Effect of fungal growth on pH and grain quality of wet-blue

By Dr Luis A. Zugno & Dr Purushottam V. Gawande, Buckman International, Memphis, TN, USA.

Introduction

There is a need for better understanding of the physical effects of fungal growth on wet-blue leather to help resolve practical processing problems. Conflicting research highlights the complexity of work in this area. For example, it is well documented that dermatophytes tend to produce an alkaline pH when grown on Sabouraud medium. This is due to deamination of amino acids and consequent formation of ammonia. The dermatophytes are pathogenic fungi that are not commonly isolated from leather. On the other hand, certain non-pathogenic moulds such as Penicillium and Aspergillus species, which are commonly found growing on wet-blue leather, are known to shift the pH of the medium towards acidity (Nickerson and Williams, 1947). The leather industry rightly questions if similar pH changes occur upon fungal growth on leather. A second example is the conflicting information regarding the influence of fungal growth on grain integrity. Zugno et al (2009) reported that the fungal growth did not result in visual damage to the grain quality of wet-blue. These results differ from recent publications where wet-blue leather samples were subjected to biodegradation test with the addition of nutrients (Fontoura et al., 2016 a and b).

Dr Luis Zugno, at the FILK Leather Days conference in Freiberg

Materials and methods

- Fungal cultures: Aspergillus niger, Penicillium spinulosum ATCC 16348, Trichoderma viride ATCC 12582, Arthrobotrys amerospora ATCC 36911 and wild type cultures from Tropical Chamber (ASTM D7584-10)
- Mould growth was performed on wet-blue according to ASTM D4576-01.
- Cow lime split pelts sourced from U.S. tanneries were chrome tanned in the Buckman lab.
- Ammonium sulphate, urea, magnesium sulphate and monopotassium phosphate used as chemically defined medium.
- Potato Dextrose Agar (PDA) and Sabouraud Agar used as complex medium.
- Yeast Nitrogen Base tested as nitrogen source growth medium.
- Various emulsified oils and commercial fatliquors were used as carbon sources.
- Media only with emulsifier without fatty material served as a control.
- Surface pH was measured using Orion 8135BN ROSS combination flat surface pH electrode; qualitative pH measurements were made using Bromocresol Green and Universal Indicators.
- Grain evaluation was made with optical microscope and Scanning Electron Microscope (SEM) Jeol JSM-6480LV.

Results

pH measurements on selected culture media are shown in Figure 1.

Fungi were grown on wet-blue at 28°C for 10 days following ASTM D4576-01 protocol. In this test, the leather pieces were embedded in Potato Dextrose Agar. Each fungal culture was spot inoculated twice on agar surface and one time on the wet-blue surface. After 10 day's incubation at 28°C, the fungal growth was observed on growth media and wet-blue. The wet-blue pH was

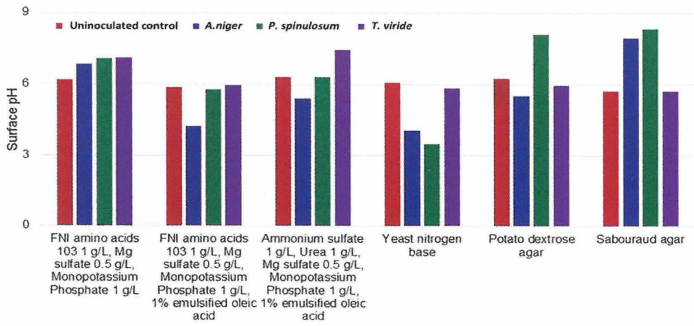

Figure 1: Effect of growth media on pH change upon fungal growth, 21 days incubation at 28°C

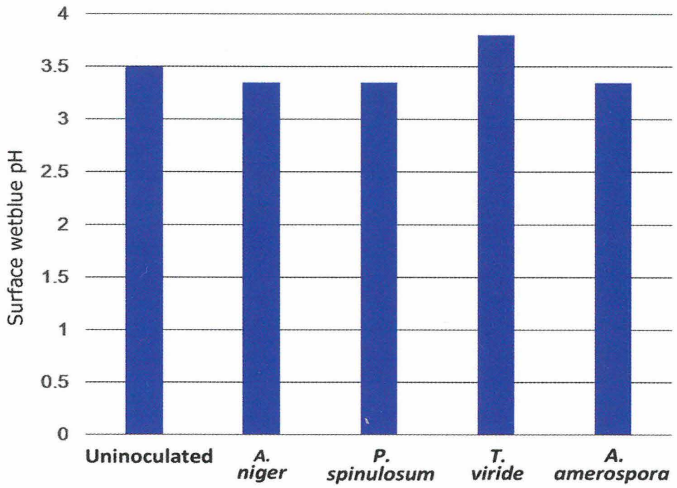

Figure 2: Effect of fungal growth on wet-blue pH, 10 days incubation at 28°C

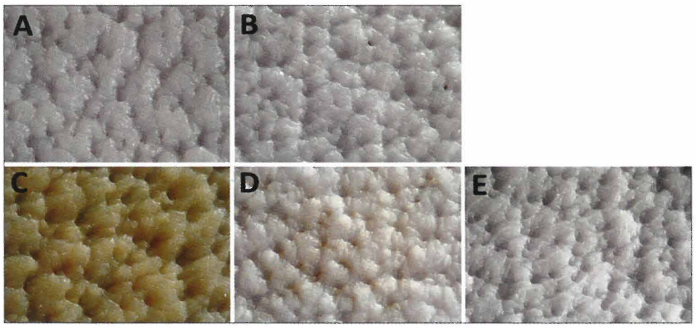

Figure 3: Optical microscope photographs: A: A. niger; B: P. spinulosum; C: T. viride: D: A. amerospora and E: Control

