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# Application of essential oils of thyme as a natural preservative in leather tanning

Justa Širvaitytė<sup>a\*</sup>, Jūratė Šiugždaitė<sup>b</sup>, Virgilijus Valeika<sup>c</sup>, and Edita Dambrauskiene<sup>d</sup>

<sup>a</sup> Department of Organic Technology, Kaunas University of Technology, Radvilenu Pl. 19, Kaunas, LT-50524, Lithuania

<sup>b</sup> Lithuanian Health Science University, Veterinary Academy, Department of Infection Diseases, Tilzes Str. 18, Kaunas, LT-47181, Lithuania

<sup>c</sup> Department of General Chemistry Technology, Kaunas University of Technology, Radvilenu Pl. 19, Kaunas, LT-50524, Lithuania

<sup>d</sup> Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Kauno Str. 30, Babtai, Kaunas Reg., LT-54333, Lithuania

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Abstract. The aim of this study was to investigate the possibility of using essential oils of *Thymus vulgaris* as an alternative preservative for chromed leather. The differences between the chemical composition of commercial and pure essential oils of thyme were determined. It was observed that these differences have an influence on the antibacterial activity of essential oils. Gram-positive bacteria were found to be more sensitive to the essential oils of thyme than Gram-negative bacteria. The bacteria *Pseudomonas aeruginosa* had a low sensitivity to the action of the selected essential oils of thyme, but the leather samples treated with the essential oil of thyme remained resistant to the action of these bacteria. As the main result of this study, it was concluded that the essential oil of thyme could be used as a preservation agent in the leather tanning industry. The leather preserved with 2-(thiocyanomethylthio)benzothiazole had weaker protection after four weeks compared to the samples treated with the essential oil of thyme when the amount of the used essential oil was not less than 3% of the wet-blue mass. The essential oil of thyme was the more active component in the mixture of essential oil and synthetic biocide used for the preservation of leather.

Key words: essential oil, thyme, leather, preservation, biocide.

# INTRODUCTION

Leather is a natural product which is decomposing over time due to the bacterial or fungal activity. Bacteria are everywhere in the environment. These microorganisms quickly colonize on surface, as well as on clothing and footwear. Most of these organisms are not dangerous to human health. However, immuno-compromized individuals, such as the elderly, people with diabetes, or infants, may be at risk of infection by these microorganisms. The natural materials, also leather, can act as carriers for microbial growth. In contact with human body, garments offer an excellent environment for microorganisms due to providing them oxygen, water, and warmth [1,2].

Antimicrobial agents (biocides) currently used in the leather industry are generally harmful to human health and the environment, and their use has been or shall be restricted or even banned. For example, the use of pentachlorophenol has been banned in the leather industry due to the toxicity of the compound, despite the fact that it has been used as a general antiseptic material in the industry [3]. Also, some countries have banned the use of polyhalogenated phenolic compounds [4]. European Union Directive 2009/251/EC banned the use of dimethylfumarate (DMF) (Fig. 1) [5]. Clinical studies have shown that this substance, which prevents leather furniture and footwear from the mould, may cause health damage. It affects consumers who are in contact with the products, penetrating through the clothes onto the consumers' skin, causing painful skin, dermatitis, including itching, irritation, redness, and burns; in some

Corresponding author, justa.sirvaityte@ktu.lt



Fig. 1. Structure of the biocide used in the leather tanning industry.

cases, acute respiratory troubles were reported. The dermatitis due to DMF is particularly difficult to treat. The presence of DMF is thus a serious risk. So, it demonstrates that biocides, which even currently are in the market, are not safe.

The fungicide 2-(thiocyanomethylthio)benzothiazole (TCMTB) (Fig. 1) became a new standard antiseptic material starting in the 1970s and remains in use up to now. TCMTB exhibits low acute oral and dermal toxicity (toxicity category III). However, it is highly irritating to the eyes and skin (toxicity category I and II, respectively) and is also considered to be highly toxic via the inhalation route of exposure (toxicity category I). TCMTB is a dermal sensitizer [6].

