CHARACTERIZATION OF THE EFFECT OF HEAT ON VEGETABLE TANNED LEATHER AND RESTORATION TRIALS THROUGH ENZYMATIC PROCESSES

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Cultural heritage artefacts made on leather may suffer from adverse condition during conservation that results in an irreversible change of their chemical and physical properties. Our research aims to develop a new restoration approach for leather having lost its flexibility after exposure to heat. The characterization of heat-damaged leather was performed by various technics such as Dynamic Mechanical Analysis (DMA) and contact angle measurement. Heat causes darkening, mass loss, shrinkage, stiffness increase and renders leather non wettable. Part of these changes can be due to an aggregation of leather proteins as a result of heat exposure. An innovative method relying on the use of biological molecules was developed in order to respect the nature of the object and preserve its past and future. Enzymes such as hydrolases able to break the protein aggregates have been used. One of the challenges was to provide water necessary for the enzyme activity without wetting the leather surface to avoid further damage of the leather. Several procedures were tested and compared to decrease water availability/activity, and first promising results were obtained with an enzymatic emulsion allowing a flexibility gain of about 20% of heated leathers. Moreover the efficiency of the enzyme in this treatment has been demonstrated. Attempts to restore will be pursued in this direction.

Keywords: leather, heat, enzymes

INTRODUCTION – BACKGROUND AND HYPOTHESIS

The research project named "BIORESTOCUIRS" focusses on cultural artifacts having leather, such as book bindings, that have been exposed to drastic conditions. Exposure to high heat during a fire is especially devastating and has for consequence to turn the items non-handable due to its rigidity and fragility. The first goal of this project is to characterize the changes induced to leather by heat in order to understand at various scales its consequences. The second objective is to elaborate an innovative treatment based on an enzymatic process to restore leather initial properties, especially its flexibility.

MATERIALS AND METHODS

Materials

Different new calf leathers tanned with vegetable sumac (hydrolysable) or mimosa (condensed) tannins were used for the experiments. Artificially altered samples were prepared by exposing the leather to dry heat at 160°C for 4 days.

Characterization Methods

To quantify the loss of flexibility after exposure to heat and the efficiency of the restoration treatment, dynamic mechanical analysis (DMA) was performed using a DMA Q800 (TA instrument) in tensile and frequency sweep mode between 0.5 and 60Hz at room temperature, under a controlled strain of 0.05 % and 0.01N static force; specimens are placed in the direction head-tail.

To determine the consequences of heat exposure on water absorbency, leather samples (unheated and heated) were immersed in pure water. Before and during the measurement (each hour), samples were weighted until an equilibrium state is reached.

To determine the sample surface hydrophobicity and wettability, a goniometer is used. A droplet of water (15µL) is deposited on the leather sample surface (grain side) and the droplet behaviour on the support is recorded. Contact angle value (θE) is determined by the software "Drop Shape Analysis". Material is defined as hydrophobic when θE is superior to 90° and wettable if the droplet can penetrate the material within 3 minutes.

RESULTS AND DISCUSSION

Characterization of Modifications Induced to Leather by Exposure to Heat

Following heat exposure, shrinkage and darkening of the sample were both accessed at a macroscopic scale.

First, dynamic mechanical analysis (DMA) was performed to quantify the loss of flexibility after heat exposure as shown in figure 1.

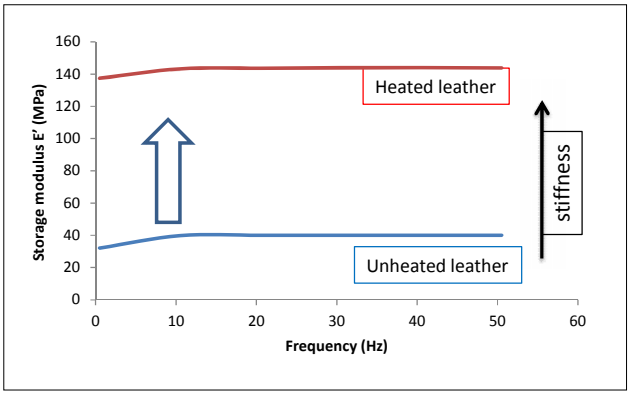

Figure 1. Storage modulus of an unheated leather and a heated leather as a function of frequency

The results show a large increase of the storage modulus (up to about 260%) after exposure to heat correlated with an increase in leather stiffness. Considering that one of the main objective of the restoration treatment is to restore leather flexibility, this method will be essential in evaluating the efficiency of the treatment.

Because the restoration treatment has to be applied on the surface through an aqueous solution, the wettable and absorbency properties of the leather with water has

to be determined. The contact angle, wettability and water absorbency measurement are shown in figure 2.

