TANNING BIOTECHNOLOGIES FOR NOVEL SUSTAINABLE AND CIRCULAR MATERIALS

Florio C.[1], Gargano M.[2], Lettera V.[3], Pagliuca I., Abbro M., Sannia G.[2]

[1] SSIP - Stazione Sperimentale per l'Industria delle Pelli e delle materie concianti - Italian Leather Research Institute
Via Campi Flegrei 34 Pozzuoli, Italy
[2] University of Naples Federico II - Department of Chemical Sciences - Complesso Universitario di Monte Sant'Angelo
Via Cintia, 21 - 80126 - Napoli Italy
[3] Biopox srl - Viale Maria Bakunin 12, 80125 Napoli
Corresponding author: c.florio@ssip.it

ABSTRACT
The Circular Economy principles are well integrated with leather production processes and with the current direction that tanning chain is following, that is becoming even more strategic as a model of development for sustainable growth; today the tanning sector has an impacting role as part of the Bioeconomy scenario, indeed.
On the other hand, the production of solid tanning waste is a critical issue to be addressed as a new strategic challenge, aimed at fostering the transformation of the international economy in a greener, more resilient and circular system. These wastes can represent a great opportunity for the development of new products and applications in different fields, however their valorisation can be affected by some critical issues, mainly connected to the presence of chemical components deriving from the production process (metal iones and/or organic tanning agents, biocides, synthetic polymers, etc.), that currently limits the possible use of wastes in some applications.
This work aims, on the one hand, to minimize the use of traditional chemicals in the production process and, on the other hand, to use sustainable biotechnological approaches for the use of wastes in the production of new generations of bio-based circular materials.
Two main biotechnological approaches were particularly considered; one which involves the selection and use of enzymatic systems for obtaining collagen hydrolysates with high added value, for its use both in the tanning process, as filler and as finishing agent, than in the production of novel bioinks in 3D printing technologies. In this approach, ad hoc enzymes were selected for the treatment of trimming and shaving of wet-white wastes, derived from different tanning systems; another approach involved the use of symbiotic cultures of bacteria and yeasts for the production of a cellulose film and its use in the creation of bio-based finishing layers.
Both approaches provide important tools to produce new generation of circular and sustainable materials.

Keywords: circular materials; circular finishing; sustainable finishing; biotechnological approaches; leather waste valorisation; bio-based fillers; bio-based polymers.

1. Introduction

Leather is one of the leading natural-based materials among the fashion and luxury supply chain; its natural and ancient origin, its high performances and qualities, guarantee a unique international leadership to the tanning industry. Leather is also a circular material, deriving from the transformation of a food industry by-product, raw leather; the purpose of the tanning process, consisting in a series of chemical and mechanical treatments, is to make the skin non-perishable, stable, resistant over time, in order to guarantee a durable and high value-added finished product, where, in recent years, many efforts have been made to improve the sustainability content of leathers, mainly through the experimentation of novel green solutions for tanning.

In addition, the leather supply chain is a crucial sector of the bioeconomy, being the most driving part of the textile and fashion industry in this area. The bioeconomy is a particularly strategic and currently remarkable sector and can be defined as the socio-economic system associated with the enhancement of renewable biological resources, for their use in the production of other goods, materials and products. Bioeconomy is closely connected to the concept of circular economy, especially with reference to the extensive use of agricultural waste and by-products of the food industry as an input for the creation of products with greater added value; bioeconomy is also intrinsically circular where carbon is sequestered from the atmosphere by plants, reused in natural transformations and recycled as soil carbon (Gowdy J. M.,et al., 1998; Leipold S.,et al., 2018).

In order to improve the sustainability and circularity content of leather products, it is necessary to foster the exploration of progressively more advanced and innovative scientific and technological approaches. Particularly interesting and promising in this sense are the approaches based on enabling technologies and particularly on Biotechnologies.

According to the definition deriving from the Convention on Biological Diversity (UNITED NATIONS 1992), "Biotechnology" means any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use. The term in the plural form (Biotechnologies) is currently more commonly used to indicate the plurality of technologies developed and the related fields of application.

Specifically, the activities of this work concern the use of innovative and sustainable approaches for the study, characterization and transformation of biological systems of tanning interest, including the engineering and innovative use of enzymes and microbiological systems for the enhancement of leather and related waste, as well as for the production of biobased materials.

The former part of the work involved the use of biotechnological approaches consisting in the selection and use of enzymes and the development of highly effective approaches for obtaining collagen hydrolysates with high added value from tanned waste (shaving and trimming of bovine hides produced with different tanning systems). From a chemical point of view, lether waste is essentially made up of protein material of which collagen represents about 90-95%. In the methods traditionally used to obtain hydrolysates, based on the use of acidic or alkaline hydrolytic agents, the products obtained are normally made up of small, deconstructed polypeptides of about 10-20 kDa, where these characteristics limit their use in numerous fields.

In this context, the former part of the present project has been aimed at enhancing solid wastes from tanning processes, through their conversion into high added value products and, particularly by developing new environmental viable methods for the extraction and reuse of collagen.

The use of enzymes is not new in the tanning sector, but for many years it has been limited to their use in the beamhouse phases (Covington A., et al., 2020); in recent times, their application has been tested, in addition to or in place of chemical approaches for the treatment of waste and for obtaining protein hydrolysates. These hydrolysates, especially if deriving from not yet tanned waste, are used for the production of fertilizers and biostimulants, as well as, in the presence of given requirements, for the production of food jellies. The treatment of tanned waste is more complex, whereas the variety of currently existing tanning systems makes it difficult to identify effective univocal approaches. The goal of this former part of the work is therefore the development of highly sustainable approaches based on enzymatic and combined treatments (enzymatic / chemical / thermal) for the transformation of these complex substrates. The hydrolysates thus obtained, with desired molecular weight ranges, will then be used, without any modification or cross-linked with casein for different applications, mainly concerning their use in the retanning and finishing phases.

