

TANNIN TREATMENT OF SHEEP WOOL

Hana Křížová¹ and Jakub Wiener²

¹Technical University of Liberec, Institute for Nanomaterials, Advanced Technologies and Innovation, Department of the Preparation and Analysis of Nanostructures, Bendlova 1409/7, Liberec, Czech Republic

²Technical University of Liberec, Faculty of Textile Engineering, Department of Material Engineering, Studentská 2, Liberec, Czech Republic
hana.krizova@tul.cz, jakub.wiener@tul.cz

Abstract: The aim of this study was to test the possibility of treating raw sheep wool with tannic acid. This treatment improves the odor of sweat wool and gives the wool antibacterial properties. The tests also suggest the possibility of anti-felt treatment. To achieve these aims, tannic acid was used as strong antioxidant. The sensory evaluation of raw wet sheep wool smell before and after tannin treatment was provided by a group of 30 assessors. This subjective assessment was supplemented by an indicative measurement of volatiles using gas chromatography. Antibacterial properties of tannic acid were evaluated by the method when eluates of treated and untreated wool were cultivated on the agar medium. The antibacterial effect of tannin has been manifested in growth of different number of bacterial colonies. Finally, electron microscope images showing some damage to wool fiber surface indicated the possibility of anti-felt treatment by using tannic acid. All results suggest that tannic acid could be an effective and ecological means of raw sheep wool treatment for use in the building or automotive industry.

Keywords: wool, tannic acid, odor, volatiles, antibacterial

1 INTRODUCTION

Historically, sheep farming in Czech Republic was a widespread agricultural activity, especially in the mountain and foothills of Bohemia and Moravia. However, the socialist past of the country and its centralized economy and planning have meant that sheep farming has almost disappeared [1]. At present, many private breeders are returning to sheep breeding. The reason is mostly the desire to return to nature and to rediscover old traditions. Modern farmers use sheep mainly for milk and meat, but due to the overall reduction of the textile industry in the Czech Republic, there is no interest in sheep wool. The wool industry has been almost completely liquidated and private sheep farmers rarely process this raw material for textile purposes [2].

Central European sheep wool is not as fine and good as fine Merino wool from New Zealand, for example. However, it is a raw material that can be used very well in building and automotive industry, for applications such as sound and heat insulation. Wool fibers absorb moisture well. It can absorb almost one-third of its own weight in water [3, 4]. Wool ignites at a higher temperature than cotton and some synthetic fibers. It has a lower rate of flame spread, a lower rate of heat release, a lower heat of combustion and does not melt. It forms a char which is insulating and self-extinguishing and it contributes less to toxic gases and smoke than other flooring products when used in carpets.

Wool has a high specific heat coefficient, so it impedes heat transfer in general [5]. In addition, wool also absorbs sound like many other textile fibers [6].

However, the basic problem that limits the use of wool as an insulating material is its odor. The odor of wool is caused by both the fatty matter covering the raw wool fiber and the fiber composition itself. The building material of the wool is the fibrous protein keratin which is the source of sulfur-containing amino acids and numerous disulphide bridges. Fats and sulphur-containing compounds are the main source of volatile organic compounds that contribute to the raw wool odor in heat and moisture [7].

1.1 Tannins

Plant tannins are water-soluble polymeric polyphenolic compounds found in a variety of plant parts such as wood, tree bark, fruit peels, pods, leaves, roots and plant balls. Through their phenolic and carboxyl groups they form complexes with various substances - mainly proteins (protein coagulation on which the skin tanning process is based), amino acids and alkaloids. Tannins also form complexes with metal ions, polysaccharides and fats. Their typical representative is tannic acid (Figure 1), which is used, for example, in textile dyeing as a staining agent for the dyeing of cellulosic and other fibers. Phenolic groups of tannins can form effective bonds with different types of fibers and dyes

to help fix dyes. In these reactions, the tannin is first drawn into the fiber or adhered to the surface of the fiber and the subsequent application of metal salts (Fe, Cr, Cu, Al, Pb or Sn ions) leads to the formation of complexes which fix the tannin to the dyed material. Subsequently, the applied dye is tied to the fiber more firmly and with higher affinity [8].

