Research article

Antimicrobial effect of lining leather fatliquored with the addition of essential oils

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Abstract: *Footwear, especially those often and intensively used, provides an ideal environment for microbial growth. An appropriate high temperature and moisture content inside it enhance bacterial and fungal colonization by microbes. These microorganisms can be potentially pathogenic to human health and responsible for destruction of shoes materials from which the parts inside the shoe are made. In previous studies it has been shown that the addition of essential oils of antiseptic activity at leather finishing operations allows a leather to get antimicrobial properties. The aim of this paper is to assess the durability of antimicrobial effect of leather fatliquored with the addition of cinnamon, thyme and oregano essential oils at concentration of 5% per leather weight. Antimicrobial activity was evaluated according to guidelines of PN-EN ISO 20645:2006 "Textile fabrics – determination of antibacterial activity – Agar diffusion plate test" after 36-month storage. The obtained results have indicated that leathers under investigations show antimicrobial activity against E. coli, S. aureus and C. albicans strains even 3 years after fatliquoring. The use of natural bioactive substances such as cinnamon, thyme and oregano oils at concentration of 5% per leather weight in the leather finishing process may be an alternative to biocides used in the tanning industry as well it can improve hygienic properties of shoes, internal parts of which are made of such finished leathers.*

Keywords: *lining leathers, essential oils, antimicrobial finish, leather hygienic properties.*

Introduction

Animal skin undergoes microbiological decay since as an organic material is a source of feed for microbes. Different bacterial and fungal species, for example the genus *Bacillus*, *Corynebacterium*, *Clostridium*, *Staphylococcus, Penicillium*, *Aspergillus*, *Paecilomyces*, *Candida* and *Cryptococcus* [1-3] are responsible for destruction of leather and leather products. The mechanism of the deterioration of leather and leather products by microbes is primarily related to enzymatic decomposition of material. This leads to leather embrittlement, loss of elasticity, decrease in collagen molecular weight, unpleasant smell is felt, and spots appear on its surface [4]. Discolourations and spotting resulting from the presence of microorganisms may be grey-white, violet, red, grey or black [5]. Among various industrial products the degree of susceptibility of natural leathers to biodegradation and microbiological interactions is estimated as medium and takes second place on a four-step scale (where 1 – low degree assigned to glass and household chemicals, while 4 – very high degree is assigned to natural textile products and wood and wood products) [6].

The presence of microorganisms on the surface leathers being processed in tanneries involves not only deterioration of raw materials at different stages of tanning or later defects visible in finished leathers. Skóra et al. [7] indicate also an increased amount of microbes in the air in tanneries as compared to atmospheric air that may have an adverse effect on the health of employees due to inhalation exposure. There are pathogenic some bacteria and fungi among many microbes coming from tanneries and identified by researches. These microorganisms may cause allergic reactions, food poisoning, toxic effect or infections in employees with weakened immune system [3].

Also finished products such as footwear may be colonized by fungi and bacteria. The carbon source for bacterial growth will be sweat compounds of footwear users and other compounds contained in shoe materials [5, 8]. Apart from the question of the deterioration of material also hygienic properties is an important item for footwear. Footwear, especially those often and intensively used, provides an ideal environment for microbial growth, including pathogenic species causing athlete's foot (*tinea pedis*) and bacterial foot infections. This is connected with an appropriate temperature and moisture content inside enhancing microbial growth [8-10]. Conferring antimicrobial properties to leather lining inside shoes, because of its long time use, require a prolonged protective effect.

Essential oils are aroma products extracted from plants mainly by steam distillation. Depending on the type and amounts of compounds contained, essential oils show various properties, e.g. they inhibit growth of infectious microbes, prevent fungal growth, accelerate wound healing and mitigate inflammations [11].

There were attempts worldwide to use essential oils as natural bioactive substances for protecting leathers in the tanning industry [12-14]. These studies confirmed the effectiveness of the oils used. It was also shown that leather protected with *Origanum minutiflorum* oil (in some cases at oil concentration of 0.5% per leather weight) [13] or *Thymus vulgaris* oil (at oil concentration of 3% per leather weight) [14] is more resistant to microorganisms than chemical biocide-protected samples (in doses recommended by manufacturers) used in the tanning industry. Also experiments carried out by the authors proved that the use of cinnamon, thyme and oregano essential oils at concentration of 5% per leather weight allows leathers to get antimicrobial activity. These studies conducted after 1 and 6 months from introducing oils into the material showed antimicrobial activity of leathers against strains of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* [15-16].

