The Durability of Antimicrobial Effect of Leathers Finished with Oregano Oil

by
Elżbieta Bielak,[1] Ewa Marcinkowska[1]* and Justyna Syguła-Cholewińska[2]
[1]*Department of Industrial Commodity Science, Faculty of Commodity Science and Product Management, Cracow University of Economics,* Sienkiewicza 4, 30-033 Cracow, Poland
[2]*Department of Microbiology, Faculty of Commodity Science and Product Management, Cracow University of Economics,* Rakowicka 27, 31-510 Cracow, Poland

Abstract

Essential oils introduced into raw materials in the technological process enable leather to gain antimicrobial properties. These properties were examined toward microorganisms potentially pathogenic to humans and causing biodegradation of leather. The article presents an assessment of antimicrobial activity of cowhide lining leathers fatliquored with the addition of oregano oil at concentrations of 1% and 3% per leather weight after one month storage. Additionally, the durability of this activity for leathers with oregano oil at concentration of 3% was confirmed after 12 months. Antimicrobial effect was tested according to PN-EN ISO 20645:2006 Textile fabrics – Determination of Antibacterial Activity – Agar Diffusion Plate Test. Furthermore, to verify the results of antimicrobial durability of leathers within one year after fatliquoring, the method according to ISO 22196:2011 Measurement of antibacterial activity on plastics and other non-porous surfaces was used.

Antimicrobial effect of finished leathers against bacteria *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli,* yeast *Candida albicans* and filamentous fungus *Scopulariopsis brevicaulis* was tested. The obtained results indicated that lining leathers enriched with oregano oil at concentration of 3% per leather weight is characterized by its good and durable antimicrobial effect persisting even one year after adding oil into leather. The results presented in this paper is a continuation of previous studies on animal leather enriching with essential oils.

Introduction

Biocides used in the tanning industry are divided into two principal groups: bactericidal and fungicidal[1] depending on kind of organisms against which leather is to be protected. They must be used due to the fact that the presence of microorganisms in raw material and during its processing, storage and transport is associated with a damage to leather. However, these substances may have a harmful effect on human health. Śpiewak[2] paid attention to a high sensitizing potential of biocides used in leathers (see Figure 1) by referencing, among other things to research 2-(thiocyanomethylthio)benzothiazole.[3]

The problem is also the presence of microorganisms in finished products made of leather, e.g. shoes. This is particularly important for people who are especially sensitive to the presence

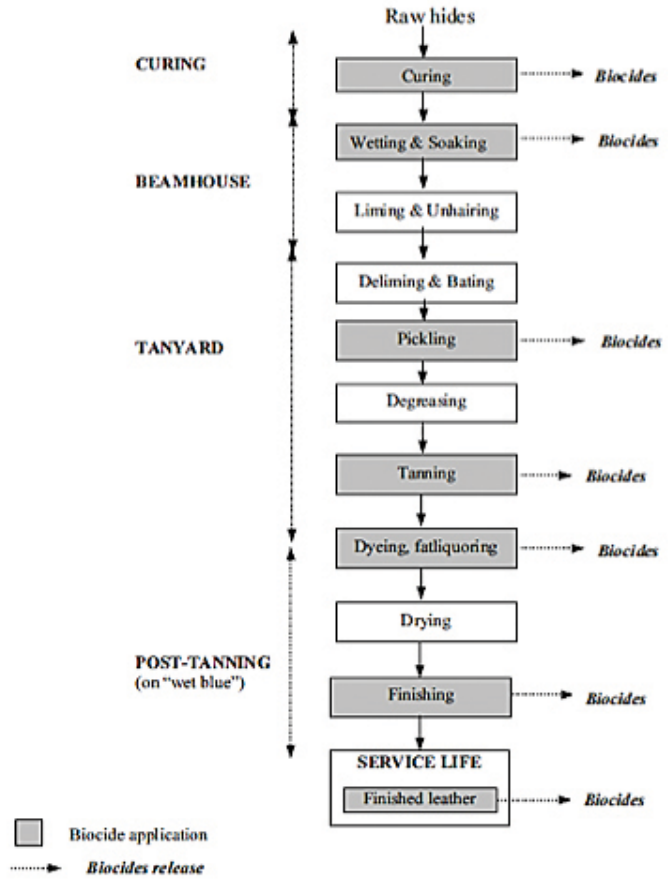

Figure 1. Main processes and releases of the "leather tanning"[4]

*Corresponding author e-mail: etmarcin@cyf-kr.edu.pl
Manuscript received April 22, 2017, accepted for publication July 28, 2017.

of bacteria and fungi as well deal with fungal skin diseases such as mycosises of the foot and toenails. The moisture content and temperature inside the shoes, especially those used for many hours a day create favorable conditions for microbial growth, thus promoting infections and reinfections. Currently there are a lot of preparations for foot and shoe protection against harmful action of bacteria and fungi available in the market.[5] They contain chemical substances of antimicrobial properties, e.g. Triclosan and some of them comprise also natural agents like tea tree oil of the same action.

