Chestnut saponins – "green" scouring agents for raw wool

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The problems of wastewater contamination with various pollutants from the textile finishing and care process require the search for a solution to this dispersed system and its complex mechanisms. In order to promote sustainability and a circular economy, environmentally friendly products are being introduced and natural products are increasingly being used in arts and crafts. In this study, a comparison was made between horse chestnut and sweet chestnut saponins as natural and green products. In the first phase of the study, an analytical evaluation of the horse chestnut and sweet chestnut was carried out based on the total active substance and pH value. In the second phase, the washing performance of domestic raw wool, the sheep breed "Lička pramenka" was tested. Finally, after the washing process, the properties of the wool fibres and the composition of the wastewater were analysed to verify the concept of functionality and sustainability.

Keywords: *"green" agents; chestnut saponins; wool scouring; wastewater*

Izvorni znanstveni rad**

Saponini kestena – "zelena" sredstva za pranje sirove vune

Problemi onečišćenja otpadnih voda različitim zagađivačima iz procesa oplemenjivanja i njege tekstilija zahtijevaju traženje rješenja za ovaj disperzni sustav i njegove složene mehanizme. Za promociju održivosti i kružnog gospodarstva uvode se ekološki prihvatljiva sredstva, a obrtništvo i rukotvorstvo sve više koriste prirodna sredstva. U ovom istraživanju provjerena je mogućnost pranja sirove vune saponinima divljeg kestena i pitomog kestena kao prirodnih i zelenih proizvoda. U prvoj fazi istraživanja provedena je analiza divljeg kestena i pitomog kestena na osnovi ukupne aktivne tvari i vrijednosti pH. U drugoj fazi ispitana je učinkovitost pranja domaće sirove vune ovce pasmine "Lička pramenka". Završno, nakon procesa pranja, analizirana su svojstva vunenih vlakana i sastav otpadne vode procesa pranja kako bi se potvrdio koncept funkcionalnosti i održivosti.

Ključne riječi: *„zelena" sredstva; saponini kestena; pranje vune; otpadna voda*

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1. Introduction

The problems of soil, water and air pollution caused by large quantities of micro- and/or nano-plastics released from primary and secondary sources have recently fuelled debates on sustainability and imposed the benefits of natural materials. Wool is a raw material that has been used for many years in the textile industry and other industrial sectors, as sheep's wool is an excellent material that can be utilised in a variety of ways [1]. According to [2] "Sheep are economically the ideal fibre factory as provide milk, meat and wool and produce minimal waste. As wool fibres are natural, renewable and biodegradable, they are perhaps the most sustainable resource of all fibres".

The development of products and technologies for processing according to the principles of sustainability has opened up new avenues for research so that wool, which is often unwanted waste, can be comercialised as a valuable sustainable raw material. The complete sustainability and safety of functional textile wool blends is confirmed by the BlueSign® label, which guarantees that production is carried out in accordance with strict safety and environmental standards [3].

However, wool contains various impurities, such as dirt and grease, which must be removed before further processing [4]. The total amount of impurities in greasy wool is approx. 35% to 40% and more of the total weight of the fleece. Greasy raw wool is cleaned by washing/scouring with about 1% surface active agent in several baths, whereby grease, dirt, sand and grime are removed from the fibre [5]. The IWTO defines wool scouring as a process in which wool is washed in hot water and detergent to remove the non-wool impurities (natural fats, waxes, proteins and other components as well as dirt, oil and other impurities) [6]. The process of washing raw wool can be carried out in different ways, and the result of the process is the load of wastewater [7,8]. Due to the differences between the various sheep breeds with regard to non-wool contaminants, no standardised process parameters can be applied [4]. Regardless of the processing details, the purpose of wool scouring is to remove impurities while preserving the physical and mechanical properties of the wool fibre as much as possible [9]. It is important to recognise the difference between the needs of industry and the needs of artisans.

This research is linked to a project to promote sustainability. One of the tasks is to investigate the possibilities of washing domestic raw wool with alternative - natural agents in order to obtain a clean wool for handicrafts.

