STUDY ON THE ANTIBACTERIAL PROPERTIES OF LEATHERS TANNED WITH NATURAL TANNINS AND THEIR INTERACTIONS WITH SHOES INHABITING BACTERIA

E. Poles1a, A. Polissi2b, A. Battaglia1, S. Giovando1, M. Gotti1

1 Silvateam S.p.a., Leather division - Via Torre, 7 - 12080 San Michele ondovì (CN) Italy

Email: epoles@silvateam.com

2 Department of Pharmaceutical and Biomolecular Sciences (DiSFeB),

University of Milan - Via Balzaretti, 9 - 20133 Milan (MI) Italy

alessandra.polissi@unimi.it

Abstract

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 agent has been known for many years among agriculture, animal nutrition, pharma and cosmetics industry. If tannins would perform an antibacterial activity in a vegetable tanned leather, this effect could be very interesting for all the applications in which the leather, being in contact with sweat and bacteria, becomes a solution to reduce more or less severe hyperhidrosis and bromhidrosis. 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. Firstly, the antibacterial activity has been evaluated and compared between leathers tanned with Chestnut, Quebracho and Tara extracts, chrome tanned leathers and synthetic material. The trial was performed *in vitro* by inoculating Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacterial strains. A later step defined the most suitable blend of tannins to obtain, after tanning and/or retanning, an antibacterial natural leather. Furthermore, the vegetable tanned leathers, made with this tannins blend, have been the target of an *in vivo* trial during which 15 panellists, have worn two differently made shoes. The lining and insole inside the right shoe have been made with vegetable tanned leathers with tannins, while the ones inside the left shoe contained only synthetic material. The shoes have been worn for 28 consecutive days, followed by a molecular and bioinformatic analysis of microbiota samples taken from the inner surface of the shoes by using a sterile swab. Lastly, a biochemical analysis of volatile short chain fatty acids has been carried out to investigate the by-products of the bacteria responsible for the unpleasant odour of shoes.

Feet Odour: A Common Problem

Feet odour is a very impacting and popular issue: the results of a 2012 US based survey carried out by 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. High 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.

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

Certain food, 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.

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 foot. The key causative bacteria are *Staphylococcus* spp, *Corynebacterium*, *Brevibacterium*, *Micrococcus*, *Kytococcus* and *Propionibacterium*, mainly found on the plantar area and 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 not able to grow and the number of VFAs produced is very limited. Therefore, the result is a barely noticeable odour, without any offensive cheese-like/acidic notes.

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/her shoes on.

High levels of hydration, as a consequence of the use of occlusive footwear and/or high environmental temperatures, lead to an increase in microbial numbers, particularly Staphylococcus, that cause hyperhidrosis.

Hyperhidrosis is an excessive sweating, 4/5 times more than normal, usually caused by emotional factors, medical conditions, use of particular 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 volatile fatty acids, responsible of the unpleasant odour.