Only a few studies made all over the world[6-9] on substituting biocides in the tanning industry with essential oils of proven antimicrobial activity indicated that it is possible to eliminate harmful effect of these agents on humans. This also indicated a possibility for improving the leather processing process with a view of ecological aspects.

An example of essential oil of strong antimicrobial action is oregano oil derived from *Origanum vulgare*.[10,11] This is a perennial herb with erect hairy stalk about 80 cm tall. *Origanum vulgare* grows in dry grassy places as well in open shrub lands, woodland edges and on hills.[12]

The results of previous experiments of enriching cowhide lining leathers with essential oils to give antimicrobial properties to them allows to select two essential oils. Those oils namely cinnamon and oregano provided the best antimicrobial effect after introducing into leather at amount of 5% both after 1 as well as after 6 months of storage.[13] The next step of this research was to determine the lowest concentrations of selected oils inhibiting microbial growth. Antimicrobial activity of leathers fatliquored with the addition of cinnamon oil at concentrations of 1% and 3% after one month of storage is presented by Bielak *et al.* (2016).[14]

The aim of the study presented in the present paper was to determine antimicrobial activity of leathers fatliquored with the addition of oregano oil at concentrations of 1% and 3% per leather weight after one month of storage and antimicrobial durability of leathers fatliquored with the addition of oregano oil at concentration of 3% per leather weight after 12 months of storage. The reference strains of bacteria *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli,* yeast *Candida albicans* and filamentous fungus *Scopulariopsis brevicaulis* were tested. These strains are recommended to the tests because they are usually active on leathers in various dressing processes and can cause foot skin and toenails infections.[15-19]

The evaluation of activity of leathers fatliquored with addition of oregano oil (1% and 3%) after one month of storage and antimicrobial durability of leather enriched with oregano oil (3%) after 12 months of storage was made according to the method presented in PN-EN ISO 20645:2006.[20] To verify the results obtained from leathers tested after one year from fatliquoring, the frontier method of the inhibition of bacterial growth according to ISO 22196:2011,[21] hitherto applied to other materials, was used.

Experimental

Materials

Leather

Wet-blue lining leather of 1.4-1.6 mm in thickness derived from raw cowhide 12-16 kg in weight by employing the standard chrome tanning process. The leather was purchased from the "TECHNO SKÓR" company.

Essential Oil

To confer antimicrobial properties to leather oregano oil, derived from *Origanum vulgare* belonging to the *Lamiaceae* family were used. The oil has been provided from Laboratorium Biologii Przemysłowej i Eksperymentalnej PWSZ im. St. Pigonia w Krośnie (Poland) – *Industrial and Experimental Biology Laboratory at PWSZ in Krosno*. The oil came from Portugal and was obtained by steam distillation. Chromatographic analysis[22] indicated that oregano oil used in the tests contained predominantly carvacrol (94.06%) with respect to all organic compounds present in the oil. This compound has antimicrobial properties,[23,24] thus is responsible for antimicrobial action of the oil containing it.

Methods

Preparing Leather for Testing

Original samples of 150x250 mm in size were cut from "wet-blue" leather purchased from the tannery. The samples were weighed and placed in triples in Wacker type drums for laboratory leather bath finishing. The following operations were performed in sequence: soaking, retanning I, rinsing, retanning II, fixation, and rinsing. During retanning II oregano oil at concentration of 1% and 3% (w/w) was added to give the leather antimicrobial properties. After the 8-hour finishing cycle the original samples were allowed to dry in hanging position for 24 hours, and then they were kept in horizontal position for 10 days to remove excess moisture. Afterwards, they were placed in paper envelopes and stored at room temperature of 22-25˚C. The control samples were leathers fatliquored as described above without the essential oil and emulsifying agent.

The original samples fatliquored with and without the addition of oregano oil were cut into laboratory specimens. According to guidelines outlined in PN-EN ISO 20645:2006[20] circular specimens of 25±5 mm in diameter, while according to recommendations given in ISO 22196:2011[21] square specimens of side 50±2 mm were prepared.

Microbiological Examination

A. Agar Diffusion Plate Test

The evaluation of antimicrobial activity of leathers fatliquored with addition of oregano oil at concentrations of 1% and 3% per leather weight after one month of storage and antimicrobial durability of leather enriched with oregano oil at concentration of 3% after 12 months of storage was made according to PN-EN ISO 20645:2006.[20]

The two bacterial species *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) recommended by PN-EN ISO 20645:2006[20] were used in tests. Besides the strains mentioned above the leather resistance against bacterial species *Staphylococcus epidermidis* (ATCC 12228), yeast *Candida albicans* (ATCC 10231) and one filamentous fungus *Scopulariopsis brevicaulis* (IHEM 2227) was also tested. These additional microorganisms used in testing were selected due its capability to cause foot skin and toenail diseases and animal leather decompositions.[15-19]

The circular leather specimens of 25±5 mm on diameter cut from the original samples previously fatliquored with and without the addition of the oil (control specimens) were tested. Before testing the specimens were conditioned at room temperature of 22-25˚C for 24 hours in sterile Petri dishes.[20] The following media were used during testing:

- TSA (Tripticase soy agar) – bacteria culture medium,
- SGA (Sabouraud-4% glucose agar) – fungal culture medium.

