey breast; essential oils; sous vide

**INTRODUCTION**

Sous vide is a professional widely used food cooking and preservation technology and it is applied in catering, food industry, and home-made food production. The sous vide technology may also be referred to as lapping, vacuum cooking, vacuum-packed cooking, or baking-cooling in vacuum (Nyati, 2000; Todd, 2014; Yikmi et al., 2018). Sous vide helps to improve food characteristics that meet customers' demands for "fresh-like" processed foods of good quality (García-Linares et al., 2004; Stringer et al., 2012). *Listeria monocytogenes* is a foodborne pathogen that causes listeriosis. Listeriosis may be characterized by serious disorders as sepsis, meningitis, meningoencephalitis in immunocompromised patients, which may result in lifelong harm and/or death. Listeriosis cases were connected with the consumption of raw milk and dairy products, meat and poultry, fish, and Ready-to-eat (RTE) products (Liu et al., 2012). Cooked chicken meat can be contaminated with *L. monocytogenes* during processing or post-processing activities (Goh et al., 2014).

The poultry meat as was shown can be contaminated with pathogens so control of pathogens is a great challenge for poultry-processing companies to avoid economic losses and minimize public health risks (Ferreira Moura et al., 2016).

Chemical food preservatives were recognized as an effective method to control spoilage and pathogenic bacteria. Nowadays, consumers demand healthy foodstuff without the addition of chemical preservatives and replace them with natural compounds. Essential oils (EOs) attracted the interest of the food industry to satisfy consumer needs. Essential oils (EOs) are aromatic oily liquids produced from different parts of plants such as leaves, seeds, flowers, or roots (Burt, 2004). Natural extracts were reported to inhibit the growth and survival of *L. monocytogenes* in meat (Mytle et al., 2006; Djenane et al., 2011).

*Thymus vulgaris* L. (thyme) is an aromatic plant of the Lamiaceae family (Solomakos et al., 2008) and its EO showed antibacterial activity (Solomakos et al., 2008). Thymol is the main antibacterial compound of *T. vulgaris* EO and comprises over 50% of its chemical composition (Rota et al., 2008; Govaris et al., 2011; Pesavento et al., 2015).

The eucalyptol is the main compound of *Rosmarinus officinalis* L. (Rosemary) is recognized for its antioxidative and antimicrobial activities (Ojeda-Sana et al., 2013) with activity against bacterial membrane (Van Vuuren and Van Vijoed, 2007).

The study aimed to examine the effect of sous vide thermal treatment on the microbiological quality of fresh turkey

breast meat with thyme and rosemary EO and the survival of *Listeria monocytogenes*.

### Scientific hypothesis

The use of the sous vide method, temperature with time combination and essential oils addition allows to reduce microbiological contamination, reduce the number of bacteria, and survival of *L. monocytogenes* in food to a safe level.

## MATERIAL AND METHODOLOGY

### Sample

Fresh turkey breast meat purchased in a commercial chain was used for the study.

### Chemicals

Buffered peptone water (BPW, pH 7.0, Oxoid code CM0509, Basingstoke, UK), Tryptone Soya Agar (TSA, Oxoid, UK), Oxford Agar with the supplement of oxford supplement (OA, Oxoid, UK).

### Animal and Biological material

*L. monocytogenes* CCM 4699 was got from the Czech Collection of microorganisms (Brno, Czech Republic).

### Instruments

MALDI-TOF MS Biotyper (Bruker, Daltonics, Bremen, Germany).

### Laboratory method

#### Microbiological analyses

There was 5 g of the turkey breast transferred into a sterile stomacher bag containing 45 mL of 0.1% buffered peptone water (BPW, pH 7.0, Oxoid code CM0509, Basingstoke, UK) and it was homogenized for 60 s at room temperature.

Appropriate serial decimal dilutions were prepared in 0.1% BPW solution for each sample. The amount of 0.1 mL of serial dilutions was spread on the surface of Tryptone Soya Agar (TSA, Oxoid, UK) for detection of total viable counts (TVC). They were counted on after incubation for 2 days at 30 °C. There was an Oxford Agar with the supplement of oxford supplement inoculated with 0.1 mL of sample. The incubation was carried out at 37 °C for 24 h.

#### Identification of the bacteria

The colonies were resuspended in 300 µL of sterile distilled water after incubation, and there was 900 µL of absolute ethanol added. The mixture was centrifuged at 10,000 x g for 2 min. The pellet was centrifuged again after discarding the supernatant. The precipitate was allowed to dry at room temperature.

Then 30 µL formic acid (70%) and 30 µL of acetonitrile were added and mixed thoroughly with the pellet. The solution was centrifuged at maximum speed for 2 min and 1.5 µL of the supernatant was spotted on a polished MALDI target plate (Bruker Daltonics, Bremen, Germany). There was 1.5 µL of the matrix solution added to each spot and allowed to dry immediately after drying.