It is known that saponins are natural surface-active substances, so they have the ability to clean various surfaces [10]. Saponins are bioactive compounds produced mainly by plants, but also by some marine organisms and insects. Such specialised metabolites are mainly found in the plant kingdom, although they have also been discovered in marine invertebrates [11,12]. A whole range of other activities is attributed to saponins, as well as various pharmacological properties attributed to them [13]. Saponins contains sugar and non-sugar (aglycone) part of glycoside molecule. Aglycone is characterised by a skeleton derived from the 30-carbon precursor oxidosqualene, to which glycosyl residues are attached [14].

The chestnuts from the chestnut tree, which is widespread in rural and urban areas of Croatia, were selected as a source of saponins in this study. The horse chestnut, *Aesculus hippocastanum*, is a deciduous plant from the *Sapindaceae* family whose chestnuts are not edible but are used for other purposes (source of starch, horse feed, medicinal preparations for veins, etc.). Chestnuts can be useful for laundry due to the saponins they contain, which many consider to be a natural detergent. This extract consists mainly of escins and a mixture of triterpenoid saponins (α- and β-escin). The saponins consist of four substances that make up 60% of the total mixture. Aescins form a rich family of saponins (*Saponosides*) and are the most active components in the seeds of *Aesculum hippocastanum.* Chestnut fruit (prickly husk) also contains aescin but in much lower quantity [15,16].

The sweet chestnut (*Castanea sativa Mill*.) is a deciduous tree from the beech family (*Fagaceae*) [17]. They are particularly valued for their high carbohydrate content and gluten-free nature. The main component of fresh chestnuts is water and depending on the type of chestnut, the dry matter contains starch and polysaccharides (up to 25%) [18]. In contrast to horse chestnut, extracts from Castanea sativa Mill. can be a potential source of natural tannins with possible application in nutrition and therapy [19].

This study investigated the washing effect of horse and sweet chestnuts as natural and green products containing saponins. In a first step, a basic analytical evaluation, extraction and isolation of the active ingredients from horse chestnut and sweet chestnut was performed. In a second step, the washing performance of both chestnuts was tested on domestic raw wool of the "Lička pramenka" sheep breed. After the washing process (scouring) the spectral properties of the washed wool samples and the composition of the wastewater was analysed in order to verify the concept of functionality and sustainability.

2. Experimental

2.1. Material

Raw wool (R-WO) of the "Lička pramenka" breed from the Lika region was selected for washing with horse chestnut and sweet chestnut suspensions.

The fruits (chestnuts) of the horse chestnut (hc) were collected in the western part of the city of Zagreb, and the fruits of the sweet chestnut (sc) in the Majur area, both in October 2024. The chestnuts were stored in dry and dark conditions.

2.2. Procedures

2.2.1. Preparation of chestnut suspension

The suspension of horse and sweet chestnuts was prepared step by step by quartering 100 g of chestnuts and placing them in a laboratory beaker to which 2 L of distilled water was added. The procedure for preparing the suspension was carried out on a magnetic stirrer at 400 rpm at 60°C for 2 hours. The suspension was cooled, covered and left to stand overnight. In the morning, the peel was removed from the chestnut, the suspension was filtered and the isolated chestnut was crushed in a blender and returned to the filtrate to obtain a suspension for the washing process.

2.2.2. Determination of wool yield

Before washing with chestnuts, the wool yield was determined using an in-house procedure of conventional washing (L) in five baths with inter-phase squeezing, Table 1.

Tab.1 Protocol for determination of wool yield

Bath	Composition	Conditions
I	2 g/L Felosan RG-N (CHT)* 0.05 g/L Na_2CO_3	50°C, 5 minutes
II	1 g/L Felosan RG-N 0.3 g/L Na_2CO_3	50°C, 5 minutes
III	0.2 g/L Felosan RG-N	50°C, 5 minutes
IV	Warm distilled water	50°C, 5 minutes
V	Cold distilled water	5 minutes

* Felosan RG-N is fatty alcohol ethoxylate

Washed samples were dried until constant mass was achieved.