Bacterial suspensions were prepared from 24 hours cultures of reference strains in sterile saline solution and brought to a 0.5 McFarland standard (1,5 x 10^8 cfu/ml) based on densitometric measurements made on DENSIMAT densitometer (bioMerieux). The yeast suspensions to 1 McFarland standard (3,0 x 10^8 cfu/ml) were prepared from cultures of *Candida albicans*. For filamentous fungi, the suspension was brought to a density of 1x10^6 cfu/ml based on counting in a Bürker's chamber. The Petri dishes containing agar were inoculated with these microbial suspensions at amount of 0.1 ml (cm^3). The disks of leather facing the grain side upwards were placed centrally in Petri dishes by using sterile tweezers.

The test was carried out on three repetitions for each microorganism by checking antimicrobial activity of leathers fatliquored with the addition of oregano oil at concentrations of

Table I
Antibacterial effect of the antibacterial treatment.

Inhibition zone (mm) Mean value	Growth[a)]	Description	Assessment
>1	none	inhibition zone exceeding 1mm, no growth[b)]	good effect
1-0	none	inhibition zone up to 1 mm, no growth[b)]	
0	none	no inhibition zone, no growth[c)]	
0	slight	no inhibition zone, only some restricted colonies, growth nearly totally suppressed[d)]	limit of efficacy
0	moderate	no inhibition zone, compare to the control growth reduced by half[e)]	insufficient effect
0	heavy	no inhibition zone, compared to the control no growth reduction or only slightly reduced growth	

[a)] The growth of bacteria in the nutrient medium under the specimen.
[b)] The extent of the inhibition shall only partly be taken into account. A large inhibition zone may indicate certain reserves of active substances or a weak fixation of a product on the substrate.
[c)] The absence of bacterial growth, even without inhibition zone, may be regarded as a good effect, as the formation of such inhibition zone may have been prevented by a low diffusibility of the active substance.
[d)] As good as no growth" indicates the limits of efficacy.
[e)] Reduced density of bacterial growth means either the number of colonies or the colony diameter.

1% and 3% after 1 month of storage. Due to repeatability of test results, the experiments concerning antimicrobial durability of leathers fatliquored with the addition of oregano oil (3%) after 12 months of storage were performed on two repetitions only.

For unfatliquored "wet-blue" leathers or fatliquored with preparations showing no or weak antimicrobial properties there is a possibility of microbial contamination during production. To reveal if leathers were contaminated with microorganisms capable to deteriorate leathers or cause harm to the user, the specimens fatliquored with oregano oil at different concentrations (one for each experiment) were placed onto sterile culture media (TSA or Sabouraud dextrose agar). Microorganisms were cultured in laboratory incubator for:

- 18 to 24 hours at 37±1°C for bacteria,
- 48 hours at 37±1°C for yeast,
- 14 days at 29±1°C for filamentous fungi.

After incubation, the presence of a zone of inhibition of microorganisms around the specimen as well the presence of microbial growth on agar under the specimen were evaluated.

Table II
Assessment schematic for the growth of filamentous fungi on the agar.

Degree of growth	Assessment
0[a)]	no visible growth observed under a microscope (at 50x magnification)
1[a)]	no visible growth without magnification devices but clearly visible under a microscope
2	visible growth without magnification devices, intensity up to 25% of the control growth
3	visible growth without magnification devices, intensity up to 50% of the control growth
4	considerable growth, intensity exceeding 50% of the control growth
5	heavy growth, the same intensity as that of the control growth

[a)]Inhibition means „no growth" on agar around the specimen. Incomplete inhibition (reduced growth) should not be considered as inhibition. If fungal growth in the vicinity of the test specimen is partially inhibited it should be recorded.

Simultaneously (on three or two repetitions) the control specimen (leather fatliquored without the addition of oil) was tested by using the procedure described above. The effect of antimicrobial activity of cowhide lining leathers fatliquored with oregano oil was assessed with respect to reference strains of bacteria and yeast (Table I) according to PN-EN ISO 20645:2006[20], and to PN-EN 14119:2005[25] for filamentous fungi (Table II).

B. Method of the Reducing of Bacterial Growth

The antibacterial effect of lining leathers fatliquored with the addition of essential oil derived from *Origanum vulgare* (at concentration of 3% by leather weight) was examined according to ISO 22196:2011.[21] The examination method was adjusted to the tested material and the growth of selected microorganisms.

The standard bacterial strains *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) were used. The leather specimens in the shape of squares 50±2 mm with an average thickness of 1.57 mm were cut from leather pieces fatliquored with the addition of oregano oil and without this oil (control samples). Microorganisms were cultured on the following media:

- TSB (Tripticase soy broth) – bacteria culture medium,
- TSA (Tripticase soy agar) – bacteria culture medium,
- Baird-Parker agar – selective medium for culture of *Staphylococcus aureus*,
- TBX (Tryptone Bile X-Glucuronide) agar – selective medium for culture of *Escherichia coli*.

Selective media were additionally used to avoid contamination of leather specimens with other microflora than that under investigation, that could occur in specimens showing no antimicrobial activity and make more difficult interpretation of test results.

