Paper A5

Investigation of Red Stains on Wetblue Leather

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Abstract

Stains on wetblue leather are a common problem. Determining the etiology and mitigation of stains can be difficult. Stains can appear during processing or may evolve after the wetblue has been shipped to far-away destinations. This can be perplexing for the tanner. Red stains are one of the more frequent problems associated with wetblue production and the subject of this investigation. The cause of red stains on wetblue has been linked with microbiological growth and with metal contamination, specifically iron. When red stains are associated with mould growth causation can be easily ascribed if evidence of fungal growth remains on the leather. When mould or other contaminants are not present, the cause and origin of the red stains can be more difficult to analyze. Red stains associated with metal contamination are frequently described in the literature as "chrome soaps" but the components are not well understood. In this paper we will evaluate the importance of metals in the formation of red stains by reacting iron and chrome with tallow and a range of free fatty acids. The generation of red stains is simulated in the laboratory and the underlying components identified. Based on the results obtained, pragmatic steps to minimize occurrence of red stains in wetblue are suggested.

Key Words: wetblue, leather, red stain, chrome soap, iron, mould

1. Introduction

Various shades of green, yellow, red, pink, brown, and black stain are found on wetblue leather. The shape, size, and ease of stain removal can be a clue to their origin and mitigation. Red or pink stains are a significant and recurring problem for wetblue producers. The etiology and composition of these stains is the subject of this paper. For brevity, we will only use the term red stain, but this includes pink stains as well.

Red stains are typically associated with the presence of fat, chrome, and iron, and may be correlated with growth of microorganisms. Practical experience points towards a higher incidence in salted hides versus fresh hides and the problem appears to increase with direct chrome recycling systems. Stains are often associated with poorly preserved raw material. Generation of red stains has also been linked to fungal growth on wetblue after production[1]. It has been observed that there is greater hydrophobicity associated with the red stained area. Red stains are found in sheep and goat, but the predominant commercial problem is experienced with bovine wetblue. Mechanical operations such as splitting and shaving may enhance the prevalence of red stains and they are sometimes observed with greater intensity surrounding blood vessels. The stains are often not readily apparent on freshly produced wetblue stock, but can appear after a few days or weeks of storage – a source of frustration for the tanner. It is common knowledge that these stains can be difficult to remove in the blue. Without good understanding of the etiology and composition of the stains, it is difficult to recommend appropriate measures for prevention.

The literature states that red stains involve chromium, iron, and fat, but the fundamental role of these components in forming the stain is not made clear[2,3,4]. Authors refer to these stains as "chrome soaps", and this suggests necessary involvement of chromium for color generation. Also, it may be logically deduced that the frequent association of the stain with bacterial or fungal activity implies a greater involvement of the metals with metabolites of fatty material and not with tallow or with pigmentation from the actual microorganism *per se*. It is perfectly feasible that exogenous bacterial or fungal lipases degrade triglycerides present in the hide to form free fatty acids (FFA) which then react with the metals to form the red stains.

However, such speculation needs to be tested, and the leather literature is not enlightening regarding the precise origin and components of these common stains.

This work was undertaken to better determine the origin of the red stains, reach some conclusions on the chemistry involved, and reflect on possible mitigation of red stains.

2. Materials and Methods:

2.1. Fat, metal, and iron analyses

Cuttings of wetblue leather were analyzed for fat and iron using pieces cut from unstained wetblue and an adjacent area showing heavy red stain. Standard fat analysis was performed using IUC/4 industry method. The samples were also analyzed for metal content using standard SEM-EDX analysis. A spot test for iron, using an acidic potassium thiocyanate solution with dilute hydrogen peroxide, was also performed on both areas.