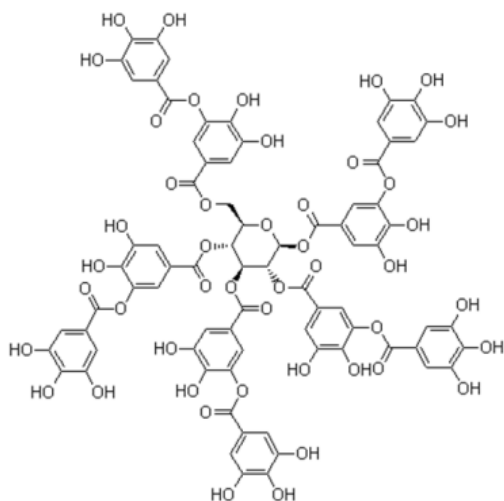


Figure 1 Acid gallic (gallotannin)

Tannins - gallotannins and condensed tannins (so-called proanthocyanidins and catechins, Figure 2) can bind to the fiber substrate by several mechanisms:

1. the ionic bond between the deprotonated phenol group and the protonated amino groups of the protein fibers (wool, silk) and polyamide,
2. the numerous hydrogen bridges that occur between pseudovacant hydrogen orbits of phenol groups (if not dissociated) with free electron pairs of oxygen or nitrogen in the fiber structure,
3. the covalent bonds that may arise between quinone and semichinone groups present in tannin and suitable reactive groups of fibers.

When tannins react with proteins (or amino acids), the crosslinking effect is also applied. E.g. when collagen is crosslinked with plant polyphenols (catechin or tannic acid), the hydroxyl group of amino acids hydroxyproline and serine, the aspartic acid carboxyl group, the amino group of lysine and the asparagine amide group are the potential site of interaction of polyphenols with collagen. The literature describes well-known tannin reactions not only with collagen but also with albumin and other proteins. E.g. the amino acid proline is also present in the sheep's wool, where it accounts for about 6.5% of all amino acids in keratin, so tannins with wool and other protein fibers can be expected to interact [9-11].

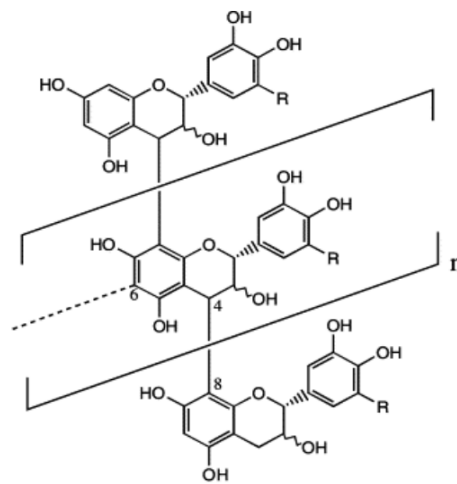


Figure 2 Condensed tannin (catechin)

Generally, plant tannins, catechins, proanthocyanidins and polyphenols have a strong affinity for proteins (and thus also for animal fibers made up of keratin - wool, rabbit hair, etc.). Protein coagulation, amino acid crosslinking, polyphenol binding to protein, change in protein configuration and thus change in functionality until inactivation or destruction (in general: protein denaturation) are the consequences of these reactions. All this is just a question of choosing the appropriate type, concentration and method of applying tannin or other polyphenol [12].

1.2 Antioxidant effect of tannins

Tannins, like polyphenols, are powerful antioxidants (reductants) having the ability to reduce other substances and oxidize themselves. They also can eliminate free radicals and ROS (reactive oxygen species). According to the antioxidant mechanism, antioxidants are divided into primary ones that reduce the activity of ROS by reacting the free electron and themselves to become a less dangerous and more stable radical and to secondary (reductants) that oxidize themselves and reduce the other substance. They are often oxidized by a dehydrogenation mechanism to form a carbonyl group. The antioxidant activity of polyphenols is a complex process that combines different mechanisms. The complexity of this process is evidenced by the fact that since 1955 about 700 articles have been published suggesting different in vivo and in vitro anti-oxidation mechanisms of polyphenols! The ability of polyphenols (PPh) to eliminate free radicals proceeds simplistically according to the equation (1) where the phenol group of polyphenols reacts with a strongly reactive radical which is "quenched" and polyphenol itself changes into a radical.



Figure 3 shows a double mechanism (dehydrogenation and radical) of a flavonoid or polyphenol reaction with hydroxyl groups at ortho positions. In fact, the result of these interactions is the oxidation of polyphenols to various complex quinoid and semiquinoid structures, depending on the pH and redox potential of these compounds [13, 14].

