# Fatty materials as nutrient for fungal growth on wetblue leather

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## Abstract

Fungal growth on wetblue leather can create many technical problems and represents significant financial impact for the tanner. Current fungal control programs are generally successful in preventing mold growth in wetblue, but there is always interest in developing more effective solutions to this problem. Most studies on fungal growth in leather have focused on the effectiveness of various fungicides on preventing mold. However, microbiology, biochemistry, and histology are scientific fields that could be more deeply studied in order to help design more effective solutions to problems caused by mold on leather. In work published by Zugno *et al* (2009) a study was done on the growth of several strains of fungi on wetblue samples that had not been treated with fungicide. The results showed that natural oils and fats were the main nutrients for the fungi. No evidence of attack on the tanned structural protein components was found. It may be concluded that the damage to the leather is mostly due to the physical-chemical changes resulting from fat breakdown and removal and ensuing differential uptake of chemicals added in retanning, dye and fatliquor operations. In the present work we study the influence of several types of oils commonly used in fatliquor operations and natural fats on fungal growth in wetblue. This may contribute to the development of more efficient fungal control programs with less aggressive eco-toxicological profiles.

**Keywords:** Fungal, Wetblue, Fats, Oils, leather.

## 1 Introduction

Every year tanneries around the world invest millions of dollars to protect their leather against fungal growth. The main line of defense are the fungicides applied during tanning, retanning and finishing operations, which are normally based on TCMTB, OIT, CHED, PCMC, and OPP, alone or in various combinations. These chemicals prevent mold from growing on leather, and consequently avoid staining, discoloration and other damages to the final article. While there is some limited understanding of the mechanism of the action of these molecules on fungal metabolism, most of the research has been practical work focused on the quantitative application of fungicides on leather and their relative performance. Therefore, there is still a lot to learn on how the fungi feed themselves when growing on a piece of wetblue, crust or finished leather. This knowledge could play an important role in improving the efficiency of fungal control programs.

The fact that fungi use natural fat as a substrate for its growth on leather, either wetblue or crust, has been previously reported by several authors, including Koppenhoefer (1951), Kanagy *et al* (1946) and Wilson *et al* (1952). Kanagy found that the fat/oils content in molded leather decreased significantly with time and that the percent of fatty material removed would vary depending on the fungal species growing on the leather. Sharma & Sharma (1980) observed that when fungi are grown on

finished leather – either vegetable tanned or chrome tanned – the total oils and fat content is reduced by 40% - 72% of the original amount. More recent studies from Zugno *et al* (2011) investigated the growth of different fungal species on wetblue leather with no fungicide and showed that natural oils and fats were the main nutrients for the fungi.

The purpose of this work is to initiate a long-term project to further investigate the relation of the type and amount of fat/oils present on leather and its susceptibility to fungal growth. In this first phase we will evaluate the qualitative influence of the fatty material present in the leather and the results should serve as a starting point for future research.

## 2 Materials and Methods

The influence of different fatliquor materials on fungal growth was evaluated using pieces of wetblue leather from the same hide that had not received any fungicide during its processing. After tanning, the leather was cut into pieces and submitted to solvent extraction for extended natural fat removal. The samples were immersed in an emulsion of fatliquor in warm water, followed by analysis for total fat content and incubation in a tropical chamber for fungal growth evaluation. The extent of mold formation was measured and recorded daily. At the end of six weeks in the tropical chamber, each sample was analyzed again for total fat content (see figure 1). Along with the test samples a blank – no fatliquor added – was also evaluated for fungal growth and fat content.

Leather samples preparation

One bovine hide was chrome-tanned using a wooden drum according to the recipe described in Table
1. After tanning, pieces of leather measuring 12 cm x 8 cm were cut from the central region of the hide and extracted with dichloromethane in a Soxhlet apparatus for a total of five hours of reflux.