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

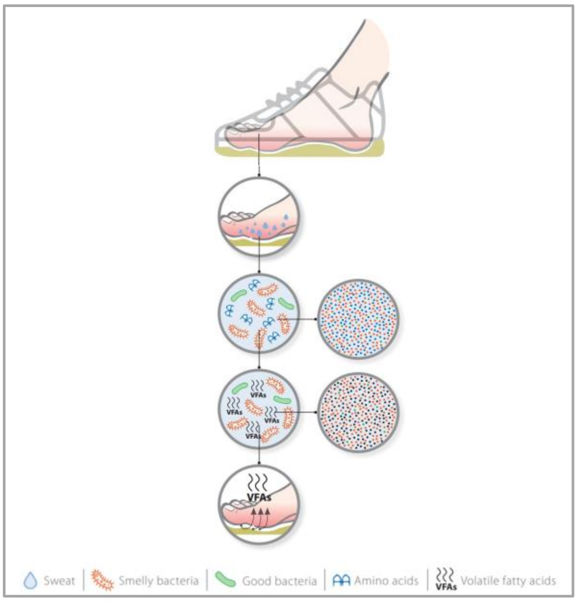
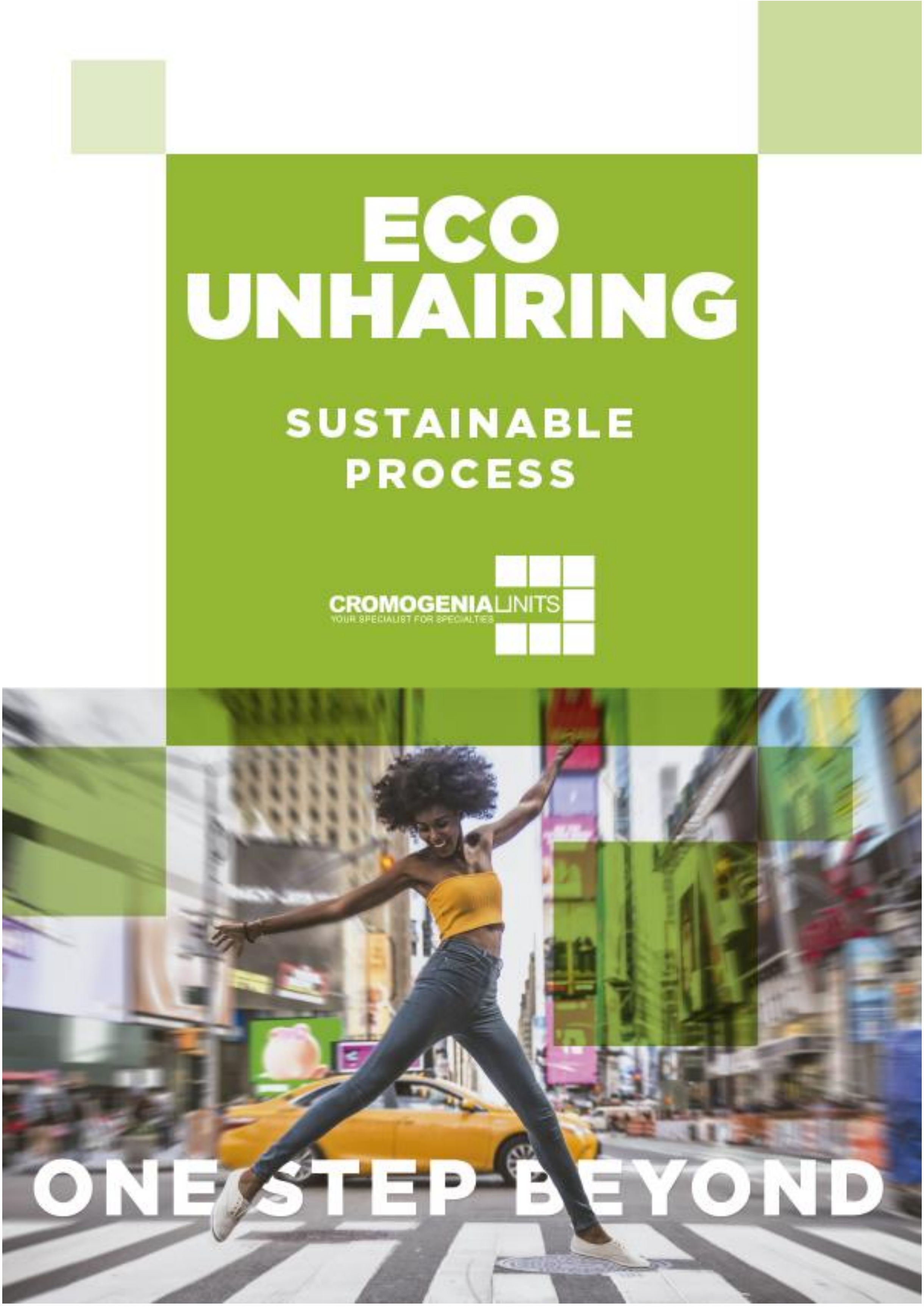


***Fig. 1**. Production of unpleasant odours in feet wearing shoes with inside made of synthetic material.*

The internal part of the shoe (insole and lining) made of synthetic material is not able 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. 1).

