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

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ABSTRACT

The use of vegetable tanning agents could be an alternative to synthetic tanning agents with regard to sustainability and reduction of fossil based materials in leather production. In recent years, a new group of secondary plant compounds, the iridoids or secoiridoids, has been investigated for its use as tanning agents. Secoiridoids are found in relevant concentrations in leaves from olive trees or privet shrubs. Extracts from these two secoridoid containing plants are investigated for their tanning characteristics. Extracts from privet *(Ligustrum vulgare)* leaves show the same high crosslinking activity as extracts of olive leaves. In addition to Oleuropein found in olive leaves, the privet extracts contain a high proportion of the secoiridoid Ligustaloside A. The tanning activity of olive and privet leaf extracts is determined by crosslinking of the model compound hide powder. A comparison to aldehyde crosslinking was investigated with regard to concentration and temperature of denaturation. After investigating the crosslinking activity by the hide powder method, a technology for the production of leather was developed that uses *Ligustrum* tanning exclusively for pre-tanning. Properties of the resulting leather are discussed, including mechanical and shrinkage characteristics during climate change tests.

Keywords: Vegetable tanning, Ligustrum vulgare, Secoiridoid

1. Introduction

Besides wool, stone and wood, leather is one of the oldest natural materials used in human history. It has always been used as protective and decorative clothing but also as technical material. Tanning methods based on natural resources have been continuously developed over centuries and ages. At the end of the 19th century, tanning technology changed due to the development of mineral tanning based on chromium (III) salts, and synthetic tannins in the early of the 20th century into an industrialised process. The use of vegetable tannins as sole or pre-tanning agents has been nearly completely displaced. Nowadays, the majority of leathers are chromium tanned (about 90 % of all leathers), followed by synthetic tanning with glutaraldehyde. Syntans are frequently used in retanning to improve haptic and mechanical properties. Due to an increasing environmental and social responsibility, ongoing efforts are needed to reduce or discharge of hazardous chemicals and minimise the use of fossil based resources in leather production.

During the production of leather from hide, the collagen is chemically crosslinked to stabilize it thermally and against enzymatic degradation by microorganisms. The hides are usually washed, dehaired and pre-tanned. These pre-tanned semi-finished products are then mechanically processed to remove all parts of the hides that do not produce high-quality leather. Mechanical processing includes the removal of side cuts as well as shavings from the thickness adjustment of the hides. These mechanical processes reduce the weight of the hide to be tanned, thus saving chemicals, energy and waste streams in the final leather manufacture. However, particularly shaving leads to a considerable temperature load of the hides (Witt *et al.*, 2021). Therefore, the hides need to be stabilised against

thermal stress, which is achieved by pre-tanning. Since pre-tanning is regularly done with chromium or aldehyde tanning, a vegetable-based tanning method would be highly desirable to omit chemicals under restriction. Chrome tanning came under pressure as a result of chromium VI residues or impurities in chrome-tanned leathers from not adequately managed processes.

Chromium VI is a known allergen and carcinogen and is restricted to an analytical limit of 3 mg/kg. Tightening of this limit is being discussed and is to be expected. However, conventional chrome-free tanning is based on glutaraldehyde, a chemical classified as a sensitizer, which is expected to be restricted in the future (European chemical agency). Within the framework of the ZDHC initiative (Zero Discharge of Hazardous Chemicals), leather manufacturers must also meet increased requirements with regard to the safety and sustainability of the chemicals used for tanning.

Although leather was tanned over centuries with vegetable tanning agents, there is still need for improvement and an increased interest in novel, biobased tanning methods. Focus is not only on known vegetable tanning agents, but also on improved product quality with regard to demands for sustainability and ecologically responsible production. The use of tanning agents from plants as the sole pre-tanning agent would be an excellent way to overcome the disadvantages of chromium or aldehyde pre-tanning. This is particularly true with regard to the degradability of the leather production waste, especially the shavings from thickness adjustment during leather production. These shavings represent a major proportion and need to be costly disposed, if classified as chemically hazardous. Consequently, there is a high need for alternative non-hazardous tanning agents. Ideally, those tanning agents would be plant based, non-competitive to food production on agricultural land and regionally available.