Changes in consumers' preferences toward more natural products than synthetic stimulate the use of natural products also in leather tanning. Humans have used plant extracts for their antifungal, antimicrobial, insecticidal, cytostatic, and therapeutic activities [7]. Essential oils are widely used as components of drugs, biologically active additives and dietary supplements, as well as in aromatherapy and the food and cosmetics industries [7–9]. They are so widely used due to their pleasant or spicy smell. In addition, numerous recent studies report biological activity of essential oils: they exhibit antibacterial, fungicidal, antioxidant, and antiradical activities [10].

Essential oils consist primarily of low-molecularweight mono- and sesquiterpene hydrocarbons, their oxygen analogues, and phenol derivatives [1,4,11]. Small sizes of molecules of essential oils allow them to easily penetrate through cell walls and affect various biochemical processes. The biological activity of essential oils depends on their composition. Essential oils that contain substituted phenols (eugenol, thymol, carvacrol, and guaiacol (Fig. 2)) exhibit strong antibacterial and antioxidant effects [12–14].



Fig. 2. Structure of phenols.

The genus *Thymus* has numerous species and varieties. The composition of their essential oils has been studied earlier [15–17]. Many species of the genus *Thymus* are aromatic and medicinal plants, whose essential oils possess strong antibacterial properties [18,19]. So, due to their wide use in various fields and their well-known antiseptic properties the essential oils of thyme were chosen for this research work.

Application of the mixture of the essential oil and a synthetic preservative for the preservation of leather was another direction of this study. The use of such a mixture could solve two problems at once: firstly, by decreasing the amount of synthetic preservative needed for leather treatment and thus reducing the irritant effect on customers, and secondly, by decreasing the amount of essential oil, which is fairly expensive.

The main goal of this work was to investigate the possibility of using essential oils of thyme as an alternative preservative for the leather tanning industry.

# EXPERIMENTAL

#### **Preparation of leather samples**

Bovine hides cured by salting were used to obtain the chromed or vegetable tanned leather samples, which were processed according to the conventional technologies. Tanned leather samples for the experiments were fatliquored under the conditions described in Table 1. The products of TFL Holding GmbH, Germany, used in this study were as follows: *Coripol GF* is an oil based combination of selected natural and synthetic fatty substances; *Coripol A* is a viscous oil based on natural phospholipids; *Borron SAF* is a clear product, based on sulphated fatty alcohols.

*Fungicide FDE*, product of KEMCOLOR S.p.a. (Italy), used in leather tanning is a biocide based on 2-(thiocyanomethylthio)benzothiazole.

The control samples of leather were tanned according to the conventional technology of the joint-stock company "Kedainiu oda", at which a commercial bactericide (*Fungicide FDE*) was added during the pickling process. Table 1. Leather processing method (% of wet-blue mass)

#### Neutralization

- (a) H<sub>2</sub>O 150%; NaHCO<sub>3</sub> 1.5%; temperature 35-40°C; duration - 0.5 h; run - continuously;
- (b) HCOONa 2.0%; duration 1.5 h; run continuously; (pH of leather ~5.6).

Drain.

# Washing

 $H_2O - 100\%$ ; temperature -40-45 °C; duration -30 min; run - continuously. Drain.

#### Fatliquoring

(a)  $H_2O - 150\%$ ; Coripol GF - 10.0%; Coripol A - 4.0%; Borron SAF - 0.2%; essential oil - 5%; temperature - 55–60 °C; duration - 1.5 h; run - continuously;

(b) HCOOH – 1%; duration – 0.5 h; run – continuously; (pH of leather ~4.0).

Drain.

#### Washing

 $H_2O - 100\%$ ; temperature - 30°C; duration - 0.5 h; run - continuously. Drain.