The samples were processed on a MALDI-TOF MS spectrometer with Flex Control software (Bruker Daltonics). Each spectrum was obtained by averaging 40 laser shots obtained in automatic mode with the minimum laser power necessary to ionize the samples. The spectra were analyzed and compared to the database according to real-time software, v3.1 classification.

### Description of the Experiments

#### Sample preparation

The samples of fresh turkey breast meat were prepared as follow (Table 1, 2):

MC: turkey was vacuum packed in polyethylene bags and stored anaerobically at 4 °C, treated at 55 – 60 °C for 5 – 30 min;

MT: turkey with 0.1% thyme EO was vacuum packed in polyethylene bags and stored anaerobically at 4 °C, treated at 55 – 60 °C for 5 – 30 min;

MR: turkey with 0.1 % rosemary EO was vacuum packed in polyethylene bags and stored anaerobically at 4 °C, treated at 55 – 60 °C for 5 – 30 min;

MB: turkey with *L. monocytogenes* was vacuum packaged in polyethylene bags and stored anaerobically at 4 °C, treated with 55 – 60 °C for 5 – 30 min;

MBT: turkey with *L. monocytogenes* and 0.1% thyme EO was vacuum packed in polyethylene bags and stored anaerobically at 4 °C, treated at 55 – 60 °C for 5 – 30 min;

MBR: turkey with *L. monocytogenes* and 0.1 % rosemary EO was vacuum packed in polyethylene bags and stored anaerobically at 4 °C, treated with 55 – 60 °C for 5 – 30 min.

The samples were prepared under sterile conditions with 800 g of turkey which was divided into 78 samples. Meat (10 ±0.2 g) was placed in knurled vacuum bags, *Listeria monocytogenes*-infected samples were packed after inoculation in a vacuum sealer (Proficook PC-VK 1015). The control sample was prepared from raw meat on 0 days. The next day, EOs were added to samples, and maceration for 24 h was performed. The samples were prepared in CASO SV1000 sous vide device. *L. monocytogenes* CCM 4699 was prepared in concentration 10<sup>8</sup> cfu and 100 µL was added to samples.

**Sample preparation:** 78

**Number of samples analyzed:** 78

**Number of repeated analyses:** 3

**Number of experiment replication:** 3

### Statistical analysis

All analyses were performed in triplicate. Statistical variability of data was processed using Microsoft-Excel@ software. Analysis of variance (ANOVA) was used to evaluate the results. Comparison of the treatment means was based on Tukey's Honest Significant Difference (HSD) test.

## RESULTS AND DISCUSSION

The present study aimed to examine the control of microbiological hazards of food with the application of heat treatment and EOs. The raw meat without any antibacterial treatment is prone to microbiological spoilage and the growth of all groups of bacteria was demonstrated. The microbiological analysis confirmed that the thyme oil had the best inhibitory effect on *Listeria* and TVC.

TVC in meat samples without the addition of EOs ranged from 2.21 ±0.02 to 8.26 ±0.02 log cfu.g<sup>-1</sup> (Figure 1).

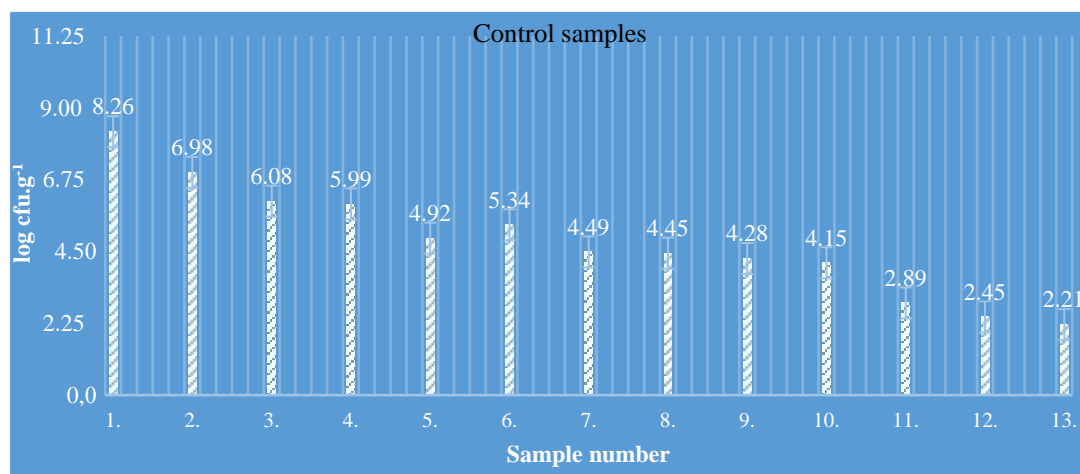
Food spoilage is a food deterioration during storage due to the proliferation of microorganisms resulting from external contamination or the proliferation of natural microbiota of meat. Common preservation methods help to prolong the shelf-life of products and delay microbial growth. There is a need to pay more attention to additional ingredients in food together with the development of food technology, that may improve the overall quality of food. Plant compounds such as EOs which have often been used in traditional and