Currently, a tanning agent from olive leaves with active crosslinking substances deriving from the secoiridoid Oleuropein is commercially available (Zotzel *et al.*, 2011). In order to extend the product range of alternative vegetable tanning agents with covalent crosslinking mechanisms by indigenous raw material, we screened a number of further plants for such covalently crosslinking active substances (Schröpfer and Meyer, 2016). Extracts from privet leaves showed a particularly high crosslinking 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, in Germany especially as a hedge plant for gardens. Secoiridoids found in privet leaves (Romani *et al.*, 2000) include Oleuropein, Ligustrosid, and Ligustalosides A and B (see Figure 1). However, the complete quantitative determination of the different tanning active secoiridoid species in privet leaves is not yet analytically possible.

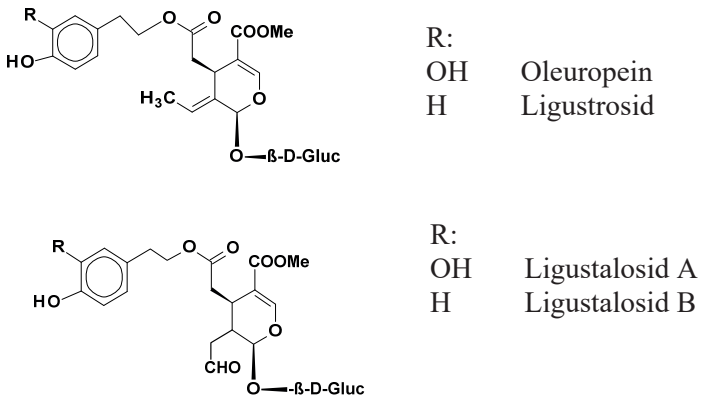


Figure 1: Main Secoiridoids found in extracts of Ligustrum leaves (Romani et al., 2000)

The glucose molecule bound to the secoiridoids stabilises the inactive form of the secoiridoid. Only after deglycosilation a ring opening and the formation of reactive aldehyde groups occurs. In plants, the deglycosilation is a started by plant-specific enzymes (ß-glucosidase), when cells are hurt or damaged. The formed aldehyde is able to crosslink proteins such as collagen. The proposed reaction mechanism for the crosslinking of collagen is a covalent Schiff base formation with basic amino groups (Konno *et al.*, 1999).

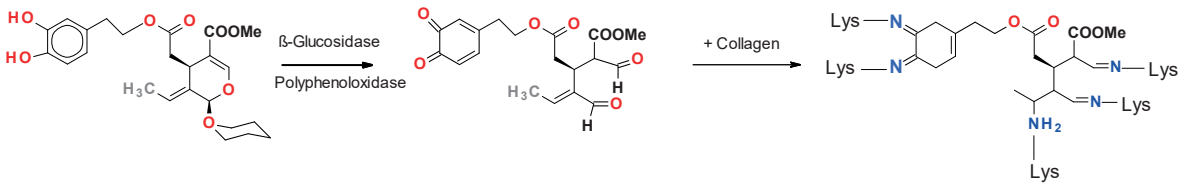


Figure 2: Proposed chemical reaction of oleuropein and collagen (Konno et al., 1999)

The reaction mechanism of secoiridoid aldehyde groups with collagen is similar to the crosslinking mechanism of collagen with glutaraldehyde. Compared to other plant based crosslinkers, crosslinking with secoiridoid results in crosslinking products with the highest chemical stability (Schroepfer and Meyer, 2016). This is a major advantage over conventional vegetable tanning agents with weak and pH unstable interactions.

Since aldehyde tanning is a well known pre-tanning method, tanning with *Ligustrum* should also lead to well pre-tanned hides with a temperature of denaturation well above 70 °C that could be readily shaved. The present paper introduces a pre-tanning method based on *Ligustrum* extracts and ground leaves as well as the physical properties of finished leathers produced with different retanning technologies.

