DEVELOPMENT OF A TANNING TECHNOLOGY WITH TANNING AGENTS FROM *LIGUSTRUM VULGARE*

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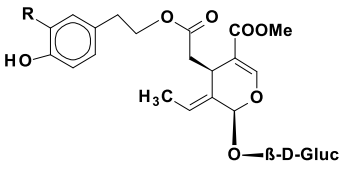
Abstract. A technology for the production of leathers was developed using exclusively privet tanning agents as pre-tanning agents. The development includes production, characterization and optimization of the plant extracts, the development of the pre-tanning technology and the adaptation of the wet end for the corresponding application areas. The leathers which have been manufactured show high shrinkage temperature and good mechanical properties. They show an inherent colouring, but seem to be suitable for use in automotive interiors, as shown by a comparison of the test results with the technical delivery conditions of automobile manufacturers.

1 Introduction

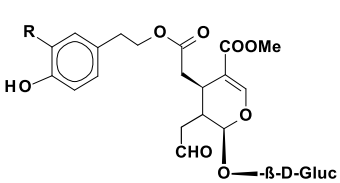
The sole use of vegetable tanning agents for pre-tanning as an alternative to synthetic or chromium-containing tanning agents is one way of improving sustainability and ecology in leather production. In recent years, a new group of secondary plant compounds, the iridoids and secoiridoids, has been discovered to be used as tanning agents.

Currently, a tanning agent from olive leaves with active cross-linking substances deriving from the secoiridoid Oleuropein is commercially available[1]. In order to extend the product range of alternative vegetable tanning agents with covalent cross-linking mechanisms by indigenous raw material, we screened a number of further plants for such covalently cross-linking active substances. Extracts from privet leaves showed a particularly high cross-linking activity.

Privet belongs to the *Oleacea* family and is common in Asia with several species. In Europe, the species *Ligustrum vulgare* can be found everywhere, especially as a hedge plant for gardens. The main Secoiridoids in privet leaves[2] are drawn in Figure 1:



R:
OH Oleuropein
H Ligustrosid



R:
OH Ligustalosid A
H Ligustalosid B

Figure 1. Main Secoiridoids found in extracts of privet leaves.[2]

In order to cross-link proteins such as collagen by secoiridoids, plant-specific enzymes (ß-glucosidase) first cleave off the glucose molecules bound to the secoiridoid. This causes a ring opening and the formation of reactive aldehyde groups. The proposed reaction mechanism for the cross-linking of

collagen is a covalent Michael addition to basic amino groups[3]. In addition, these secoiridoids contain phenolic hydroxyl groups which can also react covalently with the basic amino groups after oxidation to quinones by plant polyphenol oxidases, or via hydrogen bonds.

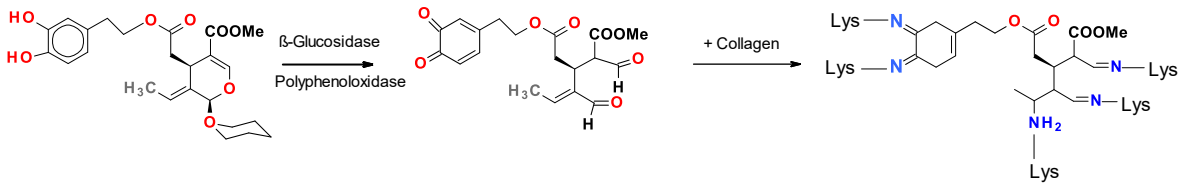


Figure 2. Proposed chemical reaction of oleuropein and collagen.[3]

The reaction mechanism is similar to the cross-linking mechanism of collagen with glutaraldehyde. Compared to other plant based cross-linkers, cross-linking with secoiridoid results in cross-linking products with the highest chemical stability.[4]

The following questions were considered in the development of tanning technology:

- use of privet leaf extracts or ground privet leaves
  The suitability of ground leaves without the need for prior extraction would result in significant economic advantages.
- During the production of extracts, the optimum production parameters need to be determined and methods found to assess cross-linking activity. These investigations were carried out with skin powder, as there are no problems with the diffusion but only the binding on the collagen can be evaluated.
- determination of the required concentrations of the tanning agent and the pH control of the tanning process. For this purpose, small pelt pieces were used on a laboratory scale.
- investigation of the interplay of diffusion and binding of the tanning agent to skins on a pilot plant scale
- the production of finished leathers with various retanning technologies.