2.2.3. The process of washing raw wool in chestnut suspension

The process of washing raw wool (scouring) with a suspension of horse and sweet chestnut was carried out in two stages, pre-washing and washing with intermediate rinsing. The pre-washing of 800 g of raw wool was carried out with tap water at a temperature of 30 °C for 15 minutes, followed by a rinsing cycle with cold tap water for 5 minutes.

The washing process was continued by gently kneading the wool in a suspension of horse and sweet chestnuts (2 litres) for 15 minutes. The samples were then drained and rinsed in tap water at a temperature of 30 °C for 3 cycles, each cycle lasting 5 minutes.

Wastewater from the pre-washing (1-w, 2-w) and washing process was collected in separate containers (1-hc, 2-sc) to analyse its composition (Table 2).

The washed wool samples were placed on a textiles mesh screen and allowed to air dry.

Tab.2 Labels of wastewaters from pre-washing and washing of wool samples

Label	Collected wastewater
1-w	after pre-washing of wool
1-hc	after washing with horse chestnut
2-w	after pre-washing of wool
2-sc	after washing with sweet chestnut

2.3. Methods

In order to evaluate as objectively as possible, the effect of horse and sweet chestnut suspensions when washing raw wool, the methods of analysis specified in Table 3 include:

(i) chestnut fruit
(ii) horse and sweet chestnuts suspensions
(iii) raw and washed wool
(iv) wastewater.

Tab.3 Analysis parameters

(i) chestnut fruit	**(ii) chestnut fruit suspensions**
Et-OH extract	
Wa- isolate	pH
(iii) wool	**(iv) wastewater**
Wool Yield (Yld)	pH
Whiteness degree (W)	Conductivity (κ)
Yellowness index (YI)	Turbidity (T)
Remission (R)	Chemical oxygen demand (COD)
Alkali solubility (S)	Total Solids (TS)
FTIR	Total Suspended Solids (TSS)
	Total Disolved Solids (TDS)

(i) Chestnut fruit analysis

The analysis of horse and sweet chestnut fruits was carried out using methods for extracting the chestnut fruits in ethanol and isolating in water. These procedures were preceded by the preparation of the chestnut fruit by dividing it into four parts in order to obtain an approximately equal mass of both chestnuts. The fruits were dried and prepared gravimetrically using a Kern balance, model ALJ 220-5DNM, Germany. After accurate weighing of the dried horse chestnut (hc-f) and sweet chestnut (sc-f), the extraction procedure in 200 mL ethanol (Et-OH) and the isolation procedure in water (Wa) were started.

Soxhlet extraction of chestnuts in Et-OH was carried out for 2 hours. Filtration and combined evaporation of extract in a Soxhlet and in a dryer were then carried out until a constant mass was obtained.

Isolation in water was carried out in an Erlenmeyer flask at 60°C for 1 hour. After this time, the contents were filtered into previously weighed Erlenmeyer flasks using a sintering funnel. The filtrate was then evaporated to dryness on a water bath. The flasks with the residue were then placed in a desiccator, cooled and then weighed. After extraction in Et-OH, isolation in water (Wa) and evaporation, the content of extracted and isolated components from the horse and sweet chestnut fruits was determined.

(ii) Horse and sweet chestnuts suspensions

The pH value of horse (hc-s) and sweet (sc-s) chestnuts suspensions was measured with a Seven Compact™ Duo S213 multimeter from Mettler Toledo, Hong Kong before and after crushing the chestnuts in a blender.

(iii) Analysis of wool

To determine the wool yield (Yld), samples of raw wool were dried and weighed before and after washing using the conventional procedure.

Samples of raw wool and wool washed by the conventional method and suspensions of horse and sweet chestnut were analysed by the reflectance spectrophotometry method on a DataColor 300, Switzerland, to determine their remission curves (R), and samples of wool washed in chestnuts suspensions were measured for whiteness (W) and yellowness index (YI).

The evaluation of the wool fiber surface chemistry was performed with a Spectrum 100 FTIR spectrometer (Perkin Elmer, Buckinghamshire, UK) using the attenuated total reflection method. Five different measurements for each fiber were evaluated, and the average value was considered. All spectra were registered from 4000 cm^{-1} to 500 cm^{-1}, with a resolution of 4 cm^{-1} and four scans.