The aim of this paper is to present the results of investigation of the durability of antimicrobial effect of cowhide lining leather fatliquored with the addition of cinnamon, thyme and oregano essential oils at concentration of 5% per leather weight. The antimicrobial activity was tested after 36 months from introducing oils into leather against reference bacterial strains of *Staphylococcus aureus*, *Escherichia coli* and yeast *Candida albicans*.

Experimental

Materials

The tests were carried out on an industrially chrome tanned cowhide leather and shaved to a thickness of 1.4 to 1.6 mm. Such "wet-blue" leather was purchased from the PPHU TECHNO-SKÓR company (Poland).

The leather was then sequentially treated at laboratory scale in a Wacker device drums (Figure 1) according to the standard processing procedure. As shown in Table 1 leather bath finishing involved the following consecutive operations: soaking, retanning I, rinsing, retanning II, fixation and rinsing. The three types of tanning agents were used during the process, namely polymeric, mineral and vegetable. The Regulan RE polymeric tanning agent effects leather filling, Chromitan B (a chrome tanning agent counted among mineral agents) gets a leather a desired softness, while vegetable tannins (mimosa) fill the leather in its weaker places and have also a softening effect [17]. Perfectol HQ fatliquor as well as baking soda (use twice to obtain the desired pH at the end of retannig I), sodium formate, ammonia water, formic acid and water were used in this experiment.

Figure 1. Wacker device with drums
Source: own research.

At the stage of retanning II the following essential oils were introduced by one hour at concentration of 5% per leather weight :

- cinnamon oil extracted from *Cinnamomum zeylanicum* and originated from Sri Lanka,
- thyme oil extracted from *Thymus vulgaris* and originated from Turkey,
- oregano oil extracted from *Origanum vulgare* and originated from Portugal.

According to the manufacturer's declarations all oil were obtained by steam distillation.

Table 1. Leather finishing bath

Process stage	Chemical compound/agent used	Percentage to leather weight %
soaking	water	250
retanning I	water	150
	Chromitan B	3
	baking soda	0.2
	sodium formate	2
	baking soda	1.7
rinsing	water	250
retanning II	water	100
	Perfectol HQ	1
	Relugan RE	3
	mimose	5
	Relugan RE	2
	water	50
	water	100
	ammonia water	0.2
	Perfectol HQ	5
	Dekalin SE	0.2
	essential oil	5
fixation	water	100
	formic acid	0.5-1.6
rinsing	water	200

Source: own research.

When fatliquoring leathers with essential oils an emulsifying agent (Dekalin SE) was additionally used to facilitate oil penetration into leather. This agent was not used in fatiquoring the samples without the addition of essential oils which were a reference material.

The fatliquored original samples (rectangles of 150 × 250 mm in size) after excess moisture removal (draining and drying) were placed in paper envelopes and stored at room temperature for 36 months. Afterwards, circular laboratory specimens of 25±5 mm in diameter were cut from original samples.

Methods

When assessing antimicrobial activity of leathers fatliquored with the addition of cinnamon, thyme and oregano essential oils at concentration of 5% per leather weight after 36 months from fatliquoring, the two standard bacterial species recommended by PN-EN ISO 20645:2006 [18], and additionally one yeast species (Table 2) were used, while adapting the methodology to leather products. All strains used in this study came from the reference strain collection.

The tests were carried out on TSA agars for bacteria and Saboraud dextrose agar for yeasts. The growth inhibition zones and microbial growth under the leather specimens were determined after incubation at 36±1°C for 18-24 hours for bacteria and 48 hours for yeasts.

Table 2. Reference microorganism strains used in the experiments

Microorganisms	Strain No.
Bacteria	
Staphylococcus aureus	ATCC 6538
Escherichia coli	ATCC 8739
Fungi	
Candida albicans	ATCC 10231

Source: own research.

Results and discussion

The results of investigation of antimicrobial activity of cowhide lining leathers finished without or with the addition or cinnamon, thyme and oregano essential oil at concentration of 5% per leather weight after 36 month-storage of leather specimens are presented in Table 3.