**Table 1** Heat treatment conditions with the sous vide method of control samples.

Control meat (MC)			Control meat with thyme EO (MT)			Control meat with rosemary EO (MR)		
No.	Temperature (°C)	Time (min)	No.	Temperature (°C)	Time (min)	No.	Temperature (°C)	Time (min)
1.	4	-	14.	4	-	27.	4	-
2.	55	5	15.	55	5	28.	55	5
3.	55	15	16.	55	15	29.	55	15
4.	55	30	17.	55	30	30.	55	30
5.	55	60	18.	55	60	31.	55	60
6.	60	5	19.	60	5	32.	60	5
7.	60	15	20.	60	15	33.	60	15
8.	60	30	21.	60	30	34.	60	30
9.	60	60	22.	60	60	35.	60	60
10.	65	5	23.	65	5	36.	65	5
11.	65	15	24.	65	15	37.	65	15
12.	65	30	25.	65	30	38.	65	30
13.	65	60	26.	65	60	39.	65	60

**Table 2** Heat treatment conditions with the sous vide method of inoculated samples.

Meat with <i>L. monocytogenes</i> (MB)			Meat with <i>L. monocytogenes</i> with thyme EO (MBT)			Meat with <i>L. monocytogenes</i> with rosemary EO (MBR)		
No.	Temperature (°C)	Time (min)	No.	Temperature (°C)	Time (min)	No.	Temperature (°C)	Time (min)
40.	4	-	53.	4	-	66.	4	-
41.	55	5	54.	55	5	67.	55	5
42.	55	15	55.	55	15	68.	55	15
43.	55	30	56.	55	30	69.	55	30
44.	55	60	57.	55	60	70.	55	60
45.	60	5	58.	60	5	71.	60	5
46.	60	15	59.	60	15	72.	60	15
47.	60	30	60.	60	30	73.	60	30
48.	60	60	61.	60	60	74.	60	60
49.	65	5	62.	65	5	75.	65	5
50.	65	15	63.	65	15	76.	65	15
51.	65	30	64.	65	30	77.	65	30
52.	65	60	65.	65	60	78.	65	60



**Figure 1** The total number of microorganisms in meat samples without addition of Eos.

natural medicine for centuries are widely used natural substitutes for food preservation.

EOs are becoming more popular because of the increased palatability of products and inhibitory properties on spoilage microbiota (Król et al., 2013). It seems that for microbial growth in Sous vide products, products stored at 3 and 10 °C have longer shelf life than 40 days, while the microbial growth started on day 9 in products stored at 20 °C (Yıkın et al., 2018).

TVC in meat samples with the addition of thyme EO ranged from  $1.96 \pm 0.02$  to  $6.38 \pm 0.02$  log cfu.g<sup>-1</sup> (Figure 2) and rosemary EO ranged from  $1.89 \pm 0.02$  to  $7.7 \pm 0.02$  log cfu.g<sup>-1</sup> (Figure 3).

This study showed that thyme EO was more effective than rosemary EO in the reduction of TVC. This fact can be related to the chemical composition of this EO which contains phenolic compounds in high concentrations.

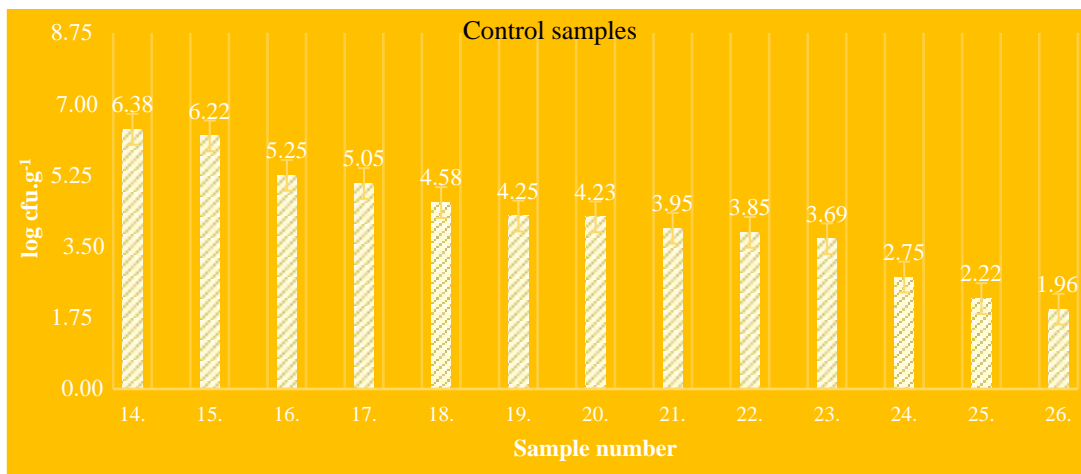


Figure 2 The total number of microorganisms in meat samples with addition of thyme EO.