To compare the FTIR spectra they were baseline corrected, smoothed and normalized on the amide I vibration at 1627 cm^{-1}.

The alkali solubility (S) test was performed according to [20] and is sensitive for the detection of acid damage, because under normal, moderately strong alkaline conditions the amount of protein degraded provides a direct measure of the hydrolysis of the main chains. The procedure is carried out to process a mass of about 1 g of finely divided wool fibres (w_1) in 100 mL of NaOH solution (0.1 M) at a temperature of 65°C for 60 minutes, shaking for 5 seconds every 15 minutes. The precipitate obtained is filtered and washed in several cycles with distilled water, twice with 200 mL acetic acid and in six cycles with water. The residue is dried (w_2) and the mass loss is determined, on the basis of which the alkali solubility (S) is calculated according to equation 1:

$$S = \frac{w_1 - w_2}{w_1} \cdot 100 \qquad (1)$$

(iv) Analysis of wash wastewater

The wastewater from the pre-washing process and the process of washing raw wool in horse and sweet chestnut suspensions was analysed by measuring and determining pH, conductivity, turbidity, chemical oxygen demand (COD), total solids (TS), total suspended solids (TSS) and TDS (total dissolved solids). The physico-chemical properties of the wastewater were monitored by determining the pH [21] and conductivity using a SevenCompact™ Duo S213 multimeter from Mettler Toledo [22]. Turbidity was measured [23] with the TU5200 turbidimeter from Hach and COD [24] with the HT200S Thermostat and DR3900 Laboratory Spectrophotometer for water analysis, both from Hach Lange GmbH, Germany. TSS, TS and TDS were determined according to in-house methods based on standard filtration (glass fibres filter, GF-pore size 0.7 μm, Ahlstrom-Munksjö, Germany) and gravimetric methods using the Kern balance, model ALJ 220-5DNM, Germany.

3. Results

Results of preliminary analyses of the applicability of horse and sweet chestnuts suspensions for the process of raw wool scouring by four analytical approaches using adapted methods are presented.

The results of extraction of substances horse and sweet chestnut fruits (f) in ethanol, Et-OH and isolation in water, Wa are presented in Table 4.

Tab.4 Isolated substances content

Sample	% extract (Et-OH)	% isolate (Wa)
hc-f	1.15	18.66
sc-f	2.26	13.44

The results of the percentage of isolates in Et-OH and water differ in quantity. The mass fraction of the isolates extracted in ethanol is significantly lower than the fraction isolated in water. It can be assumed that the isolates in alcohol consist of saponins and some other alcohol-soluble substances, as this method is used to determine surfactants in detergents. The proportion of isolates from sweet chestnut is twice as high as that from horse chestnut, while the proportion of isolates in water is many times higher than that in alcohol and the proportion from horse chestnut is higher than that from sweet chestnut. A possible explanation for the higher proportion of isolates in the horse chestnut could be a starchy substance.

The analysis of the horse chestnut and sweet chestnut suspensions included the pH of the supernatant and the suspension obtained by homogenisation of the supernatant and the crushed chestnut fruit, Table 5.

Tab.5 pH of horse and sweet chestnuts' suspensions

Sample	pH	
	supernatant	suspension
hc-s	5.89	6.11
sc-s	5.46	5.79

The results for the pH of the solution and the suspension differ by about 0.4 pH units, and higher values were measured for the horse chestnut for both the supernatant and the suspension. These relationships confirm the differences in the composition of these two fruits as described in the literature.

The yield of raw wool (Yld) washed under laboratory conditions was 63.1%, which is consistent with previous analyses of wool from other breeds [25,26]. Fig.1 shows photos of raw wool (R-WO), wool washed with chestnut fruit under field conditions (hc-WO, sc-WO) and wool washed under laboratory conditions (L-WO) to illustrate the effect achieved by the washing process [4].