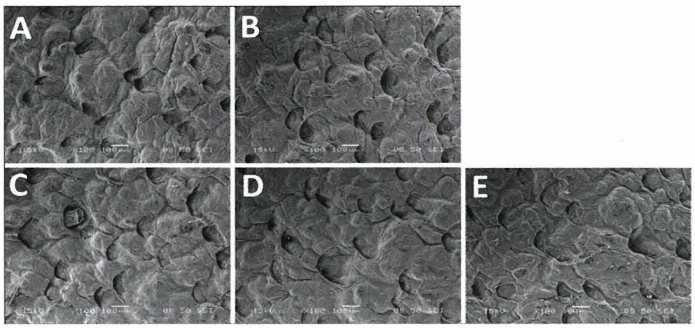

Figure 4: SEM images of the gold coated samples. A: A. niger; B: P. spinulosum; C: T. viride: D: A. amerospora and E: Control

measured using a pH probe (Figure 2). No significant change in pH was observed upon fungal growth compared to an uninoculated wet-blue sample.

The wet-blue samples were removed from PDA, washed and observed under optical (Figure 3) and SEM (Figure 4) for grain damage. No additional grain damage was observed as compared to control wet-blue (uninoculated sample) by both the microscope techniques.

The wet-blue pieces without fungicide were soaked in different growth media and incubated in a Tropical Chamber for 21 days in order to grow wild fungal cultures. After 21 days the pieces were removed from the Tropical Chamber, wiped with paper to remove the fungi and the surface pH was measured using surface pH electrode. No significant pH change was observed for either one of the nutrient combinations as compared to control without nutrients (Figure 5).

The effect of carbon sources on pH change was studied using media containing ammonium sulphate, urea, magnesium sulphate and monopotassium phosphate (recommended by in-house enzymologist Dr Jaquess - personal communications). Several carbon sources including emulsified fatty acids and commercial fatliquors (seven from shoe upper and five automotive) were used. This resulted in good fungal growth on most of the carbon sources tested; the lowest growth was observed with the C9 fatty acid. A. niger reduced pH; P. spinulosum, T. viride and wild fungal cultures increased pH as compared to uninoculated agar plates (Figure 6).

Discussion

In general, the results indicate that Aspergillus slightly decreased the pH and other fungal cultures showed moderate increase in pH as compared to uninoculated growth media. No significant pH change was observed on wet-blue samples tested following both protocols such as ASTM D4576-01 and growth in tropical chamber. This is consistent with our observations that wet-blue experiences very little pH change due to the mould growth. This could be attributed to the excellent buffering capacity of wet-blue due to presence of collagen acid groups and the formic acid/formate buffer.

No additional grain damage was observed upon fungal growth as compared to uninoculated wet-blue. The results are in accordance with many years of observation with mouldy wet-blue leathers. This could be due to several reasons such as (i) wet-blue typically resists fungal growth more than wet-white or vegetable leather, (ii) wet-blue surface pH is highly acidic for collagenase activity (typical pH optima 6.5-8.5) to show grain damage and (iii) free chromium on the wet-blue might inhibit collagenase activity

Case Study

A South American tannery had a severe case of stains on wet-blue sometimes associated with the presence of mould. The wet-blue turned intense dark blue when bromocresol green indicator was added to the surface, indicating that superficial pH was above 5.4. One sample (Figure 7) was sent to the Buckman lab in Memphis for analysis. No mould was observed and the pH on the grain surface measured 4.9. The flesh pH was measured with bromocresol green indicator and found to be in the range 3.8 to 5.4.

The stained and non-stained areas were analysed by Energy Dispersive X-Ray Spectroscopy (EDX) (Figure 8). In addition, the stained area was analysed by Thermal Scale Elemental X-ray mapping analysis for aluminium.

EDX analysis show that the stained area contained 5.6 and 1.8 times higher amount of aluminium and chrome respectively. Excess alkalinity can precipitate chromium and aluminium making punctual green stains. This could be followed by mould growth in some cases, leading to the false conclusion that the mould growth caused the stains.