2.2. Simulated tannery reactions of chromium, iron, and selected fatty materials

A series of typical industry solutions (50 ml) were prepared in 250 ml Erlenmeyer flasks. These included a standard pickle solution and a standard tanning solution containing 0.5 g chromium sulfate of 33% basicity. Iron was omitted from the first series, but 10 mg Fe^{3+} was added to the second two series. Selected fatty materials were introduced to all samples in the amount of 2 g; with the exceptions of linoleic and palmitoleic where 1.0g was used, and for linolenic the quantity used was 0.3 g. The selection of component materials was based on an analysis of typical bovine tallow and FFA composition - see Table 1.

Table 1: Analysis of Tallow (98.5% Triglycerides) showing FFA Composition, C-chain length and level of Saturation

FFA	C:Saturation	%	FFA	C:Saturation	%	FFA	C:Saturation	%
Butyric	C4 : O	0.00	Pentadecenoic	C15 : 1	0.15	Eicosadienoic	C20 : 2	0.00
Valeric	C5 : O	0.00	Palmitic	C16 : O	24.61	Homo-y Linolenic	C20 : 3	0.00
Caproic	C6 : O	0.00	Palmitoleic	C16 : O	2.87	Arachidonic	C20 : 4	0.00
Enanthic	C7 : O	0.00	Hexadecadienoic	C16 : 2	0.58	Eicosapentaenoic	C20 : 5	0.00
Caprylic	C8 : O	0.00	Hexadecatetradienoic	C16 : 4	0.12	Heneicosanoic	C21 : O	0.00
Perlargonic	C9 : O	0.00	Margaric	C17 : O	1.29	Behenic	C22 : O	0.00
Capric	C10 : O	0.05	Margaroleic	C17 : 1	0.73	Erucic	C22 : 1	0.00
Undecanoic	C11 : O	0.00	Stearic	C18 : O	18.42	Docosadienoic	C22 : 2	0.00
Undecylenoic	C11 : 1	0.00	Oleic	C18 : 1	41.50	Docosatrienoic	C22 : 3	0.00
Lauric	C12 : O	0.07	Linoleic	C18 : 2	3.05	Docosatetraenoic	C22 : 4	0.00
Dodecenoic	C12 : 1	0.00	Linolenic	C18 : 3	0.19	Docosapentaenoic	C22 : 5	0.00
Tridecanoic	C13 : O	0.00	Octadecatetraenoic	C18 : 4	0.45	Docosahexaenoic	C22 : 6	0.00
Tridecenoic	C13 : 1	0.03	Nonadecanoic	C19 : 0	0.00	Lignoceric	C24 : O	0.00
Myristic	C14 : O	3.19	Nonadecenoic	C19 : 1	0.16	Nervonic	C24 : 1	0.00
Myristoleic	C14 : 1	0.64	Arachidic	C20 : O	0.17	Other fatty acids	----	0.84
Pentadecanoic	C15 : O	0.48	Gadoleic	C20 : 1	0.40			

Decanoic acid (C10:O) and cholesterol were also tested. High purity materials were used where possible but commercial samples of oleic acids containing other FFA as impurities were also tested. Samples selected are shown in Table 2. The samples were put on a standard laboratory shaker and shaken for 72 hours at 35°C to ensure a good mixing and interaction of insoluble fatty materials with components in the simulated tannery solutions.

Table 2: Selected Fatty Materials reacted with Tannery Solutions

ID	Fatty Material	ID	Fatty Material	ID	Fatty Material
A	*Decanoic acid*	*F*	*Oleic acid 99%*	*K*	*Stearic acid*
B	*Cholesterol*	*G*	*Oleic acid 90%*	*L*	*Tallow*
C	*Linoleic acid*	*H*	*Oleic acid 80%*	*M*	*Mix - oleic, palmitic, stearic, 33% each*
D	*Linolenic acid*	*I*	*Palmitic acid*		
E	*Myristic acid*	*J*	*Palmitoleic acid*		

2.3. Quantitative reactions of iron and oleic acid

Four samples of a typical pickle solution (100 ml) were spiked with increasing amounts of iron. The samples were then split into two 50 ml aliquots. To the first split sample, 2.0 g of oleic acid 80% was added, and no oleic acid was added to the second split sample. After shaking for 72 hours, the eight samples were left for the oil to separate and 30 ml aliquots of the water phase were then taken for Fe^{3+} analysis.