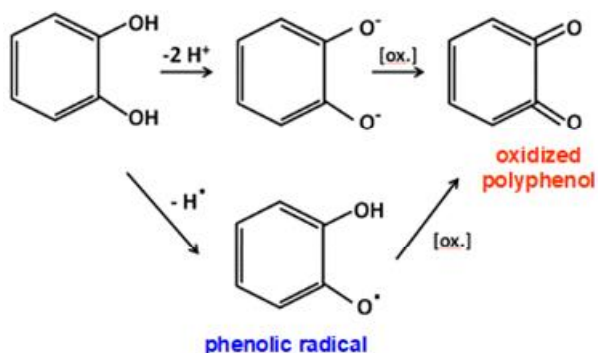


Figure 3 Double mechanism of polyphenol oxidation

1.3 Antibacterial effect of tannins

Tannic acid and gallic acid are very strongly bound to proteins and enzymes by hydrogen bonds, which is also one of the possible mechanisms of their action against microorganisms. This effect was observed when testing tannic acid, gallic acid and catechins for highly resistant MRSA (methicillin-resistant *Staphylococcus aureus*). One of the virulent factors of gold staphylococci, distinguishing it from other staphylococci, is the formation of so-called free or bacterial wall-bound plasma coagulase, which makes *S. aureus* capable of producing fibrin from plasma fibrinogen. The result is a protective sleeve (fibrin biofilm) around the bacteria itself or an infection deposit that severely impedes both the penetration of antibiotics and the penetration of macrophages [15].

S. aureus most often causes skin furuncles, skin and tissue abscesses and localized soft tissue infections, as well as osteomyelitis, mastitis, necrotizing pneumonia, endocarditis, toxic shock syndrome and sepsis. It turns out that the ability of tannins to form complexes with proteins is used here, because the tannic acid inactivates this protective shell of the bacterium, making it accessible to the bacterium to contact the antibiotic [16].

Iron and other trace elements are essential for the metabolism of aerobic microbes and therefore another mechanism of antibacterial action of polyphenols is explained by chelation of iron from the environment (in vivo from blood plasma), which makes this important micronutrient rendered inaccessible to bacteria.

The antibacterial effect of polyphenols is a complex of several mechanisms - protein binding and

inactivation, chelation of metal ions, direct biochemical pathways affecting in cells including the inhibition of some enzymes and the induction of apoptotic effects in bacteria. Thus, chelating, antioxidant and sometimes pro-oxidative effects with antibiotic effects are combined as with synthetic and polysynthetic antibiotics. Their effectiveness is also conditioned by a lot of other factors such as the resistance of a bacterial strain, the concentration and availability of the antibiotic at the site of action, etc. [17, 18].

1.4 Evaluation of odor

Several methods can be used to evaluate the odor. Volatile substances can be separated, identified and quantified by means of a separating column and inert gaseous media (gas chromatography) using a suitable detector (a flame ionization detector - FID), an analyzer identifier unit (mass spectrometer - MS) and calibration standards. At the research workplaces, the so-called electronic nose is also developed, which is a complex of sensors that identify individual chemical volatile substances. The most important evaluator, however, remains the human nose, because even if an exact analysis of the volatile content of the sample is carried out, nothing or very little tells about how that scent is perceived by humans, because the odor is a very subjective perception.

Human olfactory cells function as chemoreceptors responsive to the volatile substances contained in the flowing air passing through them, respectively, around the olfactory epithelium in the nasal cavity, where numerous protuberances of these olfactory cells are present in the mucosa. The excitement generated by their chemical irritation is led to the olfactory center of the frontal lobes and to other parts of the brain (thalamus, hypothalamus). Then there will be a resulting impression influenced by past experiences and experiences associated with certain odors. For example, human smell is not sensible in comparison to some animals. However, an untrained person is able to recognize about 4 to 10 thousand odors, trained professional up to 10 times more.

Women also generally have a better sense of smell, which is related to their maternal role. However, it is shown that the sensitivity of human smell changes considerably throughout life. The biggest olfactory perception has children around the 6th year of life and then it still falls olfactory sensitivity.