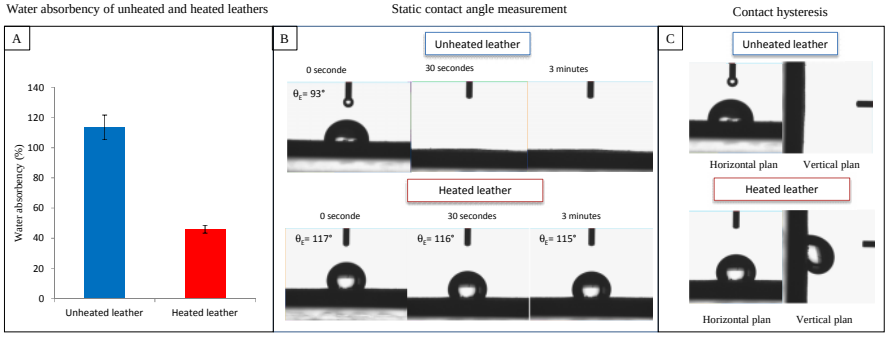

Figure 2. Water absorbency [A], hydrophobicity measurement and wettability in static mode [B] and contact hysteresis observation in dynamic mode [C] of unheated and heated leather

Results highlight that water absorbency has decreased in the heated leather compared to the unheated one (fig.2[A]). Contact angle measurement and wettability, show that after exposure to heat leather becomes more hydrophobic, with θE> 90°, and also non wettable, as the droplet does not penetrate into the leather within 3 minutes (fig.2[B]). This variation could be due to heat-induced chemical modifications of leather components, i.e. heated proteins are usually more hydrophobic than native ones (Baldwin, 1986). These results could also be attributed to the increase in rugosity after heat exposure; as shown in figure 2[C]. The droplet does not flow from the vertical surface, this "lotus effect" is due to a large rugosity at the nano- or micrometric scale. This result also predicts probable difficulties for our restoration treatment to penetrate inside the heated leather.

As largely reported in literature, heat creates protein aggregation (Wallace *et al.*, 1986). Our observations of the macroscopic properties of heated leather are in good agreement with such hypothesis. Moreover, sequential extraction experiments have revealed that some proteins (i.e. fibronectin) cannot be solubilized, even in denaturing solutions (urea, sodium hydroxide) after heating, while they are extracted before heating (data not shown). This indicates that exposure to heat leads to a rearrangement of the leather proteins.

Restoration Attempts

The restoration treatment is based on the use of enzymes to hydrolyze protein aggregates formed after heat exposure. Such approach is a challenge since, water is necessary for ensuring enzyme activity by allowing it to preserve its active three-dimensional structure and the flexibility necessary for the catalytic process. However water has also a dramatic damaging effect on leather having been exposed to heat : the shrinkage, the stiffness and the darkening observed after exposure to heat are getting worse in contact with water as shown in figure 3[A].

Thus, the treatment should provide water for the enzyme while limiting the amount of water interacting with leather.

First, the direct action of an enzyme in aqueous solution was attempted. It was expected that the enzyme action on protein aggregates could be fast enough to counteract the effects of the water on leather. The enzymatic solution in buffer (water as control, data not shown) represents the optimal environment for the enzyme as pH value can be chosen and enzyme regulators added. Moreover, in such media thermodynamic water activity (a_w) reflecting the water availability for the reaction is close to 1 which is optimal for protease hydrolysis activity (Clerjon *et al.*, 2003). Nevertheless, with this method, a very strong shrinkage of the heated sample (fig.3[B]) was observed showing that the enzymatic activity, in aqueous solution, does not permit to avoid the leather retraction.

To reduce the water addition to the leather surface, restoration tests were undertaken by the use of enzymatic polysaccharide gels. In this case, water is largely present but the gel network limits water penetration within the leather. The water activity is not lowered (from 0.91 to 1) but water is "locked" by the polysaccharides, being both physically contained in and having strong interactions with the biopolymer network. Thus gels represent a suitable media for enzymes. Nevertheless, once again, results show again a considerable shrinkage of the heated leather (fig.3 [C]), but to a lower extent than in the previous attempts.

To further reduce the water content, a water soluble co-solvent (glycerol), acting as a thermodynamic water activity depressor was used to prepare new enzyme solutions. In this case, interaction between the two solvents reduces water availability toward the leather. Water activity is strongly reduced, decreasing from 0.96 in 1 M glycerol solution to 0.61 in 10 M glycerol solution. The counterpart of this phenomenon is the large decrease in enzyme activity. For a protease, the enzymatic efficiency is reduced by 35% for 1 M glycerol solution and up to 99% for 9 M glycerol solution as compared with the usual buffer medium (Hertmanni *et al.*, 1991). Several concentrations of co-solvent were tested (fig.3[D]). At low co-solvent concentration (1M glycerol) shrinkage is still observed but to a lower extent than with polysaccharides gels. At high co-solvent concentrations (10 M) no retraction of the heated leather is observed, but no significant gain in flexibility occurred, probably due to the low or none enzyme activity. Moreover, this treatment causes a color change of the unheated leather.

The last approach consisted in the use of an enzymatic emulsion made of an aqueous phase, in which the enzyme is introduced, and a hydrophobic phase to facilitate the introduction of the enzyme on the hydrophobic leather surface. In this particular phase structuration, water is available (a_w varies from 0.91 to 0.95) for the enzymatic reaction, but due to the medium compartmentalization, it is in low contact with the leather surface.