2.4. Simulated breakdown of triglycerides and reactions with iron

Various mixtures of tallow, oleic acid, soda ash, and lipase enzymes were made and then spiked with iron to simulate reactions that might occur during leather processing. The procedures followed were:

A. Tallow + soda ash, run 24 hours on shaker, drop pH to 3.5 with acid, add 20 mg Fe^{3+}
B. Tallow + soda ash + lipase, run 24 hours on shaker, drop pH to 3.5, add 20 mg Fe^{3+}
C. Tallow + soda ash + lipase + surfactant, run 24 hours on shaker, drop pH to 3.5, add 20 mg Fe^{3+}
D. Tallow + soda ash + surfactant, run 24 hours on shaker, drop pH to 3.5, add 20 mg Fe^{3+}

2.5. Thin Layer Chromatography (TLC): Separation of selected fat compounds and comparison with red stain extracted from wetblue leather

The following samples were prepared in an effort to simulate components of the red stain area. The following mixtures were then evaluated using TLC:

A. Oleic, palmitic, and stearic acids – commercial sample
B. Bovine tallow – commercial sample
C. Wetblue leather extract - A sample of wetblue leather showing extensive red stain was extracted with dichloromethane and Soxhlet apparatus according to the standard method for fat analysis (IUC/4).
D. Bovine tallow hydrolysate (TH) – The TH was prepared by adding 5 g bovine tallow and 1g lipase enzyme to 100 ml water and agitated overnight on a laboratory shaker at 35°C. The resulting TH was cooled, acidified to pH 1.0 with 10% sulfuric acid to revert the fatty soaps to FFA's. The phases were separated in a volumetric funnel. The resulting paste was washed repeatedly and then removed from the volumetric flask for analysis.
E. TH + iron – These compounds were produced by reacting 1 g of the fatty material with 10 mg Fe^{3+} in 100 g of water for 3 days at 35 °C with agitation.
F. Oleic + iron – These compounds were prepared as for E above.

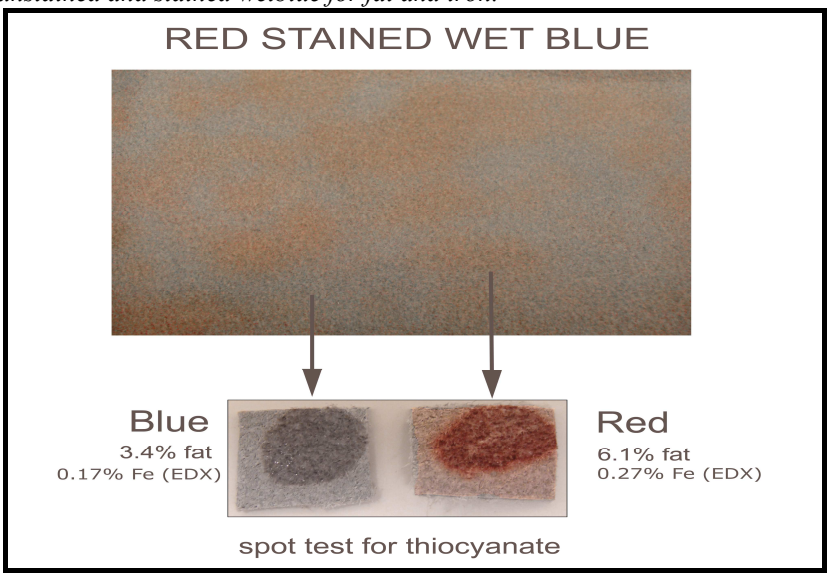
Several types of TLC plate and different solvent mixtures were evaluated to determine best system to obtain good separation of the components of iron and FFA complexes. Good results were obtained with the stationary phases 1) reversed phase silica gel absorbent of 60 Å pore size on PET foil plate, and 2) microcrystalline cellulose fiber plate (Eastman 6065 Cellulose chromatogram sheet). Solvents tested included hexane, ethyl acetate, formic acid, ethyl ether, methanol, and dichloromethane. Best results for the silica gel separation were obtained with a 1:2 mixture of ethyl acetate:hexane, and for the cellulose fiber separation with 1:1 mixture of methanol:dichloromethane. Developed TLC plates were removed from the development chamber, dried, and placed in an iodine chamber for enhanced visualization.