The 20-year-old human has 82% olfactory receptors with which he was born, at the age of 60 he drops to 38% and at age 80 to 28% of the original amount. The young people around 20-23 years of age have the best sense of smell, because they still have a high olfactory sensitivity, which is supplemented by a sufficient amount of information and experience. [19]

2 EXPERIMENTAL PART

2.1 Material and chemicals

Raw unwashed wool sheared from sheep, tannic acid (Lach-Ner, Czech), trypto-casein soya agar (TSA) (Biovendor, Czech) and natural spring water (without chlorine treatments) sterilized for 10 minutes by boiling, were used in this work.

2.2 Methods and devices

Solution of tannic acid at the concentration of 10 g/l was prepared. The raw sheep wool (4 g each sample) was dosed into hermetically sealed glasses marked A, B, C. 100 ml of tannic acid solution was added to glass A, 100 ml of spring water was added to B and C glasses. The glasses were hermetically sealed. Samples A and B were boiled for 5 minutes in a water bath. Subsequently, the samples were allowed to cool for 1 hour in this water bath. Throughout this time, cold water was left in vessel C. Samples B and C served as comparative samples. Sample B was prepared to distinguish the influence of boiling water from the tannin effect to odor decomposition. Sample C, which was left without boiling, was a comparative sample in which only cold water influenced the wool. All three glasses were shaken regularly throughout the period of wool contact with the fluid. After the samples A and B were cooled, the liquid from all three glasses was removed and the wobble wool was wrung out. The glasses with wet wool were hermetically sealed again. Thus, samples of the treated and untreated wool for olfactory (sensory) test, for quantitative bacterial test, for gas chromatography and electron microscope images (SEM) were prepared.

The sensory evaluation of the wool odor was done in the form of an olfactory test of randomly selected respondents who had the task of subjectively assessing the odor of the A, B and C samples and sorting them according to the intensity of odor from at least to the most odorous sample. Considering that women have a more sensitive sense of smell and because the sense of smell is getting worse in human life, 15 women and 15 men aged between 16 and 65 were interviewed to cover the widest possible age group. Samples A and C were also prepared for gas chromatography analysis. The vials were preheated to 45°C and a small volume of wet air from each vial was sampled directly through the rubber septum from the sample vessels into a gas chromatograph column with a mass spectrometer (GC-MS).

For the quantitative evaluation of the bacteria content in the samples, 1 g of material was removed from each wet wool fibers, 40 ml of sterilized natural water of 20°C was added and the samples were shaken vigorously for 2 minutes. The resulting eluates were each 1 ml dosed on the agar surface of the Petri dishes. Cultivation at 37°C took 24 hours. This procedure was performed on samples which

were treated the same day and repeated after 5 days when still wet samples were left in hermetically sealed glasses at room temperature. After 24 hours of cultivation, colonies (CFUs) were counted in each Petri dish.

3 RESULTS AND DISCUSSION

3.1 Sensory evaluation of wool odor

Three samples in glass sealed containers (A, B, C) were presented to the respondents for snooze and to sort by intensity of odor from the least smelly to the most odorous. Sample A = wool treated with tannic acid at boiling, sample B = wool treated only with boiling water, sample C = untreated wool, washed only with cold water. Fifteen women aged 16 to 65 and fifteen men aged 17 to 62 were interviewed (Figure 4). The interviewees were selected randomly, but there were also 4 people (1 man and 3 women) who had or recently have had a valid certificate for sensory analysis and can be considered the professional assessors.

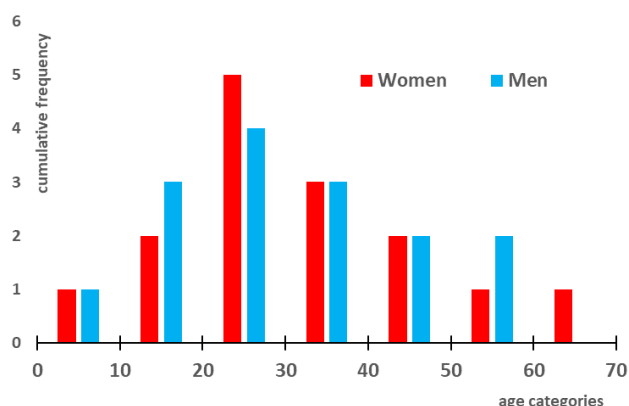


Figure 4 Age representation of evaluators

Twelve women and fourteen men (including four professional sensory evaluators) ranked samples in the same order A, B, C. One man and one woman evaluated the order of samples B, A, C, two other evaluators with different results ranked as A, C, B. In the sensory analysis, 87% of respondents (93% of women and 93% of men) identified a tannin-treated sample as the sample with the smallest odor intensity and the same percentage of women and men identified the sample of wet untreated wool as the worst smell. Subsequent questioning revealed that both respondents who placed a tannin-treated sample on the second or even third place in the order did so because the smell of the tannin-treated sample was not pleasant for them, not because they compared the samples with the increasing intensity of the odor. While the wet tannin-treated wool aroma resembled most of the respondents "the smell of wool after washing in washing machine", these two differently evaluating respondents described

this odor as a "resembling varnish" or "lemon-like". The majority, however, said the odor of the wool had the lowest intensity and its aroma was not unpleasant.