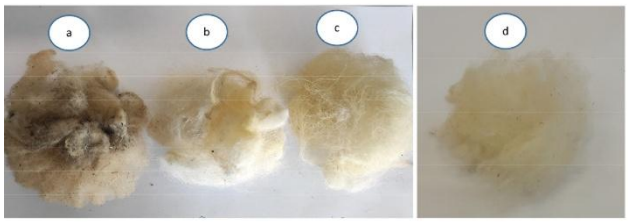

Fig.1 Photos of wool samples: a. R-WO, b. hc-WO, c. sc-WO; d. L-WO

It can be seen that impurities and dirt were removed during the washing processes. The wool sample washed in laboratory conditions using a stepwise process (L-WO) contained less residual vegetable impurities than the wool samples washed in horse chestnut and horse chestnut suspensions.

The remission curves of the unwashed wool samples and the wool washed in laboratory and field conditions are shown in Fig.2, while the degree of whiteness, yellowing index and alkaline solubility of the wool samples washed in field conditions are shown in Table 6.

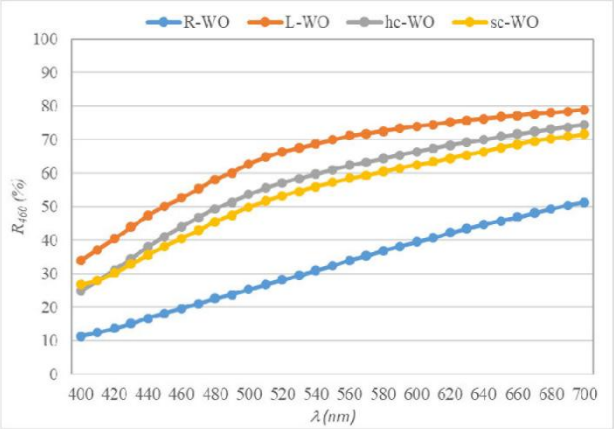

Fig.2 Remission curves of raw wool (R-WO), wool washed with chestnut fruit under field conditions (hc-WO, sc-WO) and wool washed under laboratory conditions (L-WO)

The remission curves of the wool washed under laboratory and field conditions in horse chestnut and sweet chestnut suspensions in Fig.2 have the same shape in the entire spectral range (400-700 nm). The remission values of the wool sample washed in the laboratory are the highest, confirming a better effect compared to the samples washed in horse chestnut suspension. Considering the conditions of the washing procedures in the laboratory and under field conditions, the differences obtained are as expected. The remission of the wool sample washed in horse chestnut suspension is about 5 units higher in the 460 to 700 nm range than the values for the wool sample washed in sweet chestnut suspension.

Tab.6 Whiteness, yellowness index and alkali solubility of washed wool samples

Sample	W	YI	S (%)
hc-WO	-42.2	39.24	11.87
sc-WO	-46.9	40.07	15.10

The spectral values (whiteness and yellowing index) in Table 6 confirm the yellowish tone of the washed samples. The differences in the yellowness index are small, while the whiteness values of the wool samples washed in horse chestnut suspension are about 4 units

higher than those of the samples washed in sweet chestnut suspension, which is consistent with the remission values in the blue part of the spectrum.

Fig.3 shows the FTIR spectrum of the wool sample washed under laboratory conditions (L-WO) and Fig.4 shows the FTIR spectra of the raw wool samples (R-WO) and the wool washed in horse chestnut (hc-WO) and sweet chestnut (sc-WO) suspension.

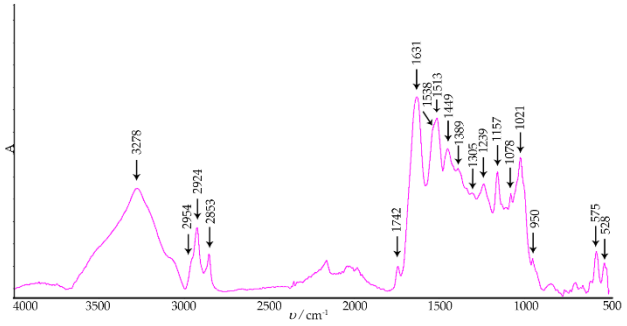

Fig.3 FTIR spectrum of wool sample washed in the laboratory conditions (L-WO)