2. Material and Methods

Collecting and comminution of plant material

The plant material was collected from the cut of privet garden hedges and dried at ambient temperature. Leaves and stems were separated. Olive leaves were privately collected in Italy. Dry leaves were milled to an average size of 50 µm with a turbo rotor mill (Görgens Mahltechnik GmbH, Germany).

Extraction of plant material

The preferred method was as follows: 100 g of milled leaves were soaked in 10 l water at 40°C and stirred for 4 h. The dispersion was filtrated and the supernatant was concentrated using a rotary evaporator (Büchi, Labortechnik AG) and finally freeze dried (Epsilon 2-4 LSC Plus, Martin Christ Gefriertrocknungs GmbH, Germany).

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 acetonitrile/water. Calibration was performed with the reference substance oleuropein (Sigma-Aldrich).

Determination of crosslinking activity

Hide powder was prepared from limed, air-dried cattle pelts, which were milled on a cutting mill (Retsch, SM 2000) to a particle size of approximately 2 mm. It was ensured that no denaturation occurred during milling. For the trials commercially available extracts of the respective tanning agents (Valonea: SCRD, France; Wattle: SetaSun, Brazil) were used. Glutaraldehyde (25%) was purchased from Carl Roth, Germany. Cross-linked hide powder was prepared at different pH: Hide powder was initially soaked in a defined volume of 0.4 M McIlvain buffer at different pH in the range of pH 2-9. The extract was solved in water and added to the buffered hide powder in a volume ratio of 1:1 (final concentration 5 % weight crosslinker/ weight solution). Samples were shaken for 6 h at 30 °C, than centrifuged. The supernatant was discarded. Crosslinking with glutaraldehyde was done at 1% final concentration of pure glutaraldehyde as a reference.

Production of leathers in pilot scale

Ground leaves and extracts from ground leaves were used as tanning agents. The production of leathers was carried out in a drum Dosemat VGI. Cattle hides of mass class 20-25 Kg were croupated and halved. They were limed, delimed and pickled to pH 3. A leather with glutaraldehyde tanning was produced as reference. The concentration

of the tanning agent was 30 % (w/w pelt) for *Ligustrum* and 6 % (w/w pelt) for glutaraldehyde; resp. Tanning time was 48 h. After tanning, pH was set to 4.3. The tanning time including basifying was maximum 52 h.

For wet end, two technologies tailored for automotive chrome-free interiors were used (wet end 1 and wet end 2). Syntans and tara were used as post-tanning agents and fatliquors used were based on natural oils and lecithin. All leathers were worked to crusts without colouring, dried, stacked and milled. The thickness of the crusts was 1.5 mm.

Differential Scanning Calorimetry (DSC) measurement

The DSC measurements of were performed with a DSC 1 device (Mettler-Toledo).

Hide powder samples: Approximately 6 mg (calculated on dry weight) of wet cross-linked hide powder (pH corresponds to the pH during crosslinking) were placed in an aluminum pan and hermetically closed. Temperature scans were run from 10 - 125 °C with a rate of 5 K min^{-1}. From the endotherms T_{peak} was calculated. The results for the different pH are expressed as the difference (ΔT) between the denaturation temperature (T_{peak}) of a cross-linked sample and a sample without any crosslinking at the same pH.

Tanned pelts: The wet semi-finished tanned pelts were cut into pieces of appr. 10 mg (dry mass) and soaked in a phosphate-buffer at pH 7 for 16 h. The excess buffer was removed with a tissue. Samples were then placed in aluminum pans (pan volume 40 µl) and hermetically sealed. Temperature scans were run from 10 – 125 °C with a rate of 5 $Kmin^{-1}$. From the endotherms, T_{onset} was calculated.