3.2 Quantitative bacterial evaluation of wool samples

The number of bacterial colonies that grew on agar from 1 ml of eluate of one- and five-days old samples A, B and C after 24 hours is shown in Figure 5 and Table 1. It is evident that most of the bacteria contained an untreated raw wool eluate in both measurements, where the number of colonies was so high that they were uncountable. A sample of wool that had undergone heat treatment (5 minutes boiling in water) contained a noticeably lower number of bacteria. The eluate contained 825 CFU/ml, but after 5 days in wet and at room temperature, the bacteria on this fiber substrate significantly increased and 1 ml of the eluate contained almost two orders more of bacteria, which was at the limit of CFU counting on the agar surface. The tannin heat treated sample was initially almost sterile (4 CFU in 1 ml of eluate), after 5 days in a sealed container in wet and at room temperature, 212 CFU from 1 ml of eluate grew on

the agar plate. The difference in the number of bacteria also corresponded to the odor of the samples: while sample A did not change the odor, sample B in the closed glass subjectively smelled more than the first day just after the heat treatment.

Table 1 Number of CFU in 1 ml of aqueous extract of wool

	sample A	sample B	sample C
1 day	4	825	uncountable
5 days	212	cca 10 ⁴	uncountable

3.3 Gas chromatography

Analysis of gaseous substances in the samples A and C was performed using gas chromatography. The analysis was only indicative without the use of calibration standards. Identification of some volatiles was performed by a mass spectrometer using a software library to identify chemical substances. The presence of, for example, acetone, 3-methylpentane, heptanaldehyde, 2-ethylhexanol, methylheptyl acetate and others has been observed from sulfur and nitrogen-free volatile substances (Figure 6).

