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# Antimicrobial activity of thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*) essential oils against *Listeria monocytogenes* in fermented sausages

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**Abstract.** The aim of this study was to investigate antibacterial effects of oregano and thyme essential oils on *Listeria monocytogenes* in fermented sausages and their effect on the sensory characteristics of these sausages. For testing purposes, sausages contaminated with *L. monocytogenes* were produced. Changes in the microbiological status of fermented sausages and physicochemical properties were monitored during ripening. Essential oils exhibited antibacterial activity against *L. monocytogenes*, and in the groups with a high concentration (0.6%) of oregano or thyme essential oils (KLO2 and KLT2), the number of *L. monocytogenes* was below the detection threshold on day 14 of ripening, with a stronger effect of oregano. In groups with 0.3% essential oil of oregano or thyme added, the number of *L. monocytogenes* was reduced to below the detection threshold on day 21 of ripening. During the ripening, the  $a_w$  and pH of all test groups of fermented sausages decreased. Experimental sausages with 0.3% thyme essential oil had acceptable smell and taste, while in other experimental groups, sausage smell and taste were very intense, uncharacteristic and unacceptable.

## 1. Introduction

Foodborne listeriosis is one of the most serious and severe foodborne diseases, caused by the bacterium *Listeria monocytogenes*. This pathogen has been isolated from various ready-to-eat (RTE) food products, including fermented dry and semi-dry sausages [1]. The number of *L. monocytogenes* decreases during fermentation and drying of sausages because of the set of hurdles created in the manufacturing process (low pH and water activity ( $a_w$ ) and high salt concentration). However, this microorganism can be isolated from fermented sausages because of its ability to adapt to its environment and because of its presence in raw meat [2]. The manufacturing process is not effective enough to reduce or eliminate this microorganism from the finished product [3, 4].

Because of the growing popularity of natural and organic food, there has been a consumer shift away from chemical preservatives in food, as these compounds exhibited many adverse effects [5]. There is a new trend in the meat industry, where there is no place for artificial preservatives with possible



carcinogenic and toxic properties [6, 7, 8]. Essential oils obtained from a variety of plant materials impart distinctive flavours, exhibit antimicrobial activity in meat products and possess antioxidative properties [9]. Oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) are aromatic plants with important antioxidant and antimicrobial properties. Oregano essential oil is known to possess antibacterial, antiviral, antifungal, antiparasitic and antioxidant activities. Thyme has been used medicinally for thousands of years. Beyond its common culinary application, it has been recommended for a myriad of indications, based upon proposed antimicrobial, antitussive, spasmolytic and antioxidant activity [10]. Carvacrol and thymol are the two main phenols that constitute oregano and thyme essential oils, as well as the monoterpene hydrocarbons *p*-cymene and  $\gamma$ -terpinene [11].

The aims of this study were to investigate antibacterial effects of thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*) essential oils on *L. monocytogenes* in fermented sausages and to examine their effect on the sensory properties of these sausages.

## 2. Materials and Methods

### 2.1. Sausage manufacture

Five different formulations of sausages were prepared: one of control sausages with no essential oil, two formulations with thyme (0.3% and 0.6%) and two formulation with oregano (0.3% and 0.6%) essential oil. In half the sausages with essential oil and in control sausages, an inoculum of *L. monocytogenes* was added. The other half of the sausages were *Listeria*-free, and they were used for sensory analysis. Fermented sausages were manufactured in the experimental laboratory at the Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, University of Belgrade, following the traditional formulation. All batches were prepared with the same raw components: 60% lean pork meat, 40% pork backfat, 5% water, 2% sodium chloride, 1% lactose, 0.05% sodium ascorbate, 0.015% sodium nitrite, 0.03% potassium nitrate, 0.05% black pepper and 0.05% white pepper. The meat and fat were minced at 4 °C through a 6 mm grinder plate (Mainca PM-98, Equipos cárnicos S.L., Granollers, Barcelona, Spain) and mixed with the other ingredients. This paste was stuffed in synthetic sausage casings of 33-35 mm diameter with a Mainca EM-12 (Equipos cárnicos S.L., Granollers, Barcelona, Spain) stuffing machine. Thereafter, sausages were fermented for 24 h (22-24 °C, 94-98% RH) followed by 28 days of dry-curing (16-18 °C, 80-85% RH) in a controlled dry-cured chamber (Tarré, STA-15-HXA, Noain, Navarra).