#### Chemical analysis of essential oils

Quantitative and qualitative analyses of essential oils were performed on a gas chromatograph GC-2010 (Shimadzu, Japan) with a mass spectrometric detector GCMS-OP2010 (Shimadzu, Japan). The mass spectrometer was programmed in the electron impact ionization mode at 70 eV, the mass range was m/z 29–550. Volatile compounds were separated using an RTX-5MS column (length 30 m, i.d. 0.25 mm, film thickness 0.25 µm) (Restec, USA). The carrier gas, helium, was adjusted to 1.2 mL min<sup>-1</sup> volumetric flow. Split mode was used at a ratio of 1:10; the injector temperature was 240°C. The oven temperature was maintained at 60°C for 3 min, then raised at a heating rate of 2 °C min<sup>-1</sup> to 70°C and held for 2 min, then raised at a heating rate of 1°C min<sup>-1</sup> to 120°C and held for 2 min, and finally raised at a heating rate of 20  $^{\circ}\mathrm{C}$  min  $^{-1}$  to 220  $^{\circ}\mathrm{C}$  and held for 5 min. Three replicates of each sample were run three times by GC-MS.

#### Antibacterial studies

The following bacteria ATCC and NCTC strains were used as control: Gram-negative rod-like, lactose-fermenting bacteria *Escherichia coli* (ATCC 8739) belonging to the family Enterobacteriaceae; Gram-negative, motile, obligate aerobic rods *Pseudomonas aeruginosa* (NCTC 6750); Gram-positive pathogenic cocci *Staphylococcus aureus* (ATCC 9144); and Gram-positive spore-forming rods *Bacillus cereus* (ATCC 11778).

Antimicrobial susceptibility was determined according to the technique by Kirby–Bauer [20]. Cultures of microorganisms were re-inoculated to saline solution. A concentration of  $10^8$  CFU mL<sup>-1</sup> (colony forming units) is generally considered to be the starting point, corresponding to McFarland's standard 0.5. The density of microorganisms was evaluated by a unit of McFarland with Mini Shaker MS 1 (*Crystal Spec*, USA). Bacterial suspensions of 0.25 mL were inoculated on plates with Mueller Hinton II agar (*Oxoid*, England). Leather specimens (diameter 19 mm) were placed onto the agar. Inoculated Petri plates were incubated at 35–37°C for 18–24 h. Resistance to essential oils was evaluated by measuring the inhibition zones. Leather specimens without essential oil were used as control.

In the case of essential oil test, the test paper wells (diameter 5 mm) were impregnated with essential oil and placed onto the agar. Petri plates were incubated at 35-37 °C for 18-24 h. The inhibition zones (mm) were measured and antimicrobial activity was estimated (Table 2).

#### Plant materials and distillation of essential oils

Thymes (*Thymus vulgaris* and *Thymus serpyllum*) were cultivated in the experimental fields of the Lithuanian Institute of Horticulture for two consecutive years, 2009 and 2010. The distance between the plants in the rows was 30 cm; the distance between the rows was 45 cm; the length and width of each testing field were 3 and 3.15 m, respectively (total area 9.45 m<sup>2</sup>). The soil was sod gleic clay loam on clay loam with the pH 6.1–7.4.

The harvested herbs were dried at ambient temperature in the dark. The yield of essential oil in fresh and dried thyme was determined by hydrodistillation in a Clevenger-type apparatus [21]. Essential oils of *Thymus vulgaris* (TV1) and *Thymus serpyllum* (TS) were obtained. Additionaly, the company "Meta" (Lithuania) provided commercial essential oil of *Thymus vulgaris* (TV2), which was also used for experiments.

Table 2. Assessment of antibacterial activity

Growth description	Notation
Specimen with growth (not resistant)	_
No inhibition zone, but no growth on the sample (resistant)	0
Specimen free of growth,	Size of inhibition zone,
visible inhibition zone	mm
Specimen free of growth; clean petri-dish – no growth	*

# Leather quality indexes

The amount of chrome compounds in leather was determined by methods described in the literature [22]. The shrinkage temperature of the pelt was measured with a special instrument [23]. The tensile strength and elongation rate of leather were measured with a dynamometer [24]. The matter soluble in dichloromethane and free fatty acid content were determined according to standard [25].

