Paper B6

Antifungal leather finishing with biological extracts

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1. Introduction

The risk of mould growth is given in nearly all process stages of leather production as well as the subsequent processing and use [1]. Leather as a natural product containing grease, dyes and tanning agents is an ideal nutrient source for moulds. Their enzymes digest proteins, fats and carbohydrates of the leather structure. Optimum conditions for moulds are temperatures above 25 °C and a water content of about 15%. Under these conditions the moulds germinate, grow and destroy the leather [2].
Due to the globalisation of the leather market and the resulting export and import of raw hides, semi-finished products, leather and leather goods over long distances, it is necessary to protect the package against a growth of moulds [3].
Adequate protection of leather is currently ensured solely by using toxic and ecologically critical preservatives such as benzothiazoles or phenol derivatives [4, 5]. This paper presents an antifungal treatment for leather and its semi-finished products on the basis of biological and environmentally friendly agents without toxic effects for humans. During the search for appropriate agents that possess well-known antifungal effects and have not yet been mentioned in context with leather and semi-finished products, especially carboxylic acids with 8 to 11 carbon atoms were noted as effective. These also include caprylic acid (C_8), pelargonic acid (C_9) and undecylenic acid (C_{11}). The carboxylic acids destroy cell walls, denature proteins of fungi, take part in fatty acid synthesis and pigmentogenesis and prevent the growth of moulds, e. g. by suppressing their respiration. Carboxylic acids are classed as environmentally friendly, physiologically safe and non-toxic. They can be easily synthesised with a good availability [6]-[10].
The antifungal finishing should work during the transport of packaged goods and should prevent the germination of existing mould spores on the materials regardless of the microclimate.

2. Materials and method

2.1. Materials

Ricinoleic acid with 13 carbon atoms and sodium salts of different carboxylic acids were used in addition to caprylic-, pelargonic- and undecylenic acid. The fatty acids and their salts were purchased by Sigma-Aldrich (Germany) and VWR International (Germany).
The following relevant leather moulds from DSMZ (German Collection of Microorganisms and Cell Cultures) and CBS (Netherlands Fungal Biodiversity Centre) were used for microbiological tests: *Aspergillus niger* DSM 1957, *Chaetomium globosum* DSM 1962 und *Hormoconis resinae* CBS 115963. The moulds were plated on malt extract medium and cultured either at 25 °C or at 30 °C for 14 days prior to the commencement of the experiments. The mould suspensions were prepared in physiological saline with 0.1 vol% polysorbate 80.

2.2. Determination of the antifungal efficacy of the fatty acids and their salts

Direct examination was carried out with the suspension test. Carboxylic acids and their corresponding sodium salts were added to malt extract medium in the concentrations of 0.3 wt%, 3 wt% and 10 wt%. *A. niger* was plated and grown at 30 °C for 14 days. The

assessment of efficacy was done with mould growth on untreated medium compared to medium containing the antifungal agents.

2.3. Finishing of leather and its semi-finished products

Leather and semi-finished products were treated either with the carboxylic acids or with the sodium salts to test the effectiveness on the materials. Then, the following important parameters such as concentration and exposure time of the antifungal agents, the stability of the finishing at different physical conditions and the impact on the material, like appearance and surface feel were investigated.

For the experiments, 4 wt% (based on material weight) sodium caprylate was added to the wash liquor. Subsequently, crust leather specimens (diameter 3.5 cm) were added and treated for 30 minutes. After finishing, the specimens were sammed, spores of *A. niger* were applicated and the test specimens were incubated for 14 days at 30 °C in a moist chamber. Each week, the specimens were examined regarding mould growth. Growth was expressed in percentage of the specimen surface that was covered with the mould.