3. Results and Discussion

3.1. Fat, metal and iron analyses

Figure 1 shows the fat content of the unstained wetblue was approximately half the value of the fat level in the red stain area. The amount of iron present in the unstained area, determined as a semi-quantitative ratio of the elements identified by SEM-EDX, was also approximately half of the amount of iron present in the stained area. The KSCN spot test provides good visual confirmation of higher iron content. Clearly higher iron and fat are associated with the red stain area.

Figure 1: Analysis of unstained and stained wetblue for fat and iron.

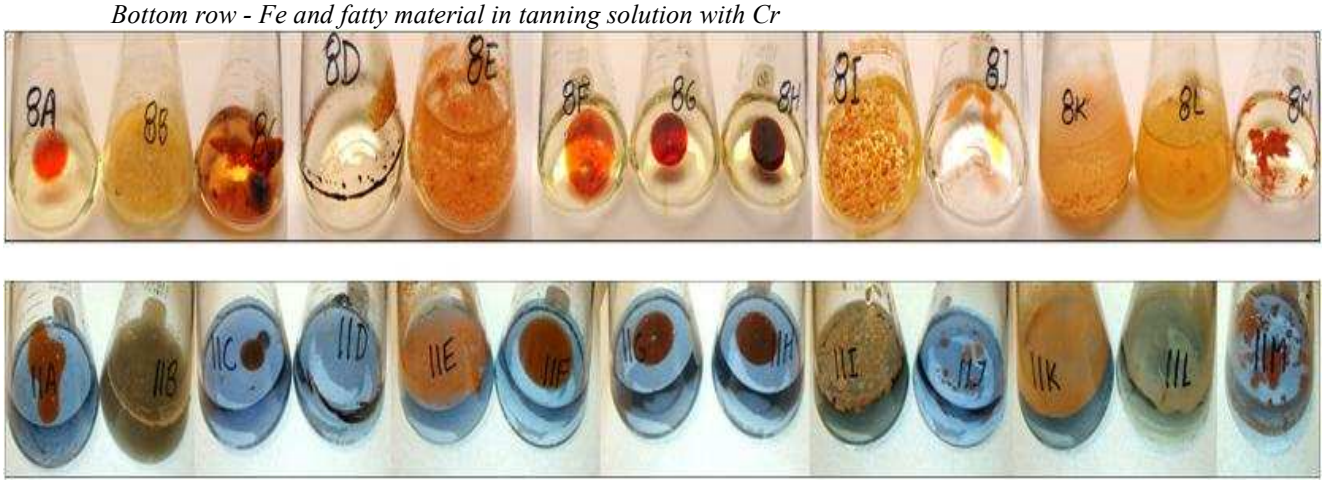


3.2. Reaction of selected fatty materials in pickle and tanning solutions, with and without Fe^{3+} added

Reaction of the tanning solution, which contained chromium but no iron added, resulted mostly in colorless solutions. The exceptions were linoleic and linolenic acid (highly unsaturated FFA's) where a dark blue to charcoal matter was observed sticking to the sides of the flask. No red or brown stains were observed in any of the samples. This is an indication that chrome may not play a role in color formation.