### 2.2. Essential oils

The essential oils extracted by the steam distillation method (year 2012) were purchased from the manufacturer Herba doo (Belgrade, Serbia). Essential oils were kept in dark glass bottles at 4 °C.

### 2.3. Detection and isolation of *L. monocytogenes*

Samples were taken from each batch after 0, 7, 14, 21 and 28 days of production. For *Listeria* enumeration, 25 g of fermented sausages was weighed out aseptically and transferred into sterile Stomacher bags and homogenized with 225 ml of buffered peptone water (BPW; Merck, Germany) that was added to each sample. The bag contents were homogenized in a Stomacher blender (Stomacher 400 Circulator, Seward, Worthing, UK) for 2 min. Serial decimal dilutions were prepared and 1 ml or 0.1 ml of appropriately diluted suspension was inoculated directly on the surface of the appropriate media for *L. monocytogenes* enumeration. Inoculated fermented sausages were analysed for *L. monocytogenes* on days 0, 7, 14, 21 and 28 of maturation (ripening of sausages). *L. monocytogenes* was enumerated on the Agar *Listeria* acc. to Ottaviani and Agosti (ALOA, Oxoid, Hampshire, UK) and plates were incubated for 24-48 h at 37°C according to ISO 11290-1:2017. After incubation, plates were examined visually for typical colony types and morphological characteristics associated with each growth medium, the number of colonies was counted, and results were recorded as colony forming units per gram (cfu/g).

#### 2.4. pH and $a_w$ of sausages

The pH of sausages was measured using the portable pH meter (WTW 340i, Germany) according to methods recommended by International Organization for Standardization (ISO 2917:2004). Means of three measurements are presented. Water activity ( $a_w$ ) of sausages was determined using aw-Wert Messer, GBX Scientific Instruments, Fa-St/1, according to ISO 21807:2004E, at a constant temperature of 20°C.

#### 2.5. Sensory evaluation

A panel consisting of seven trained members performed sensory evaluation (ISO 8586-2:2008) in the laboratory that was designed according to the requirements of standard SRPS EN ISO 8589:2012. The panellists were asked to evaluate the dry fermented sausages for the following characteristics: external appearance and/ or condition of the packaging, appearance and composition of cut surface, colour and stability of colour, smell and taste and texture and/or juiciness. Evaluations were performed according to a 5-point scale descriptive system, from 1 to 5, where each mark represented a certain level of quality. The overall sensory quality of sausages was multiplied by an appropriate coefficient of significance (external appearance of sausage x2 + appearance and composition of cut surface x5 + colour and colour maintenance on the cutting x3 + door and taste x7 + texture and juiciness x3).

#### 2.6. Statistical analysis

Statistical analysis of the results was carried out using GraphPad Prism v6 (GraphPad, San Diego, CA, USA) software. Since the data were homogeneous (coefficient of variation <30%), groups were compared using two-way ANOVA with repeated one factor measures followed by Tukey's multiple comparison tests.

### 3. Results and Discussion

Approximately equal numbers of *L. monocytogenes* were added to the stuffing of fermented sausages of all groups at the beginning of production (day 0). In the groups with the addition of a higher concentration (0.6%) of oregano or thyme essential oils (KLO2 and KLT2), *L. monocytogenes* numbers were below the detection threshold on day 14 of ripening, with a stronger effect of oregano. In sausage groups with 0.3% oregano or thyme essential oil, the numbers of *L. monocytogenes* were below the detection threshold on day 21 of ripening. In the control group (KL), the number of *L. monocytogenes* decreased below the detection threshold (<2) after day 28 of maturation (Table 1). Analysing the number of *L. monocytogenes* in the experimental groups of fermented sausages during ripening, no significant differences were observed on day 0 ( $p > 0.05$ ). On day 7 of maturation, the lowest number of *L. monocytogenes* was found in KLO2 (0.6%) ( $2.51 \pm 0.01$ ), while the highest number was found in the control group KL ( $5.01 \pm 0.01$ ). On day 7 of ripening, there was a statistically significant difference between all examined groups ( $p < 0.01$ ).