2.4. Determination of the application concentration of fatty acid salt

To determine the concentration of fatty acid salt, which is necessary to ensure an adequate antifungal protection for 28 days, crust leather specimens (diameter 3.5 cm) were treated with five different concentrations of sodium caprylate (0.8 to 4 wt%) in the wash liquor for 30 minutes, sammed, inoculated with spores of *A. niger* and then incubated for 28 days at 30 °C in a moist chamber. The examination of mould growth was carried out as described above.

2.5. Determining the duration of treatment with fatty acid salt

Depending on the performed leather technology the washing steps in the process can vary between 10 to 30 minutes. Therefore, it was important to find out, whether the sodium salts of fatty acids can also protect the leather or its semi-finished product by treatment shorter than 30 minutes. For this purpose, the wet blue and crust leather specimens (diameter 3.5 cm) were treated 10, 20 and 30 minutes with 4 wt% of either sodium caprylate or sodium undecanate in the wash liquor. After samming of test specimens and inoculation with *A. niger* the test specimens were incubated for 28 days at 30 °C in a moist chamber. The mould growth was examined as described above.

2.6. Determination of the antifungal efficacy of the fatty acid salt in depending on the intensity of the spore contamination

Leather and its semi-finished products can be contaminated to different extent by moulds or fungal spores during manufacture and transport. Sammed wet blue and crust leather specimens (diameter 3.5 cm) treated either with 4 wt% sodium caprylate or sodium undecanate were inoculated with three different concentrations of a spore suspension of *A. niger*. In addition to the commonly used concentration of 10^8 spores/ml, suspensions of 10^4 and 10^{16} spores/ml were tested. After application of the spores, test specimens were incubated for 28 days at 30 °C in a moist chamber. The tests were evaluated by determining the percentage of mould growth on the test specimens. The aim of the experiment was to determine whether the antifungal finishing is still effective at a higher infestation of mould spores.

2.7. Determination of the antifungal efficacy of the fatty acid salt in depending on the contamination by various fungi

To determine the efficacy of the fatty acid salt against various moulds, the two mould species *C. globosum* and *H. resinae* were additionally included in this study. For these experiments, wet blue and crust leather specimens (diameter 3.5 cm) were treated for 30 minutes either with 4 wt% sodium caprylate or undecanate in the fleet, sammed and then inoculated with the three different fungi. In contrast to the experiments with *A. niger*, the moulds were incubated at 25 °C for 42 days. Mould growth was controlled weekly and expressed as above.

2.8. Determination of the antifungal efficacy of the fatty acid salt after influence of different physical conditions

Many different environmental influences act on leathers and its semi finished products during manufacture and transportation. Environmental conditions can affect the stability of the fatty acid salt and impair its effectiveness. To verify this, wet blue and crust leather specimens (diameter 3.5 cm) were treated with 4 wt% sodium caprylate in the fleet, sammed, stored for 14 days under various light intensities (light / dark), temperatures (20 °C / 40 °C) and humidity conditions (22% relative humidity / 95% relative humidity), then inoculated with *A. niger* and incubated for 28 days at 30 °C in a moist chamber. This physical parameters refelct an exemplified overseas shipment in containers from Germany to India. The examination of mould growth was carried out weekly and was specified in percentage growth on the surface samples.

2.9. Determination of the antifungal efficacy of the fatty acid salt in aldehyde tanned leather

The efficacy of the new preservatives were not only tested on chrome tanned leathers but also on free of chrome leathers (FOC), especially aldehyde tanned leathers.

For these experiments, 4 wt% sodium caprylate were added to the washing liquor and the wet white crust leather specimens (diameter 3.5 cm) were treated over 30 minutes. Subsequently, the specimens were sammed, spores of either *A. niger*, *C. globosum* or *H. resinae* were applied and the specimens were incubated for 28 days at 30 °C or 42 days at 25 °C, respectively, in a moist chamber. The investigation of mould growth was controlled weekly and expressed as above.

2.10 Quantification of fatty acids

The content of fatty acids of material samples was measured by using head space vials. They were heated at 160 °C using the ThermoDesorptionSystem TDS 2 (Gerstel GmbH, Germany) and analysed by GC-MS (Agilent Technologies, Germany).

