STUDY REGARDING THE RESISTANCE OF WET-WHITE LEATHER TANNED WITH TITANIUM – ALUMINUM TO THE GROWTH OF FUNGI

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In the tanning industry the problem of micro-organisms able to grow on leather during the different processing phases is well known. Wet-white leathers are excellent substrates for fungal growth: storage temperature, acid pH, presence of water, proteins and fats constitute the most important conditions for the development and growth of a lot of moulds such as *Penicillium* spp, *Aspergillus* spp. and *Trichoderma viride*. The fungal contamination appears as coloured stains on the leather, usually permanent. Microbiological testing has been performed on wet-white leather tanned with Ti-Al based tanning agent using an inoculum with 4 species of fungi. This study revealed that the Ti-Al-tanned wet-white leather is attacked by all types of fungi studied.

Keywords: wet white leather, microbiological analysis, Ti-Al tanning agent

INTRODUCTION

At present, chrome tanning is the most widely used technique for leather tannage, accounting for more than 90% of leathers tanned worldwide. However, chrome tannage involves serious environmental risks resulting from the possible oxidation of chromium to a hexavalent state, although tanners are aware of the carcinogenic effect, in accordance with the International Agency for Research on Cancer (IARC). For this reason, the market has shown a growing demand for "ecological" products, especially regarding the development of tanning processes using alternative tanning agents different from chromium.

Alternative free of chrome (FOC) tanning technologies include the use of tanning agents based of titanium-aluminum, which in combination with other retanning agents of vegetable or synthetic origin, allow for obtaining quality leathers that may be used by footwear and upholstery industries (Kleban, 2004; Adiguzel Zengin *et al.*, 2012; Mutlu *et al.*, 2012).

Wet-white refers to partially processed leathers that have been tanned with titanium and/or aluminium, but not dyed, dried or fat-liquored yet (Crudu *et al.*, 2012a; 2014; 2012b; 2010).

This study aims at testing fungal resistance of wet white leather tanned with titanium-aluminium (Crudu *et al.*, 2013).

The most common fungi found on leather are *Aspergillus niger* and *Aspergillus flavus*, moulds that, in addition to the destructive effect they have on leather objects, are also harmful for human health. *Aspergillus flavus* is dangerous because of the alfatoxin it produces, one of the most carcinogenic substances in the living world, while *Aspergillus niger* may cause aspergillosis in immune-compromised patients.

Aspergillus niger is a widespread mould in the environment which may develop on almost anything: coffee, various foods, textiles, wood, paper and leather goods, which is why this mould was used to test resistance of leather to fungi. Mould is invasive, developing quicker than *Penicillium* or other types of fungi, with the tendency of extending to the detriment of other species.

Aspergillus niger strain ATCC 6275 was first isolated in a laboratory in The United States, from a leather sample. The strain is characterized by the presence of genes for

carboxymethyl cellulase, citric acid, glucosidase, xylanase, lipases and resistance to copper.

Trichoderma viride is a green coloured mould used in the antifungal treatment of soil and seeds due to its ability to inhibit growth of other fungi, but can also be pathogenic for certain plants, such as onion. The mould produces cellulases and chitinases and develops on wood and parasitizing other fungi, which is why it damages mushrooms. This mould may also grow on tanned leather.

Aspergillus oryzae is characterized by the presence of orange globular conidia, and it is used in Asian cuisine for soybean fermentation. The mould produces amylases and carboxypeptidases. Unlike *Aspergillus flavus* and *Aspergillus niger*, *Aspergillus oryzae* has not been identified on leather so far, but the possibility of its development on chromium-free tanned leather samples was taken into account.

Mucor pusillus develops in soils and decomposes organic matter of vegetable origin. No growth of this mould has been yet reported on tanned leather, but this study aimed at discovering whether fungi can damage wet-blue and wet-white tanned leather through the proteases they produce.

MATERIALS AND METHODS

Materials

Samples: specimens of wet white leather were obtained after tanning stage of bovine hides using a tanning agent based on Ti-Al according to Crudu *et al.* (2013).

Biological material: 4 fungi strains were used: *Aspergillus niger* ATCC 6275, *Trichoderma viride*, *Aspergillus oryzae* 153 and *Mucor pusillus.*

Culture medium: Potato Dextrose Agar (PDA) culture medium was used.

Methods

Microbiological tests were performed according to ASTM standard D 4576-08 (2013) - Test Method for Mold Growth Resistance of Wet Blue (Tarlea *et al.*, 2009). Four samples of each type of leather were inoculated with each of the 4 fungi strains and were incubated at 28 ± 1°C for 28 days. Leather samples were evaluated after 7, 14, 21 and 28 days to determine fungi growth, ranking them from 0 to 4, as follows: mark 0 for leathers not covered by mould, mark 0.5 for growth on less than 12% of the sample surface, mark 1 for growth ranging between 12 and 25% of the surface, mark 2 if 50% of the sample surface is covered by fungi, mark 3 if mould grew on 75% of the sample surface and mark 4 if the sample is entirely covered by mould.

Chemical tests of wet-white leathers were performed according to EN ISO standards.