Determination of bound amino groups after acid hydrolysis

The samples were hydrolysed with 6 N HCl at 110 °C for 20 h, dried and resolved in lithium citrate buffer. Amino acid composition was determined from crosslinked and non-crosslinked samples by an amino acid analysator (Biochrom 30+) with 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 under the lysine, hydroxylysine or arginine peaks and normalised to the area under the peaks from alanine and valine (not involved in crosslinking). The resulting factor was related to the same factor calculated from non-crosslinked sample. All analytical results are averages of a duplicate.

Leather Testing Mechanical properties

Tensile strength and elongation were performed according to DIN EN ISO 3377 and tear propagation load was performed according to DIN 3376-1. Stitch tear resistance was determined according to DIN EN ISO 23910.

Shrinkage behaviour

Area shrinkage was performed according to VW-TL 52064. The testing area of 20 cm x 20 cm was marked on leather samples of a size of 25 cm x 25 cm. The samples were subjected to 20 cycles of a climate change test under the specified conditions. Area shrinkage was determined by calculating the percentage of the area after the climate change test in relation to the area before.

Isometric shrinkage force was measured according to PPV 52002.

3. Results and Discussion

Determination of crosslinking ability and stability of crosslinking

Hide powder was used as a model substance in order to investigate the stability of collagen crosslinking by secoiridoids from the new vegetable crosslinking agents from *Ligustrum*. A comparison was made between different plant based tannins, and glutaraldehyde as a conventional reference.

Crosslinking stabilises the supramolecular structure of the collagen against denaturation (Reich, 2007; Schroepfer and Meyer, 2017). Consequently, the temperature of denaturation of collagen is increased by crosslinking. The stability of crosslinks between collagen and plant based tanning agents depends on the nature of the chemical groups present in the tanning agent. Tannins of vegetable origin can be divided into three classes according to their chemical structure: Hydrolysable tannins, condensed tannins and tannins with iridoid or secoiridoid structure. Hydrolysable tannins are esters of gallic acid and glucose moieties, which are able to form hydrogen bonds between the polyphenolic-OH groups and the peptide oxygen of the collagen molecule (Mavlyanov *et al.*, 2001; Gustavson, 1956) or between the protonated amino-groups and the oxygen atom of the phenolic-OH groups (Spencer *et al.*, 1988). These bonds are not stable against pH changes. Condensed tannins are

polymerised flavonoids, mostly based on catechine monomers (Haslam, 1966; Roux *et al.*, 1975). The flavonoid macromolecules of condensed tannins can additionally be oxidised in the presence of the plants-own polyphenol oxidases. The resulting quinones react covalently via a nucleophilic Michael addition with amino-groups of the collagen (Peter, 1989; Endres, 1964) resulting in more stable bonds compared to hydrogen bonds. Iridoids and secoiridoids undergo a ring opening after enzymatic deglycosilation, creating two aldehyde groups that can interact with amino groups in amino acids of collagen (Konno *et al.*, 1999) forming covalent bonds.

Crosslinking of collagen by tannins of the different classes and with glutaraldehyde as a reference was investigated with hide powder as a model substance. Hide powder was crosslinked and the resulting temperature of denaturation was measured. Figure 3 shows the difference of the temperature of denaturation of the crosslinked hide powder in dependence of the pH compared to non-crosslinked hide powder. Valonea (hydrolysable tannin) crosslinking is not stable against pH changes, as can be seen by the decreasing difference in the temperature of denaturation with increasing pH. In contrast, the temperature of denaturation of hide powder crosslinked by extracts of Wattle (condensed tannin), Olive leaves, *Ligustrum* leaves (secoiridoids), or glutaraldehyde does not change significantly with pH changes.

Another method to evaluate the stability of covalent bonds in crosslinking reactions between tannins and collagen is to determine the percentage of bound amino groups after acid hydrolysis. As can be seen in Figure 3, conventional glutaraldehyde tanning show a high percentage of bound amino groups after acid hydrolysis. From the plant based tanning agents, only secoiridoid tanning form stable bonds under this conditions. This finding illustrates the similar nature of bonding by aldehyde moieties both in secoiridoid tanning agents and glutaraldehyde. It underlines that crosslinking of collagen with secoiridoids would result in a sufficient stabilisation of pre-tanned hides for mechanical operations and the associated thermal stress.