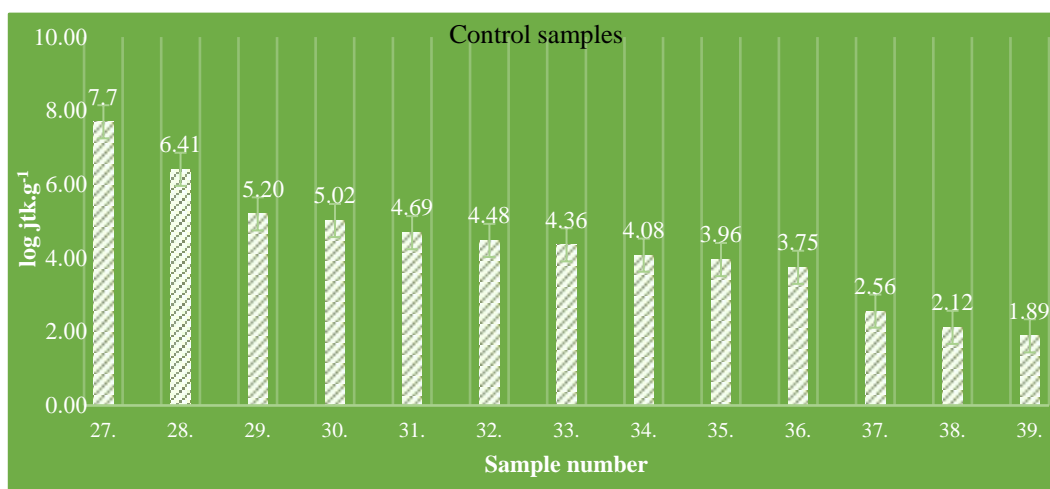


Figure 3 The total number of microorganisms in meat samples with addition of rosemary EO.

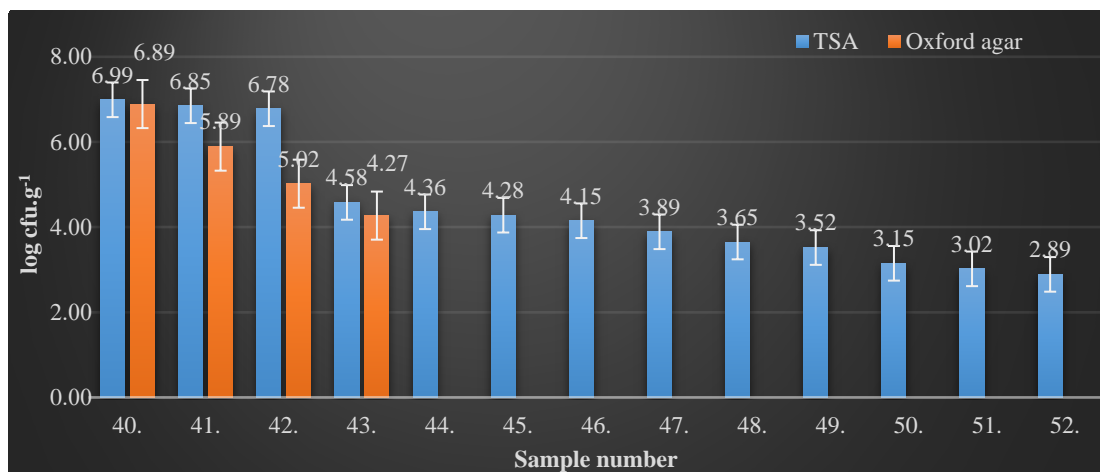
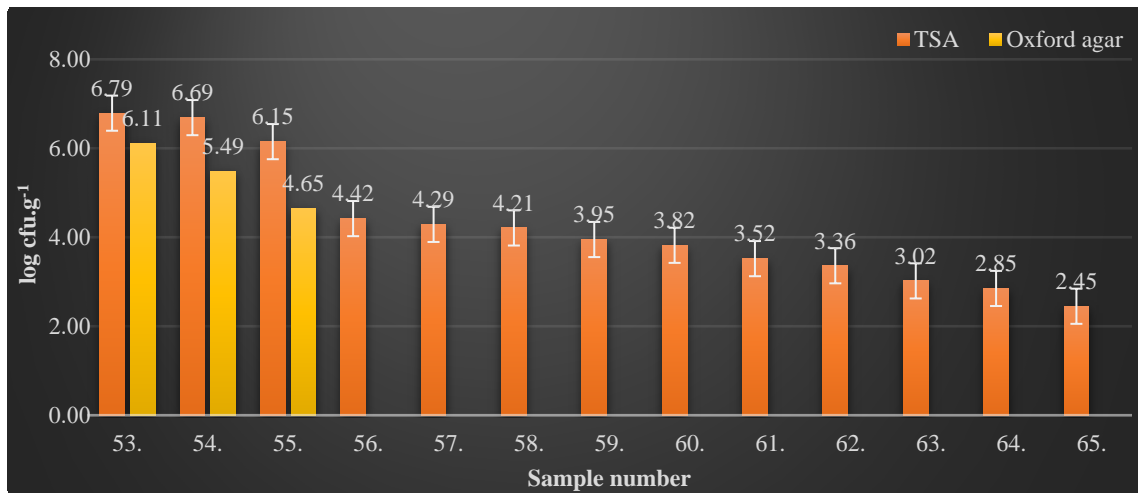


Figure 4 The total number of microorganisms and *L. monocytogenes* in meat samples without addition EO.