Fatliquor emulsion preparation

After natural fat extraction, the leather samples were immersed in an emulsion containing one of the five different types of oils: palm oil, palm kernel oil, refined tallow, synthetic oil 1 and synthetic oil 2. The vegetable and animal oils were supplied by Miracema-Nuodex (Brasil) and the synthetic oils from Buckman India, and their characteristics are described on Tables 2 and 3.

The fatliquor emulsions were prepared in the ratio of 1 part of fatliquor to 10 parts of water in a glass beaker and then heated to 50o C for 10 minutes. Each wetblue leather sample and its duplicate were immersed in the respective fatliquor emulsion for 45 minutes at 50o C, and stirred.

Fat content and fungal growth analysis

Following immersion in each fatliquor emulsion, the natural and total fat content of the samples were determined according to Method ABNT–NBR11030/1997– “Determination of Dichloromethane Extractable Substances”. After that, each sample was incubated in a tropical chamber for fungal growth evaluation according to the ASTM D7584-10 – “Standard Test Method for Evaluating the Resistance of the Surface of Wetblue to the Growth of Fungi in an Environmental Chamber”.

After 42 days in the tropical chamber, each sample and its duplicate was analyzed once more for natural and total fat, using the same method ABNT-NBR11030/1997.

**Table 1** – Chrome tanning recipe starting from fresh Brasilian hides

| **Process/Product** | **Dosage, % (based on hide weight)** | **Running time, minutes** |
|---|---|---|
| Water | 100 | |
| Sodium Carbonate | 0.35 | |
| Fatty Alcohol Ethoxylated | 0.15 | |
| Lipolytic Enzyme | 0.08 | |
| Proteolytic Enzyme | 0.10 | |
| Bactericide | 0.10 | 120 - Drain |
| | | |
| Water | 40 | |
| Fatty Alcohol Ethoxylated | 0.05 | |
| Proteolytic Enzyme | 0.07 | |
| Lime | 0.5 | 60 |
| | | |
| Sodium Sulfide (50%) | 0.6 | 90 |
| | | |
| Lime | 1.0 | |
| Proteolytic Enzymes | 0.03 | 60 |
| | | |
| Water | 80 | |
| Lime | 2.5 | |
| Fatty Alcohol Ethoxylated | 0.1 | 60 |
| | | Run 5 / Stop 55 – o/n |
| | | Drain, wash and flesh |
| Water | 100 | |
| Ammonium Sulfate | 0.3 | 20 – Drain |
| | | |
| Ammonium Sulfate | 1.5 | |
| Dicarboxylic Acids | 0.5 | 60 – Drain |
| | | |
| Water | 60 | |
| Ammonium Sulfate | 1.5 | |
| Dicarboxylic Acids | 1.0 | 60 |
| | | |
| Water | 40 | |
| Bating Enzyme | 0.10 | 60 – Drain/wash 2x |
| | | |
| Water | 30 | |
| Sodium Chloride | 6.0 | 15 |
| Sodium Chlorite (30%) | 0.6 | 15 |
| Formic Acid (85%), 1:10 | 1.0 | 45 |
| Sulfuric Acid (98%), 1:20 | 1.2 | 180 |
| | | |
| Chromium Sulfate (25%, 33% basicity) | 5.5 | 240 |
| Sodium Formate | 0.5 | 45 |
| Magnesium Oxide | 0.3 | 60 |
| Magnesium Oxide | 0.3 | 480 |
| | | Boiling test - unload |

**Table 2** – Fatty acid composition of vegetable and animal oils: palm oil, palm kernel oil and tallow.

| Fatty acid composition | Palm Oil | Palm Kernel Oil | Tallow |
|---|---|---|---|
| Caprylic (C8:0) | 0.0 | 2.9 | --- |
| Capric (C10:0) | 0.0 | 2.7 | --- |
| Lauric (C12:0) | 0.0 | **49.1** | --- |
| Myristic (C14:0) | 0.9 | **14.2** | 2.1 |
| Palmitic (C16:0) | **41.6** | 7.5 | **25.4** |
| Palmitoleic (C16:1) | --- | --- | 2.1 |
| Stearic (C18:0) | 3.18 | 0.7 | **29.1** |
| Oleic (C18:1) | **41.5** | **16.0** | **28.2** |
| Linoleic (C18:2) | **12.0** | 6.3 | 1.1 |
| Linolenic (C18:3) | 0.2 | 0.4 | 0.5 |
| Others | 0.7 | 0.2 | **12.1** |

