

Antibacterial properties of leathers, natural tannins and their interactions with shoe bacteria

A. Battaglia, E. Poles, S. Giovando, M. Gotti
Silvateam S.p.A.

Tannins are high molecular weight polyphenols, naturally synthesised by plants to defend themselves against biotic and abiotic stress factors. Their role as antioxidant, antibiotic and antibacterial agents has been known for many years among agriculture, animal nutrition, pharma, and cosmetics industries. If tannins can also perform antibacterial activity in vegetable-tanned leather, this effect could be very interesting for all the applications in which the leather, when it comes into contact with sweat and bacteria, becomes a solution to reduce hyperhidrosis (excessive sweating) and bromhidrosis (unpleasant odour).

The goal of the study was the assessment of the antibacterial activity of vegetable tanned leathers with natural tannins to produce articles in direct contact with human skin and, therefore, their effect on sweat, bacterial growth and metabolite production. Currently, in the European Union, it is prohibited to claim tannins as antibacterial substances. In view of the scientific evidence presented, Silvateam has started the authorisation process for the registration of tannins as antibacterial active substances, in accordance with the Regulation (EU) N.528/2012 concerning making them available on the market and the use of biocidal products.

1. Foot Odour: A Common Problem

Foot odour is a common problem and can usually be traced to a combination of perspiration (foot sweat) and bacteria. Moist and warm feet, socks, dead skin, and footwear provide the perfect environment for the bacteria to breed and multiply and cause foot odour. The results of a 2012 US-based survey carried out by the Institute for Preventive Foot Health (IPFH) over 1,456 panellists showed that it affects 16% of the population over 21.

Most consumers do not know what happens to their feet while they are wearing shoes. Excessive sweating and unpleasant odours are just part of their daily life, something people usually take for granted but that makes them feel guilty, embarrassed, and helpless.

Foot odour is a common condition, especially among people who are very active and whose feet tend to sweat a lot because of emotions (e.g. anxiety, stress, and embarrassment), use of particular drugs or hereditary predispositions.

Certain foods, drinks, caffeine, and nicotine can also trigger sweating in a way that is anything but normal. While it is natural, for instance, to sweat when you eat especially hot or spicy food, people with gustatory hyperhidrosis may do so when they eat something cold. In some cases, even smelling or thinking of food can elicit a response.

1.1 Production of unpleasant odours in feet

Thermoregulation is a process that allows the body to maintain its core internal temperature. Whenever the body temperature rises, the hypothalamus tries to lower it by

producing sweat through eccrine glands. Sweat is an odourless solution that mainly consists of water (99%) and a mixture of inorganic salts, vitamins, glucose, lactic acid, urea, and amino acids, such as leucine, isoleucine and valine. These organic molecules also originate from the keratin-rich thickened stratum corneum of plantar skin, called callus.

Bacteria inhabiting the foot skin flora feed on sweat, especially by biodegrading the branched-chain amino acids into volatile fatty acids (VFAs). VFAs are responsible for the production of unpleasant cheesy/acidic notes usually associated with feet. The key causative bacteria are *Staphylococcus spp.*, *Corynebacterium*, *Brevibacterium*, *Micrococcus*, *Kytococcus* and *Propionibacterium*, mainly found on the plantar area and in the toe clefts.

The microbiota, the totality of microorganisms that populate a certain ecologic niche, can vary depending on different environmental, topographical, and biochemical conditions as well as within person variability, without impacting the total amount of microorganisms. It is therefore important to increase the beneficial bacteria, essential for human well-being, while reducing the dangerous ones in the feet.