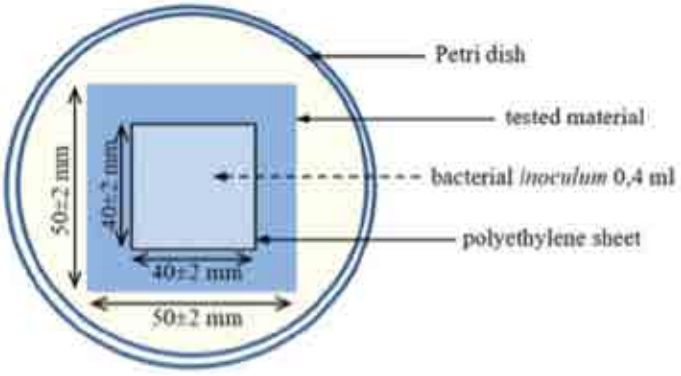

Figure 2. Schematic diagram of the test system based on ISO 22196:2011[21]

The bacterial suspensions of *Staphylococcus aureus* and *Escherichia coli* were prepared from the 24 hours broth culture incubated at 35±1˚C. The prepared bacterial *inoculum* in sterile saline solution (0.9% NaCl) was brought to McFarland Standard No. 2 (6 x 10^8 cfu/ml) based on densitometric measurements made on the DENSIMAT (bioMerieux) instrument. The suspensions were then diluted on TSB to obtain the recommended final concentration of 6 x 10^5 cfu/ml.

The test samples were inoculated according to the adopted test method ISO 22196:2011.[21] The specimens of leather fatliquored with addition of oregano essential oil and fatliquored without this oil were placed into Petri dishes with sterile tweezers. Bacterial suspension at amount of 0.4 ml was applied with sterile pipette onto the prepared samples. The leather coated with *inoculum* was then covered with polyethylene sheet (square of 40 mm±2 mm sides) that gently pressed against the tested material to spread the suspension uniformly over the leather surface and avoid seepage beyond the sheet edges. Schematic diagram of the test system is shown in Figure 2.

Some of specimens were rinsed just after inoculation, while the others only after 24 hours incubation at 35±1˚C and relative humidity of 90%. In both cases the leather and the sheet were rinsed thoroughly with sterile distilled water by using a sterile pipette. The obtained bacterial suspensions were diluted with sterile saline solution (0.9% NaCl). After a series of 10-fold dilutions the bacterial suspensions were applied onto basal media (TSA) and selective media for individual bacteria in two repetitions to obtain surface cultures. The inoculated media were incubated at 35±1˚C for 48 hours (TSA and Baird-Parker) or 44±1°C for 24 hours (TBX). After incubation, the obtained bacterial colonies were counted automatically with the automatic colony counter with the software EasyCount 2 model 7510/AES AES CHEMUNEX and the number of surviving colonies (CFU/cm^2) on tested materials was determined according to ISO 22196:2011.[21] The experiments were carried out by using the described procedure for specimens of leather fatliquored with addition of oregano essential oil and fatliquored without this oil.

Results and Discussion

The results of the study of antimicrobial activity of leathers fatliquored with the addition of oregano oil at concentrations of 1% and 3% per leather weight after one month of storage and antimicrobial durability of leathers after 12 months of storage assessed according to PN-EN ISO 20645:2006[20] are presented in Table III.

The most intensive antimicrobial activity of leathers enriched with oregano oil at concentration of 1% was observed with respect to the fungus *Scopulariopsis brevicaulis* and yeast *Candida albicans*, where inhibition zones for the growth of these microorganisms were 10 mm and 7 mm, respectively. A weaker but still considered as good antimicrobial effect was assigned to leather specimens reducing the growth of the bacteria *Staphylococcus epidermidis* (inhibition zone of 2 to 3 mm), *Escherichia coli* (inhibition zone of 1 mm) and *Staphylococcus aureus* (inhibition zone of 0 to 1 mm). No growth under any specimen was observed for all microorganisms.

As follows from the data presented in Table III, satisfactory results were obtained by using oregano oil at a concentration of 3% per leather weight. In tests carried out after one month of specimen storage, the inhibition zone for the growth of the fungus *Scopulariopsis brevicaulis* (Figure 3a) was at least 32 mm, i.e. this fungus was completely eliminated from the culture medium, while for the yeast *Candida albicans* (Figure 3b) was 16 mm, for the bacteria *Staphylococcus aureus* (Figure 3c) and *Staphylococcus epidermidis* (Figure 3d) was 10 to 11 mm, and for the strain *Escherichia coli* (Figure 3e) was 9 mm. No microbial growth under specimens was observed.