**Table 3** – Physical-Chemical characteristics of synthetic and animal oils

| Characteristic | Tallow | Synthetic Oil 1 | Synthetic Oil 2 |
|---|---|---|---|
| Active matter, % | > 99.0 | 37.0 – 38.0 | 64.0 - 66.0 |
| pH (1:10) | NA | 6.5 - 8.5 | 65. – 7.5 |
| Ashes, % | NA | 1.0 – 2.0 | NA |
| Stearic/palmitic acid content, % | 50.0 – 60.0 | < 0.2 | NA |

## 3 Results and Discussions

1.1. Fat content of wetblue

The fat content on each sample pair before and after incubation in a tropical chamber are reported in Table 4. Based on these results, it should be noted that:

- The fat distribution within the samples was not very uniform, which could have impacted the results. Ununiformity was identified after degreasing both of the "blank" samples as well.
- The biggest difference in fat content - before and after fungal growth - was observed on the samples treated with palm kernel oil, tallow and palm oil.
- The content of synthetic oil 1 after fungal growth was the same as before incubating the samples in the tropical chamber.

**Table 4** – Fat content on leather samples before and after incubation in tropical chamber.

| Sample # | Oil used | Fat content after immersion, % | Fat content after 6 weeks in Tropical Chamber, % | Fat content variation, % |
|---|---|---|---|---|
| 1A | Synthetic 1 – A | 2.24 | 2.08 | 0% |
| 1B | Synthetic 1 – B | 2.08 | 2.25 | |
| 2A | Synthetic 2 - A | 1.66 | 1.29 | -23% |
| 2B | Synthetic 2 – B | 2.00 | 1.51 | |
| 3A | Palm Oil – A | 0.49 | 0.23 | -50% |
| 3B | Palm Oil – B | 0.52 | 0.28 | |
| 4A | Palm Kernel Oil - A | 1.49 | 0.18 | -90% |
| 4B | Palm Kernel Oil – B | 2.01 | 0.18 | |
| 5A | Tallow - A | 1.08 | 0.32 | -78% |
| 5B | Tallow – B | 1.55 | 0.26 | |
| 6A | Blank - A | 0.24 | 0.20 | 33% |
| 6B | Blank - B | 0.06 | 0.20 | |

1.1. Fungal growth assessment

The evaluation of fungal growth after incubation in a tropical chamber is reported in Table 5, and the average of the ratings is expressed in a graphical form in figure 1. The interpretation of the results shows that:

- All samples resisted up to four days in the tropical chamber without fungal growth.
- After 21 days in the tropical chamber, all samples presented a very similar rate of fungal growth.
- The samples treated with synthetic oil 2 presented the slowest growth, resisting up to nine days while all others failed at day 7. This same trend was observed until the 17th day of the experiment.
- For the two synthetic oils used in the study it can be observed that the higher the amount of oil on the wetblue the faster the fungal growth.
- This relation was less evident, although still noticeable, on the blank samples (natural fat), where the sample with 0.06% of natural fat resisted 2 weeks longer than its duplicate with 0.24% of natural fat.
- In the case of tallow, palm and palm kernel oils no relation could be observed between the fat content and the speed of fungal growth on the wetblue.