The first trials with enzymatic treatment (fig.3 [E]) do not induce color change of the unheated leather neither shrinkage of the heated leather were observed.

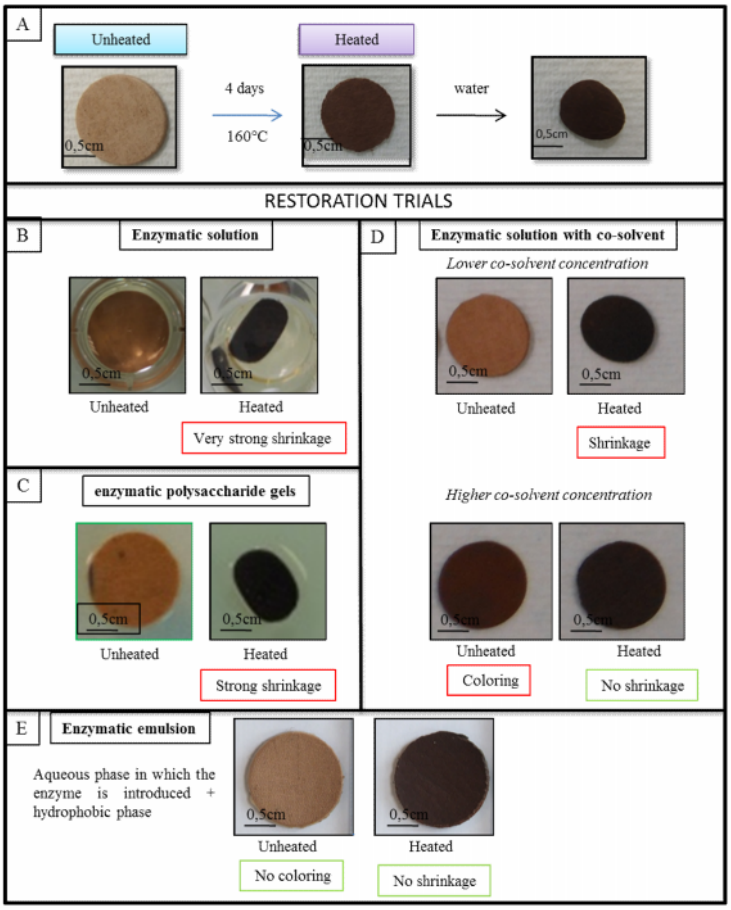

Figure 3. Degradation of a leather exposed artificially to heat, and then placed in contact with water [A]; enzymatic restoration treatments tested on heated leather and on unheated leather as a control, [B] to [E]

Moreover dynamic mechanical analysis (DMA) performed on heated leather treated with the enzymatic emulsion highlighted a flexibility gain. Three days after the enzymatic treatment, a lower storage modulus was measured corresponding to about 30% of flexibility gain as shown in figure 4[A]. This flexibility gain is well due to the enzymatic reaction since without enzyme, the gain reaches is much lower (about 5 %) as shown in figure 4[B].

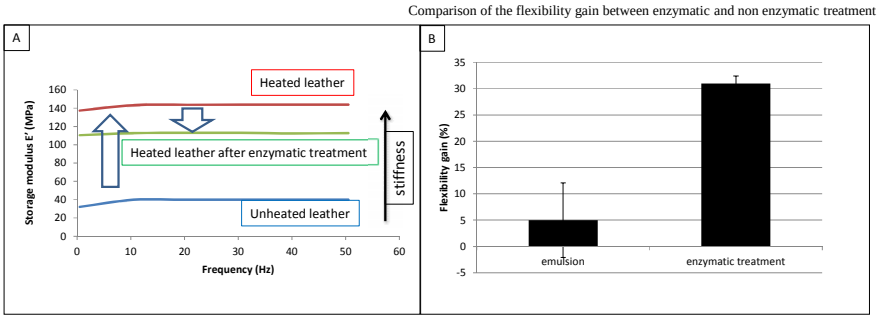

Figure 4. [A] Storage modulus as a function of frequency for unheated leather, leather after exposure to heat and the same sample after enzymatic treatment; [B] Flexibility gain (%) after application of emulsion without enzyme and enzymatic treatment

CONCLUSION

The characterization of heated compared to unheated leathers highlight changes of the leather at various scales. At a macroscopic scale, exposure to heat induces a darkening combined with a stiffness increase and changes in the surface hydrophobicity, wettability and rugosity as well as in water absorbency. All these parameters are consistent with a protein aggregative process. Restoration trials aim to break the protein aggregates by using hydrolases. Various approaches were attempted in order to bring the enzyme to the leather. The results of restoration tests highlighted the need to limit the water in contact with the leather. Since water is necessary, we applied an emulsion that allows enzyme activity but limits the risks of water damage. No color change of the leather was observed while a gain in flexibility was noticed. The various tests carried out show the feasibility and efficiency of this restoration technique which seems promising. Work will continue in this direction.

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