## Statistical analysis

All data were expressed as the mean±standard error of triplicate measurements. Confidence limits were set at P < 0.05. Standard deviations did not exceed 5% for the majority of the values obtained.

### **RESULTS AND DISCUSSION**

It is known that genetic constitution and environmental conditions influence the yield and composition of volatile oils produced by thyme plants. The composition of essential oils of thyme and the percentage of major components from a particular species of the plant can differ depending on the harvesting season, geographical sources, and even on the function of the vegetal part of the same plant [26].

#### Composition of essential oils of thyme

The chemical composition of essential oils of thyme is given in Table 3. It is known that the biological activity of essential oils depends on their chemical composition, which is influenced by environmental and agronomic conditions [27]. A total of 22 constituents were identified in essential oil TV1, 21 in essential oil TS, and 18 in essential oil TV2. The major components were as follows:  $\beta$ -cymene (8.64%),  $\gamma$ -terpinene (9.43%), thymol (38.53%), and carvacrol (10.11%) in TV1;  $\beta$ -cymene (16.53%),  $\gamma$ -terpinene (22.19%), and thymol (37.89%) in TS;  $\alpha$ -pinene (14.06%),  $\beta$ -cymene (10.29%), D-limonene (11.72%), thymol (11.01%), and 1,8-cineole (20.02%) in TV2.

Antimicrobial properties of the essential oils of thyme depend mostly on their phenolic constituents. The quantitatively most important compounds are the phenols thymol and carvacrol. Two different monoterpene phenols with similar properties are isomeric molecules. It is known that these two phenolic compounds have strong antimicrobial properties [28–30]. The determined content of these components in different samples of the essential oils of thyme was also different: 48.64% in TV1, 39.18% in TS, and only 14.89% in TV2.

High antimicrobial activity of the essential oils of thyme is also characterized by a high content of monoterpenes, such as hydrocarbons  $\gamma$ -terpinene and  $\beta$ -cymene [30]. The former constituent was detected in samples

TV1		TS		TV2	
Constituent	%	Constituent %		Constituent	%
α-Thujene	3.23	α-Thujene	3.12	α-Pinene	14.06
α-Pinene	1.46	α-Pinene	1.53	Camphene	0.38
Camphene	1.01	Camphene	0.82	β-Pinene 2	
Sabinene	0.33	β-Phellandrene	0.21	β-Myrcene	0.54
β-Pinene	0.31	β-Pinene	0.33	α-Phellandrene	0.14
1-Octen-3-ol	3.59	1-Octen-3-ol	1.37	(+)-4-Carene	8.35
β-Myrcene	2.62	β-Myrcene	2.93	β-Cymene	10.29
α-Phellandrene	0.34	α-Phellandrene	0.26	D-Limonene	11.72
(+)-4-Carene	2.00	(+)-4-Carene	2.33	1,8-Cineole	20.02
β-Cymene	8.64	β-Cymene	16.53	Linalool	0.04
Ocimene	0.66	1,8-Cineole	0.53	Terpinen-4-ol	1.54
γ-Terpinene	9.43	γ-Terpinene	22.19	Borneol	3.83
trans-Sabinene hydrate	0.84	trans-Sabinene hydrate	1.12	Terpinyl acetate	3.22
Borneol	1.45	Linalyl acetate	2.38	Linalyl acetate	1.19
Terpinene-4-ol	0.32	Borneol	0.94	Thymol	11.01
Methyl thymol ether	1.80	Terpinene-4-ol	0.33	Carvacrol	3.88
Thymol	38.53	Methyl thymol ether	1.96	Caryophyllene	2.72
Carvacrol	10.11	Thymol	37.89	Caryophyllene oxide	1.08
Caryophyllene	0.68	Carvacrol	1.29		
Germacrene	1.07	Caryophyllene	0.83		
β-Bisabolene	3.61	Menthol	0.48		
β-Phellandrene	4.77				

Table 3. The constituents of thyme essential oils TV1, TS, and TV2

TV1 and TS; the highest amount was obtained in TS (22.19%).  $\beta$ -Cymene was determined in all analysed samples, and its amount varied from 8.64% to 16.53%.