Figure 2 shows reaction of the fatty materials in pickle and tanning solutions with Fe^{3+} added. Cholesterol and tallow (Samples B and L respectively) resulted in formation of a brown to slightly orange color. A slight brown to orange coloration was also observed in the saturated FFA's myristic, palmitic, and stearic acid (Samples E, I & K). An intense orange to red coloration was observed with decanoic acid and the remaining unsaturated FFA's represented by linoleic, linolenic, oleic, palmitoleic acid (Samples C, D, F, G, H, J) and a mixture of the three most common FFA's present in beef tallow (Sample M). Iron produces a more intense color with increasing level of unsaturation of the carbon chain. It is also interesting to compare the samples of oleic acids at higher impurity levels, the intensity of color was highest with the least pure commercial oleic acid (i.e. F<G<H) which suggests the impurities are FFA's with higher levels of unsaturated carbon chain.

Figure 2: Top row - Fe and fatty material in pickle solution.
Bottom row - Fe and fatty material in tanning solution with Cr

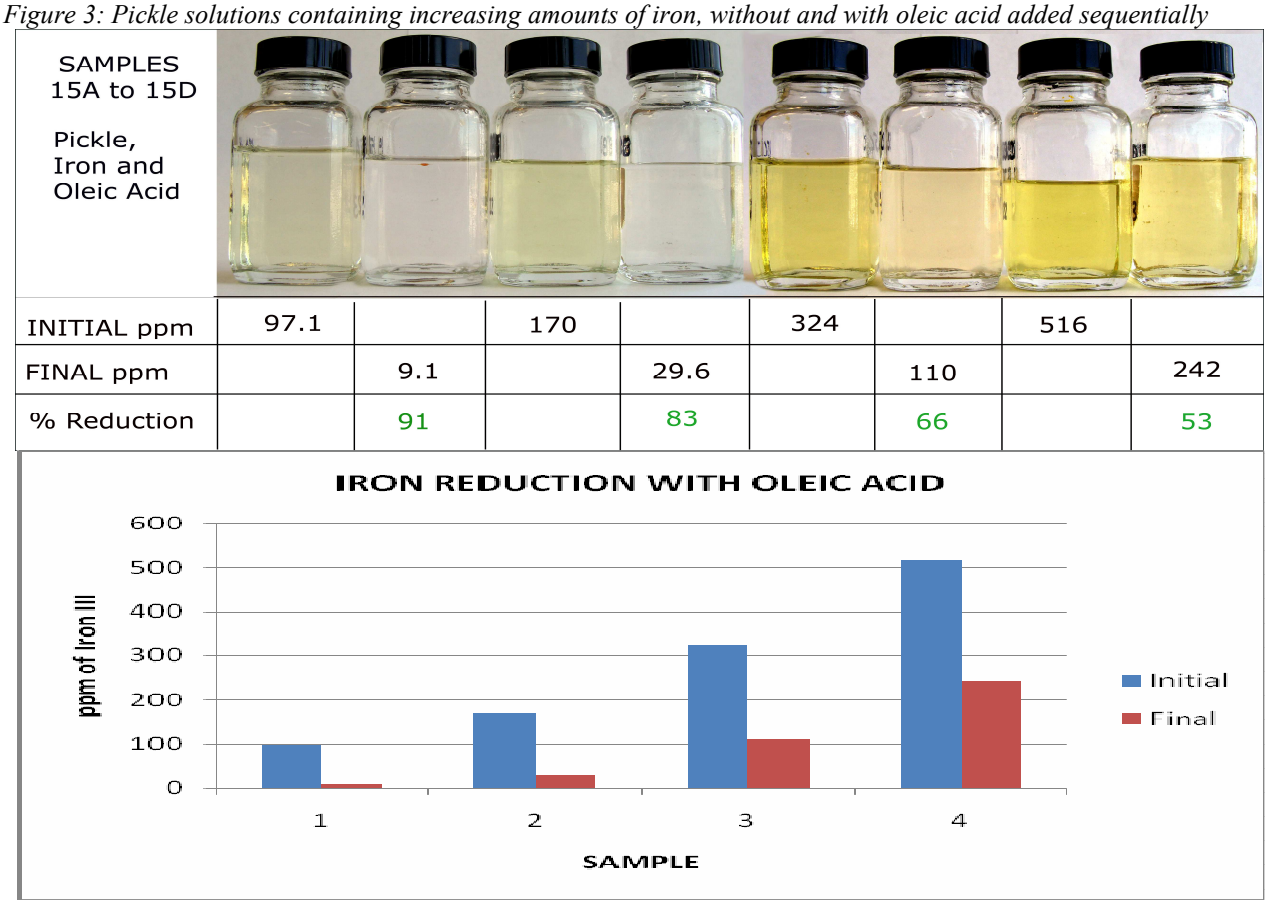


No significant difference in the intensity of the color or the sequence of color formation was observed from samples with chrome and/or those without chrome. This is a further indication that chromium may not play an important role in the development of red stain coloration.

3.3 Quantitative reactions of iron and oleic acid:

Figure 3 shows from the color of the samples that oleic acid has a substantial ability to remove Fe^{3+} from solution (yellow intensity increases with higher iron content). As the iron concentration increases in four stepwise intervals, the absolute amount of iron removed by the oleic acid increases, although the percentage removal declines. This is shown visually and graphically in Figure 3.