Usually worn shoes host up to 10 million of those “smelly” bacteria responsible for odour per cm2, especially in toe clefts. Every time a person puts shoes on, the clean feet will be contaminated by the bacterial population


ECO
UNHAIRING
SUSTAINABLE
PROCESS
CROMOGENIAUNITS
YOUR SPECIALIST FOR SPECIALTIES
ONE STEP BEYOND

growing inside the shoes. So it is not important how many times somebody washes his/her feet. Washing one's feet every day is necessary but it is not a sufficient condition to defeat feet odour. Day after day, the bacterial population inhabiting the shoe expands, more VFAs are produced and a strong cheesy/acidic odour sticks 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 fairly limited, not permanent and very expensive. In some cases, they are harmful since they irritate the skin or alter the body thermoregulation mechanism.

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 and 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, basis or certain enzymes), hydrolysable tannins can be classified in 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 tannin that has been shown to be predominantly composed of oligomers of 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 raised 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 disease.
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.
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 are able to 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 chestnut wood and is mainly used for the production of sole leathers, with high yield in weight, which are compact, firm and flexible, and 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 provides to the leathers excellent properties, such as fullness, softness, good resistance to light and to heat.

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.
Today a retreat towards more sustainable lifestyle and a slowdown to the use of chemicals, favoured by various deep changes in research, make tannins an important natural component in the tanning industry.

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.

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 of tannins favour the absorption of the excess perspiration produced by the eccrine glands, along with the bacteria inhabiting the foot skin. Thanks to tannins, leather insole and lining become hydroscopic. Once in contact with tannins within the leather, bacteria are immediately killed and destroyed (Fig. 2). 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 his/her shoes off. So when he/she puts them 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.

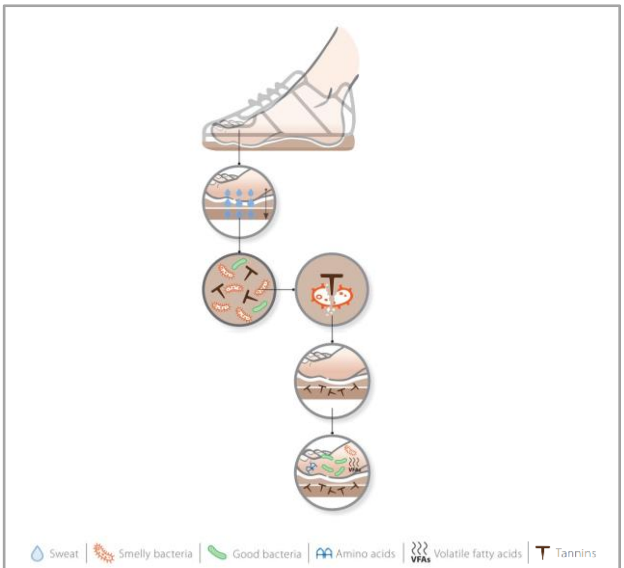


Fig. 2. *The role of tannins in reducing the unpleasant odour of feet.*

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 diverted in 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.

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.

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 activity was tested at two different time points, time zero and after 6 hours, following contact with the bacterial cells (Fig. 3).