3. Results and discussion

3.1. Antifungal activity of fatty acids and their salts in the suspension test

The direct determination of the antifungal potential of fatty acids was carried out with suspension tests. As implied by Fig. 1, caprylic acid, pelargonic acid and undecylenic acid showed already at a concentration of 0.3 wt% and the corresponding sodium salts at a concentration of 3 wt% a pronounced antifungal activity against *A. niger* for at least 14 days. In contrast, ricinoleic acid did not suppress the germination and growth of spores of *A. niger*.

3.2. Finishing leather and its semi-finished products

Having shown that fatty acids and their salts prevent germination and growth of moulds on culture medium, the effectiveness of treated leather and wet blue were tested. The aim of these tests was to establish a method to finish wet blue, wet white and leather during their manufacture with the antifungal agents, without affecting the common operations of the manufacturing process. Preservation was applied in the final aqueous washing step. The highly water soluble fatty acid salts sodium caprylate and sodium undecanate were used in subsequent investigations. By treatment of crust leather with 4 wt% sodium caprylate growth of A. niger was prevented for 14 days minimum.

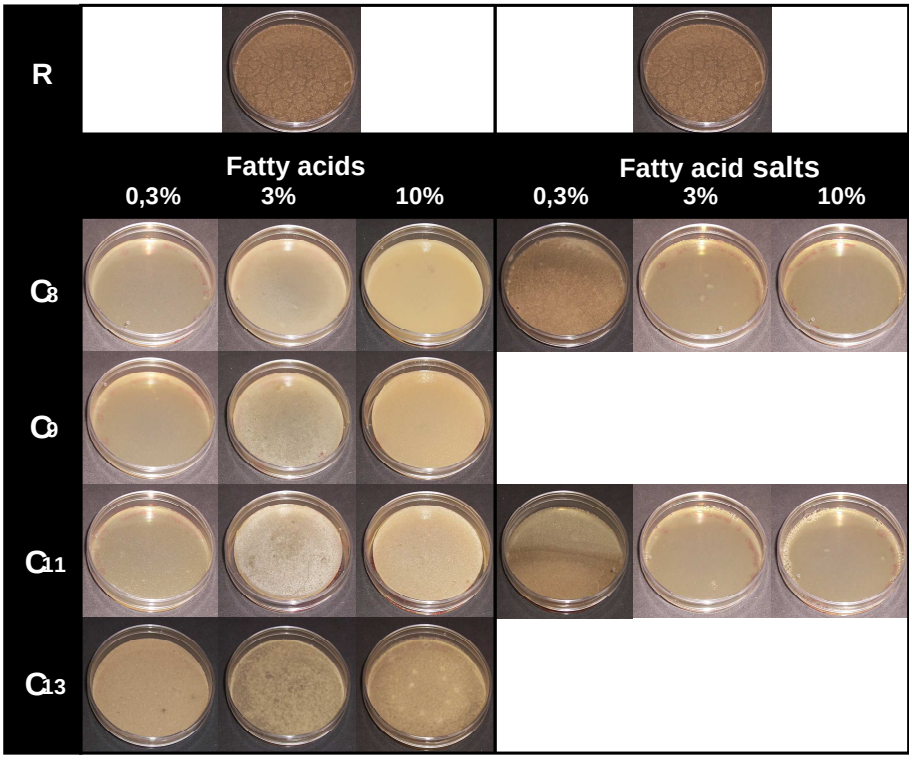

Fig. 1: Antifungal efficacy of different concentrations of fatty acids and sodium salts in suspension tests after inoculation with *A. niger* (R = reference, culture medium without addition of fatty acids, C_8 = caprylic acid, C_9 = pelargonic acid, C_{11} = undecylenic acid, C_{13} = ricinoleic acid, arrows: growth of *A. niger*, nutrient medium: clear, yellow)

3.3. Application concentration of fatty acid salt

To determine the minimal inhibition concentration (MIC) of the fatty acid salt, crust leather was finished with different concentrations of sodium caprylate. As shown in Fig. 2, a concentration of 4 wt% sodium caprylate is necessary to protect leather sufficiently against any growth of *A. niger* for a period of 28 days. Therefore, application of less than 4 wt% is not recommended.