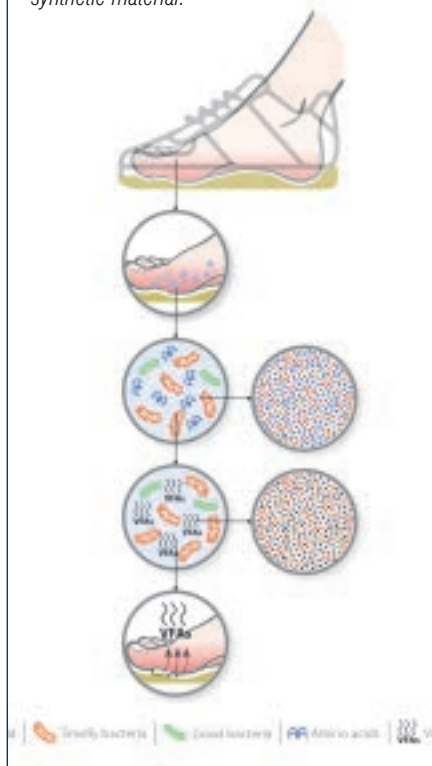
Humans were born to walk barefoot, the ideal situation for a perfect foot thermoregulation. Without shoes, the foot temperature barely rises, and the eccrine glands do not have to produce much sweat to lower it. With less sweat, bacteria are unable to grow and the number of VFAs produced is very limited. Therefore, the result is a scarcely noticeable odour, without any offensive cheese-like/acidic notes.



Fig. 1: Degradation of amino acids to volatile fatty acids in feet.



Fig. 2: Production of unpleasant odours in feet wearing shoes with inside made of synthetic material.



1.2 What happens inside a shoe with a synthetic insole and lining?

The foot experiences an increase in temperature, pH and humidity together with a minor pitting of the stratum corneum every time a person puts his or her shoes on.

High levels of hydration, because of the use of occlusive footwear or high environmental temperatures, lead to an increase in microbial numbers, particularly staphylococcus, that cause hyperhidrosis.

Hyperhidrosis is an excessive sweating, four to five times more than normal, usually caused by emotional factors, medical conditions, the use of drugs and hereditary predispositions. This condition nurtures the secretion of high levels of peptides and amino acids in the sweat which are biodegraded by bacteria into VFAs, that are responsible for the unpleasant odour.

Distinct cheese-like/acidic notes are generated by the high microbial load fostering the biotransformation of amino acids and callus into VFAs, such as isovaleric acid, butyric acid and isobutyric acid. The chronic condition of human feet producing excessive sweat with foul odour is called bromhidrosis.

The internal part of the shoe (insole

and lining) made of synthetic material is unable to counteract the overproduction of foot sweat. This leads to the unpleasant odours that cause social embarrassment and reduce self-confidence, especially among women. Moreover, shoes remain wet and humid even when a person is not wearing them, for example throughout the night. Therefore, the shoe becomes the perfect habitat for bacteria proliferation (Fig. 2).

Frequently worn shoes host up to 10 million per cm² of those "smelly" bacteria responsible for odour, especially in the toe clefts. Every time a person puts shoes on, the clean feet become contaminated by the bacterial population growing inside the shoes. It is not important how many times somebody washes their feet - washing the feet every day is necessary, but it is not enough to defeat foot odour. Day after day, the bacterial population inhabiting the shoe expands, more VFAs are produced and a strong foul odour persists with the shoe.

Common disinfectants or do-it-yourself home remedies in the long run can cause rashes, allergies, and other problems with direct impact on human well-being. The odour issue comes from the shoe, not from the foot.

All these solutions are limited, not

permanent and very expensive. In some cases, they are harmful since they irritate the skin and alter the body's thermoregulation mechanism.