Table 5 – Fungal growth on wetblue incubated in tropical chamber

| ID | Oil | Day 1 | | Day 2 | | Day 3 | | Day 4 | | Day 7 | | Day 8 | | Day 9 | | Day 10 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | G | F | G | F | G | F | G | F | G | F | G | F | G | F | G | F |
| 1A | Synthetic 1 - A | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 | 9 | 8 | 9 | 8 | 9 | 7 | 9 |
| 1B | Synthetic 1 - B | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 4 | 7 | 4 | 6 | 3 | 6 | 3 | 6 |
| 2A | Synthetic 2 - A | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 | 10 | 9 | 9 | 9 | 8 | 8 | 7 |
| 2B | Synthetic 2 - B | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| 3A | Palm - A | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 5 | 5 | 4 | 4 | 4 | 4 | 3 | 4 |
| 3B | Palm - B | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 8 | 8 | 7 | 8 | 7 | 8 | 7 | 7 |
| 4A | Palm Kernel - A | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| 4B | Palm Kernel - B | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 7 | 7 | 7 | 6 | 7 | 5 | 6 | 5 |
| 5A | Tallow - A | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 7 | 5 | 6 | 5 | 6 | 5 | 5 | 5 |
| 5B | Tallow - B | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| 6A | Blank - A | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 5 | 5 | 4 | 4 | 4 | 4 | 4 | 4 |
| 6B | Blank - B | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 | 9 | 7 | 9 | 6 | 8 | 6 | 8 |

| ID | Oil | Day 11 | | Day 14 | | Day 15 | | Day 16 | | Day 17 | | Day 18 | | Day 21 | | Day 22 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | G | F | G | F | G | F | G | F | G | F | G | F | G | F | G | F |
| 1A | Synthetic 1 - A | 7 | 9 | 6 | 8 | 6 | 8 | 5 | 7 | 4 | 6 | 3 | 6 | 3 | 6 | 3 | 6 |
| 1B | Synthetic 1 - B | 2 | 6 | 1 | 5 | 0 | 4 | --- | 4 | --- | 3 | --- | 2 | --- | 1 | --- | 1 |
| 2A | Synthetic 2 - A | 8 | 7 | 7 | 7 | 6 | 6 | 5 | 5 | 4 | 4 | 3 | 3 | 2 | 2 | 1 | 2 |
| 2B | Synthetic 2 - B | 9 | 9 | 8 | 8 | 8 | 8 | 7 | 7 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| 3A | Palm - A | 3 | 4 | 2 | 3 | 2 | 3 | 2 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| 3B | Palm - B | 7 | 7 | 5 | 6 | 4 | 6 | 3 | 6 | 3 | 6 | 3 | 6 | 3 | 6 | 3 | 6 |
| 4A | Palm Kernel - A | 7 | 7 | 6 | 7 | 5 | 7 | 4 | 6 | 3 | 6 | 3 | 6 | 2 | 6 | 2 | 6 |
| 4B | Palm Kernel - B | 6 | 5 | 5 | 5 | 4 | 4 | 4 | 4 | 3 | 3 | 2 | 3 | 2 | 3 | 2 | 3 |
| 5A | Tallow - A | 4 | 5 | 4 | 4 | 3 | 4 | 3 | 4 | 3 | 4 | 3 | 4 | 3 | 3 | 3 | 3 |
| 5B | Tallow - B | 5 | 6 | 5 | 5 | 4 | 4 | 4 | 4 | 3 | 4 | 2 | 4 | 1 | 4 | 1 | 4 |
| 6A | Blank - A | 3 | 4 | 2 | 3 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| 6B | Blank - B | 6 | 8 | 6 | 7 | 5 | 6 | 5 | 6 | 5 | 6 | 5 | 6 | 5 | 6 | 5 | 6 |