**Table 1.** Number of *L. monocytogenes* (log CFU/g) during the ripening and maturation of sausages

Group	Day of during the ripening and maturation of sausages				
	0	7	14	21	28
KL	6.22±0.01	5.01±0.01 <sup>ABCD</sup>	4.22±0.01	2.15±0.01	< 2
KLO1 (0.3%)	6.26±0.01	4.04±0.05 <sup>AE</sup>	2.02±0.01	< 2	< 2
KLO2 (0.6%)	6.26±0.01	2.51±0.01 <sup>BEFG</sup>	< 2	< 2	< 2
KLT1 (0.3%)	6.23±0.01	4.23±0.01 <sup>CF</sup>	2.44±0.01	< 2	< 2
KLT2 (0.6%)	6.23±0.01	3.73±0.92 <sup>DG</sup>	< 2	< 2	< 2

Statistical significance is presented in the same letters: a -  $p < 0.05$ ; A -  $p < 0.01$

KL - Control group - fermented sausages without added essential oils + L.m.

KO1 - fermented sausages with 0.3% oregano essential oil

KLO1 - fermented sausages with 0.3% oregano essential oil + L.m.

KO2 - fermented sausages with 0.6% oregano essential oil

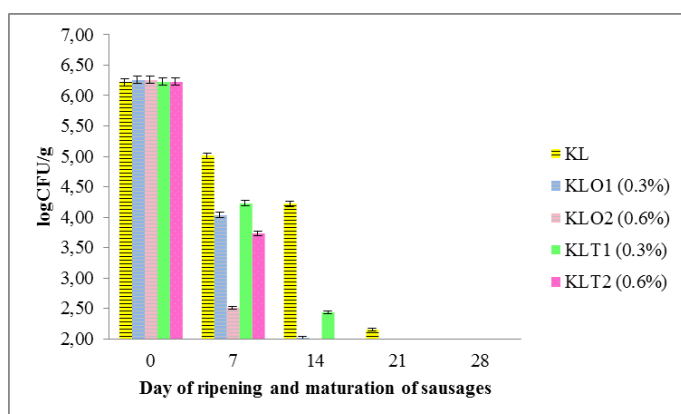
KLO2 - fermented sausages with 0.6% oregano essential oil + L.m

KT1 - fermented sausages with 0.3% thyme essential oil

KLT1 - fermented sausages with 0.3% thyme essential oil + L.m

KT2 - fermented sausages with 0.6% thyme essential oil

KLT2 - fermented sausages with 0.6% thyme essential oil + L.m



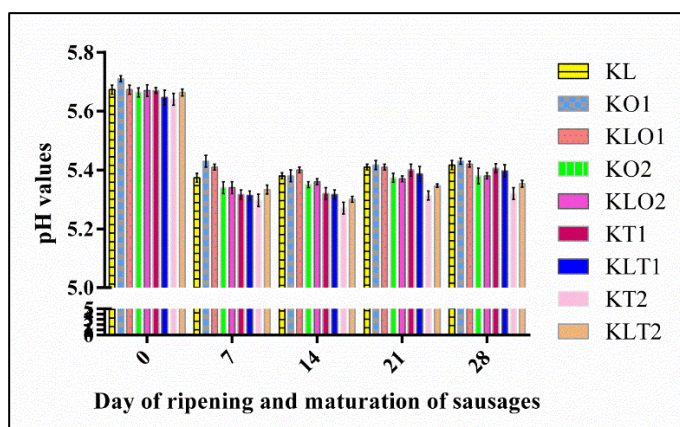
**Figure 1.** Number of *L. monocytogenes* (log CFU/g) during the ripening and maturation of sausages

Examination of the pH on day 0 of ripening showed the highest pH was in the control group with added oregano essential oil KO1 (0.3%) ( $5.71 \pm 0.01$ ), and the lowest was in the control group with thyme essential oil KT2 (0.6%) ( $5.64 \pm 0.02$ ). Further analysis revealed significant differences in almost all examined groups except KLO1, KLO2, KT1 (0.3%), ( $p < 0.05$ ;  $p < 0.01$ ). By day 7 of ripening, the highest pH was in KO1 (0.3%) ( $5.43 \pm 0.02$ ), and the lowest in KT2 (0.6%) ( $5.30 \pm 0.02$ ). On day 14, the highest recorded pH occurred in group KLO1 ( $5.40 \pm 0.00$ ), while the lowest was in the group KT2 (0.6%) ( $5.27 \pm 0.02$ ). On day 21 of ripening, the control group with oregano essential oil KO1 (0.3%) ( $5.42 \pm 0.02$ ) had the highest pH, and the lowest pH occurred in the group with thyme essential oil KT2 (0.6%) ( $5.31 \pm 0.02$ ). Also, at the end of ripening (day 28), the highest pH was found in the control group ( $5.43 \pm 0.01$ ), and the lowest in the group with thyme essential oil KT2 (0.6%) ( $5.32 \pm 0.01$ ). Statistically significant differences occurred within all examined groups ( $p < 0.05$ ;  $p < 0.01$ ).