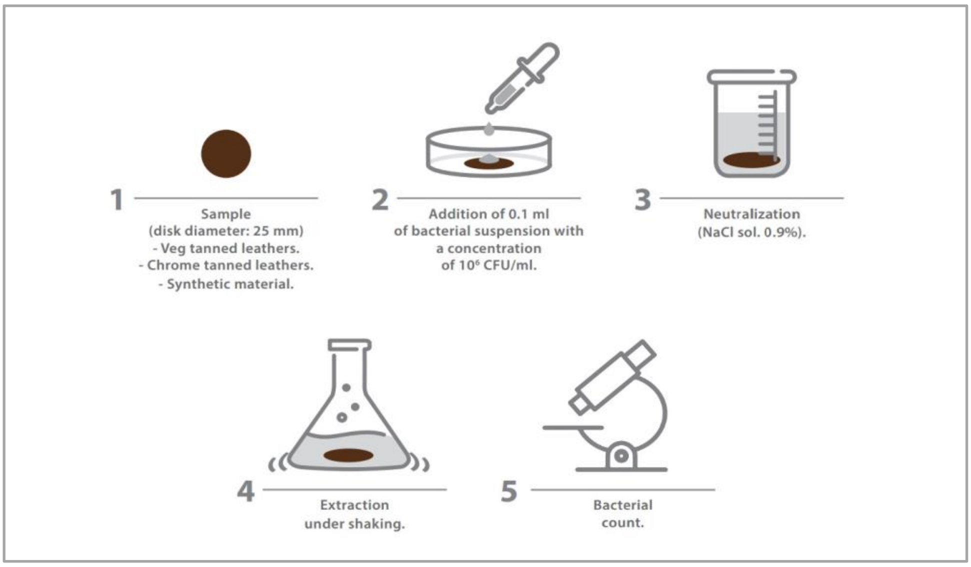


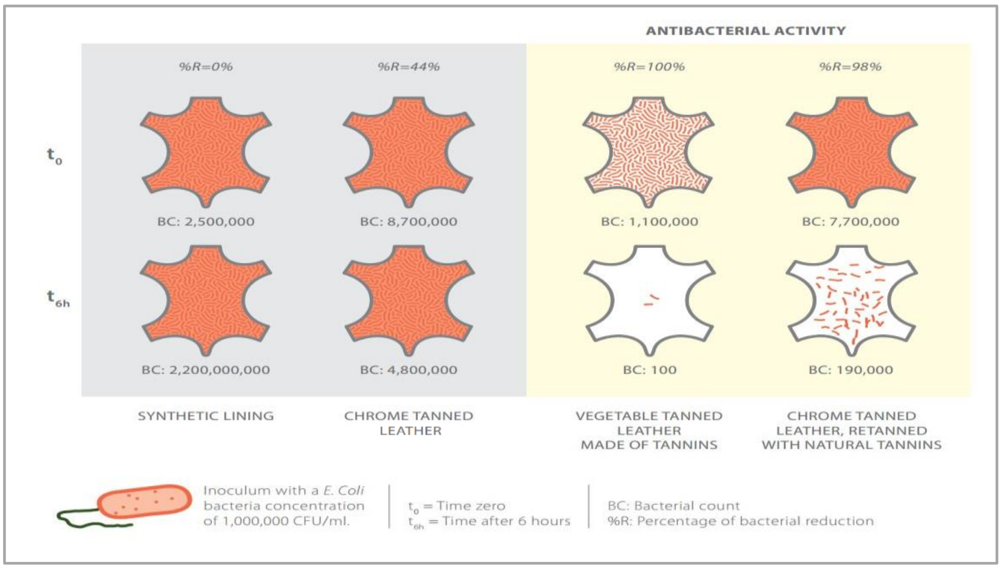
***Fig. 3.** Experimental conditions of the in vitro trial.*

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 get counted using the following formula:
%R = (A-B)/Ax100 where A = CFU/ml at t0 and B = CFU/ml at t6h
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 ≥ 90%.

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 opposite, no antibacterial activity was observed for the synthetic lining nor for the chrome tanned leather sample (Fig. 4).
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 of tannins may offer a solution to prevent or decrease the development of unpleasant odours caused by the microbial biotransformation of body secretions in feet.