For leather specimens finished without the use of essential oils no growth inhibition zone was observed around the leather for any microbe under investigation. A poor growth of yeasts *Candida albicans* and moderate growth of *Staphylococcus aureus* was observed under specimens. However, no growth of *Escherichia coli* was recorded, that was probably caused by too strong adhesion of specimens to the substrate, thus avoiding bacterial growth, which was observed in previous study.

For all leathers enriched with essential oils no microbial growth under specimens was observed (Table 3). The strongest antimicrobial effect of leather was recorded against yeasts *Candida albicans*, wherein sharply outlined inhibition zones extending up to dozen of millimetres were observed depending on type of oil used for leather finishing. For specimens treated with thyme oil the zone of 5 mm in size was recorded (Figure 2a), 5-12 mm for cinnamon oil (Figure 2b) and 7-13 mm for oregano oil (Figure 2c), depending on the sample. Comparable results for antimicrobial activity of leather with these oils against *Candida albicans* were obtained for specimens analysed after 1 and 6 months from introducing oils into lining leathers [15], thus indicating its long-lasting action.

Table 3. An assessment of antimicrobial effect of leathers fatliquored without and with essential oils added (at concentration of 5% per leather weight) after 36-month storage

Microorganisms	**Growth inhibition zones around specimens [mm] Microorganism growth evaluation according to the scale[a/]**			
	leather fatliquored without oil	**leather fatliquored with addition**		
		cinnamon oil	**thyme oil**	**oregano oil**
Staphylococcus aureus	0	0-1	0	0-1
	0 moderate growth under the specimen	0-1 no growth under the specimen	0 no growth under the specimen	0-1 no growth under the specimen
Escherichia coli	0	0-1	1	0-1
	0[1/] no growth under the specimen	0-1 no growth under the specimen	>1 no growth under the specimen	0-1 no growth under the specimen
Candida albicans	0	5/12	5	7/13
	0 poor growth under the specimen	>1 no growth under the specimen	>1 no growth under the specimen	>1 no growth under the specimen

[a/] PN-EN ISO 20645:2006 [18] for bacteria and yeasts.
[1/] strong specimen adhesion.

Source: own research.

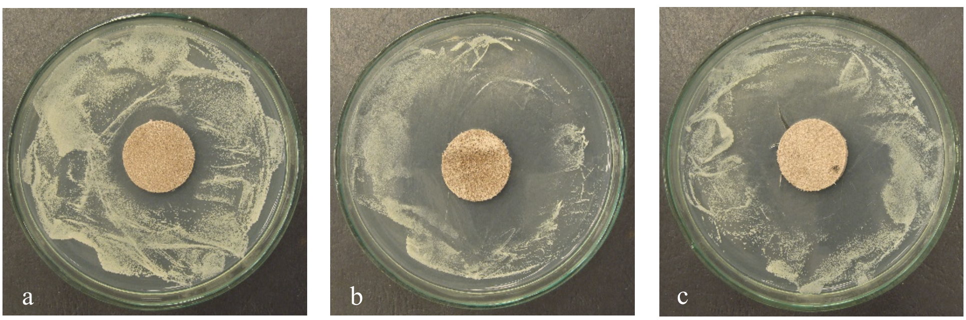

Figure 2. Leather fatliquored with (a) thyme oil, (b) cinnamon oil, (c) oregano oil (5%), view from the flesh side – growth inhibition zone for the yeast *Candida albicans* (after 36-month storage)

Source: own research.

Smaller inhibition zones than those of yeasts growth, were still considered as a good antimicrobial effect according to PN-EN ISO 20645:2006 [18], which were recorded against *Escherichia coli* and *Staphylococcus aureus* (Table 3). For the first of strains mentioned above the growth inhibition zone of 1 mm in size was observed around specimens fatliquored with thyme oil (Figure 3a). The zones recorded for leathers with cinnamon oil (Figure 3b) and oregano oil (Figure 3c) were up to 1 mm in size. Compared to the antibacterial activity tested after a shorter storage time (1 and 6 months after fatliquoring) the obtained effect was weakened. It was showed as a smaller growth inhibition zones (even up to few millimeters), especially around the samples with oregano oil [15].