Monoterpene hydrocarbons of pinene type (a-pinene and  $\beta$ -pinene) are well-known chemicals with antimicrobial potentials [31]. Also, borneol has been reported to have significant antimicrobial activity [32,33]. A high content of borneol was established in sample TV2 (3.83%). The amount of  $\alpha$ -pinene and  $\beta$ -pinene in this sample was very high: 14.06% and 2.64%, respectively. Caryophyllene has a very high activity as an antiinflammatory and anti-bacterial material [34-37]. Caryophyllene oxide, an oxygenated terpenoid, well known as a preservative in food, drugs, and cosmetics, has been tested in vitro as an antifungal against dermatophytes and it passed the test. Its efficiency is comparable with the different antifungal drugs [38]. The presence of caryophyllene was determined in all samples, but caryophyllene oxide only in TV2.

# Antimicrobial properties of the essential oils of thyme

The next step was to investigate the antibacterial properties of selected essential oils. The results are presented in Table 4.

The essential oil of TV1 had the highest antimicrobial activity. The high sensitivity to oils was observed in the case of Gram-positive bacteria *S. aureus* and *B. cereus*. The Gram-negative bacteria *E. coli* were sensitive to all selected essential oils of thyme. However, none of the essential oils of thyme had affected *P. aeruginosa*. It was determined in previous studies that this bacterium is sensitive to the action of the essential oils of thyme [39,40], but in our investigation such effect was not observed. The commercial essential oil (TV2) was chosen for the further investigations due to the simpler technological application and sufficient antimicrobial properties.

**Table 4.** Dependence of the antibacterial activity on the type of the essential oil of thyme

Microorganism	Inhibition zone, mm			
	TV1	TV2	TS	
Staphylococcus aureus	52	50	38	
Bacillus cereus	35	12	21	
Escherichia coli	22	10	12	
Pseudomonas aeruginosa	-	-	-	

Note. See Table 2 for the notation of antibacterial assessment.

# Practical use of the essential oil of thyme as a preservative for wet-blue

The main aim of this investigation was to determine the possibility of using the essential oil of thyme as an antibacterial agent in leather processing. It is known that essential oils contain low molecular mass compounds, which could easily penetrate into the system [14], have a good solubility in fats, and are poorly miscible with water [41]. Fatliquors are offered to leather as emulsions and they are incorporated into the structure, influencing its properties. Also, the leather fatliquoring emulsion contains surfactants in its composition, which should better emulsify the essential oil in the system if added to the fatliquoring emulsion. This may lead to better distribution (and deeper penetration) of essential oils in the leather matrix during the treatment with emulsion in comparison with simple painting of leather surface and, presumably, ensure a longer preservation effect on leather.

The treatment with fatliquor–essential oil emulsion was carried out under the conditions described in Table 1. The commercial product TV2 was used as the essential oil in the treatment. The amount of the essential oil varied from 0.05% to 5% of the wet-blue mass. The results obtained in this experiment confirmed Gram-positive bacteria to be more sensitive to the essential oil of *Thymus vulgaris* (TV2) than Gramnegative bacteria. The results also show that the amount of essential oil used had to be higher than 3%. The lower amounts (0.05% and 1.0%) did not show inhibition zones, although the leather samples stayed resistant to the action of the selected bacteria. The conventionally used synthetic preservative did not protect better than treatment with 3-5% of the essential oil of TV2.

Resistance of treated leather against bacteria during the storage of the samples was estimated. The increase (Table 5) in the amount of the used essential oil intensified the resistance of leather to bacteria's action. The highest resistance of samples was observed to the Gram-positive bacteria S. aureus and B. cereus. Also, it can be noted that inhibition zones increased over time. This fact can be explained by the migration of fat through the derma matrix [42]. Treatment with 5% of essential oil increased the sensitivity of samples to E. coli. None of the treatment methods increased chromed leather resistance to P. aeroginosa. Still, such result is acceptable because the bacteriostatic effect was achieved. This means that the growth of this bacterium over the samples was stopped. The use of the synthetic preservative (Table 5) ensured leather samples resistance to P. aeruginosa only for 2 weeks.