2 Experimental

Extraction of plant material

The plant material was collected from the cut of garden hedges and dried. Leaves and stems were mechanically separated. The extraction was carried out on a laboratory and pilot scale. The parameters used were part of optimization process (see results).

Investigation of extract composition

The extracts were analysed by reverse phase liquid chromatography (LC, Shimadzu) with a photodiode array detector (PDA).

- Stationary phase: C18 (Supelco)
- Mobile Phase: Gradient ACN/ Water

Calibration was performed with the reference substance oleuropein (Sigma-Aldrich). The substances ligustaloside A and ligustrosid were identified by fractionation of the extract after separation via the stationary phase and determination of the characteristic mass peaks with a mass spectrometer (QTrap400, Episciex).

Test of cross-linking activity

In order to assess the cross-linking activity of the extracts and leaves, an indirect method must be used, since the cross-linking active substances (deglycosylated and degraded secoiridoids) cannot

be determined analytically. Therefore, hide powder was treated with the extracts according to a standardized procedure and the cross-linking parameters of the hide were determined. The use of hide powder offers the advantage over the use of intact pelts that diffusion is of minor influence and only information on cross-linking activity is obtained.

Hide powder was soaked in a defined volume of 0.4 M McIlvain buffer at pH 7. The plant extract was solved in water and added to the buffered hide powder in a volume ratio of 1:1 (final concentration 10 % mass $_{Extract}$/mass $_{buffer}$). Samples were shaken for 6 h at 30 °C, than centrifuged. The supernatant was discarded and the samples were washed 3 times with excess of water and finally soaked in phosphate buffer at pH 7.

The cross-linking degree of the treated hide powder was measured via the increase of denaturation temperature and by the determination of the amount of bound amino-groups. The denaturation temperature was measured with a DSC 1 device (Mettler-Toledo). Approximately 6 mg (calculated on dry weight) of wet cross-linked hide powder at pH 7 were placed in an aluminum pan and hermetically closed. Temperature scans were run from 10 – 125 °C with a rate of 5 $Kmin^{-1}$. From the endotherms T_{onset} and T_{peak} were calculated.

The amount of bound amino-groups was measured by amino-acid-analysis (Biochrom 30+). The samples were hydrolysed with 6 N HCl at 110 °C for 20 h, dried and resolved in lithium citrate buffer and analyzed by pre-column derivatisation with ninhydrin according to standard protocols.
The percentage of amino groups, that formed an acid-stable bond, was calculated from the area below the lysine, hydroxylysine or arginine peaks and normalised to the area under the peaks from alanine and valine (not involved in cross-linking). The resulting factor was related to the same factor calculated from a non-cross-linked sample.

Development of a tanning technology on a laboratory scale

The laboratory-scale tanning tests were carried out on pelt pieces (de-limed cow skin, standard liming protocol) of approx. 200 g and 1,8 mm thickness in a parallel dyeing machine (diameter approx. 50 cm). Pelt mass, treatment time (36 h) and float volume were kept constant. The tanning results were evaluated by the determination of denaturation temperature of the cross-linked pelts with DSC. Therefore samples were thoroughly washed with water and buffered to pH 7 to ensure comparability of the denaturation temperature.

Production of leathers on a pilot scale

The crusts were produced in a 1 m-diameter tanning drum (Dosemat). Half croupons were used. Cow hides of mass class 20-25 kg were limed according to a standard protocol. Technologies with and without pickle were investigated.