FTIR spectra of washed wool is presented in Fig.3 and show peaks characteristic for the peptide bond that is a basis for protein chains. Bands within the range from 3700 cm^{-1} to 2700 cm^{-1} are attributed to N-H, O-H, $-CH_3$ and $-CH_2$ groups, while CH groups are determined with 1449 cm^{-1} and 1389 cm^{-1} peaks. Peaks attributed to CH groups are more pronounced in w-WO than in hc-WO or sc-WO, which may reflect changes in CH-containing side groups during the washing process. Peaks at 1742 cm^{-1} and 1305 cm^{-1} may be associated with lanolin-related functional groups, while the disappearance of bands at 1462–1468 cm^{-1} and 1174 cm^{-1} may indicate a reduced contribution of lanolin or other lipid-related components compared with chestnut-washed wool (Fig.4). The stretching vibration of amide I is appeared at 1631 cm^{-1} while amide II peaks are visible at 1538 and 1513 cm^{-1}. Peak at 1239 cm^{-1} is visible in all tested samples and is assigned to the C-N group from amide III [1].

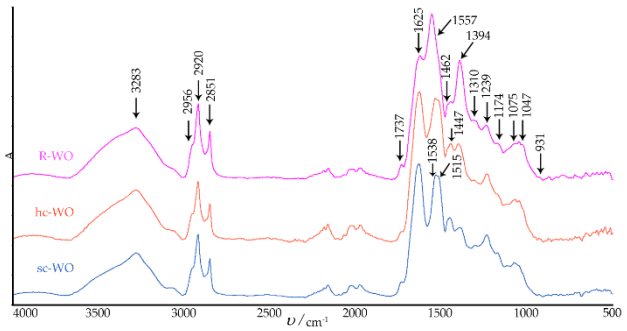

Fig.4 FTIR spectra of R-WO, hc-WO and sc-WO samples

Peaks within the range 1200-1000 cm^{-1} are assigned to sulphur-oxygen groups of keratin. C-H bending is attributed to peak at 950 cm^{-1}. S-S stretching vibrations corresponds to peak at 528 cm^{-1} while peak at 575 cm^{-1} is attributed to C-S stretching from disulphide [27,28].

FTIR spectra of wool fibers show bands associated to the peptide bond (-CONH-) which is characteristic for the polypeptide chain structure [27].

A broad absorption band is visible within the range 3500-3200 cm^{-1} and is attributed to the overlapping of N-H and O-H groups [28].

Peak at 2956 cm^{-1} is attributed to CH_3 group. Intensity of this band is highest for R-WO sample and lowest for hc-WO sample. Peaks at 2920 and 2851 cm^{-1} are attributed to the symmetrical and asymmetrical stretching of the CH_2 group [29]. FTIR spectra showed the lowest intensity of these bands in the hc-WO sample, which may be consistent with a lower contribution of surface-associated organic impurities. Bands observed in the 2700–1900 cm^{-1} region are not commonly assigned to the characteristic vibrations of wool keratin and may therefore be related to non-fibrous constituents present in raw wool. This interpretation is supported by the progressive decrease in band intensity from R-WO to sc-WO and hc-WO.

Peaks at 1735–1737 cm^{-1}, 1462–1468 cm^{-1}, 1303–1312 cm^{-1} and 1174 cm^{-1} are associated with lanolin-related functional groups. These bands are more pronounced in the R-WO sample and less intense in the hc-WO sample, which may indicate a lower contribution of residual lanolin after washing with horse chestnut extract [30,31].

The amide I band shifted towards higher wave-numbers, from 1625 cm^{-1} in R-WO to 1631 cm^{-1} in hc-WO and sc-WO. Amide II peaks appeared at 1557 cm^{-1} for R-WO and at 1538 and 1515 cm^{-1} for other tested samples. Bending vibrations of C-N-H from amide II is assigned to 1515 cm^{-1} band. It is obvious shifting of peaks to lower wave numbers compared to unwashed (raw) wool. Peak at 1239 cm^{-1} is visible in all tested samples and is assigned to the C-N group from amide III [27].