1.3 Tannins: classification and their role in leather making

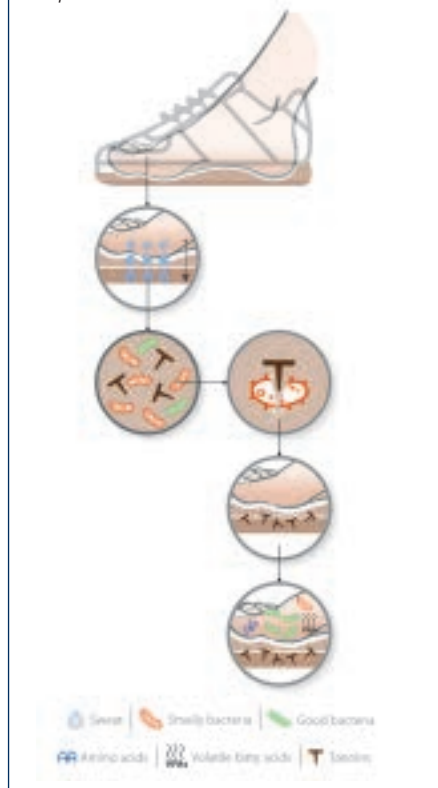
Tannins are a complex family of water-soluble polyphenolic compounds, synthesised as secondary metabolites by many plants. Tannins are present in numerous types of trees and plants and they can be found in barks, leaves, wood but also in fruits and roots. From a chemical point of view, it is difficult to define tannins due to their heterogeneity in terms of chemical compositions and molecular weight (MW). Traditionally, tannins have been divided into two large groups: hydrolysable and condensed tannins. Hydrolysable tannins are composed of a carbohydrate core whose hydroxyl groups are esterified with phenolic acids with a MW ranging from 300 to 5,000 Dalton. Depending on the substances that are produced following hydrolysis (by acids, bases, or certain enzymes), hydrolysable tannins can be classified as gallotannins (yielding gallic acid) or ellagitannins (yielding ellagic acid). Tara pod tannin and chestnut wood tannin are representative of gallotannins and ellagitannins, respectively. Condensed tannins are oligomers of flavonoids units with a MW ranging from 1,000 to 20,000 Dalton. Quebracho extract is among the most industrially produced tannins that has been shown to be predominantly composed of oligomeric profisetinidins.

Natural tannins act by inhibiting the growth of many bacteria. In fact, tannins are the main protection agents against bacteria in the plant kingdom. Their antimicrobial properties have attracted particular interest and have been exploited in several fields such as food science and animal nutrition. Indeed, tannins have been used in the food industry as additives, natural colourants, and preservatives. More recently tannins are replacing the use of antibiotics in livestock production to enhance animal health and performance due to their ability to improve feed digestion and to protect from microbial-caused intestinal diseases.

The abundance of tannins in nature together with their chemical and biological properties and their ability to form complexes with proteins (mainly) and polysaccharides has led to their widespread use in the leather industry.



Fig. 3. The role of tannins in reducing the unpleasant odour of feet.

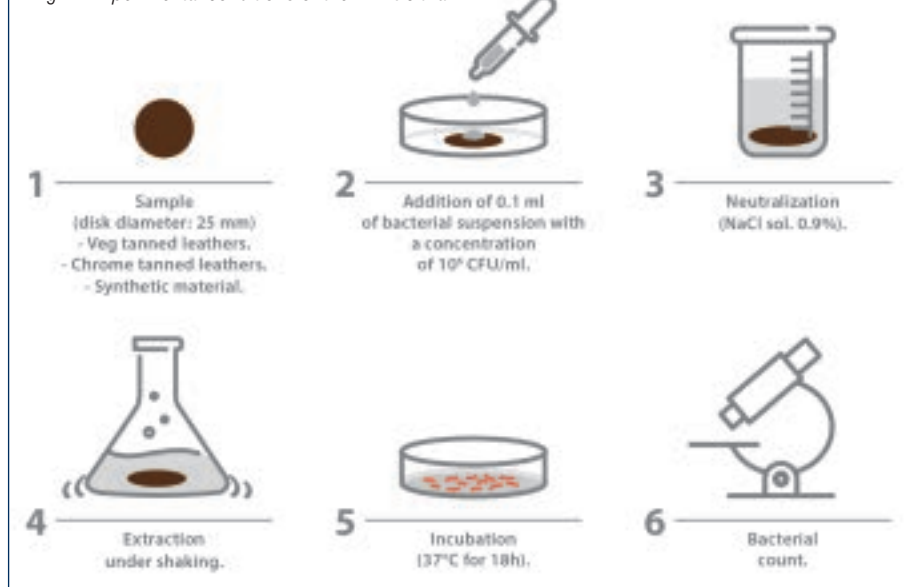


Tannins bind to the collagen proteins inside the hide and coat them, causing them to become less water-soluble and more resistant to bacterial attack.

This process also causes the hide to become more flexible. Only tannins can impart to tanned leather these unique characteristics that make them so special and distinguishable. The “smell of leather” is something typical and unique.