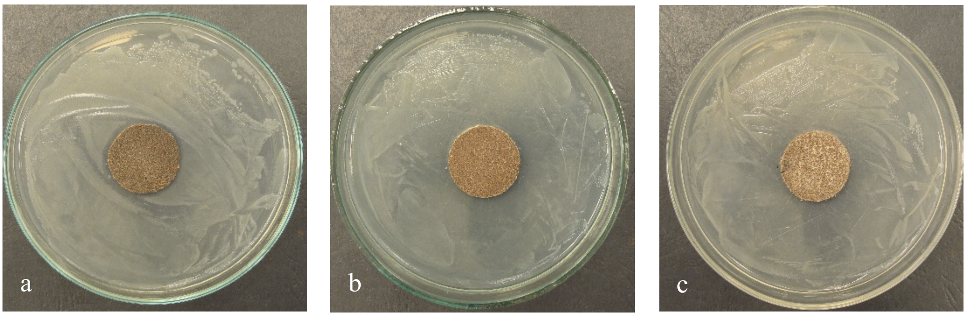

Figure 3. Leather fatliquored with (a) thyme oil, (b) cinnamon oil, (c) oregano oil (5%), view from the flesh side - growth inhibition zone for the bacteria *Escherichia coli* (after 36-month storage)

Source: own research.

As shown in Table 3 the leather enriched with cinnamon oil showed antimicrobial activity against the strain *Staphylococcus aureus* visible as the inhibition zone of 1 mm in size (Figure 4a). For leathers with oregano oil no growth of this bacterium was found within 1 mm from specimens (Figure 4b). However, no growth inhibition zone was recorded for *Staphylococcus aureus* for leathers treated with thyme oil (Figure 4c), while there was no growth under the specimen. As in case of *Escherichia coli*, the growth inhibition zone of *Staphylococcus aureus* were smaller compared to those obtained after shorter storage periods; although this effect is still considered as a good according to a standardized assessment [15].

Some tests aimed at an evaluation of possible contamination of leather specimens fatliquored with the addition of essential oil, which could take place during the manufacturing process or storage were carried out. No microbial contamination was observed after 48-hours incubation fatliquored leather samples on sterile media. This indicates that oils may show protective action against other than investigated strains, that could contaminate specimens during storage, and postulated also effective activity of oils against microflora present in the production environment.

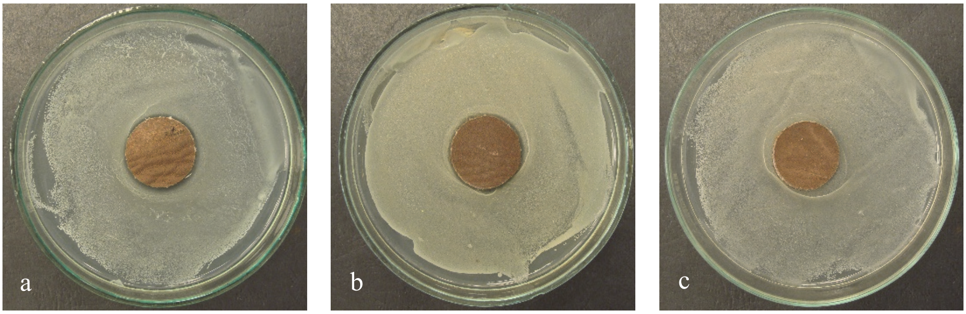

Figure 4. Leather fatliquored with (a) cinnamon oil, (b) oregano oil, (c) thyme oil (5%), view from the grain side. Growth inhibition zone (a, b) and no growth inhibition zone (c) for the bacteria *Staphylococcus aureus* (after 36-month storage)

Source own research.

Summary

The studies carried out and the obtained results indicate that lining leathers finished with the addition of cinnamon, oregano or thyme oils at concentration of 5% per leather weight show antimicrobial activity against strains of *E. coli*, *S. aureus* and *C. albicans*. This effect is long-lasting as confirmed by tests conducted after 12-month (in previous experiments) and even 36-month storage of materials.

The use of mentioned bioactive substances at the stage of leather fatliquoring can be an alternative to biocides used in the tanning industry as well contribute to improving hygienic properties of footwear with internal parts made of such finished materials. Leathers enriched with essential oils showing antimicrobial activity for the period of 3 years can be used in manufacturing special footwear and having a supporting effect in treating foot ailment.

The use essential oils in leather finishing is novel technological solution to possess leather hygienic properties. Introducing this kind of substances which has a volatile nature to the leather is very difficult but it was achieved with success. What is more, such durability of antimicrobial leather finishing after long storage has not been confirmed in another tests before.

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