3.4. Duration of treatment with fatty acid salt

Furthermore, the treatment time with fatty acid salts was investigated, which is necessary to prevent mould growth. In these experiments, wet blue and crust leathers were treated for three different time periods with sodium caprylate and sodium undecanate.

The tests documented that drumming of wet blue and crust leather for 10 minutes with sodium caprylate is sufficient. However, for a full antifungal protection with sodium undecanate, a reaction time of 30 minutes is required during washing of the crust leather. Sodium undecanate only provides low protection for a maximum of 14 days on wet blue (Fig. 3).

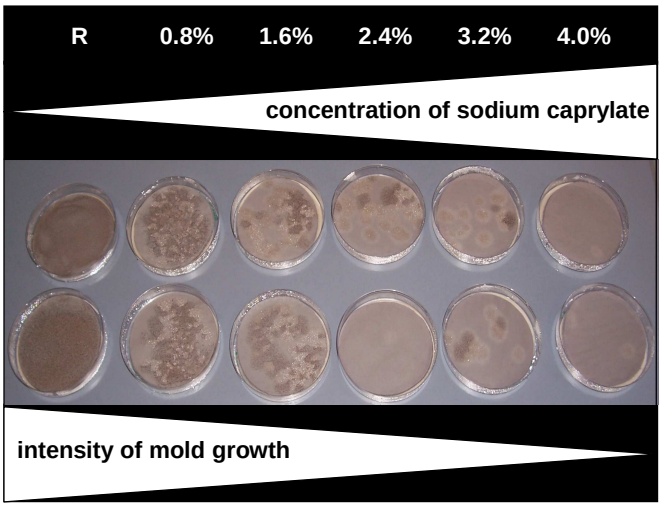

Fig. 2: Antifungal efficacy of different concentrations of sodium caprylate on crust leather and inoculation with *A. niger* (R = reference, crust leather without addition of fatty acids, inoculated with *A. niger*, two times)

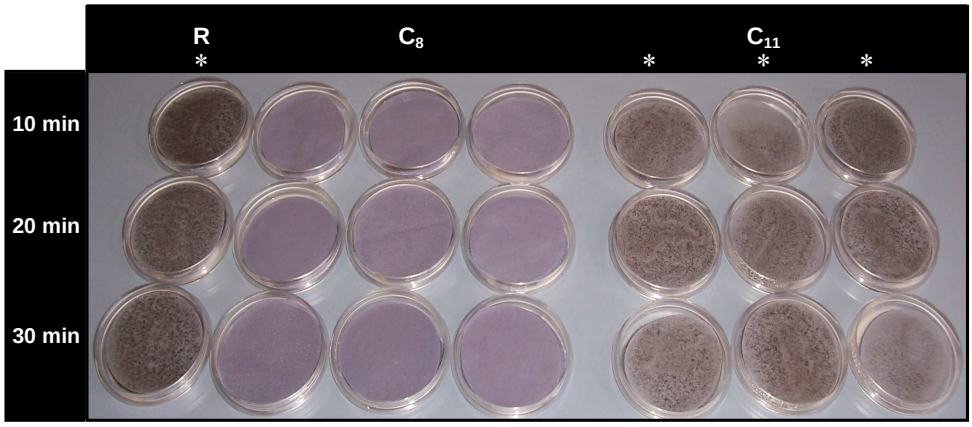

Fig. 3: Antifungal efficacy of wet blue after treatment over three different time periods with sodium caprylate (C_8) and undecanate (C_{11}), respectively. Inoculation with *A. niger* (R = reference, wet blue without addition of fatty acids, inoculated with *A. niger*, three times, *100% of the surface covered with mould)