**Table 5.** Dependence of the inhibition zone (mm) of leather samples on storing duration: control – chromed leather treated with a mixture of fatliquors and *Fungicide FDE*; treatments – chromed leather treated with an emulsion containing fatliquor and different percentages of essential oil of *Thymus vulgaris* 

Micro- organism	Duration of sample storage, weeks			Dura sto	ation o orage,	of sam week	ple s	
	1	2	3	4	1	2	3	4
		Control				% ess	ential	oil
S. aureus	5	3	0	0	0	0	0	0
B. cereus	4	2	0	0	0	0	0	0
E. coli	5	0	0	0	0	0	0	0
P. aeruginosa	3	3	0	0	0	0	0	0
	0.5% essential oil				1%	b esse	ntial c	oil
S. aureus	0	0	0	0	2	3	0	0
B. cereus	0	0	0	0	0	0	0	0
E. coli	0	0	0	0	0	0	0	0
P. aeruginosa	0	0	0	0	0	0	0	0
	3% essential oil				5%	b esse	ntial c	oil
S. aureus	10	17	*	*	11	19	*	*
B. cereus	5	3	10	4	11	11	20	20
E. coli	2	2	0	0	4	4	4	4
P. aeruginosa	0	0	0	0	0	0	0	0

Note. See Table 2 for the notation of antibacterial assessment.

One of our objectives was to investigate the possibility of reducing the use of the synthetic preservative by replacing it with the essential oil of *Thymus vulgaris*. The active agent in *Fungicide FDE* is TCMTB. This heterocyclic compound was first used on leather in the early 1970s [43,44], and in the 1980s it was tested (in the form of a commercial product containing 30% of TCMTB) both in the laboratory and in tanneries [44]. The greatest disadvantage of antimicrobial agents used nowadays in the leather tanning industry is their poor stability. They are very sensitive to moderate pH and humidity alteration.

The authors supposed that application of a mixture of essential oil and synthetic preservative could solve two problems at once: first, decrease the amount of the synthetic preservative for leather treatment and thus reduce an irritant effect to customers, and second, decrease the amount of essential oil, which is fairly expensive.

The manufacturers proposed that the amount of *Fungicide FDE* should be 0.2% of the tanned leather mass. The chromed leather samples were treated using the following methods (% of wet-blue mass):

1 – only 0.2% Fungicide FDE;

2-1.25% essential oil and 0.15% Fungicide FDE;

3 - 2.5% essential oil and 0.10% Fungicide FDE;

4 – 3.75% essential oil and 0.05% Fungicide FDE;

5 – only 5% essential oil.

The obtained results are presented in Table 6.

 Table 6. Dependence of the inhibition zone of leather samples

2

Δ

12

0

10

0

Microorganism	Inhibition zone, mm				
	1	2	3	4	5
S. aureus	0	0	0	2	0
B. cereus	3	2	3	15	5

on leather treatment methods 1-5

E. coli

P. aeruginosa

Note. See Table 2 for the notation of antibacterial assessment.

0

Δ

0

0

It is evident that leather samples were preserved better when more essential oil and less *Fungicide FDE* was used (Table 6, methods 4 and 5). When the amount of essential oil was smaller, the resistance of leather samples to bacteria's action decreased. It is also possible to state that essential oil was a more active antimicrobial agent than synthetic one in this composition. The inhibition zone in the case of *P. aeruginosa* was not detected, but leather samples remained resistant to this bacterium (bacteriostatic effect).

Also, it was observed that the effiency of *Fun-gicide FDE* was lower when it was added into the fatliquoring emulsion in comparison with its addition into the pickling solution. This result confirms the statement that TCMTB is accumulated by fats, which leads to the weakening of the antibacterial protection of the leather samples [42].