In the test carried out after one year of storage (Table III) the antifungal activity of leather with oregano oil (3%) to filamentous fungi reduced. After 1 month of leather storage the fungus *Scopulariopsis brevicualis* was completely eliminated from the agar plate. Similar effects were recorded after 6 months of storage of leathers.[26] However, after 12 months from fatliquoring this effect weakened (Table III), and only a zone of inhibition equal to 0 mm and no fungal growth under and on the specimen were observed (Figure 4a). Antifungal effect, although highly reduced was considered as sufficient. In comparison for the leather specimens without oregano oil the fungal growth under the disks amounted to approximately 30% of surface area, after the same storage periods. For the yeast *Candida albicans* antimicrobial effect attained after one year of storage (inhibition zone of 15 to 17 mm) was comparable to that observed after one month of specimen storage (inhibition zone of 16 mm) (cf. Figures 3b and 4b).

For the bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis* the inhibition zones were equal 4 mm (Figure 4c and 4d), so it was less about 6 to 7 mm compared to effect observed 11 months ago (Table III). For the bacterium *Escherichia coli* both after one month and one year of storage the inhibition zones of similar size (9 to 10 mm) were recorded (cf. Figures 3e and 4e). No microbial growth was observed under the leather specimens. It has been recognized that antibacterial effect was slightly reduced over time of storage, but it should be still considered as very strong, especially in view of the prolonged period after which its activity was verified.

For control specimens prepared without the use of oregano oil no typical inhibition zone around leather disks was observed

Table III

An assessment of antimicrobial effect of leathers fatliquored with the addition of oregano oil at concentrations of 1% and 3% per leather weight after 1 and 12 months of storage.

Microorganisms	Growth inhibition zones around specimens [mm] Microorganism growth evaluation according to the scale[a/]		
	leather fatliquored with oregano oil		
	1%	3%	
	after 1 month		after 12 months
Staphylococcus aureus	0-1	10-11	4
	0-1 no growth under the specimen	>1 no growth under the specimen	>1 no growth under the specimen
Staphylococcus epidermidis	2-3	10-11	4
	>1 no growth under the specimen	>1 no growth under the specimen	>1 no growth under the specimen
Escherichia coli	1	9	10
	>1 no growth under the specimen	>1 no growth under the specimen	>1 no growth under the specimen
Candida albicans	7	16	15-17
	>1 no growth under the specimen	>1 no growth under the specimen	>1 no growth under the specimen
Scopulariopsis brevicaulis	10	>32	0
	0	0	0

[a/] PN-EN ISO 20645:2006[20] for bacteria and yeasts, PN-EN 14119:2005[25] for filamentous fungi

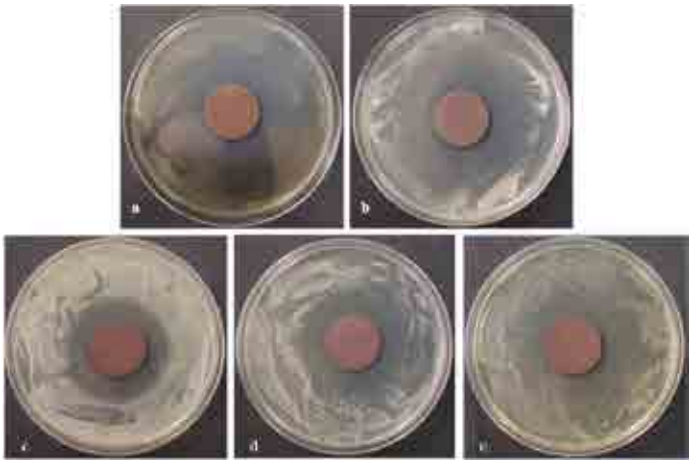

Figure 3. The leather fatliquored with the addition of oregano oil (3%) after 1 month of storage, view on the grain side, growth inhibition zones of *Scopulariopsis brevicaulis* (a), *Candida albicans* (b), *Staphylococcus aureus* (c), *Staphylococcus epidermidis* (d), *Escherichia coli* (e).

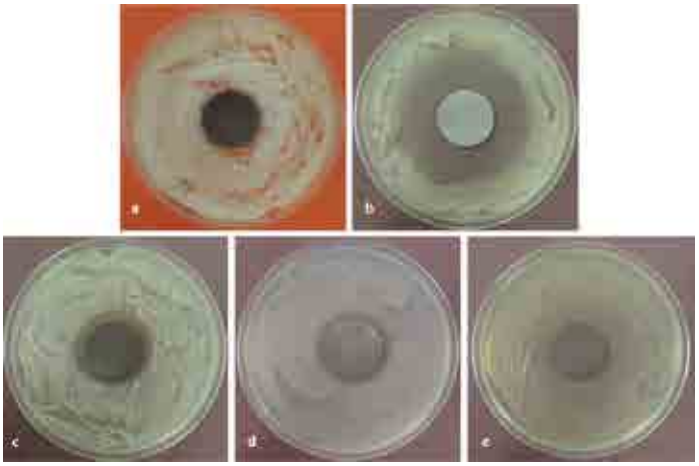

Figure 4. The leather fatliquored with the addition of oregano oil (3%) after 12 months of storage, view on the grain side, growth inhibition zones of *Scopulariopsis brevicaulis* (a), *Candida albicans* (b), *Staphylococcus aureus* (c), *Staphylococcus epidermidis* (d), *Escherichia coli* (e).

both after 1 and 12 months of storage. The lack or weak fungal growth under specimens (inter alia for the fungus *Scopulariopsis brevicualis* about 30% of surface area under the specimen), or moderate growth of reference strains was recorded.