In today's leather making processes, tanners use vegetable tannins in both liquid and powder form. The most famous and ancient extract is obtained from the wood of the chestnut tree and is mainly used for the production of sole leathers, which are compact, firm, flexible and with high yield in weight, as well as the retanning of chrome-free leathers. Another tannin comes from the quebracho wood, a tree that grows in the Chaco region in Argentina. In this case the extract imparts to the leathers an unmistakable reddish shade, a warm touch and a bright appearance, required characteristics to make leather goods. In addition to chestnut and quebracho, Tara tannin is mainly used within the car and upholstery industries. This extract, originating from Peru, gives the leathers excellent properties, such as fullness, softness, good resistance to light and to heat.

Fig. 4. Experimental conditions of the in vitro trial.



Tannins can also be used in the retannage of chrome tanned leathers to improve their “cold, impersonal feel” characteristics, by imparting the typical warm and natural feeling of vegetable tanned leathers.

The use of leather tanned with vegetable tannins may offer a solution to prevent or decrease the development of unpleasant odours caused by the microbial fermentation of body secretions in feet.

1.4 How can tannins make the difference inside a shoe made with vegetable tanned leather?

Unlike what happens inside a shoe made of synthetic material, leathers made with tannins favour the absorption of the excess perspiration produced by the eccrine glands, along with the bacteria on the skin of the foot.

Thanks to tannins, leather insoles and linings become hygroscopic.

Once in contact with tannins within the leather, bacteria are immediately killed and destroyed (Fig. 3). Science has just proven that the right formula of tannins kills 99% of bacteria in a very short time.

The moisture absorbed by the leather made with tannins evaporates every time a person takes off their shoes. So, when they are put back on, the foot encounters a fresh and dry environment, free from unpleasant bacteria that could otherwise contaminate them while respecting and protecting the skin of the feet.

Tannins act as skin flora mediators because they foster the development of “good bacteria” and prevent the foot microbiota from being attacked by the “smelly” bacteria population of the shoe.

2. Assessment of the antibacterial activity of tannins in leathers

Recently, prestigious universities have assessed the antibacterial activity of natural tannins contained in vegetable tanned leather articles, especially shoes, that come in contact with human skin in comparison with chrome tanned leathers and synthetic materials. In particular the research was designed to follow two different paths:

- *In vitro* assessment on the antibacterial activity of different types of tanned leathers and a synthetic material against a selection of gram-negative and gram-positive strains.
- *In vivo* research on the antibacterial activity of vegetable tanned leathers made with tannins within shoes (insole and lining) compared to using synthetic material.

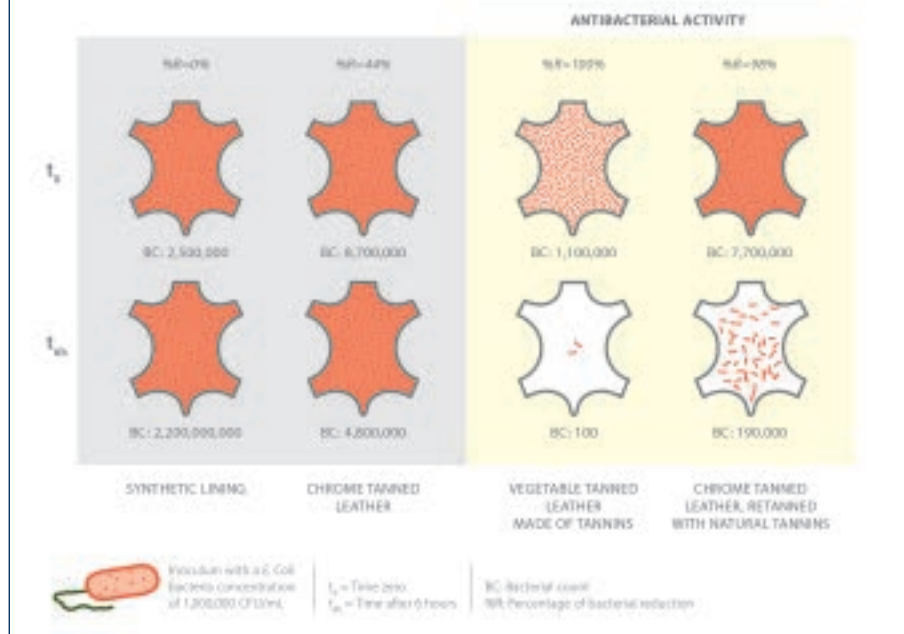
2.1 *In Vitro* research of the University of Milan

The antibacterial activity of vegetable tanned leathers made with natural tannins, such as chestnut, quebracho and tara extracts, was assessed in comparison with chrome tanned leathers and synthetic material.