Figure 3: Pickle solutions containing increasing amounts of iron, without and with oleic acid added sequentially



INITIAL ppm	97.1		170		324		516	
FINAL ppm		9.1		29.6		110		242
% Reduction		91		83		66		53

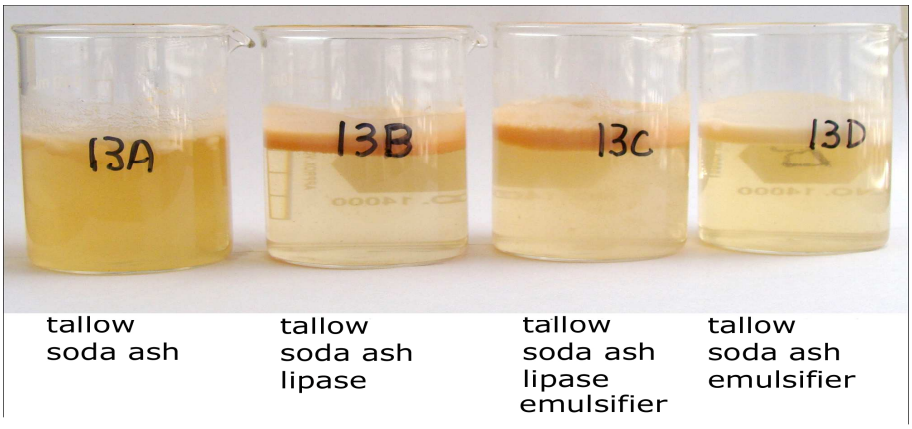
3.4 Simulated breakdown of tallow and reactions with iron

The initial results after 24 hours show a separation of phases with the top fatty layer exhibiting a pink color on the samples B and C and slight color in sample D – see Fig. 4. The acidic aqueous phase was reasonably clear. The aqueous phase in Sample A remained yellow in color indicating that the iron remained in solution. The samples were then put back on the shaker and shaken for a week to simulate the aging of the reaction. After one week, the intensity of samples B, C, and D were of similar intensity. This indicates that the iron present is sequestered by FFA's forming pinkish red complexes, and that the surfactant does little to delay or prevent the formation of the complex when FFA's are present. The aqueous phase of sample A remains intense yellow. This implies that the triglyceride is not involved in sequestration of the iron as it does not appear to remove Fe^{3+} from solution.

3.5. TLC separation of fat components and comparison with red stain extracted from wetblue leather

Selected results of different TLC scans are shown in Figure 5a and 5b. Initial work on TLC separation of oleic, palmitic, and stearic acid was difficult and they appeared as a single band in this system. It was decided to work with oleic acid only as a common representative of the FFA's.

Figure 4: Top – simulated formation of FFA's. Bottom – same samples after 1 week ageing.