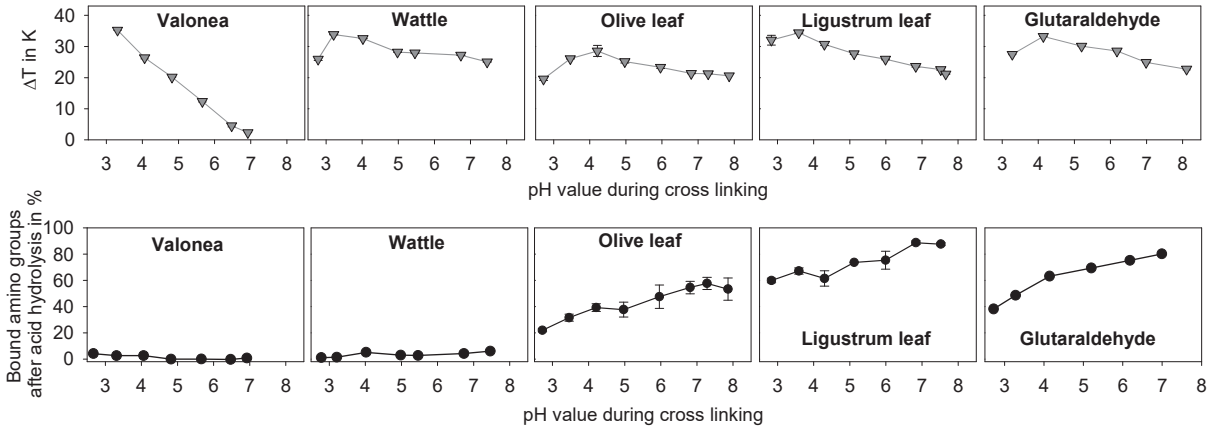


Figure 3: Increase in the temperature of denaturation of crosslinked hide powder over the temperature of denaturation of a non-crosslinked reference with extracts from Valonea, Wattle, Olive leaf, and Ligustrum leaf. For comparison, hide powder was also crosslinked with glutaraldehyde (upper row). Percentage of bound amino groups after acid hydrolysis of the crosslinked hide powder samples (lower row).

Proof of activation by plant based enzymes

The stability of crosslinking of collagen by olive and *Ligustrum* leaf extracts indicated that the reaction follows the proposed mechanism of aldehyde formation and subsequent covalent reaction with the amino groups of collagen. In order to proof the proposed activation mechanism of secoiridoids by plant based enzymes, the extraction temperature of the leaves was increased up to 90 °C, which would be above the deactivation temperature for enzymes. The crosslinking activity of the extracts yielded was tested by treating hide powder as model substance for collagen crosslinking. The hide powder crosslinked with extracts from different extraction temperatures was examined for the amount of bound amino groups after hydrolysis as a measure for crosslinking. With this method, covalently bound amino groups are determined and a percentage is calculated compared to non-crosslinked hide powder. The results are presented in Figure 4. A sharp drop of the percentage of bound amino

groups bound amino groups can be seen at extraction temperatures above 50 °C for both olive and *Ligustrum*, indicating that no active species are available in these high temperature extracts. The HPLC-chromatograms in Figure 5 shows the inactive glycosylated precursor substances in the extracts made at 90°C. This finding can be attributed to an inactivation of the enzymes necessary for deglycosilation of the secoiridoids and thus transferring them into the activated form. The chromatograms of the extracts obtained at 40°C show that the precursors are no longer present. This means that they have been degraded by the plant's own enzymes. However, the tanning active degradation products cannot be analysed with the selected analytical HPLC method. The chromatograms of the extracts at 90°C also show that the secoiridoid composition of the olive leaf and *Ligustrum* leaf extracts differ: *Ligustrum* contains larger amounts of ligustaloside A in addition to oleuropein.