| ID | Oil | Day 23 | | Day 28 | | Day 35 | | Day 42 | |
|---|---|---|---|---|---|---|---|---|---|
| | | G | F | G | F | G | F | G | F |
| 1A | Synthetic 1 - A | 3 | 6 | 3 | 6 | 3 | 6 | 3 | 6 |
| 1B | Synthetic 1 - B | --- | 1 | --- | 1 | --- | 1 | --- | 1 |
| 2A | Synthetic 2 - A | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| 2B | Synthetic 2 - B | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| 3A | Palm - A | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| 3B | Palm - B | 3 | 6 | 3 | 6 | 3 | 6 | 3 | 6 |
| 4A | Palm Kernel - A | 2 | 6 | 2 | 6 | 2 | 6 | 2 | 6 |
| 4B | Palm Kernel - B | 2 | 3 | 2 | 3 | 2 | 3 | 2 | 3 |
| 5A | Tallow - A | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 0 |
| 5B | Tallow - B | 1 | 4 | 1 | 4 | 1 | 4 | 1 | 4 |
| 6A | Blank - A | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| 6B | Blank - B | 5 | 6 | 5 | 6 | 5 | 6 | 5 | 6 |

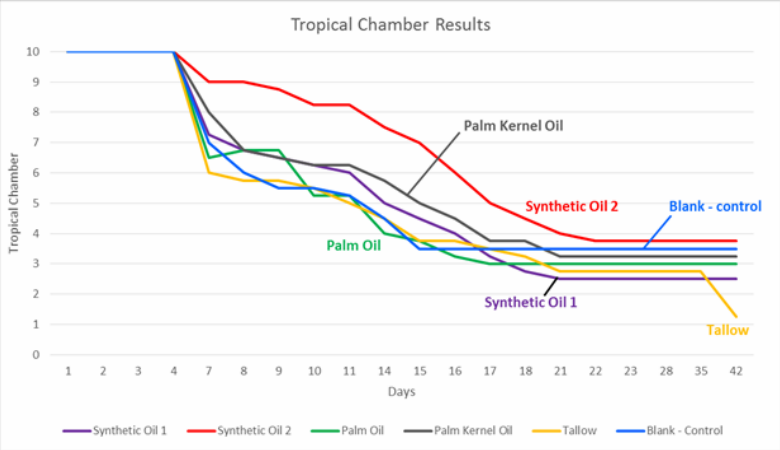


*Figure 1 – Graphical display of average Tropical Chamber results*

## 4 Conclusion

All samples presented intense mold growth in a very short incubation time due to the absence of fungicide during leather processing. The results showed a significant reduction of total fat/oils content in all the samples, except those samples treated with synthetic oil 1. These observations reinforce the conclusion of Zugno et al (2011) that fatty materials are one of the main substrates for fungal growth on wetblue leather. The results also indicate that some types of synthetic oils may not be susceptible to fungal degradation; since the leather treated with this type of oil also presented severe mold contamination after 8-9 days of incubation in a tropical chamber, it is reasonable to conclude that in this case the fungi found another substrate.

It is clear that the fungi that grew on the surface of the wetblue samples displayed a higher metabolism with natural oils (palm kernel, tallow and palm) than with synthetic oil. Nevertheless, after two weeks in a tropical chamber all samples were severely molded. This shows that without fungicide even leathers with no significant content of natural fat (blank) or with synthetic fatliquoring only, would be more easily targeted by fungi.

It was observed that, while some fatliquors presented a clear relationship between fat content in the leather and speed of fungal growth, others did not show the same pattern. This could be related to the fatliquor uptake, its distribution on the leather, and its susceptibility to fungal degradation.
In the sample treated with synthetic oil 1 there was no reduction on the fatty material after tropical chamber, although the fungal growth was very intense after 8 days. In the case of synthetic oil 2, the fungal growth was less intense compared to synthetic oil 1, but the fatty material was reduced by 23% after 6 weeks in the tropical chamber. These results indicate that for the synthetic oils used in this study, there was no direct relationship between the fatliquor and mold.

The results of this first study identified some areas that could be further investigated in order to improve our knowledge of fungal growth and fatty material, including:

- Explore the differences among synthetic oils and their impact on fungal growth

- Study the relationship between fatliquor concentration and ionic character and fungal metabolism
- Identify which other hide components are significantly degraded by fungi (elastin, other non-tanned proteins, sugars, etc.)

## 5 Acknowledgments

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