3.5. Impact of spore concentration on the effectiveness of the fatty acid salt

In environment, the spore concentration during contamination can vary widely. Therefore, the efficacy of the fatty acid salts with increasing spore contamination had to be clarified. Treated wet blue was inoculated with three different concentrations of a spore suspension of *A. niger*. The experiments clearly revealed that mould growth is successfully avoided even at high spore concentrations when using 4% caprylate (Fig. 4). Furthermore, it was shown that sodium undecanate only poorly protects against mould growth.

3.6. Antifungal spectrum of the fatty acid salt to various moulds

All previous experiments were conducted with *A. niger*, a fungus isolated from leather. To verify a general activity against fungi, the spectrum of moulds was broadened by the two species *H. resinae* and *C. globosum*. Like *A. niger* in previous studies, these two fungi showed no growth on wet blue and crust leathers treated with sodium caprylate (Fig. 5). However,

sodium undecanate prevented only the growth of *C. globosum* on wet blue, while it did not act as antifungal agent against *H. resinae*.

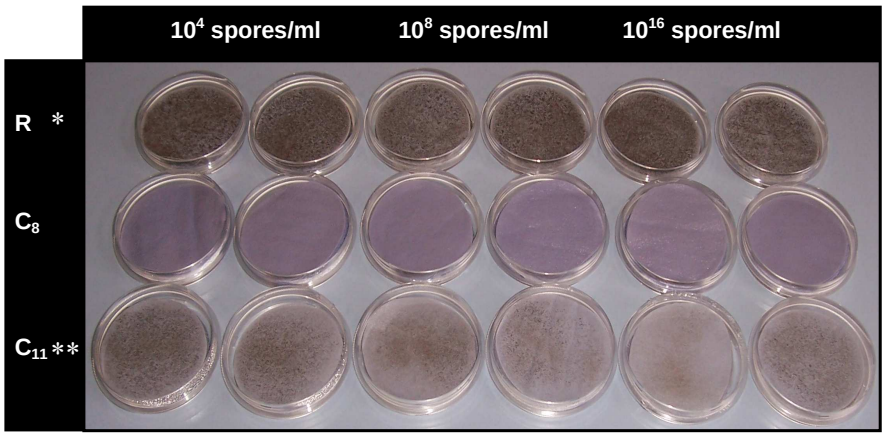

Fig. 4: Antifungal efficacy of wet blue after treatment with sodium caprylate (C_8) or undecanate (C_{11}), followed by inoculation with different concentrations of *A. niger* spore suspensions (R = reference, wet blue without addition of fatty acids, inoculated with *A. niger*, two times, *100% of the surface covered with mould, ** < 100% of the surface covered with mould)

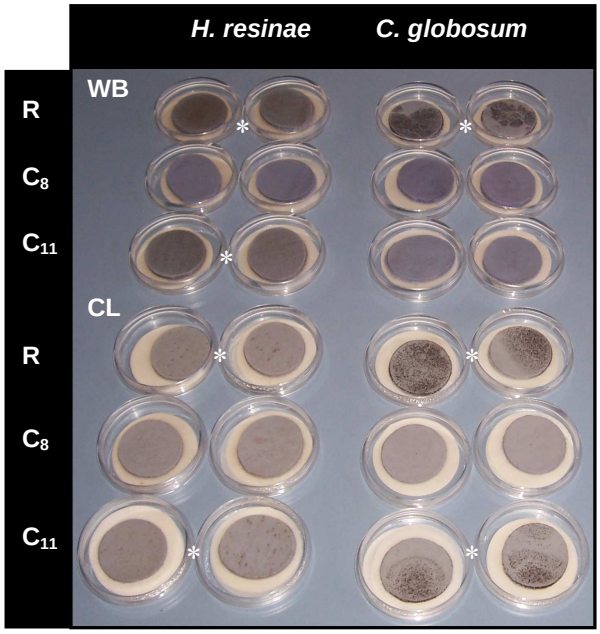

Fig. 5: Antifungal efficacy of sodium caprylate and undecanate against *H. resinae* or *C. globosum* used for preservation of wet blue and crust leather (R = reference, without addition of fatty acids, inoculated with moulds, WB = wet blue, CL = crust leather, two times, *surface covered with mould)