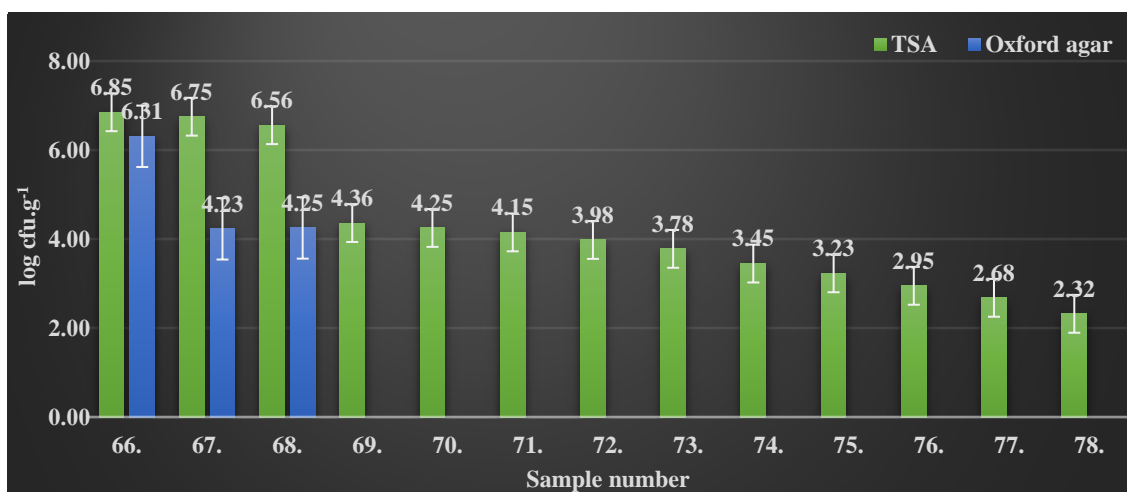
Thyme EO exhibited an antimicrobial effect on the growth of *L. monocytogenes* and these results were in agreement with **Pesavento et al. (2015)** who reported a higher antimicrobial effect against *L. monocytogenes* with the addition of thyme EO at different concentrations in minced meat stored at 4 °C.

The use of essential oils in food inhibits the growth of pathogenic microorganisms (**Nazzaro et al., 2013**). There

were different effects of EO on the growth of Gram-positive and Gram-negative bacteria observed with a more pronounced effect of EOs on Gram-positive bacteria than Gram-negative bacteria were identified due to differences in the cell wall structure (**Król et al., 2013**).



**Figure 5** The total number of microorganisms and *L. monocytogenes* in meat samples with addition of thyme EO.



**Figure 6** The total number of microorganisms and *L. monocytogenes* in meat samples with addition of rosemary EO.

Moura-Alves et al. (2020) used sage EO and sous vide treatment of beef meat with addition of *L. monocytogenes* and they investigated, that sage as a natural preservative, must be combined with other agents to control microbial growth more effectively.

TVC in meat samples with the addition of *L. monocytogenes* ranged from  $2.89 \pm 0.02$  to  $6.88 \pm 0.02$  log cfu.g<sup>-1</sup> (Figure 4). TVC in meat samples inoculated with *L. monocytogenes* and thyme EO ranged from  $2.45 \pm 0.02$  to  $6.79 \pm 0.02$  log cfu.g<sup>-1</sup> (Figure 5) and *L. monocytogenes* and rosemary EO ranged from  $2.32 \pm 0.02$  to  $6.85 \pm 0.02$  log cfu.g<sup>-1</sup> (Figure 6).

Significant differences ( $p < 0.05$ ) were between samples stored at 4 °C, but differences were not found between control samples with *Listeria* and samples with *Listeria* with thyme EO, between control samples with *Listeria* and samples with *Listeria* with rosemary EO and between samples with *Listeria* with thyme EO and samples with *Listeria* with rosemary EO. Significant differences ( $p < 0.05$ ) were noted between samples treated at 55 °C during 15'. No significant differences were between control samples and samples with *Listeria* with thyme EO, control samples with thyme EO and control samples with rosemary, and between control samples with *Listeria* and samples with *Listeria* with rosemary EO. There were significant

differences ( $p < 0.05$ ) between samples treated at 55 °C during 30', no significant differences were only between the control group with thyme EO and the control group with rosemary EO. Significant differences ( $p < 0.05$ ) were noted between samples treated at 55 °C during 60', no significant differences were only between samples with *Listeria* and thyme EO and samples with *Listeria* and rosemary EO. Significant differences ( $p < 0.05$ ) were noted between samples treated at 60 °C during 5', no significant differences were between the control group with *Listeria* and control group with thyme EO and between the control group with thyme EO and samples with *Listeria* with thyme EO. Significant differences ( $p < 0.05$ ) were found between samples that were treated at 60 °C during 15' and 30'. There weren't significant differences only between samples with *Listeria* and thyme EO and samples with *Listeria* and rosemary EO. There were significant differences ( $p < 0.05$ ) between all analyzed samples which were treated at 55 °C during 5', 60 °C during 60' and 65 °C during 5', 15', 30' and 60'.