Usually, the efficiency of leather processing technologies can be shown through the qualitative properties of variously treated leather and through comparison of these properties. In conformity with previously received results, sufficient antimicrobial protection of tanned leather samples was reached with using 5% TV2 (of the wet-blue mass). So, leather was treated according to two methods: 1 – leather was fatliquored with addition of 5% TV2; 2 – leather was processed conventionally by adding *Fungicide FDE* into the pickling solution (control). The qualitative indexes of treated leather are presented in Table 7.

Table 7. Indexes of the leather after fatliquoring

Index	Method of leather tanning				
	1	2 (control)			
Tensile strength, N/mm <sup>2</sup>	16.7	19.5			
Relative elongation of chromed	66.5	54.0			
leather under 10 N/mm <sup>2</sup> load, %					
Content of Cr <sub>2</sub> O <sub>3</sub> in leather, %	4.32	4.39			
Content of matter soluble in	3.57	3.40			
dichloromethane, %					
Content of moisture, %	12.4	12.4			
Shrinkage temperature, °C	113	113			

matrix evenly due to decreased stability of the fatliquoring emulsion after the addition of the essential oil. Uneven distribution of fats in derma has no influence on the chemical properties of leather but it reduces the tensile strength of the sample.

## CONCLUSIONS

The essential oil of *Thymus vulgaris* could be used as a preservation agent in the leather tanning industry. Analyses have shown differences between commercial and pure essential oils of thyme. The composition of essential oils influences their antibacterial activity. Gram-positive bacteria were more sensitive to the essential oils of thyme compared to Gram-negative bacteria. *Pseudomonas aeruginosa* was the least sensitive to the action of all selected essential oils. If essential oils are used as a preservative for chromed leather, their amount should not be less than 3% of the wet-blue mass.

The essential oils of thyme can be used as a preservative in a mixture with a synthetic biocide. In this case the amount of the necessary synthetic biocide can be reduced from 0.2% to 0.05% of the wet-blue mass. In the effectual mixture the amount of the biocide should be 0.05% and that of the essential oil of thyme 3.75% of the wet-blue mass.

Treatment of leather with the essential oil of thyme simultaneously with fatliquoring has an influence on the strength properties of the leather. Addition of essential oil into the fatliquoring emulsion changes the stability of the fatliquoring emulsion and the tensile strength of the leather becomes lower. On the other hand, such addition of the essential oil of *Thymus vulgaris* into fatliquoring emulsion does not change the chemical quality indexes of leather.

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# Tüümianiõlide kasutamisest loodusliku säilitusainena nahaparkimisel

Justa Širvaitytė, Jūratė Šiugždaitė, Virgilijus Valeika ja Edita Dambrauskiene

Uuriti võimalust kasutada tüümiani eeterlikke õlisid alternatiivse säilitusainena naha kroomparkimisel. Selgitati kaubanduslike ja puhaste tüümianiõlide keemilise koostise erinevused. Täheldati, et need erinevused mõjutavad eeterlike õlide antibakteriaalset toimet. Tulemused näitavad, et grampositiivsed bakterid on tüümiani eeterlike õlide suhtes tundlikumad kui gramnegatiivsed. *Pseudomonas aeruginosa* on madala tundlikkusega valimi eeterlike õlide osas, kuid samade õlidega töödeldud nahaproovid on nende bakterite suhtes vastupidavad. Põhiliseks järelduseks on tüümiani eeterlike õlide kasutatavus säilitusainena nahaparkimisel. Tüümiani eeterlike õlidega töödeldud nahaproovid (kui õli on parkimissegus vähemalt 3%) osutusid nelja nädala järel paremini kaitstuks kui 2-(tiotsüanometüültio)bensotiasooliga töödeldud nahk. Segus sünteetilise biotsiidiga on tüümiani eeterlik õli naha konserveerimise seisukohast aktiivsem komponent.