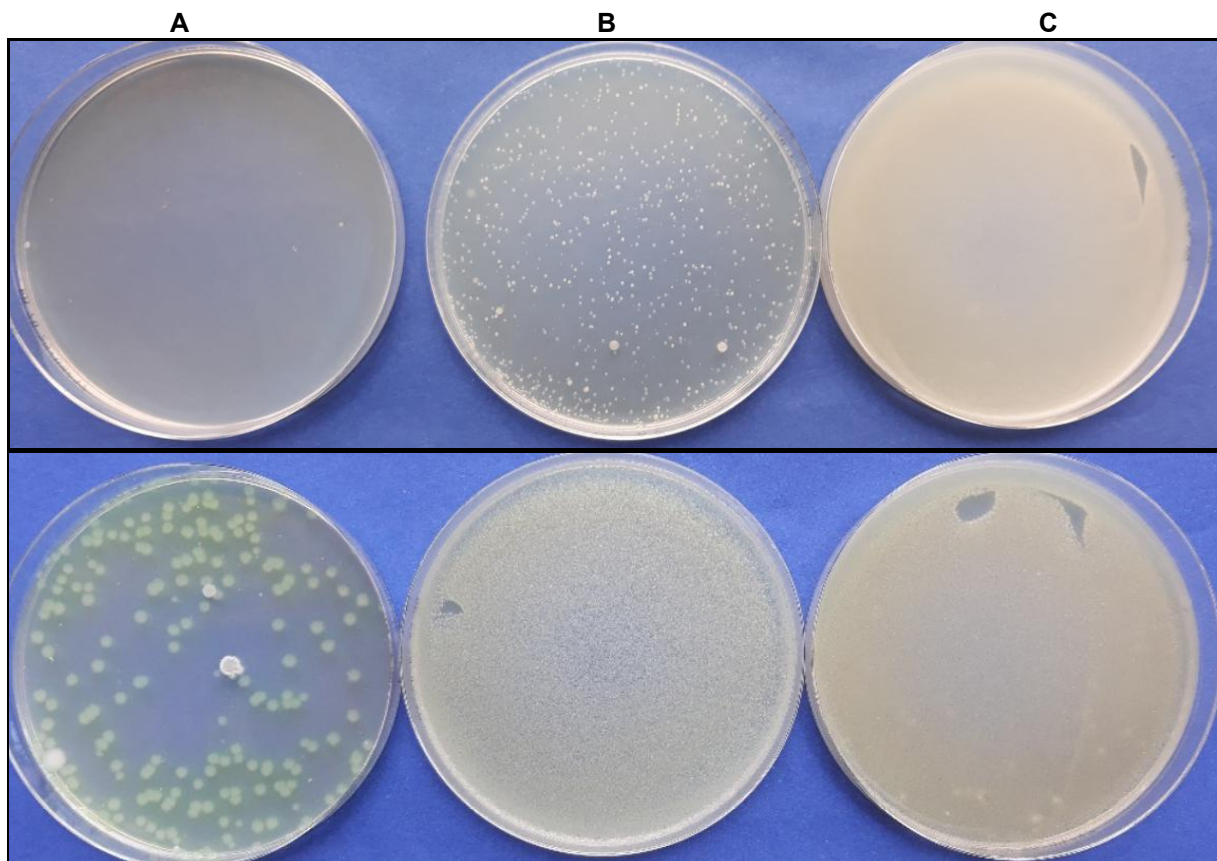


Figure 5 CFUs grown from wool eluates after 24 hours of cultivation from samples 1 day (top) and 5 days old (below)

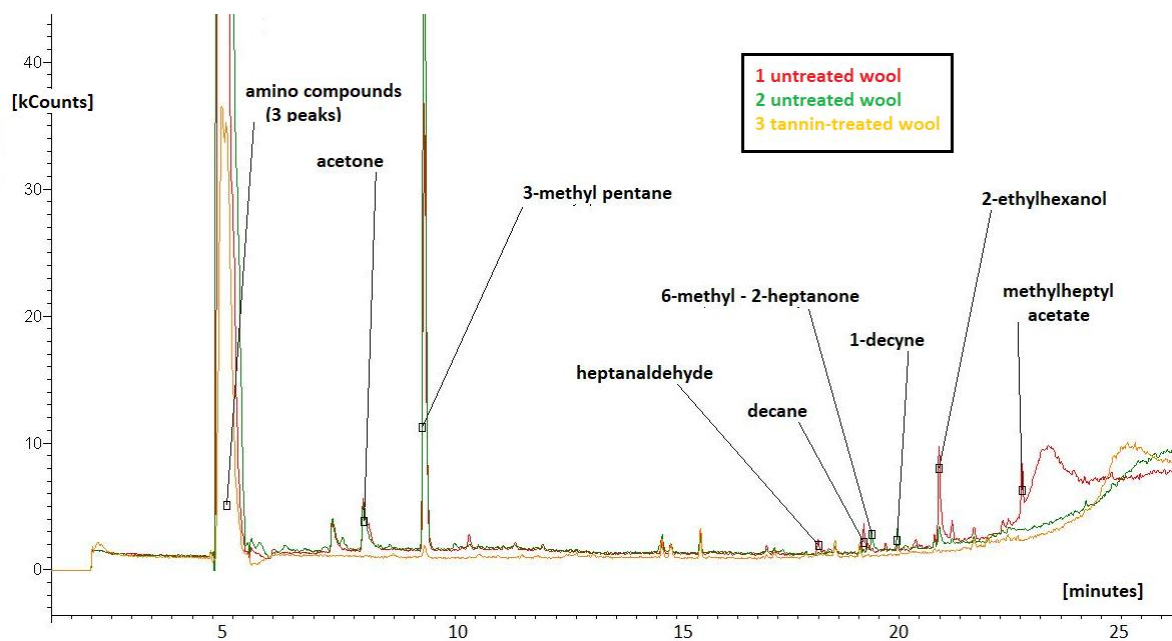


Figure 6 Some volatiles released from tannin-treated and untreated sheep wool (GC-MS)