Simultaneously a test aimed at an evaluation of possible contamination of leather specimens fatliquored with the addition of oregano oil at a concentration of 1% and 3% per leather weight was carried out during the manufacturing process. After 48 hours no bacteria or yeasts were observed on sterile substrates where leather disks fatliquored previously with oregano oil at a concentration of 1% per leather weight were placed. After two weeks both bacterial and fungal contaminations were found in the TSA substrate, while in Sabouraud agar the fungus *Aspergillus flavus* that covered about a half of the specimen surface area was identified. *Aspergillus flavus* is one among various fungal species which can appear on animal leathers.[27] For specimens of leathers fatliquored with the addition of oregano oil (3%) in tests performed after one and twelve months as well after 48 hours and two weeks no signs of contamination were found on each of substrate under investigation. The obtained results indicate that the material could become contaminated (where contamination was observed) during the manufacturing process. An increased oil concentration allowed more effective reduction especially for fungi that require low moisture for growth than bacteria. When summarizing the obtained results it should be concluded that leathers fatliquored with the addition of oregano oil at a concentration of 3% per leather weight were accepted as a good and durable antimicrobial effect lasting even after one year of storage.

At the second stage of this study an experiment aimed at confirming the effectiveness and durability of antibacterial effect of leathers fatliquored with the addition of oregano oil 3% after one year of storage was carried out according to ISO 22196:2011.[21] The assessment of antimicrobial activity in this method is based on the reduction of the number of bacteria due to the contact with a surface finished with an antibacterial agent onto which a bacterial suspension in nutrient medium is subjected to 24 hours culture under optimal conditions. The degree of reduction of the number of bacteria is expressed on logarithmic scale with respect to the control specimens which are analogous cultures done on the material without introducing an antibacterial agent. If the degree of reduction is greater than 2, and the method validation conditions are met, it is assumed that the antimicrobial finish use is effective.

The results of quantitative research of the bacterium *Escherichia coli* survive on leather fatliquored with the addition of oregano oil (3%) in one year after introducing the oil into leather are presented in Table IV. The number of bacteria was determined from the counts of colonies grown after the culture of bacteria specimens being in contact with leather, while considering corrections in counter software.

Table IV
Antibacterial activity of the lining leather fatliquored with oregano oil at amount of 3% (per leather weight) to *Escherichia coli*.

Determined parameter	**S-0[a] directly after inoculation**		**S-1[b] directly after inoculation**	
Number of bacteria [cfu/cm²]	2.06×10^4	1.27×10^4	2.19×10^3	1.01×10^3
The decimal logarithm of the number of bacteria	4.31	4.10	3.34	3.01
Mean value	**$U_o = 4.21$**		**3.17**	
	S-0[a] after 24 hours incubation		**S-1[b] after 24 hours incubation**	
Number of bacteria [cfu/cm²]	1.04×10^7	4.37×10^6	1.25	1.25
The decimal logarithm of the number of bacteria	7.02	6.64	0.10[c]	0.10[c]
Mean value	**$U_t = 6.83$**		**$A_t = 0.10$**	

[a](S-0) leather fatliquored without oil
[b](S-1) leather fatliquored with addition of oregano oil
[c]The decimal logarithm of the number of bacteria under assumption that the number of bacteria [cfu/cm²] could be 1.

According to ISO 22196:2011[21] the antibacterial activity was evaluated based on the following formula:

$$R = (U_t - U_0) - (A_t - U_0) = U_t - A_t$$

where:

R – antibacterial activity;

U_0 – mean value of the decimal logarithm from the number of surviving bacteria (cfu/cm^2), derived from specimens untreated with antibacterial agent (leathers fat liquored without addition of oregano oil) directly after inoculation;

U_t – mean value of the decimal logarithm from the number of surviving bacteria (cfu/cm^2), derived from specimens untreated with antibacterial agent (leathers fat liquored without addition of oregano oil) after 24 hours incubation;

A_t – mean value of the decimal logarithm from the number of surviving bacteria (cfu/cm^2), derived from specimens treated with antibacterial agent (leathers fat liquored with addition of oregano oil) after 24 hours incubation.

Antibacterial activity of leather fatliquored with addition of oregano oil at a concentration of 3% (w/w) to *Escherichia coli*:

$$R = 6.83 - 0.10 = 6.73$$

The results of quantitative research on the bacterium *Staphylococcus aureus* alive on the tested material are presented in Table V.