In Figure 5a, the first two samples show good mobility and separation of the fatty material oleic and tallow, with the tallow travelling furthest from origin, close to the solvent front. The center sample shows partial hydrolysis of the tallow with good separation of the components into two distinct bands aligned with oleic acid (FFA's) and intact tallow (triglyceride). When oleic is reacted with iron (last sample), the oleic + iron complex remained immobile near the origin. The extracted sample of red stain leather separated into three components. There was an immobile band similar to the oleic + iron complex near the origin, a diffuse band with similar mobility to the oleic or FFA's, and a distinct band in line with the tallow. All three of these components may be expected in the fatty extract from the stained wetblue leather.

Using a less polar plate and suitable mobile phase, it was possible to get the hydrophobic complex to migrate. Figure 5b shows the developed cellulose plate where 1) the tallow hydolysate + iron and 2) the red stain leather extract show a diffuse pattern near the origin but distinct and comparable spots that move near the solvent front. To visualize these spots, the first plate is shown sprayed with acidic potassium thiocyanate enhanced with dilute hydrogen peroxide to visualize iron, and the second plate is then exposed to iodine to highlight fat material. It is assumed that the indicated spots are an "iron + FFA" complex.

Figure 5a: TLC separation of oleic acid, tallow, tallow hydrolysate, leather extract, and oleic reacted with Fe^{3+}

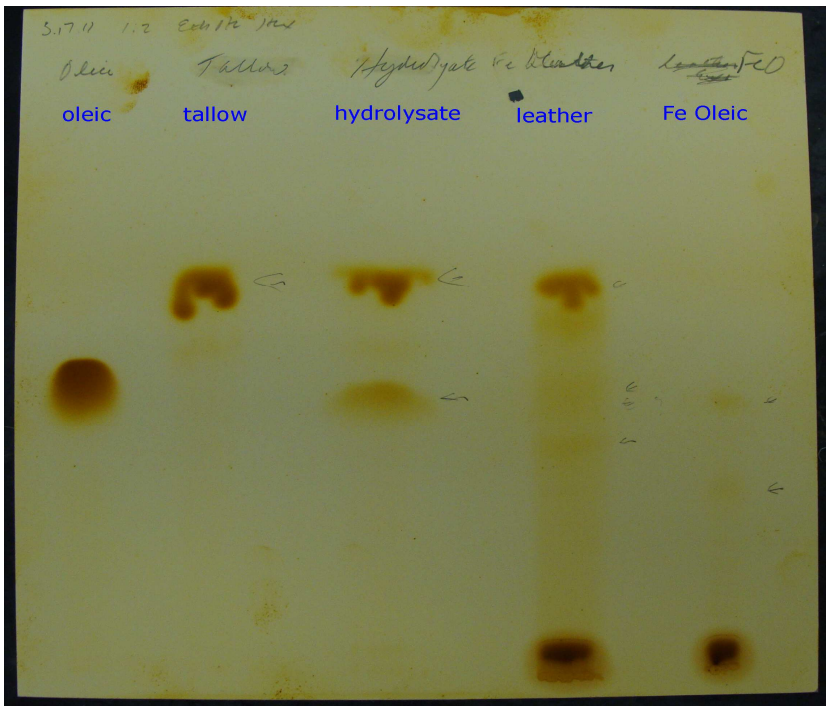
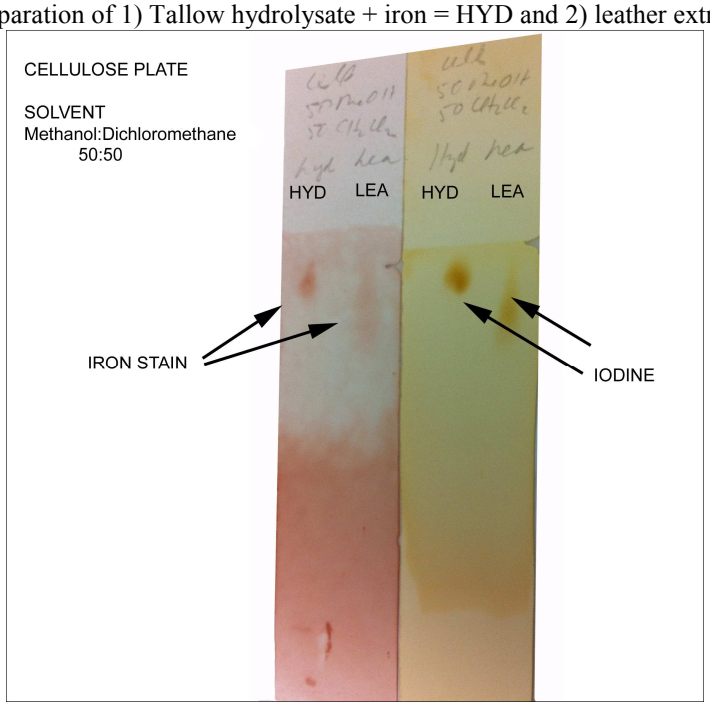