Pre-tanning was carried out with privet extracts from privet leaves ground to various degrees and ground privet leaf powder. The quantity of used tanning agent was kept constant. The degree of cross-linking across the cross-section of the skin was estimated by DSC measurement. Crusts with two different wet-end technologies (wet end 1 and wet end 2) from the automotive sector were produced from the semi-finished products. The crusts were dried, staked and milled. Leathers with glutaraldehyde pre-tanning with the same wet end technology were produced as references. Thickness was average 1,5 mm. The mechanical and chemical properties of the crusts were tested with standardized testing norms.

3 Result and discussion

Optimization of plant extraction procedure

Optimization parameters were

- plant part
- temperature
- extracting agent

- degree of grinding of plant material
- time
- amount of extractant
- number of extraction steps
- drying process

The main influence parameters on cross-linking activity of extracts of *ligustrum vulgare* are the temperature of extraction, the solvent, the part of the plant used for extraction and its grinding degree. In contrast to privet leaves, the stems show no cross-linking activity.

Figure 3 shows the dependence of cross-linking activity on hide powder on the temperature of extraction (A + B). The dependence of the Oleuropein and Ligustalosid A content of the respective privet leaf extract on the temperature of extraction is shown in Figure ***3***C. As can be seen, the cross-linking activity drops rapidly to near zero at extraction temperatures above 60 °C. Correspondingly, the non-cross-linking glycosylated secoiridoids Oleuropein and Ligustalosid A were detected at extraction temperatures above 60 °C. Since the cross-linking active deglycosylated form of the secoridoids could not be observed by chromatography, the rise of the glycosylated form above 60 °C gives evidence for deglycosylation below this temperature, hence cross- linking should be observable as proved by the rise in denaturation temperature and crosslinked lysine.

We assume that under suitable conditions a spontaneous activation of the secoiridoids (deglycosylation, oxidation to quinoid structures) takes place during extraction by the plant's own enzymes such as glucosidases or polyphenoloxidases.[3] It is assumed that the enzymes are inhibited if the extraction temperatures are too high. Unfortunately, no substances could be analyzed in the activated extracts (e.g. aglycones or their degradation products) to which a cross-linking activity could be assigned.

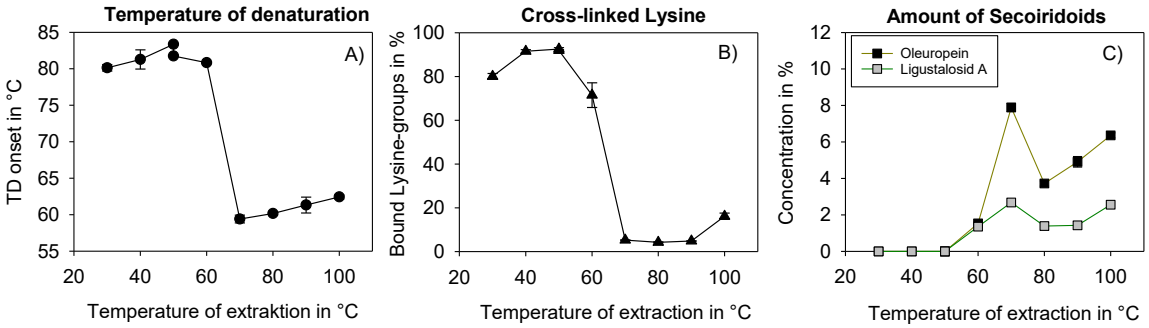


Figure 3. Denaturation temperature (A) and degree of bound Lysine-groups (B) in hide powder tanned with privet leaf extract, and amount of Oleuropein and Ligustalosid depending on the temperature of extraction.

A similar result is observed when using ethanol or mixtures of ethanol and water as extractants: the higher the proportion of non-aqueous extractant, the higher the cross-linking activity and the lower the content of secoiridoides.

The degree of grinding of the privet leaves also plays a role in the cross-linking activity. The leaves were ground and the particle size distribution was determined by sieve analysis. The categories "coarse" and “fine” include particles with diameters between 300 and 1500 µm and between 30 and 90 µm, respectively. Figure 4 shows the cross-linking activity of the extracts from the various ground leaves and the cross-linking activity of the unextracted leaves as a function of the used cross-linker concentration.