The study of Abel et al. (2020) investigated the heat inactivation efficiency of *L. monocytogenes* in nutritive solution (BHI) and under sous vide heating conditions of game meat. The results showed that the heat inactivation was strongly affected.

**Table 3** Isolated bacterial strains from turkey without addition of EOs.

MC		MR		MT	
1.	<i>Escherichia coli</i> , <i>Proteus mirabilis</i>	14.	<i>Escherichia coli</i> , <i>Proteus mirabilis</i>	27.	<i>Escherichia coli</i> , <i>Proteus mirabilis</i> , <i>Stenotrophomonas maltophilia</i>
2.	<i>Proteus mirabilis</i>	15.	<i>Proteus mirabilis</i>	28.	<i>Escherichia coli</i>
3.	<i>Escherichia coli</i>	16.	<i>Escherichia coli</i>	29.	<i>Rhizobium radiobacter</i> , <i>Stenotrophomonas maltophilia</i>
4.	<i>Escherichia coli</i> , <i>Proteus mirabilis</i>	17.	<i>Escherichia coli</i>	30.	<i>Proteus mirabilis</i> , <i>Serratia liquefaciens</i>
5.		18.	<i>Escherichia coli</i>	31.	<i>Serratia liquefaciens</i>
6.	<i>Escherichia fergusonii</i>	19.	<i>Escherichia coli</i>	32.	<i>Escherichia coli</i>
7.	<i>Escherichia coli</i>	20.	<i>Escherichia coli</i>	33.	<i>Serratia liquefaciens</i>
8.	<i>Escherichia coli</i>	21.	<i>Serratia liquefaciens</i> , <i>Stenotrophomonas maltophilia</i>	34.	<i>Proteus mirabilis</i> , <i>Stenotrophomonas maltophilia</i>
9.	<i>Escherichia coli</i>	22.	<i>Proteus mirabilis</i> , <i>Staphylococcus saprophyticus</i>	35.	<i>Escherichia coli</i>
10.	<i>Stenotrophomonas maltophilia</i>	23.	<i>Escherichia coli</i> , <i>Proteus mirabilis</i>	36.	<i>Escherichia coli</i>
11.	<i>Serratia liquefaciens</i>	24.	<i>Enterococcus faecalis</i> , <i>Escherichia coli</i>	37.	<i>Serratia liquefaciens</i>
12.	<i>Serratia liquefaciens</i>	25.	<i>Escherichia coli</i>	38.	<i>Stenotrophomonas maltophilia</i>
13.	<i>Escherichia coli</i>	26.	<i>Stenotrophomonas maltophilia</i>	39.	<i>Escherichia coli</i>

Farag proved that thyme and caraway EOs have the strongest antibacterial activity compared to other EOs (Farag et al., 1989). A study of inhibitory properties of 60 different EOs on *P. putida* strain isolated from meat showed that oregano oil had high antibacterial activity (Oussalah et al., 2006). The anise extracts also characterized by strong antibacterial activity against *P. aeruginosa* and *Candidia albicans* (Chanwitheesuk et al., 2005). Karyotis et al. (2017) described log-linear inactivation kinetics for *L. monocytogenes* and *Salmonella* in marinated chicken breast heated at different temperatures between 55 °C and 60 °C in another study.

Betts and Gaze (1995) studied the growth and heat resistance of bacteria in sous vide products and found the relationship between the temperature and time of processing and the growth of bacteria during storage. An increase of temperature up to 90 °C may significantly reduce the number of microorganisms (Betts and Gaze, 1995).

Lee et al. (2017) compared in their study the growth curves of *L. monocytogenes* inoculated in beef with storage temperatures between 5 and 25 °C. There was the development of *L. monocytogenes* at 5 °C observed.

The addition of rosemary EO at 1.25% (v/w) was found to be effective against *L. monocytogenes*. However, no statistical differences were observed after the addition of rosemary EO at 0.2% (v/w) in poultry fillets stored at 4 °C after 7 days of storage (Kahraman et al., 2015). The antimicrobial effect of rosemary EO could be associated with eucalyptol which is the main chemical compound. The oxygen groups of eucalyptol can also disrupt the cell membrane structure even in subinhibitory concentrations (Sousa et al., 2015).

Mizi et al. (2019) reported that the combined usage of sage (powder) and high-pressure processing in beef burgers using two concentrations of sage (0.3% and 0.6%) does not result in any antimicrobial activity against *L. monocytogenes*.