The study was conducted by the Department of Pharmacological and Biomolecular Sciences of the University of Milan.

2.1.1 Experimental conditions

The trial was performed in vitro by inoculating *Staphylococcus aureus* and *Escherichia coli* strains, representing gram-negative and gram-positive bacteria, respectively. The antibacterial

Fig. 5: Antibacterial activity of the materials under assessment against *E. coli*.

activity was tested at two different time points, time zero and after 6 hours, following contact with the bacterial cells (Fig. 4).

In order to be “antibacterial” a substance needs to be able to kill or inhibit the development of bacteria. The antibacterial power of a substance is the result of a quantitative assessment (AATCC Test Method 100-2012), evaluating the number of bacteria destroyed by the substance under evaluation, calculated in Colony Forming Unit (CFU).

The bacteria were counted using the following formula:

$$\%R = (A-B)/A \times 100 \text{ where } A = \text{CFU/ml at } t_0 \text{ and } B = \text{CFU/ml at } t_{6h}$$

The bigger the amount of killed bacteria, the stronger the antibacterial power of the substance under analysis. The antimicrobial activity was considered efficient when observing $\%R \geq 90\%$.

2.1.2 Results

Vegetable tanned leather samples made of tannins showed an excellent antibacterial activity against *Escherichia coli*, as witnessed by a 4-log reduction in bacterial viability following 6 hours contact. On the contrary, no antibacterial activity was observed for the synthetic lining nor for the chrome tanned leather sample (Fig. 5).

The same chrome tanned leather, if retanned with natural tannins, presented

a good antibacterial activity, with more than 1 log reduction in bacterial viability following 6 hours contact. Similar results have been achieved against *Staphylococcus aureus*.

Therefore, the use of vegetable tanned leather made using tannins may offer a solution to prevent or decrease the development of unpleasant odours caused by the microbial biotransformation of body secretions in feet.

2.2 In Vivo Research of Wellmicro

A molecular and bioinformatic analysis was performed to analyse the microbiota of the samples collected from the inside of a set of shoes worn by a selected panel.

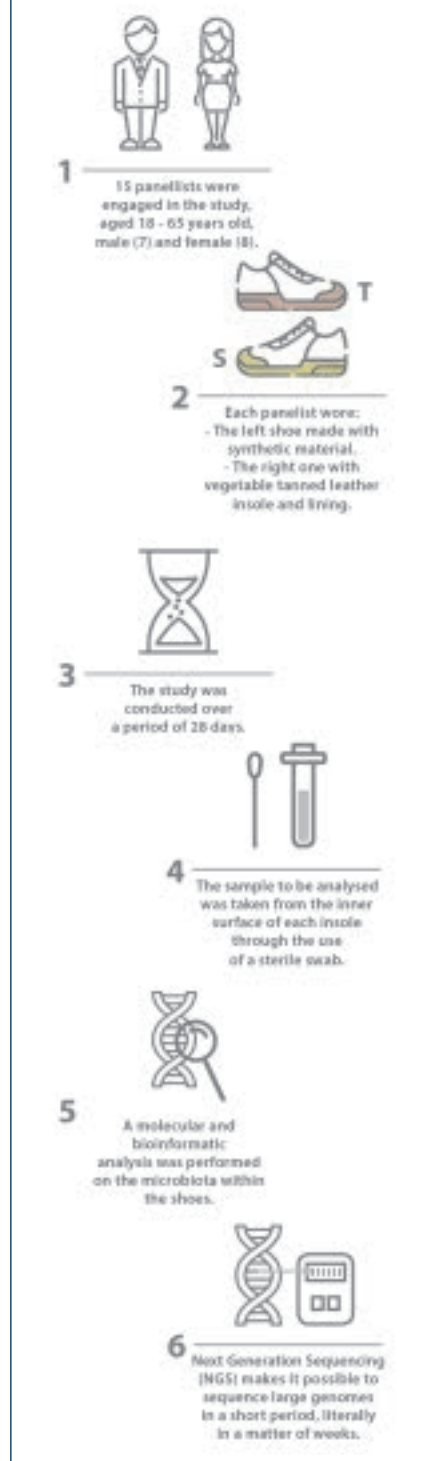
The study was conducted by WellMicro, a spin-off subsidiary of Alma Mater Studiorum, University of Bologna.