Figure 5b: TLC separation of 1) Tallow hydrolysate + iron = HYD and 2) leather extract = LEA.



Discussion on origin, composition, and mitigation of red stains

It is clear that FFA's and iron form red stains. Other components tested did not produce red or pink colors on their own. It is proposed from this work that the red stains in wetblue are present primarily as hydrophobic clusters of FFA's coordinated with iron. The literature suggests these types of complexes are not simple. For example, the common iron complex formed with stearic acid has been elucidated in work on biological transport of Fe^{3+}, and it typically forms a trimeric nuclear compound structure with 6 coordinated stearic acid

ligands[5]. This forms a stable pink compound. It is unlikely that tallow (triglycerides) are part of the color complex, but they may be associated with the stain area as hydrophobic compounds. We also cannot categorically exclude the involvement of chromium, (various heteronuclear metal complexes that involve Fe and Cr are known[6]), but it is possible that the presence of chromium is coincidental.

From practical experience, once red stains are present in wetblue, they cannot be washed out using surfactant, are only partially removed with strong oxidizing agents or bleach, and do not respond well to traditional sequestrants such as EDTA or phosphonates. In general they tend to be less noticed after retan, dye, and fatliquor operations, but it is likely that they are responsible at some level for uneven uptake of chemicals and non-uniform dyeing. It is suggested that part of the problem is the hydrophobicity of the complex and difficulty in wetting the stained area.

It would appear that the best solution for mitigation is prevention. FFA's are present in hides and skins at low concentration, but the breakdown of triglycerides through action of bacterial exo-enzymes, lipases, and saponification as a result of processing at high pH will increase the amount present. FFA's are more water soluble in aqueous systems, so good washing after liming and during deliming is normally sufficient to effectively remove these contaminants, or at least reduce them to levels that are not significant for red stain formation. Post production formation of FFA's from natural fat in wetblue by action of fungal organisms is prevented by appropriate use of fungicides. Eliminating iron from chemicals used, removal of iron from process water, and reduced contact with iron from machinery is also useful to help minimize red stains. This is especially important in recycle systems where metals and FFA's can concentrate.

4. Conclusions

This work has clearly demonstrated that free fatty acids and iron are required to form red or pink stains. Pure tallow or triglycerides do not form red stains. FFA's are naturally present in the hide or skin, but are also formed by saponification reactions during processing, through the action of lipases, or through microbial activity. Iron is present in the hide or skin at low levels, but may be introduced with chemicals, process water, or by contact with equipment. Chrome is often found associated with the stain area, but it cannot be concluded from this study that chromium is indeed part of the iron + FFA complex. The presence of chrome could be coincidental.

To prevent red stain formation, good preservation of hides and skins before and after wetblue production is important, and FFA's produced as a result of manufacturing conditions should be removed by adequate washing. Steps should also be taken to reduce levels of iron.

It is suggested by the authors that these common red or pink stains on wetblue, heretofore called "chrome-soaps" by the leather industry should in future be more accurately referred to as "iron-FFA complexes".

5. References:

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