Antibacterial activity of leather fatliquored with addition of oregano oil at a concentration of 3% (w/w) to *Staphylococcus aureus*:

$$R = 6.52 - 0.10 = 6.42$$

The aggregated test results for leather specimens fatliquored without the addition of essential oil compared to the growth of the fungus *Staphylococcus aureus* (Figure 5a) and *Escherichia*

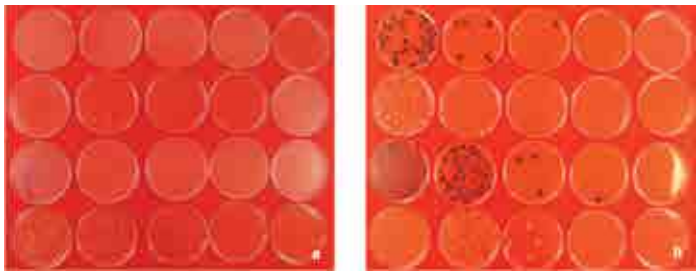

Figure 5. Bacterial colonies growing in substrates with suspensions of *Staphylococcus aureus* (a) and *Escherichia coli* (b) recovered from leather without the addition of oregano oil. The two upper rows present the results obtained for specimens directly after inoculation (TSA and selective media respectively) and the two lower after 24 hours of inoculation. The dilution order from 10^{-1} (from left) up to 10^{-5} ml of the initial sample.

Table V
Antibacterial activity of the lining leather fatliquored with oregano oil at amount of 3% (per leather weight) to *Staphylococcus aureus*.

Determined parameter	**S-0[a] directly after inoculation**		**S-1[b] directly after inoculation**	
Number of bacteria [cfu/cm^2]	1.88 x 10^4	1.22 x 10^4	1.19 x 10^3	9.52 x 10^2
The decimal logarithm of the number of bacteria	4.27	4.09	3.07	2.98
Mean value	**U_o = 4.18**		**3.03**	
	S-0[a] after 24 hours incubation		**S-1[b] after 24 hours incubation**	
Number of bacteria [cfu/cm^2]	1.54 x 10^6	7.23 x 10^6	1.25	1.25
The decimal logarithm of the number of bacteria	6.19	6.86	0.10[c]	0.10[c]
Mean value	**U_t = 6.52**		**A_t = 0.10**	

[a](S-0) leather fatliquored without oil
[b](S-1) leather fatliquored with addition of oregano oil
[c]The decimal logarithm of the number of bacteria under assumption that the number of bacteria [cfu/cm^2] could be 1.

coli (Figure 5b) are shown in Figure 5. Petri dishes present the results for dilutions from 10^{-1} (from left) up to 10^{-5} ml of the initial sample. No similar specification was not provided for leather with the addition of oregano oil after 24 hours of incubation, since no growth of bacteria under investigation in substrates was observed.

It is assumed that antibacterial activity of a tested material is recognized if R>2. The values of coefficients R found in this experiment indicate that the lining leather fatliquored with the addition of oregano oil at concentration of 3% by leather weight has strong antibacterial properties after one year from fatliquoring to bacteria under examination, i.e. *Staphylococcus aureus* and *Escherichia coli*. The validation conditions set forth in the test method were fulfilled.

Conclusions

Based on the performed study of antimicrobial activity and durability of leathers finished with the addition of oregano oil the following conclusions can be derived:

1. Leathers enriched with oregano oil at amount of 3% per leather weight are characterized as a good and durable effect of antimicrobial treatment lasting even within 12 months after fatliquoring. This enables us to conclude that leather hygienic properties can be significantly improved by introducing oregano oil into raw materials.

2. The use of oregano oil at amount of 3% per leather weight allowed leather to gain antimicrobial properties to microorganisms that can cause infections and deteriorating leathers such as the yeast *Candida albicans*, filamentous fungus *Scopulariopsis brevicaulis* and bacteria *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus epidermidis*, durable even within one year after leather fatliquoring with the addition of this oil.

3. Antimicrobial effect of leathers fatliquored with the addition of oregano oil (3%) by using the traditional diffusion disc method (PN-EN ISO 20645:2006[20]) used for textile testing, was confirmed by using the contact growth inhibition method (ISO 22196:2011[21]) used in testing of non-porous materials. Thus, by using the two methods mentioned above it is possible to investigate microbiological resistance of leather with antimicrobial finish.

Acknowledgements

This publication was co-financed from the funds of the Ministry of Science and Higher Education granted to the Faculty of Commodity Science at the Cracow University of Economics for research projects implemented by young scientists and doctoral research fellows and for maintaining the research potential.