The relative intensities of the amide I and amide II bands appeared more similar in the washed samples than in raw wool. The similar intensities of the amide I and amide II bands in the washed samples may reflect structural changes in wool keratin following the washing treatment [28].

Peaks at 1447 cm^{-1} and 1386-1400 cm^{-1} are attributed to CH group and the less intensity of these peaks can be observed in sc-WO sample. It could be associated with changes in side-chain related vibrations and thus more close packing of the protein molecules [31].

Peaks at 1047 cm^{-1} and 1075 cm^{-1} are assigned to the sulphur-oxygen groups (i.e. S=O stretching vibrations from cysteic acid [27,29].
Peak at 931-930 cm^{-1} is broad peak of low intensity assigned to C-O-C bond which decrease in order R-WO, sc-WO and hc-WO [29].

cations. The high COD values of the wastewater from the wool washing process are caused by the emulsified wool grease, while water-soluble components, the so-called suint, have a lower influence on the COD [7]. The COD values shown in Table 7 are extremely low for all wastewaters and are not consistent with other physico-chemical parameters and previously published data [4,7].

Tab.7 Physico-chemical parameters of pre-wash and wash wastewaters

Wastewater sample	pH	κ (μS/cm)	M (FNU)	COD (mg O_2/L)	TS (mg/L)	TSS (mg/L)	TDS (mg/L)
1-w	8.86	2019.10	1004	472	4036	485.3*	2552.5
1-hc	5.59	518.06	366	460	2091	521.6	1094.3
2-w	9.23	2606.03	972	120	4778	724.7*	3110.0
2-sc	6.29	480.06	222	74	1880	1178.6	662.0

pH value, conductivity (κ), turbidity (M), chemical oxygen demand (COD), total solids (TS), total suspended solids (TSS), total dissolved solids (TDS)

When analysing scoured wool, the alkali solubility of the scoured samples was taken into account, although this method is more suitable for bleached wool samples. Undamaged wool has a value of 9 to 12% and the solubility increases with the degree of damage. The alkali solubility of wool washed in horse chestnut suspension (S=11.87%) is lower than the value obtained for wool washed in sweet chestnut suspension (S=15.10%).
The analysis of wastewater from the process of pre-washing in water (1-w, 2-w) and washing in suspensions of horse chestnut (1-hc) and sweet chestnut (2-sc) included physico-chemical indicators in Table 7.
The obtained different values of the wastewater from pre-washing and washing in suspensions of horse chestnut and sweet chestnut were expected. The pH value in the pre-wash cycles confirms the alkalinity of the wastewaters. However, the measured differences of around 0.4 pH units were not to be expected, as the same fleece was treated in water in a prewash. These differences can be attributed to the conditions of the manual treatment with gentle kneading and defibration of the wool strand, which was carried out by different hands. The pH values of the wastewater resulting from the washing of wool with horse chestnut and sweet chestnut are significantly lower and correspond to the values determined for pure suspensions.
A similar ratio of conductivity and turbidity values was determined for wastewater from the pre-washing and washing of wool in suspensions of horse chestnut and sweet chestnut.
According to [4] the environmental effect of various wastewaters is usually described by chemical oxygen demand (COD), which can be as high as 45 000 mg/L for wool scouring wastewater in industrial appli-

The total solids (TS) in the pre-wash wastewaters are extremely high, but the TS of the two wash wastewaters (1-hc, 2-sc) correspond to results of previous research [4]. The TDS value is extremely high in the pre-wash wastewater and many times lower in the wash wastewater. Some technical problems occurred during the determination of the TSS (*), so that a real explanation of the values obtained was not possible.

4. Conclusions

The preliminary results of the analysis of the scouring/washing process of raw wool of the "Lička pramenka" breed with suspensions of horse chestnut and sweet chestnut showed the potential in the application, but also the need for further research and in-depth analysis of the fruits, the suspension, the properties of the washed wool and the wastewater. Considering the nutritional value of sweet chestnut, further research will focus only on horse chestnut. In addition, the fleece of other Croatian sheep breeds, especially in the Majur region, will be selected. A comparison of this natural and green agent with products available on the market is also planned.

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