***Fig.4.** Antibacterial activity of the materials under assessment against E. coli*



In Vivo Research of the 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, spin-off subsidiary of Alma Mater Studiorum - University of Bologna.

Experimental conditions

A group of 15 panellists worn for 28 consecutive days the same pair of shoes: one shoe showed a vegetable tanned insole and lining made of tannins; while the other one presented a synthetic insole and lining (Fig. 5).

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.

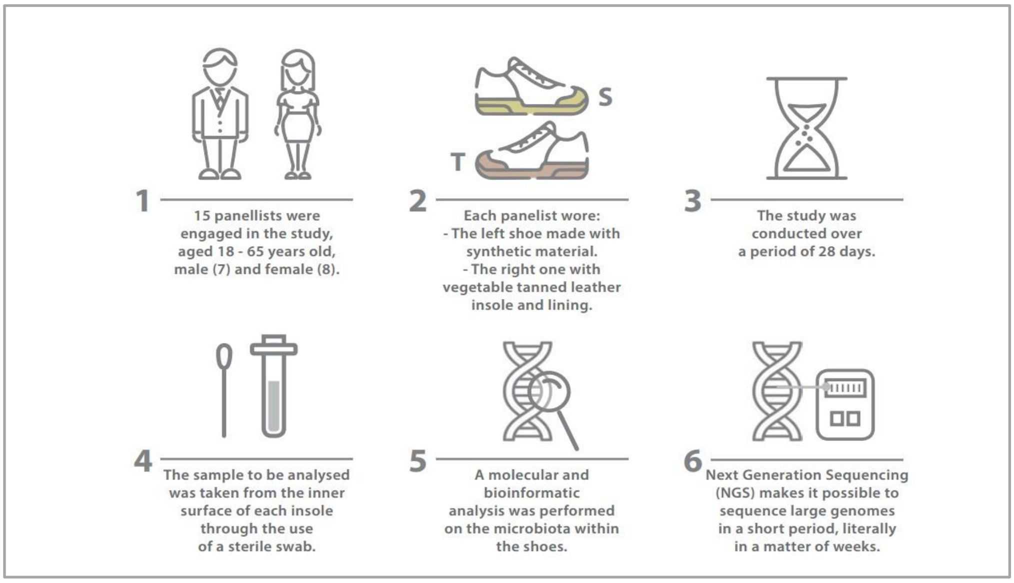


Fig. 5. *Experimental conditions of the in vivo trial.*

Results

The different type of material used to make the shoes brought to a significant difference in the selection of their bacterial population after the 28 days period (Fig. 6).

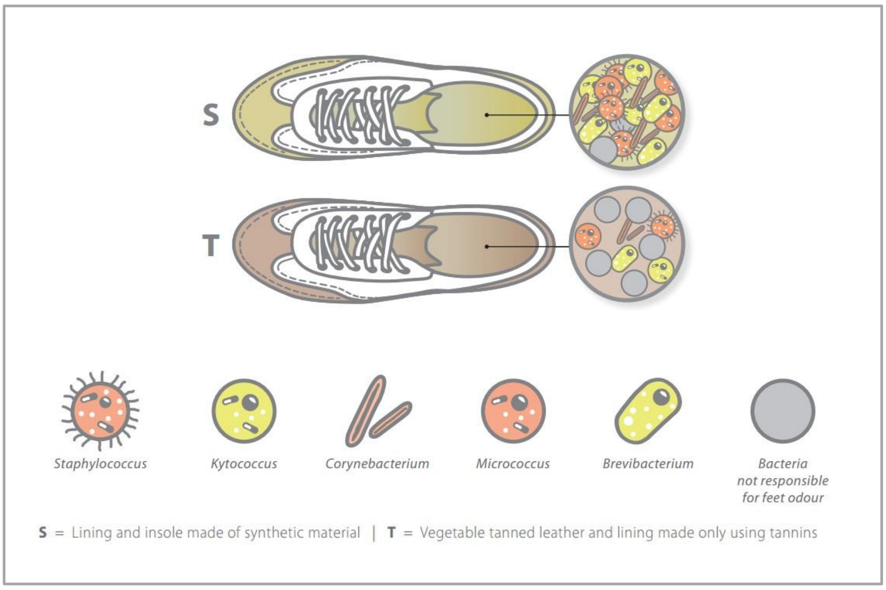


Fig.6. *Bacterial population difference between the shoes made with different materials.*