References

1. Rydin, S., Black, M., Scalet, B.M., Canova, M.; Best Available Techniques (BAT) Reference Document for the Tanning of Hides and Skins. Joint Research Centre, Institute for Prospective Technological Studies Sustainable Production and Consumption Unit European IPPC Bureau, 2013.
2. Śpiewak, R.; Alergia na materiały obuwia. W: Sadowski, T. (red.), Obuwie, badania i innowacyjne technologie wytwarzania, Instytut Przemysłu Skórzanego w Łodzi Oddział w Krakowie, Kraków, 155-161, 2010. (in Polish)
3. Nardelli, A., Taveirne, M., Drieghe, J., Carbonez, A., Degreef, H., Goossens, A.; The relation between the localization of foot dermatitis and the causative allergens in shoes: a 13-year retrospective study. *Contact Dermatitis* **53**(4), 201-206, 2005.
4. Tissier, Ch., Chesnais, M.; Supplement to the methodology for risk evaluation biocides Emission scenario document for biocides used as preservatives in the leather industry (product type 9) INERIS-DRC-01-25582-ECOT-CTi-n°01DR0165.doc. Institut National de l'Environnement Industriel et des Risques, 2001.
5. Bielak, E.; Disinfectant and deodorizing products for footwear. W: Wieczorek, D. (ed.), Current trends in Commodity Science: Household and personal care products, Poznań University of Economics, Faculty of Commodity Science, Poznań, 110-118, 2013.
6. Bayramoğlu, E.E., Gülümser, G., Karaboz, I.; Ecological and innovate fungicide for the leather industry: essential oil of *Origanum minutiflorum*. *JALCA* **101**, 96-104, 2006.
7. Bayramoğlu, E.E.; Unique biocide for the leather industry; essential oil of oregano. *JALCA* **102**, 347-352, 2007.
8. Širvaitytė, J., Šiugždaitė, J., Valeika, V.; Application of commercial essential oils of eucalyptus and lavender as natural preservative for leather tanning industry. *Revista de Chimie* (Bucharest) **62**(9), 884-893, 2011.
9. Širvaitytė, J., Šiugždaitė, J., Valeika, V., Dambrauskiene, E.; Application of essential oils of thyme as a natural preservative in leather industry. *P. Est Acad. Sci.* **61**(3), 220-227, 2012.
10. Costa, A.C. da, Santos, B.H.C. dos, Santos Filho, L., Lima, E. de O.; Antibacterial activity of the essential oil of *Origanum vulgare L. (Lamiaceae)* against bacterial multiresistant strains isolated from nosocomial patients. *Revista Brasileira de Farmacognosia* **19**, 236-241, 2009.
11. Jnaid, Y., Yacoub, R., Al-Biski, F.; Antioxidant and antimicrobial activities of *Origanum vulgare* essential oil. *IFRJ* **23**(4), 1706-1710, 2016.

12. Szewdler, I., Sobkowiak, M.; Spotkania z przyrodą ROŚLINY. MULTICO Oficyna Wydawnicza, 1998. (in Polish)
13. Bielak, E., Marcinkowska, E., Syguła-Cholewińska, J., Golonka J.; An examination of antimicrobial activity of lining leathers fatliquored with essential oils. *JALCA* **111**, 213-220, 2016.
14. Bielak, E., Marcinkowska, E., Syguła-Cholewińska, J.; Antimicrobial activity of lining leathers fatliquored with addition of cinnamon oil. *Polish Journal of Commodity Science* **4**(49), 153-162, 2016.
15. Birbir, M., Ilgaz, A.; Isolation and identification of bacteria adversely affecting hide and leather quality. *Journal of the Society of Leather Technologists and Chemists* **80**(5), 147-153, 1996.
16. Kayalvizhi, N., Anthony, T., Gunasekaran, P.; Characterization of predominant bacteria in cattle hides and their control by a bacteriocin. *JALCA* ***103***, 182-187, 2008.
17. Orlita, A.; Microbial biodeterioration of leather and its control: a review. *International Biodeterioration & Biodegradation* **53**, 157-163, 2004.
18. Moreno, G., Arenas, R.; Other fungi causing onychomycosis. *Clinics in Dermatology* **28**(2), 160-163, 2010.
19. Kędzia, B., Hołderna-Kędzia, E.; Badanie wpływu olejków eterycznych na bakterie, grzyby i dermatofity chorobotwórcze dla człowieka. *Postępy Fitoterapii* **8**(2), 71-77, 2007. (in Polish)
20. PN-EN ISO 20645:2006 Textile fabrics – Determination of Antibacterial Activity – Agar Diffusion Plate Test.
21. ISO 22196:2011 Measurement of antibacterial activity on plastics and other non-porous surfaces.
22. Bielak, E., 2016, The effect of lining leather finishing with essential oils on its hygienic properties, unpublished doctoral thesis, thesis supervisor Prof. Ewa Marcinkowska, Cracow University of Economics, 2016.
23. Nostro, A., Sudano Roccaro, A., Bisignano, G., Marino, A., Cannatelli, M.A., Pizzimenti, F.C., Cioni, P.L., Procopio, F., Blanco, A.R.; Effects of oregano, carvacrol and thymol on *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms. *Journal of Medical Microbiology* **56**(4), 519-523, 2007.
24. Chavan, P.S., Tupe S.G.; Antifungal activity and mechanism of action of carvacrol and thymol against vineyard and wine spoilage yeasts. *Food Control* **46**, 115-120, 2014.
25. PN-EN 14119:2005 Testing of textiles - Evaluation of the action of microfungi.
26. Bielak, E., Marcinkowska, E., Syguła-Cholewińska, J.; The investigation of the use of essential oils for finishing leather lining inside shoes, in: Olszewski, P., Sadowski, T. (eds.), Tanning industry in the light of tchnological and environmental issues, Instytut Przemysłu Skórzanego w Łodzi, Oddział w Krakowie, Kraków, 63-75, 2017. (in Polish)
27. Ravindran, J., Rajeswari, N.; Assessment of paracholorometacresol (PCMC), a fungicide against three common fungal species in leather manufacturing. *International Journal of Pharmacy and Biological Sciences*, **7**(1), 127-136, 2016.