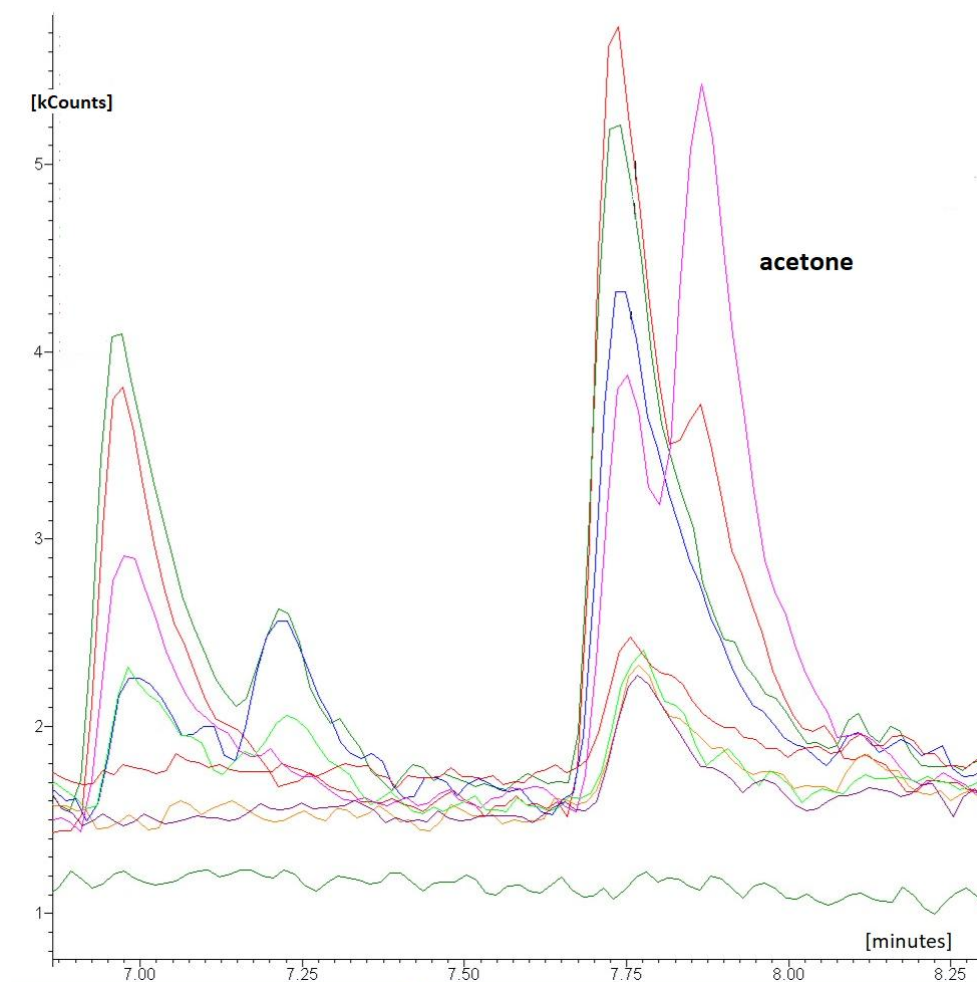


Figure 7 Zoomed peaks corresponding to acetone (GC-MS)

The chromatogram contains several peaks. The highest being identified as indeterminate volatiles containing amino groups, and 3-methyl pentane and 2-ethyl hexanol. Unfortunately, volatiles in the first half of the chromatogram were not reliably identified. The record shows that the most volatile small molecules with shorter retention times such as hydrogen sulphide (H₂S), carbon disulphide (CS₂), ethanol, sulphur dioxide (SO₂) or carbonyl sulphide (COS) are beyond the sensitivity of this chromatographic column, which is primarily determined and calibrated to analyze soil contaminated by higher hydrocarbons. However, it is clear from the record that the air sample from the tannin-treated wool follows the untreated wool peaks with noticeably lower values. Figure 7 shows an increased peak from a retention time of 7.7 minutes, identified as acetone. It is a composite record of 4 tannin-treated and 4 untreated wool samples, and it is clear to see the difference in the values of these two groups of samples because all four tannin-treated samples are near the baseline.

3.4 SEM images

The cause of the felting of animal fibers is their structure. Animal fiber has tiny scales on the surface that make the fibers captured, intertwined and interconnected. Fully reinforced fabric (felt) is produced by moisture, heat and chemicals that open the scales. The motion leads to fibers interconnections.