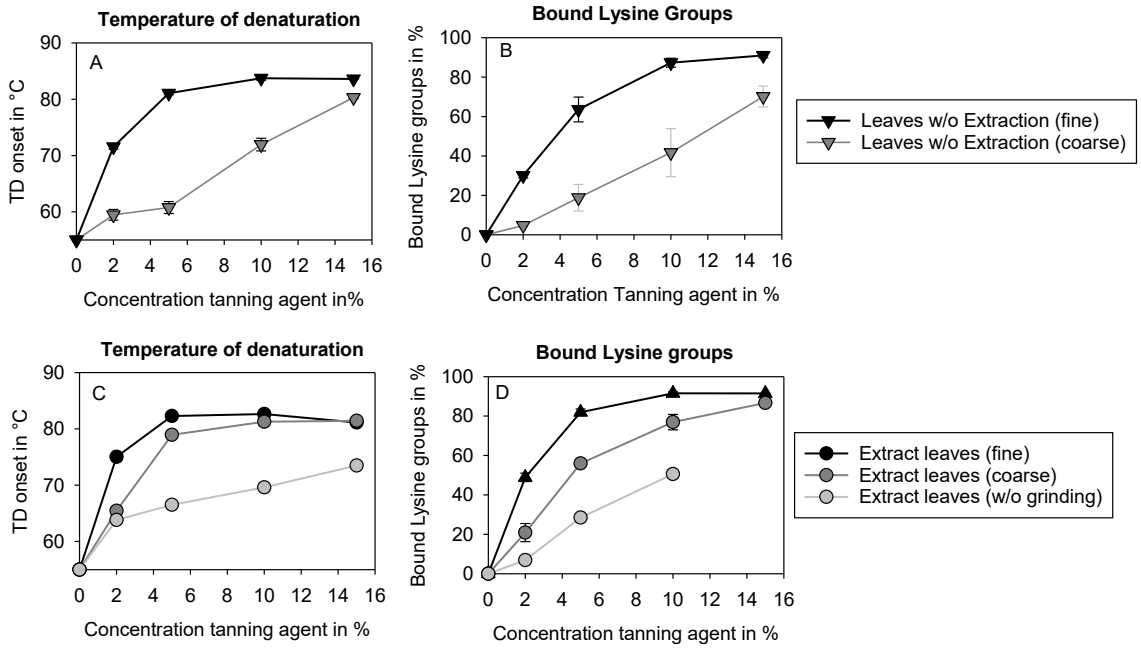


Figure 4. Cross-linking of hide powder with privet leave tanning agents: Influence of the degree of grinding of the extracted leaves on denaturation temperature TD (A, C) influence of the concentration on the proportion of cross-linked lysine groups in tanned hide powder (B,D).

The cross-linking activity increases with increasing degree of comminution. However, the colour intensity of the extract solutions and of the tanned skin powder is also increased.

Interestingly, the cross-linking activity of the finely ground leaves is almost as high as that of the extracts produced from them. This makes it possible to tan with the finely ground leaves without any additional upstream extraction step.

The influence of the other optimization parameters was less significant. In summary, the following parameter ranges were defined for scaling up the extraction:

- Temperature < 60 °C
- Extraction agent Water
- m_{water}: m_{leaves} 20:1
- one extraction step
- Time 4h
- Drying by means of freeze drying or spray drying

The production of extracts was scaled up to the technical scale. The achieved average yield is 35 % of the leaf mass. To check the extract quality, cross-linking tests were carried out by the hide powder method with each batch.

Optimization of tanning process in lab scale

When tanning hide, the diffusion of the tanning agent inside of the skin is a main optimization parameter to be considered beside the cross-linking activity. The progress of diffusion and binding is usually controlled and regulated by the pH value. The investigation of the dependence of the cross-linking activity of privet leaf extract on pH revealed a high cross-linking activity in all pH ranges[4]. This could be an indication that not only the covalent bond (nucleophilic Michael addition) is responsible for cross-linking, but also electrostatic interactions with phenolic hydroxyl groups, which are more active in the acidic range. This cross-linking ability over a wide pH-range could have a negative effect on diffusion and uniform distribution of the tanning agent.