In order to save extra chemicals for subsequent activation of the secoiridoid tanning agents, extraction should be carried out at temperatures below 50 °C.

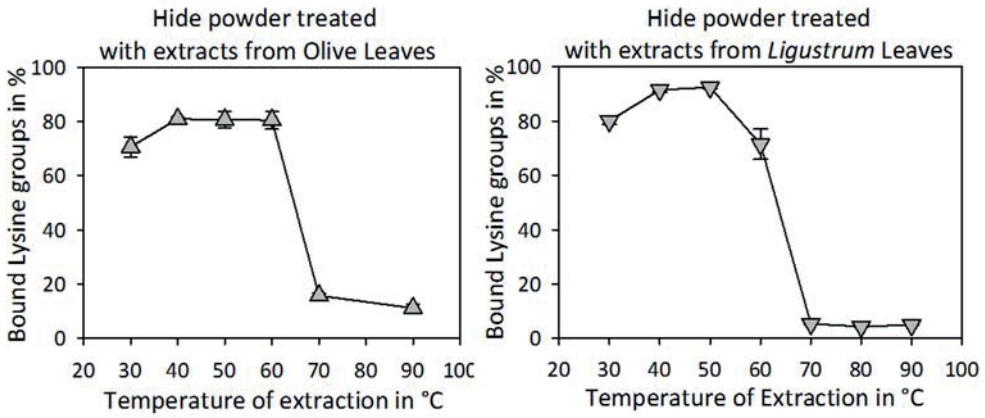


Figure 4: Percentage of bound amino groups of crosslinked hide powder after acid hydrolysis. Crosslinking was performed with extracts received from extractions at different temperatures.

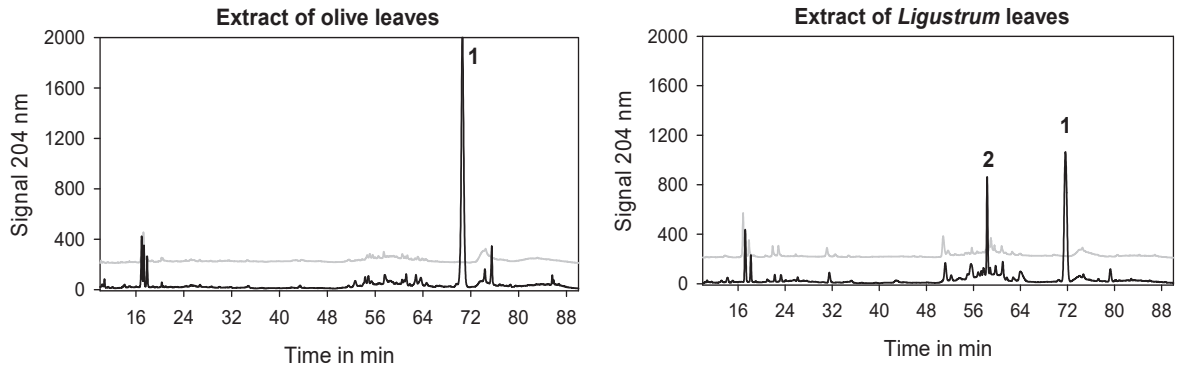


Figure 5: HPLC-Chromatograms of olive leaf extract (left) and Ligustrum leaf extract (right) grey: Extraction at 40°C, black: Extraction at 90°C, 1: Oleuropein, 2: Ligustalosid A

Tanning of hides with Ligustrum

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. Tanning of hides was successful with both extracts from leaves as well as ground leaves without extraction. Omitting the extraction step is favourable particularly in regard to sustainability of the production

process of the tanning agent. Drying is a very energy consuming process, especially for low concentrated extracts. By using ground leaves it is possible to reduce the energy consumption and thus the carbon footprint of the tanning agent production to a great extent.