**Table 2.** pH values during the ripening and maturation of sausages

Group	Day of during the ripening and maturation of sausages				
	0	7	14	21	28
KL	$5.67 \pm 0.02$	$5.37 \pm 0.02$ <sup>ABCD</sup>	$5.38 \pm 0.01$ <sup>ABCD</sup>	$5.41 \pm 0.01$ <sup>AB</sup>	$5.43 \pm 0.01$ <sup>AB</sup>
KO1 (0.3%)	$5.71 \pm 0.01$ <sup>aABb</sup>	$5.43 \pm 0.02$ <sup>AEFGHIJ</sup>	$5.38 \pm 0.01$ <sup>EF</sup>	$5.42 \pm 0.02$ <sup>abCD</sup>	$5.41 \pm 0.01$ <sup>CDEF</sup>
KLO1	$5.67 \pm 0.02$	$5.41 \pm 0.01$ <sup>KLMNOP</sup>	$5.40 \pm 0.00$ <sup>JKLM</sup>	$5.41 \pm 0.01$ <sup>EF</sup>	$5.42 \pm 0.01$ <sup>GH</sup>
KO2 (0.6%)	$5.66 \pm 0.02$ <sup>a</sup>	$5.34 \pm 0.02$ <sup>EKa</sup>	$5.35 \pm 0.01$ <sup>INO</sup>	$5.37 \pm 0.02$ <sup>aG</sup>	$5.38 \pm 0.03$ <sup>CI</sup>
KLO2	$5.67 \pm 0.02$	$5.34 \pm 0.02$ <sup>FLb</sup>	$5.36 \pm 0.01$ <sup>aPQ</sup>	$5.37 \pm 0.01$ <sup>bH</sup>	$5.38 \pm 0.01$ <sup>DJ</sup>
KT1 (0.3%)	$5.67 \pm 0.01$	$5.32 \pm 0.01$ <sup>BGM</sup>	$5.32 \pm 0.02$ <sup>AEJR</sup>	$5.40 \pm 0.02$ <sup>IJ</sup>	$5.41 \pm 0.02$ <sup>KL</sup>
KLT1	$5.65 \pm 0.03$ <sup>A</sup>	$5.31 \pm 0.02$ <sup>CHN</sup>	$5.32 \pm 0.02$ <sup>BFKa</sup>	$5.39 \pm 0.03$ <sup>K</sup>	$5.40 \pm 0.02$ <sup>Ma</sup>
KT2 (0.6%)	$5.64 \pm 0.02$ <sup>B</sup>	$5.30 \pm 0.02$ <sup>DIOab</sup>	$5.27 \pm 0.02$ <sup>CGLNPR</sup>	$5.31 \pm 0.02$ <sup>ACEGHJK</sup>	$5.32 \pm 0.01$ <sup>AEGIJKM</sup>
KLT2	$5.66 \pm 0.01$ <sup>b</sup>	$5.33 \pm 0.01$ <sup>JP</sup>	$5.30 \pm 0.01$ <sup>DHMOQ</sup>	$5.35 \pm 0.01$ <sup>BDFJ</sup>	$5.35 \pm 0.01$ <sup>BFHLa</sup>

Statistical significance is presented in the same letters: a -  $p < 0.05$ ; A -  $p < 0.01$



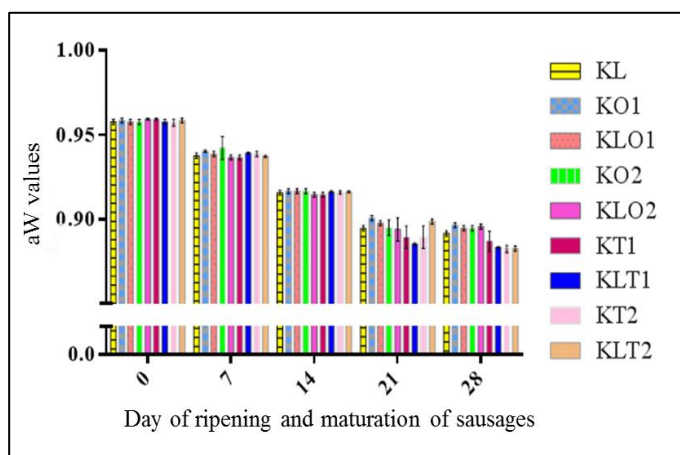
**Figure 2.** pH values during the ripening and maturation of sausages

Statistical analysis of the  $a_w$  values in the experimental groups of fermented sausages during ripening revealed the same or almost the same mean  $a_w$  on all examined ripening days. Significant differences within the examined groups were not found on days 0, 7 or 14 of ripening ( $p > 0.05$ ), but they were recorded on day 21 and 28 of ripening within all examined groups ( $p < 0.05$ ;  $p < 0.01$ ).