Full penetration and tanning across the cross-section of the skin was evaluated by the denaturing temperature and the shape of the DSC-Peak of the semi-finished products. If tanning is incomplete, the denaturing peaks are wide. Sometimes the scans show two peaks, which corresponds to a more strongly tanned outer area and a less tanned inner area (Figure 5). The distance between the lowest and highest denaturing peaks can be used as a rough evaluation criterion for tanning, whereby the differently tanned areas are not necessarily clearly separated as shown in Figure 5B. A good homogeneous tanning result is represented as single sharp denaturation peak (Figure 5A).

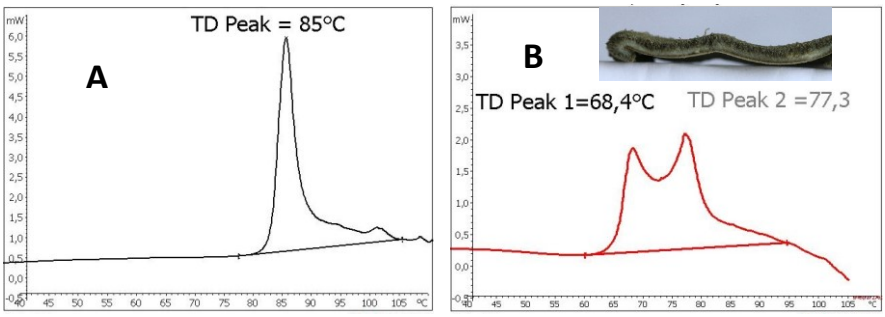


Figure 5. Example of denaturation Peaks of homogeneous A) tanned hide (Glutaraldehyde) and B) non homogeneous tanned hide (Privet leaf powder), x-axis: Temperature in °C, y-axis: Heat-flow in mW).

For optimisation, the following parameters were varied with small pelt pieces on a laboratory scale:

- concentration of the privet tanning agents
- pH value at the beginning and end of the tanning process
- Extracts or leaves (finely ground)

The tanning agent was added in two steps. Figure 6 shows the lowest peak temperatures of denaturation of the tanned pelts as a function of the concentration of tanning agent.

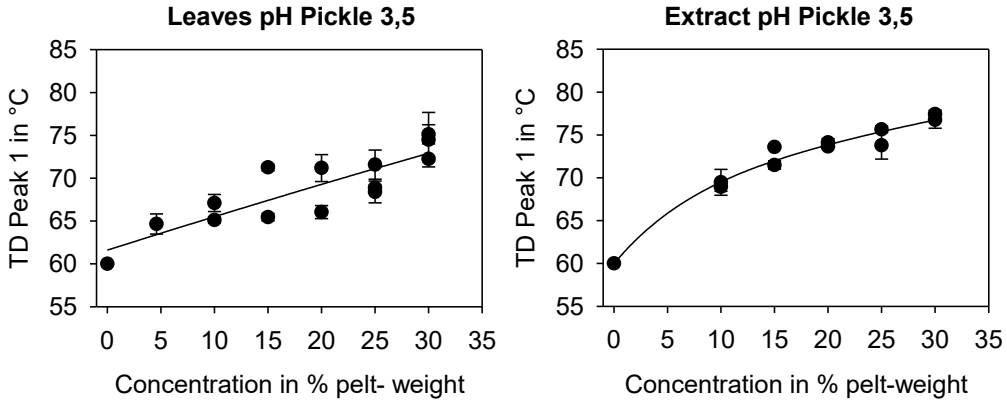


Figure 6. Denaturing temperatures inside the tanned pelts with extracts from privet leaves and ground leaves as a function of concentration (pH pickle 3.5, pH end 4.1), total tanning time: 36 h, (n=3 DSC measurements per semi-finished product, washed and bufferd to pH 7.