Tanning with *Ligustrum* resulted in half-finished leathers with a temperature of denaturation of approx. 75 °C or higher by using concentrations of tanning agents 25 % (pelt weight) minimum. The hides where shaved after pre-tanning. For wet end, two technologies tailored for automotive interiors were used. The technologies contain syntans, tara and fat liquor based on natural oils and lecithin. All leathers were worked to crusts, dried, stacked and milled. The colour of the semi-finished products before wet end differs depending on the *Ligustrum* tanning agent used. It could be brightened by the use of syntans in the wet end. Leathers tanned with ground leaves are slightly green.

Numerous mechanical parameters of the *Ligustrum* tanned leathers were tested and compared to glutaraldehyde tanned leathers as a reference.

The tensile strength of leather tanned with ground leaves is significantly higher than that of glutaraldehyde leather and leather tanned with *Ligustrum* 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. Selected parameters are shown in Figure 6.

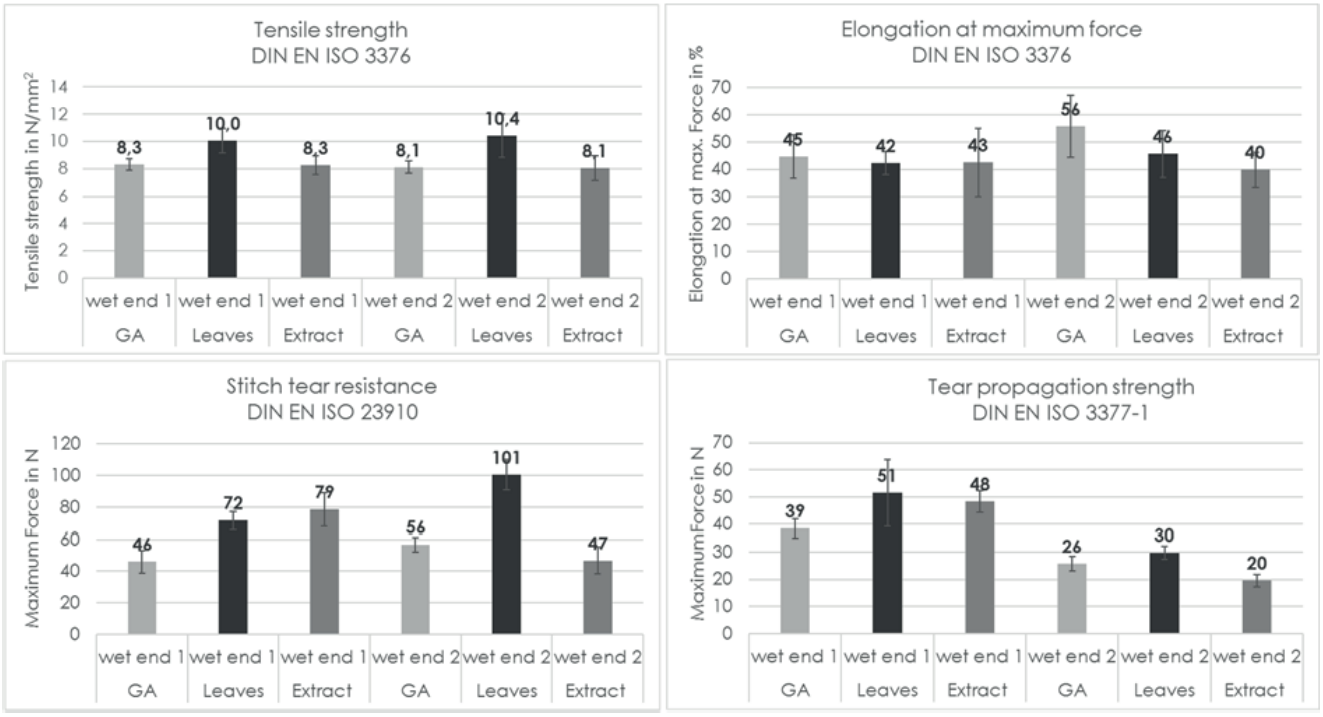


Figure 6: Mechanical parameters of leathers tanned with either glutaraldehyde (GA), Ligustrum leaves, or Ligustrum extract as pre-tanning. Wet end treatment 1 and 2, resp., was the same for all samples.