Conclusions

On lab growth media it has been shown that A. niger slightly reduced pH, P. spinulosum, T. viride and wild cultures increased

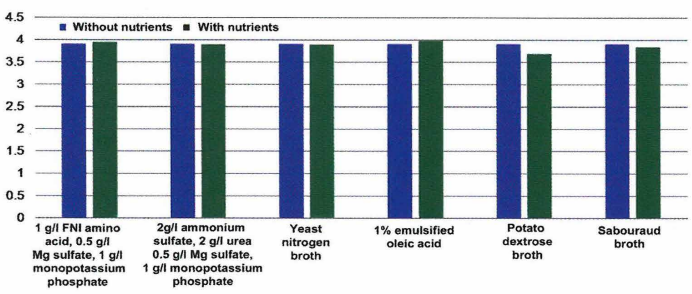

Figure 5: pH measurements on wet-blue exposed to different culture media and incubated in Tropical Chamber for 21 days

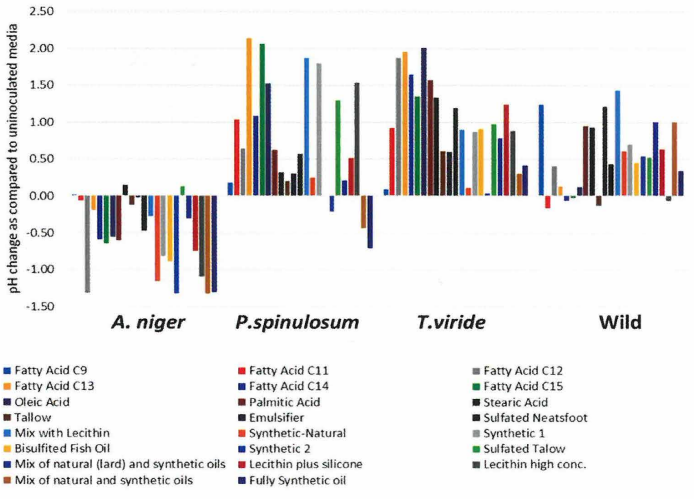

Figure 6: Evaluation of carbon sources (fatty acids & fatliquors) on growth using lab growth media, 21 days at 28°C

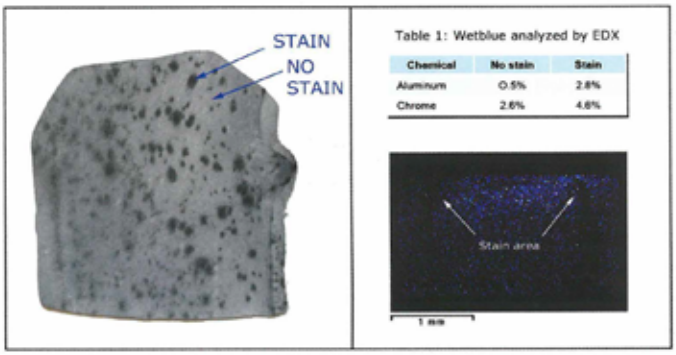

Figure 7: Wet-blue sample with stains and Figure 8: Thermal Scale Elemental X-ray mapping analysis for Aluminium with indication of stained area

pH. It is well known that fungal cultures produce various metabolites under different growth conditions and it is concluded that this leads to the noted pH changes. Unlike lab growth media, no change in pH was noticed after mould growth on wet-blue contaminated by both lab and wild fungal cultures. This could be because there is a lack of readily metabolizable substrates on wet-blue and due to the buffering capacity of wet-blue leathers. Even with careful observation, grain damage was not observed in wet-blue contaminated by both lab and wild cultures (at least no more damage than evident in the control sample).

The case study highlights the need for careful analysis of industry problems. Dark green/green spots and pH change noticed by tanners on wet-blue leather accompanied by mould growth was most likely not caused by mould growth, but by process issues resulting in precipitation of processing materials and changes in chemical composition of the wet-blue over time.

Acknowledgement

The authors thank Elton Hurlow, Dr Percy Jaquess, Dr Bernard Janse and Dr Tom McNeel for helpful suggestions. This paper is based on the presentation made during the 5th FILK Leather Days, held in Freiberg, Germany, June 15-16, 2016 by Dr Luis Zugno.

Reference: ASTM Designation: D 4576-01 Standard Test Method for Mould Growth Resistance of Wet-blue.
ASTM Designation: D7584-10 Standard Test Method for Evaluating the Resistance of the Surface of Wet-blue and Wet White to the Growth of Fungi in an Environmental Chamber.
Fontoura, JT, Ody, D. and Gutterres, M (2016a) Performance of antimicrobial agents for the preservation of chrome leather. JALCA (111) 221-229.
Fontoura, JT, Ody, D. and Gutterres, M (2016b) Performance of microbicides for the preservation of vegetable tanned leather JALCA (111) 259-265.
Nickerson, WJ and Williams, JW (1947) Nutrition and metabolism of pathogenic fungi. Chapter in Biology of Pathogenic fungi (Nickerson, ed.) Waltham Mass., Chronica Botanica Co., pp. 148-150.
Zugno, L., Hurlow, E. and Oppong, D. (2009) Fungal growth on wet-blue: Methods to measure impact on leather quality, 30th IULTCS Congress, Beijing, China.