RESULTS AND DISCUSSIONS

Wet- white leathers are excellent substrates for fungal growth: storage temperature, acid pH, presence of water, proteins and fats constitute the most important conditions for the development and growth for a lot of moulds, such as *Penicillium* spp., *Aspergillus* spp. and *Trichoderma viride*. The fungal contamination appears as coloured stains on the leather, usually permanent.

Wet white leathers were characterized for chemical characteristics shown in Table 1.

Table 1. Wet-white leather chemical characteristics

No.	Characteristics	Wet-white Ti-Al tanned
1	Ti/Al oxides, %	3.12
2	Shrinkage temperature, °C	75-78
3	Grease, %	2.1
4	pH	3.6
5	Moisture, %	66.33
6	Ash, %	12.59
7	Total nitrogen, %	13.84
8	Digestibility, %	61.3

Table 2 presents the appearance and assessment (marks) of leather samples inoculated with fungi after 7, 14, 21 and 28 days. According to the working standard, samples were evaluated ranking them from 0 to 4 depending on the growth of mould on the sample (Table 2). The following were found:

After 7 days *Aspergillus niger* grew on less than 25% of the Ti-Al-tanned leather sample.

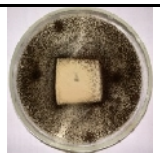
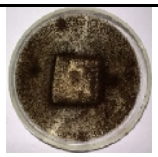
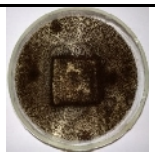
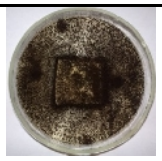
Aspergillus oryzae grew on the surface of the Ti-Al-tanned wet-white leather sample without damaging them. *Trichoderma viride* grew in the culture medium; approximately 10% of the Ti-Al-tanned wet-white leather sample is covered by *Trichoderma*. *Mucor pusillus* only grew in the culture medium around the wet-white leather samples.

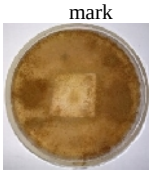
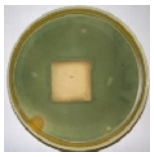
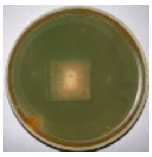
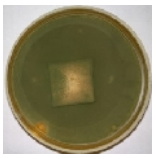
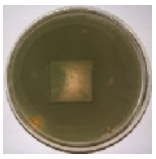
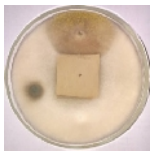
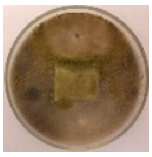
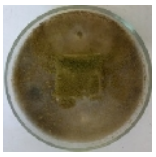
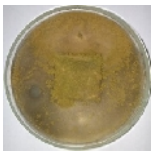
After 14 days, *Aspergillus niger* grew on wet- white leather, while *Aspergillus oryzae* covered the Ti-Al-tanned wet-white samples almost entirely. *Trichoderma viride* grew on 75% of the Ti-Al tanned wet-white leather samples. Wet-white leather samples were entirely covered by *Mucor pusillus*. On the Ti-Al-tanned wet-white leather sample inoculated with *Mucor pusillus*, a colony of *Aspergillus flavus* also appeared. The colony is very invasive, quickly covering the entire surface of the sample.

After 21 days, all leather samples inoculated with *Aspergillus niger* were entirely covered by mould; the situation of samples inoculated with *Aspergillus oryzae* remained unchanged. The appearance of the Ti-Al-tanned wet-white leather sample inoculated with *Trichoderma viride* did not change much.

After 28 days the appearance of samples inoculated with *Aspergillus niger* and *oryzae* did not change.

Table 2. Appearance and assessment of leather samples after 7, 14, 21 and 28 days

Fungus type	7 days / mark	14 days / mark	21 days / mark	28 days / mark
	Ti-Al-tanned wet-white leather			
A. niger	 0.5	 3	 4	 4

Fungus type	7 days / mark		14 days / mark		21 days / mark		28 days / mark	
A. oryzae		2		2		2		3
T. viride		0.5		2		3		3
M. pusillus		0		4		4		4

CONCLUSIONS

Ti-Al-tanned wet-white leather is attacked by all types of fungi studied. *Aspergillus niger* and *Aspergillus flavus* are the most aggressive and invasive species, developing very rapidly, much quicker than *Trichoderma viride*, *Mucor pusillus* and moulds from the *Penicillium* genus, the latter having the slowest growth rate. Although it produces chitinases with antifungal effect, *Trichoderma viride* does not inhibit development of *Aspergillus niger* and *flavus* species.

Acknowledgements

This work has been financed by the European Fund for Regional Development and the Romanian Government in the framework of Sectoral Operational Programme under the project INNOVA-LEATHER: «Innovative technologies for leather sector increasing technological competitiveness by RDI, quality of life and environmental protection» – contract POS CCE-AXA 2-O 2.1.2 nr. 242/20.09.2010 ID 638 COD SMIS – CSNR 12579.

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Study Regarding the Resistance of Wet-White Leather Tanned with Titanium – Aluminum to the Growth of Fungi