For leathers in automotive application, shrinkage behaviour in climate change tests is an important quality criterion. Leathers tanned with *Ligustrum* extract as well as tanned with ground *Ligustrum* leaves perform very well in comparison with the conventionally glutaraldehyde tanned reference.

Figure 7 shows area shrinkage according to VW TL52064 and isometric shrinkage force according to PPV 52002. Leathers tanned with *Ligustrum* show less area shrinkage compared to the glutaraldehyde-tanned reference in both wet end technologies tested. The isometric shrinkage force is equal or less for the *Ligustrum* tanned leathers compared to the reference.

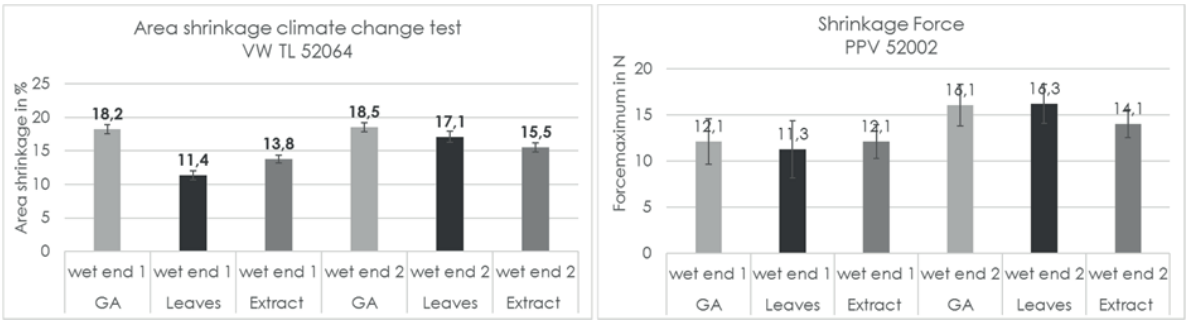


Figure 7: Shrinkage behaviour of leathers tanned with either glutaraldehyde (GA) Ligustrum leaves or Ligustrum extract as pre-tanning. Wet end treatment 1 and 2, resp., was the same for all samples.

4. Conclusion

Tanning agents from *Ligustrum* leaves contain secoiridoids, which can be used to produce leather. It was shown, that it is possible to pre-tan leathers solely by the plant based tanning agents from *Ligustrum* leaves. The *Ligustrum* leaves could be either extracted or used as finely ground powder for tanning. Extraction should be performed at temperatures below 50 °C in order to avoid an inactivation of the plant's own enzymes, which are necessary for activation of the secoiridoids. Using low extraction temperatures allows to omit an extra activation step for the *Ligustrum* secoiridoids. It has been shown that *Ligustrum* leaves that are finely ground are just as suitable for tanning as extracts from ground leaves. Since extraction and drying of extracts is an energy-consuming step, using ground leaves for tanning saves a lot of energy and thus may improve the carbon footprint of the final product.

The pre-tanning results in half-fabricates with a temperature of denaturation >75 °C, which is sufficiently high for the thermal stress associated with mechanical processing, particularly shaving. *Ligustrum* tanned leather shavings are free of hazardous chemicals and do not need to be disposed of as problematic waste. The final leathers produced with two different wet end technologies show equal, in some cases even better, mechanical properties in comparison with glutaraldehyde pre-tanned leathers processed with the same wet end technologies. Some mechanical properties like the tensile strength could be improved by tanning with the finely ground leaves. Due to a similar or less pronounced shrinkage behavior compared to aldehyde-tanned leather, the *Ligustrum* pre-tanned leather is also suitable for automotive applications. *Ligustrum* is a plant based tanning agent, which is non-competitive to food production on agricultural land and would be regionally available in middle Europe. However, collecting and harvesting *Ligustrum* on an industrial scale is still a challenge and scope of ongoing research projects.

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