Acceptable denaturation temperatures inside the pelt (> 70 °C) are achieved at concentrations of 25 % extract based on pelt weight and higher, and 30 % ground leaves, resp. The tanning is not perfectly uniform in most cases. The reproducibility of the tanning results is significantly better with extracts than with ground leaves.

Figure 7 shows the tanning results in dependence of pH before tanning. The pH was adjusted during pickling. In the tests with pH < 4.5, the pH value was raised to 4.2 after tanning.

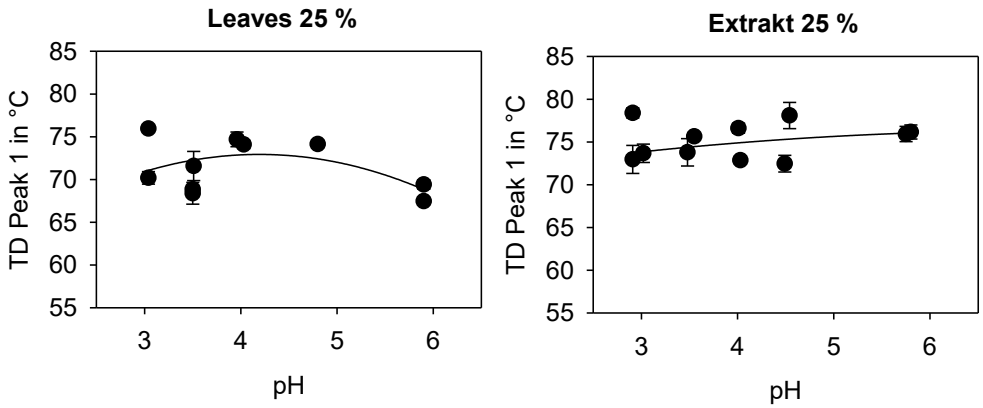


Figure 7. Denaturation temperatures inside the tanned pelts with extracts from privet leaves and ground leaves depending on the pH of the pickle, C $_{Extract}$ = 25 %, total tanning time: 36 h, (n=3 DSC measurements per semi-finished product), washed and bufferd to pH 7.

The pH of pickle has only minor influence on the cross-linking results as shown by the denaturing temperature of the semi-finished products considering the variation of the results from triplicate tests. In the case of extracts, the denaturing temperatures increase slightly with increasing pH, since the proportion of unprotonated amino groups as reaction partners increases with increasing pH. During the production of the leather, however, dead-tanned leather with partial grain breakage could result if the process was carried out without pickling or with a high starting pH. Additionally, the pH value has an influence on the intensity of the coloring of the semi-finished products: the higher the pH value, the darker the color and the more pronounced the grain. If extracts are used, the color of the pelts is brown, if ground leaves are used, the semi-finished products are green.