The shoe with synthetic insole and lining fostered the development of the bacteria typical of sweat and responsible for feet odour, such as Brevibacterium, Micrococcus and Kytococcus. In fact, the shoe became an incubator of those bacteria biodegrading the amino acids present in the eccrine sweat and callus into volatile fatty acids, the cause of cheesy / acidic notes of foot.

The shoe with vegetable tanned leather insole and lining 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 “safe” from bad bacteria.

Conclusion

The in vitro study, conducted by the University of Milan, shows that tanning of leather with chestnut, tara and quebracho tannins leads to materials with excellent antibacterial activity, especially against Gram-positives bacteria. All leathers tanned with the vegetable tannins act as non-leaching antimicrobial material, a property that makes these natural substances suitable for the 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 S. aureus a representative of Gram-positive bacteria.

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

The result of the in vivo study, conducted by Wellmicro, reports that the vegetable tanned leather made with tannins performs a selective antibacterial action, balancing the various element 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.

References

1. Ara K., Hama M., Akiba S., Kenzo K.: 'Foot odordue tomicrobial metabolism and its control', Can J Microbiol, 52(4):357-64, 2006.
2. Bojar R. A., Holland K. T.: 'Review: the human cutaneous microflora and factors controlling colonisation', World J Microb Biot, 18:889-903, 2002.
3. Caroprese A., Gabbanini S., Beltramini C., Lucchi E., Valgimigli L.: 'HS-SPME-GC-MS analysis of body odor to test the efficacy of foot deodorant formulations', Skin Res Technol, 15(4):503-10, 2009.
4. Costello E. K., Lauber C. L., Hamady M.: 'Bacterial community variation in human body habitats across space and time', Science, 326(5960):1694-7, 2009.
5. Findley K., Oh J., Yang J.: 'Topographic diversity of fungal and bacterial communities in human skin', Nature, 498(7454):367-70, 2013.
6. Fogarty A. L., Barlett R., Ventenat V.: 'Regional foot sweat rates during a 65 minute uphill walk with a backpack', In: Mekjavic IB, Kounalakis SN, et al. (eds). Environmental Ergonomics XII, Ljubljana: Taylor NAS, 266-9, 2007.
7. Frutos P., Hervás G., Giráldez F. J., Mantecón A. R.: 'Review. Tannins and ruminant nutrition', Spanish J of Agricultural Research 2, 191-202, (2004).
8. Gotti M., Castagnetti A., Poles E., Battaglia A.: 'Caratterizzazione molecolare e analisi bioinformatica de lmicrobiota rilevato da campioni prelevati dalla superficie interna della scarpa e caratterizzazione biochimica diacidi grassi a corta catena', Wellmicro, Università degli Studi di Bologna, 2018.
9. Grice E. A., Segre J. A.: ' The skin microbiome', Nat Rev Microbiol, 9(4):244-53, 2011.
10. Grice E. A., Kong H. H., Conlan S.: 'Topographical and temporaldiversity of the human skin microbiome', Science, 324:1190-2, 2009.
11. Hagerman A. E., Butler L. G.: 'The specificity of proanthocyanidin-protein interactions', J Biol Chem 256, 4494-4497, 1981.
12. Harker M., Harding C. R.: 'Amino acid composition, including key derivatives of eccrine sweat: potential biomarkers of certain atopic skin conditions', Int J Cosmetic Sci, 35:163-8, 2012.