3.7. Antifungal efficacy of the fatty acid salt after influence of different physical conditions

It was shown in previous experiments that especially sodium caprylate protected against mould growth for at least 28 days at a concentration of 4 wt%, regardless of the duration of the washing process, the type of mould and spore concentration. To obtain an unlimited use of the fatty acid salt in the manufacturing process of leather and during transport or storage, the

influence of some important physical parameters was investigated. Beside the influence of light, temperature and humidity on the antifungal potential, the influence to surface feel and appearance of wet blue and leather was examined.
Except for a more intense blue color of the wet blue, which appears immediately after the treatment with sodium caprylate, a change in material properties could not be observed. The preservation did not change feel, grip and the look of the final leathers.
Results of the studies are summarised in Fig. 6. The light intensities had no effect on the stability and, thus, the effectiveness of sodium caprylate. In both cases, sodium caprylate prevented against germination and growth of *A. niger* on wet blue and crust leather. In the temperature range from 20 to 40 °C, sodium caprylate was not destroyed and protected the wet blue and crust leather. Despite the storage of the wet blue and crust leather in high humidity conditions, the effectiveness of sodium caprylate was not affected. Growth of *A. niger* was observed only on specimens which were not previously treated with the fatty acid salt.

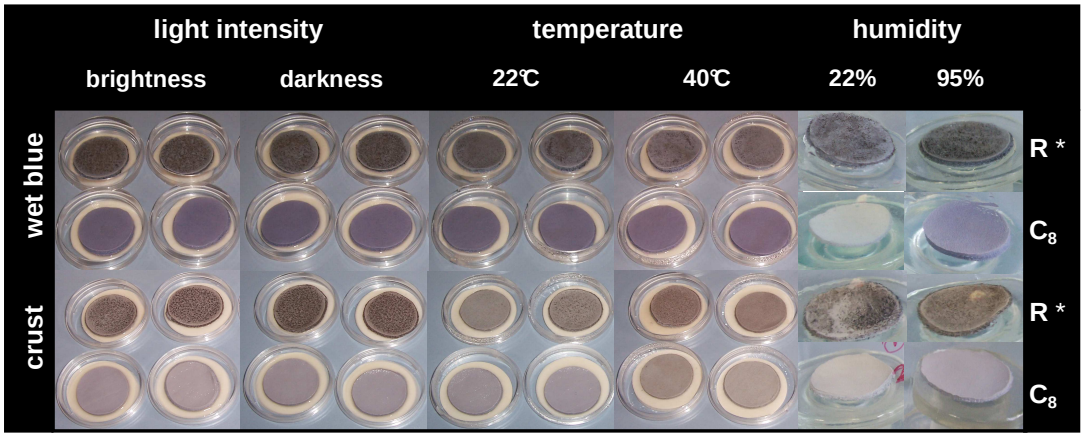

Fig. 6: Antifungal efficacy of sodium caprylate when used for wet blue and crust leather; storage at different physical conditions and inoculation with *A. niger* (R = reference, without addition of SC; inoculated with *A. niger*, WB = wet blue, CL = crust leather, two times, *surface completely covered with mould)

3.8. Antifungal efficacy of the fatty acid salt with aldehyde tanned leather

Beside chrome tanning, free of chrome leathers (FOC) mainly tanned with glutaraldehyde are often used to manufacture leather products. To exclude the influence of the type of tanning on the effectiveness of the fatty acid salt, FOC leathers were incubated with the moulds *A. niger*, *C. globosum* and *H. resinae*.
While semi finished as well as crust leathers were highly susceptible to growth of fungi, no growth was observed on preserved material. This indicated that preservation with sodium caprylate is also effective in case of aldehyde tanned leather.