The differences between the present and previous reports were explained with *L. monocytogenes* strain characteristics and the main compounds of EOs (Abdollahzadeh et al., 2014). The antimicrobial effect of EOs is related to intrinsic factors such as food composition, as well as extrinsic factors such as including temperature and presence of oxygen (Hayouni et al., 2008).

**Table 4** Isolated bacterial strains from turkey without addition of EOs and *L. monocytogenes*.

MB		MBR		MBT	
40.	<i>Listeria monocytogenes</i>	53.	<i>Listeria monocytogenes</i>	66.	<i>Listeria monocytogenes</i> , <i>Acinetobacter</i> <i>dijkshoomiae</i> , <i>Proteus mirabilis</i>
41.	<i>Acinetobacter pittii</i> , <i>Listeria monocytogenes</i>	54.	<i>Listeria monocytogenes</i>	67.	<i>Escherichia coli</i> , <i>Listeria monocytogenes</i>
42.	<i>Acinetobacter pittii</i> , <i>Escherichia coli</i> , <i>Listeria</i> <i>monocytogenes</i>	55.	<i>Listeria</i> <i>monocytogenes</i> , <i>Escherichia coli</i>	68.	<i>Listeria monocytogenes</i> , <i>Proteus mirabilis</i>
43.	<i>Listeria</i> <i>monocytogenes</i> , <i>Escherichia coli</i>	56.	<i>Escherichia coli</i>	69.	<i>Escherichia coli</i>
44.	<i>Enterococcus faecalis</i> , <i>Escherichia fergusonii</i>	57.	<i>Escherichia coli</i>	70.	<i>Serratia liquefaciens</i>
45.	<i>Escherichia fergusonii</i>	58.	<i>Serratia liquefaciens</i>	71.	<i>Escherichia fergusonii</i>
46.	<i>Enterococcus faecalis</i> ,	59.		72.	<i>Serratia liquefaciens</i>
47.	<i>Escherichia coli</i>	60.	<i>Serratia liquefaciens</i> , <i>Stenotrophomonas</i> <i>maltophilia</i>	73.	<i>Stenotrophomonas</i> <i>maltophilia</i>
48.	<i>Stenotrophomonas</i> <i>maltophilia</i>	61.	<i>Proteus mirabilis</i> , <i>Staphylococcus</i> <i>saprophyticus</i>	74.	<i>Escherichia coli</i>
49.	<i>Serratia liquefaciens</i>	62.	<i>Escherichia coli</i> , <i>Proteus mirabilis</i>	75.	<i>Stenotrophomonas</i> <i>maltophilia</i>
50.	<i>Serratia liquefaciens</i>	63.	<i>Enterococcus faecalis</i> , <i>Escherichia coli</i>	76.	<i>Serratia liquefaciens</i>
51.	<i>Stenotrophomonas</i> <i>maltophilia</i>	64.	<i>Stenotrophomonas</i> <i>maltophilia</i>	77.	<i>Stenotrophomonas</i> <i>maltophilia</i>
52.	<i>Serratia liquefaciens</i>	65.	<i>Bacillus</i> spp.	78.	<i>Serratia liquefaciens</i>

**Moura-Alves et al. (2016)** found out that the counts of *L. monocytogenes* in beef samples with rosemary EO stored at 2 and 8 °C decreased about 2 log<sub>10</sub> cfu.

There were some results in the study **Giarratana (2016)** which revealed that the mixture of rosemary and thyme EOs had a bacteriostatic activity against *L. monocytogenes* and both 0.025 and 0.05% of tested EOs significantly inhibited *L. monocytogenes* growth compared with the control sample.

Additionally, in a study carried out by **Raeisi et al. (2016)**, the effects of sodium alginate coating with nisin, cinnamon, and rosemary EOs individually and in combinations on the fate of *L. monocytogenes* in chicken meat during 15 days of refrigeration were studied. The control sample and the sample coated with alginate solution had the highest growth rate of *L. monocytogenes*, while other treated samples, especially those with the combined use of tested antimicrobial agents, resulted in the inhibition of *L. monocytogenes*, whereas the combination of cinnamon and rosemary EOs, rosemary EOs and nisin, and cinnamon EOs and nisin had the lowest final population, respectively,

indicating the synergistic effect of these EOs and nisin in controlling *L. monocytogenes*.

Similarly, **Pavli et al. (2019)** found out that the incorporation of oregano EO into sodium alginate edible films in ham slices led to a 1.5 log cfu.g<sup>-1</sup> decrease in population of *L. monocytogenes* at the end of the storage (40 days) at 8 and 12 °C and an approximately 2.5 log cfu.g<sup>-1</sup> reduction at 4 °C. They finally indicated that a significant reduction or absence of *L. monocytogenes* was achieved in ham slices by application of high hydrostatic pressure and an edible film containing oregano EO, together.