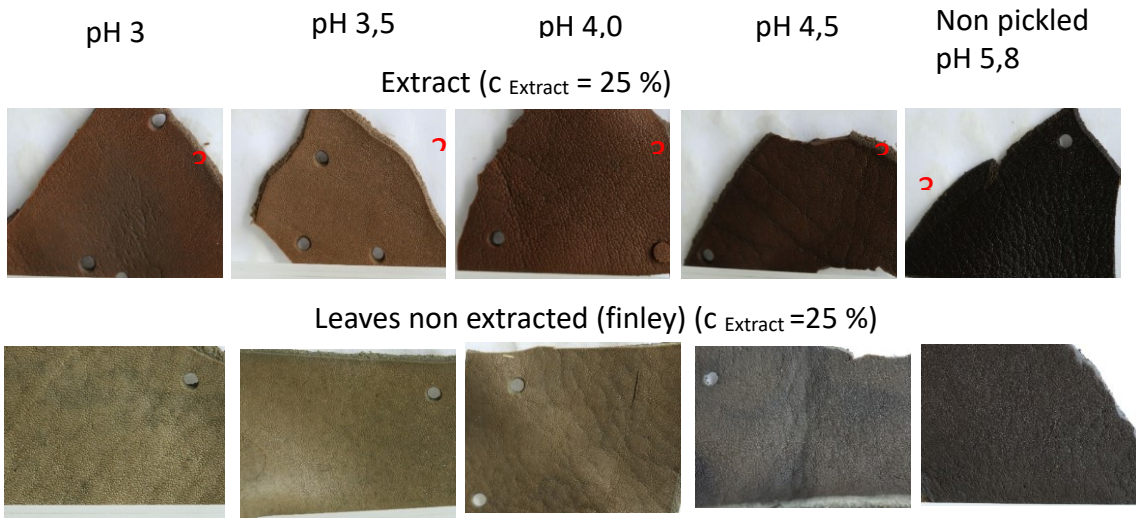


Figure 8. Colours of pelts, tanned with different tanning agents from privet leaves at different pH values, semi-finished.

Production of leathers in pilot scale

The production of leathers was performed with half croupons limed, delimed and pickled after standard protocols in a drum for labscale (Dosemat VGI). Finely ground leaves and extracts from finely ground leaves were used as tanning agents. The diffusion progress was estimated by determination of the denaturing temperatures in dependence of the tanning time. Figure 9 shows exemplarily the temporally course of the first peak temperature of the denaturation peak for leave powder and extract.

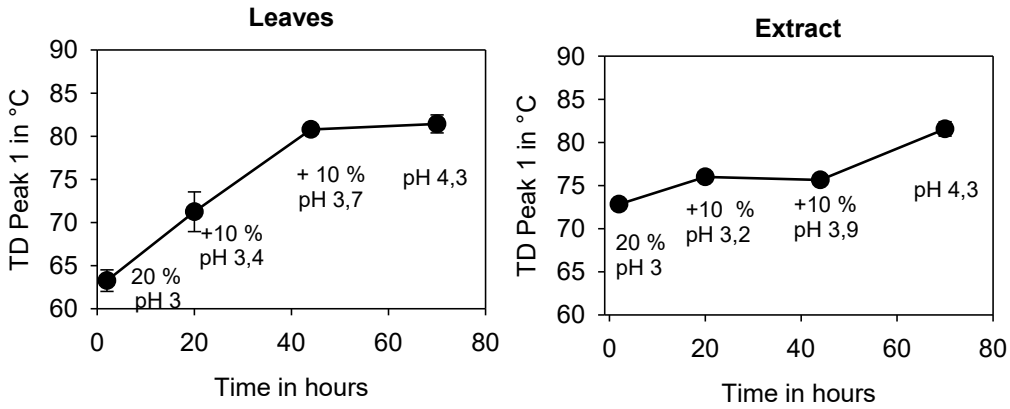


Figure 9. Time course of the denaturing temperatures of inner (TD Peak 1) areas of the hide during tanning privet leaves finely ground, and extract from finely ground leaves, adding point of different rates of tanning agent and the pH during tanning, samples are buffered to pH 7 before DSC measurement.

The colour of the semi-finished products before wet end differs depending on the privet tanning agent used. It could be brightened by the use of syntans in the wet end. The resulting colours are shown in Figure 10. The leathers produced with extract of finely ground leaves are slightly brown, whereas the leathers produced with extract of coarsely ground leaves are lighter. Leathers tanned with ground leaves are slightly green.

Figure 10. Photos and microscopic images (50x) of leathers of tanning agents from privet leaves and glutaraldehyde.

Various mechanical and chemical parameters of the privet tanned leathers were tested, and the chemical composition determined.

The tensile strength of leather tanned with ground leaves is significantly higher than that of glutaraldehyde leather and leather tanned with privet leaf extract.

Parameters such as tear load, stitch tear resistance, static and permanent elongation, bending stiffness, density, weight per unit area and softness show good values that meet the requirements for leather in automotive interiors.

4 Conclusion

Tanning agents from privet leaves contain Secoiridoids, which can be used to produce leather. The leathers show good mechanical properties, in some cases even better, as the leather tanned with glutaraldehyde and the same wet end technologies. Furthermore, it has been shown that privet leaves that are finely ground are just as suitable for tanning as extracts from ground leaves. Some mechanical properties like the tensile strength could be improved by tanning with the finely ground leaves.

5 References

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