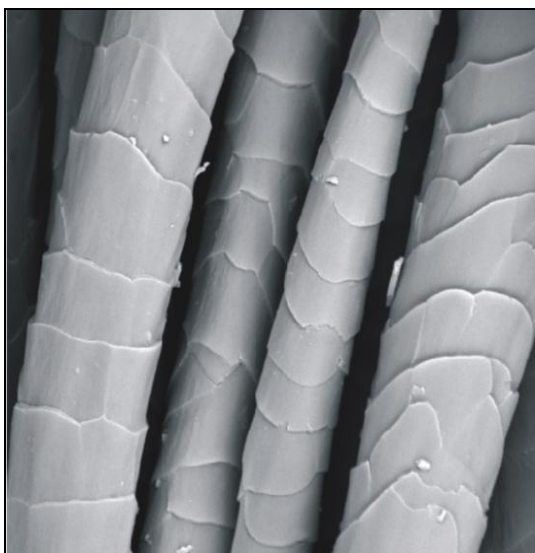


Figure 8 SEM image of untreated wool fibers

However, locally and spontaneously, this phenomenon occurs, for example, by mechanical action, resulting in fuzziness (pilling) that degrades the appearance of the fabric. This can be prevented by a non-felt treatment, which means preventing the scales on the surface of the fibers from catching

each other. The scales can be removed from the fiber or masked (polymeric coatings) so that they are firmly glued to the fiber. All or part of them can be removed chemically, physically (single, cutting) or enzymatically.

Figure 9 shows the wool fiber after heat treatment with a tannin solution having damaged and partially chipped scales. Since tannins denature proteins, i.e. interact with amino acids using the mechanisms described in section 1.1., which leads to the loss of the original function of the protein, the change of its structure and eventually its destruction. We will continue to study this effect.

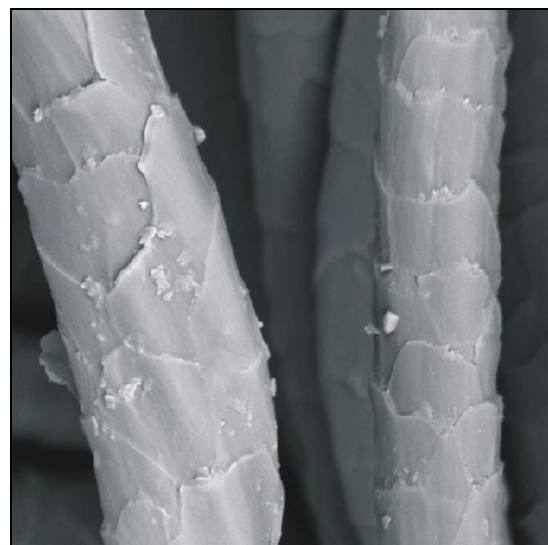


Figure 9 SEM image of tannin-treated wool fibers

4 CONCLUSION

In this work the antioxidant and antimicrobial properties of tannic acid were used in the treatment of raw sheep wool. The heat treatment of fibers using tannin solution not only significantly reduced the unpleasant odor of the raw wool, but also reduced the bacterial growth on fibers surface very significantly. It is evident that the main cause of the unpleasant wool odor are bacteria on the surface of wool fibers that decompose fat, keratin and other organic substances to form volatile substances that are the source of odor. Through a panel of evaluators who carried out a sensory analysis of raw wool odor, we have shown that heat treatment by the tannin solution (tannic acid respective) significantly reduces the intensity of the raw wool odor. At the same time, the treated wool, due to tannin, acquires some antimicrobial resistance, although this treatment did not imply a perfect sterility of the fibrous surface. Since the number of bacteria on the surface of the wool was very low even after 5 days of wet fibers in a sealed container at room temperature, the tannin-treated wool did not have an unpleasant

smell unlike wool without heat treatment or wool that was cooked only in water. The lower content of some volatile substances in the tannin-treated wool was recorded even in gas chromatography. Moreover, the electron microscope image of wool fibers treated with tannin at the boiling point suggests that the tannin wool treatment led to some fibers to morphological changes. Surface structure has been disturbed, damaging and breaking the flakes on the surface of the fibers has been observed. This can be used, for example, for an anti-felt treatment of wool.

We have demonstrated that plant tannins can be used as an inexpensive, ecological and effective means of treating raw wool that is not suitable for garment processing but could be used very well, for example, in buildings or automobiles as an insulating material.

ACKNOWLEDGEMENT: *This study is a result of the research funded by the project No. LO1201 with a financial support from the MEYS (Czech Rep.) under the NPU I program. Special thanks to Dr. Pavel Hrabák and all willing respondents from Technical University of Liberec.*

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