Increased heat treatment temperature harmed the sensory properties of food and may reduce digestibility. Lower storage temperature of heat treatment reduces the storage time (**Vaudagna et al. 2002**).

The results of **Kluz et al. (2016)** study suggest the possibility of using caraway and anise EO as natural food preservatives and a potential source of antimicrobial ingredients for chicken breast meat.

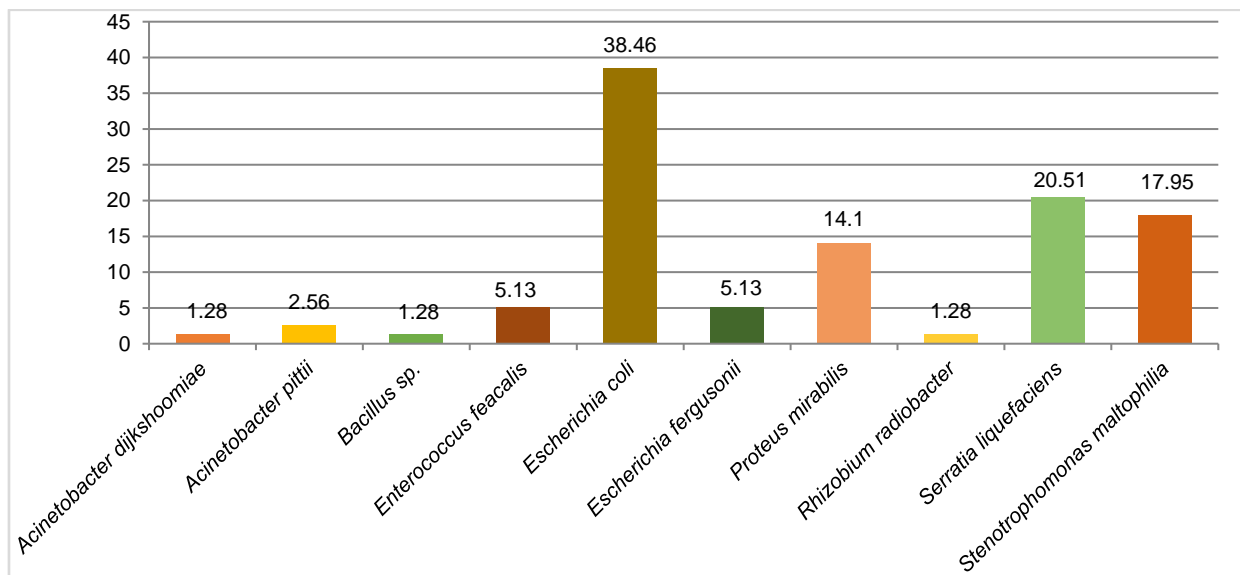


Figure 7 Isolated bacterial strains (%).

*Pimpinella anisum*, *Mentha spicata* var. *crispa*, *Thymus vulgaris* L., *Origanum vulgare* L. EOs exhibited promising results in the study of Kačaniová et al. (2016) for natural food preservatives and potential sources of antimicrobial ingredients for the food industry for chicken meat.

There were *Acinetobacter dijksboomiae* (1.28%), *Acinetobacter pittii* (2.56%), *Bacillus* spp. (1.28%), *Enterococcus faecalis* (5.13%), *Escherichia coli* (38.46%), *Escherichia fergusonii* (5.13%), *Proteus mirabilis* (14.1%), *Rhizobium radiobacter* (1.28%), *Serratia liquefaciens* (20.51), and *Stenotrophomonas maltophilia* (17.95%) isolated from turkey in the present study (Tables 3 – 4, Figure 7).

Kunová et al. (2017) isolated *Staphylococcus warneri* from the control group stored in the air conditions, *Kocuria rhizophila* from control vacuum-packed control samples, *Staphylococcus warneri*, *Aeromonas salmonicida* and *Aeromonas popoffii* from control group treated with EDTA, *Staphylococcus hominis*, and *Staphylococcus epidermidis* from meat treated with caraway EO. There were in total, 15 genera identified from meat after EO and vacuum packaging: *Aeromonas*, *Aromatoleum*, *Buttiauxella*, *Clostridium*, *Enterobacter*, *Hafnia*, *Lactobacillus*, *Lysinobacillus*, *Pantotea*, *Pseudomonas*, *Rahnella*, *Raoultella*, *Serratia*, *Staphylococcus*, and *Yersinia* Kačaniová et al. (2019).

## CONCLUSION

Turkey meat supports microbial growth including pathogenic microbiota. Storage and processing of meat may influence the microbiological contamination of turkey meat. Our study shows that the sous vide method is an effective method for the treatment of fresh turkey breast meat to protect the meat from spoilage. There were evaluated in our study that sous vide method with combination with essential oils is effective against the total count of microorganisms and *L. monocytogenes*. The best results were found by treatment samples with rosemary EOs. Temperature and application of EOs have a positive effect against the total count of bacteria and especially against *L. monocytogenes*.

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