2.2.1 Experimental conditions

A group of 15 panellists wore, for 28 consecutive days, the same pair of shoes: one shoe contained a vegetable tanned insole and lining made with tannins while the other one presented a synthetic insole and lining (Fig. 6).

Next Generation Sequencing (NGS) system was used to massively sequence DNA and RNA of bacteria inside the shoes. The parallel sequencing technology has revolutionised the biological sciences and it is less expensive and less time-consuming than a traditional sequencing.

Fig. 6: Experimental conditions of the in vivo trial.



2.2.2 Results

The different types of material used to make the shoes brought about a significant difference in the selection of their bacterial population after the 28 day period (Fig. 7).

The shoe with the synthetic insole and lining fostered the development of the bacteria typical of sweat and responsible for foot odour, such as *Brevibacterium*, *Micrococcus* and



Kytococcus. In fact, the shoe became an incubator of those bacteria that were biodegrading the amino acids present in the eccrine sweat and callus into volatile fatty acids, the cause of foul odour on feet.

The shoe with vegetable tanned leather insoles and linings made with tannins created a dry and fresh environment, reducing the development of those bacteria responsible for foot odour and promoting the development of endogenous bacteria, the good ones for the foot microbiota. The foot is "shielded" from bad bacteria.

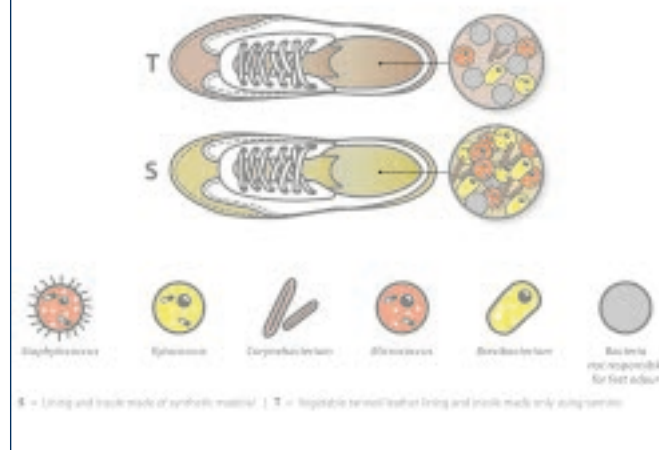
3. Conclusions

The *in vitro* study, from the University of Milan, showed that tanning of leather with chestnut, tara and quebracho tannins leads to materials with excellent antibacterial activity, especially against gram-positive bacteria. All leathers tanned with the vegetable tannins act as non-leaching antimicrobial material, a property that makes these natural substances suitable for contact with human skin. Therefore, the use of leather tanned with vegetable tannins may offer a solution to prevent or decrease the development of unpleasant odours caused by the microbial fermentation of body secretions in feet.

On the other hand, chrome tanned leather has a partial antibacterial property, only against *Staphylococcus aureus*, a representative of gram-positive bacteria.

The same chrome tanned leather, if retanned with natural tannins, reaches complete antibacterial activity against all tested bacteria.

Fig. 7: Bacterial population difference between the shoes made with different materials.



The result of the *in vivo* study, by Wellmicro, reported that the vegetable tanned leather made with tannins performs a selective antibacterial action, balancing the various elements within the bacterial ecosystem of the inner part of a shoe. In fact, it prevents the development of those bacteria responsible for shoe odour, such as *Brevibacterium*, *Micrococcus* and *Kytococcus* while fostering the growth of other bacteria that positively impact the feet skin flora. 🌱

HEUSCH BLADES

SIT BACK AND RELAX

For more information please contact us: Tel + 49 (0)241 . 168 95 - 211 www.heusch.de