4. Conclusions

Carboxylic acids with chain lengths of C_8 to C_{11} were discovered to act as effective preservatives for leather and its semi-finished products. The most effective substance was found to be caprylic acid and its sodium salt. Advantageously, caprylic acid has a biological origin, is environmentally friendly and non-toxic to humans. It shows no mutagenic potential, it is readily biodegradable with no potential for bioaccumulation and is further environmentally safe when used as recommended [11]. The fatty acid is extracted from the fruits of the palm tree and it is a bulk product of the lipolysis of fats. Futhermore, it is relatively cheap with good availability. Both, caprylic acid and its sodium salt were demonstrated to protect leather and its semi-finished products in a concentration of 4 wt% for at least 28 days, independent of the physical

conditions of storage and the inoculum concentration. Except for a minimal blue staining after the treatment of wet blue, surface feel or appearance of the materials as well as physical parameters were not affected. The application of this new preservative was feasible within 10 minutes in the last washing step, without impairment of the production process of leather.

5. Acknowledgements

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6. References

[1] John, G. (1996): Fehlermöglichkeiten bei der Lederherstellung. Druck Partner Rübelmann GmbH, Hemsbach.
[2] Hauber, C. (2005): Micobicides applications in the leather industry. In: Pauls, W.: Directory of microbiocides for the protection of materials.
[3] Volkmann, F.; Petzoldt, J.D.; Leppchen, K.; Meyer, M. (2009): Schimmelvermeidung durch prozessspezifische Risikoanalyse (HACCP) bei Herstellung, Lagerung und Transport von Packgut aus Leder und Verpackungen aus Wellpappe. Schlussbericht zum AiF-Forschungsvorhaben Nr. 15210 BG.
[4] Wallhäußer, K.H. (1995). Praxis der Sterilisation, Desinfektion – Konservierung, Keimidentifizierung – Betriebshygiene. Georg Thieme Verlag, Stuttgart.
[5] Gattner, H.; Lindner, W.; Neuber, H.U. (1988): Mikrobieller Befall bei der Lederherstellung und seine Kontrolle mit modernen Konservierungsmitteln. Das Leder, 39, 66-73.
[6] Vicher, E.; Lyon I.; White, E.L. (1959): Studies on the respiration of *Trichophyton rubrum*. Mycopathologia, 11 (3), 185-195.
[7] Vicher, E.E.; Kostiw, L.L.; Lyon, I.: Bolewicz, B.M. (1968): The effect of sodium propionate and sodium caprylate on the fatty acid content of *Trichophyton rubrum*. Mycopathologia, 35 (3-4), 208-214.
[8] Avis, T.J.; Belanger, R.R. (2001): Specifity and mode of action of the antifungal fatty acid cis-9-Heptadecenoic acid produced by *Pseudozyma flocculosa*. Appl. Environ. Microbiol., 67, 956-960.
[9] Adams, J.N.; Painter, B.G.; Payne, W.J. (1963): Effects of sodium caprylate on *Candida albicans*. I. Influence of concentration on ultrastructure. J. Bacteriol., 86(3), 548-557.
[10] Payne, W.J.; Bannister, E.R. (1963): Effects of sodium caprylate on *Candida albicans*. II. Influence of various concentrations on biochemical changes. J. Bacteriol. 86(3), 558-562.
[11] EG-Sicherheitsdatenblatt für Natriumcaprylat (Stand 2004) und Merck-Sicherheitsdatenblatt für Caprylsäure (Stand 2003), gemäß 91/155/EWG.