**Table 3.**  $a_w$  values during the ripening and maturation of sausages

Group	Day of during the ripening and maturation of sausages				
	0	7	14	21	28
KL	0.96±0.002	0.94±0.002	0.92±0.002	0.89±0.002 <sup>A</sup>	0.89±0.002 <sup>ABC</sup>
KO1 (0.3%)	0.96±0.002	0.94±0.001	0.92±0.002	0.90±0.002 <sup>BCD</sup>	0.90±0.002 <sup>DEFG</sup>
KLO1	0.96±0.002	0.94±0.002	0.92±0.002	0.90±0.002 <sup>EFG</sup>	0.89±0.002 <sup>aHIJ</sup>
KO2 (0.6%)	0.96±0.002	0.94±0.02	0.92±0.002	0.90±0.005 <sup>H</sup>	0.89±0.002 <sup>bKLM</sup>
KLO2	0.96±0.001	0.94±0.001	0.91±0.002	0.89±0.007 <sup>I</sup>	0.90±0.002 <sup>NOPR</sup>
KT1 (0.3%)	0.96±0.001	0.94±0.01	0.92±0.001	0.89±0.001 <sup>BEJK</sup>	0.88±0.001 <sup>DabN</sup>
KLT1	0.96±0.002	0.94±0.00	0.92±0.001	0.89±0.001 <sup>ACFHIL</sup>	0.88±0.001 <sup>AEHKO</sup>
KT2 (0.6%)	0.96±0.002	0.94±0.002	0.92±0.002	0.90±0.001 <sup>DGM</sup>	0.90±0.002 <sup>BFILP</sup>
KLT2	0.96±0.002	0.94±0.001	0.92±0.001	0.90±0.002 <sup>JKLM</sup>	0.88±0.002 <sup>CGJMR</sup>

Statistical significance is presented in the same letters:  $a - p < 0.05$ ;  $A - p < 0.01$



**Figure 3.**  $a_w$  values during the ripening and maturation of sausages

In sensory examination of experimental groups of sausages to which oregano or thyme extracts were added in concentrations of 0.3% and 0.6%, external appearance, cut surface appearance and composition, texture and juiciness and colour and stability of colour were highly rated in all groups

(from 4.2 to 4.8). However, the smell and taste were only acceptable only in the experimental group with 0.3% thyme (3.2), while in the other experimental groups it was very intense, uncharacteristic and unacceptable (grade 2 and lower).

**Table 4.** Sensory evaluation of sausages

Group	External appearance	Cut surface appearance and composition	Colour and stability of colour	Smell and taste	Texture and juiciness	Overall sensory quality
KO1 (0.3%)	4.8 ± 0.3	4.5 ± 0.0	4.3 ± 0.3	3.2 ± 0.3	4.3 ± 0.3	80.3 ± 6.0
KO2 (0.6%)	4.8 ± 0.3	4.4 ± 0.2	4.2 ± 0.4	2.0 ± 0.3	4.2 ± 0.4	70.6 ± 4.7
KT1 (0.3%)	4.7 ± 0.4	4.3 ± 0.4	4.4 ± 0.2	1.9 ± 0.2	4.3 ± 0.3	70.3 ± 4.5
KT2 (0.6%)	4.7 ± 0.3	4.4 ± 0.2	4.2 ± 0.4	1.3 ± 0.3	4.2 ± 0.4	65.7 ± 5.2

#### 4. Conclusion

The technological process of production of fermented sausages, without added essential oils of oregano or thyme leads to a decrease in the number of *L. monocytogenes* to below the detection level after day 28 of ripening. However, some concentrations of these essential oils reduce numbers or completely eliminate *L. monocytogenes* before the end of the technological process. With the addition of a higher concentration (0.6%) of essential oils, the number of *L. monocytogenes* was below the detection threshold on day 14 of ripening, with a slightly stronger effect of oregano. In groups with 0.3% essential oil, after day 21, *L. monocytogenes* was below the detection threshold. Examination of the sensory properties of fermented sausages to which essential oils were added showed that only sausages with 0.3% thyme essential oil are acceptable.

#### Acknowledgement

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