Concerning the finishing phase, given the need to diversify the features of the articles, particularly leather products for the fashion sector, in order to have a wide range of product customization options, biotechnological solutions have also been explored for the creation of bio-based films of a different chemical nature than collagen and its derivatives. More in detail, the production of cellulose films from symbiotic microbiological systems, capable of metabolizing sugars, was tested. This possibility can lead to the development of methods to produce finishing films, through the biotechnological transformation of natural sources of sugars, such as deriving from agri-food waste; in other terms, this approach, in addition to producing a wider range of products for bio-based finishing, would allow to foster virtuous mechanisms of Industrial Symbiosis.

2. Material and Methods

Leather and waste samples

Vegetable tanned bovine shavings, organic tanned bovine shavings, mineral and chromium tanned bovine shavings were collected from tanneries from Veneto District for enzymatic treatments.

Sheep and goat leathers in crust for footwear and leather goods, were selected from Campania District, for finishing applications.

Chemicals and biological reagents

Enzymatic treatments of leather waste: Enzymes were purchased from Sigma-Aldrich (Saint Louis, Missouri, USA). NaOH has been used for chemical pre-treatment.

Cellulose film bio-synthesis: Microorganisms symbiotic culture: *Acetobacter xylinum, Saccharomyces cerevisiae* / Culture media of the inoculum: solid plate in 40% distilled water solution, 40% C. sinensis infusion (for 20 min at 80 ° C) and 20% D-glucose; Culture media for biofilm growth: 20% w / v of sucrose, 20% v / v production surplus of vinegar, 40% v / v extract of C. sinensis in infusion.

Main Equipment

MB Line stirred incubators; Rod stirrer 2,200 rpm / 40 l; Nüve Ot 40L; Binder stove model ED SolidLine Main diagnostic techniques

- *Spectrum One ATR-IR (Attenuated Total Reflection Infrared)* Spectrometer (Frequency range: 4000- 600 cm^{-1}).
- *Wild Heerbrugg* Stereoscope equipped with *OPTIKA* Room B3 Digital Camera.
- *Zeiss - Phoni 3* - Optcal Microscope (0.01 - 1 mm - Resolving power: 0,001 mm).
- *Zeiss EVO MA10* Scanning Electron Microscope (Magnification: 10x - 200,000x Resolving power: 2µm) equipped with *INCA X-act ENERGY-250XT* detector.
- Gas-chromatograph *TRACE 1300-THERMO SCIENTIFIC* coupled to a *TSQ 9000-THERMO SCIENTIFIC* mass spectrometer detector and equipped with *ATOM XYZ-TELEDYNE TEKMAR* autosampler for analysis of volatiles on solid and liquid samples.
- *STDQ600 TA* Instrument for DSC/TGA (Differential Scanning Calorimetry/Thermogravimetric Analysis equipped with a *THERMOSTARTM* Mass Spectrometer.
- *FIBRO System AB - Mod. PGX pocket goniometer.*
- *Akta Pure* - Size Exclusion Chromatography (SEC) - *Superose 6 HR 10/300 column/ Gel Filtration Calibration Kit HMW/ Global Life Sciences Solutions, USA LLC*

3. Results and Discussion

3.1 Enzymatic treatment and enhancement of leather waste

Enzymatic approaches for the extraction of collagen have been developed allowing, when compared to the traditional extraction methods, to: *i*) increase the amount of extracted collagen up to 10% (w/w); *ii*) reduce the reaction time (at least two times); *iii*) reduce use of chemical agents up to 10 times; and *iv*) recover a high quality product, thus expanding its range of applicability. The developed enzymatic extraction methods resulted effective on wastes from different types of tanning, regardless of the nature of the tanning agents used.

According to literature, (Godwin Jenifa et al., 2020; Covington A., 2020; Taylor et al., 1996), the most efficient enzymes for collagen extraction from leather solid wastes are alkaline proteases and trypsin. In this work several hydrolytic enzymes were tested, combined with chemical and/or physical pre- treatments.

The recovered collagen has been characterized in terms of protein identification, content of substances of eco-toxicological interest, such as polyphenols and metals, and molecular weights. The diagnostic investigations on the obtained hydrolysate samples confirmed the collagenic nature of the matrix, highlighting the presence of traces of metals and polyphenols, depending on the type of tanning considered. The in-depth analysis using ATR-IR spectroscopy, on the other hand, highlighted a high degree of integrity of the collagen structure: Figure 1, in this sense, highlights how the spectroscopic profiles of collagen extracted by the enzymatic process are

comparable to those of the native collagen, suggesting that the extraction process does not affect the structure of collagen, unlike as found downstream of traditional hydrolysis processes (based on the exclusive use of acid or alkaline agents).

The extracted collagen was further characterized by molecular weights and scanning electron microscopy (SEM) analyses, confirming that it preserves an intact structure (Fig.2) and that its quality is such that no subsequent purification step is needed; furthermore, the extracted collagen displays the typical features of native collagen, such as gelling properties. Concerning molecular weights, Enzymatic method allowed to rise from molecular weights up to 6 times.

The use of these hydrolysates, without modifications, or cross-linked with casein has been experimented on a laboratorial scale for retanning and finishing, where collagen cross-linking with casein was optimized through enzymatic mediation, and where further characterizations of the performance of the materials obtained is under investigation.

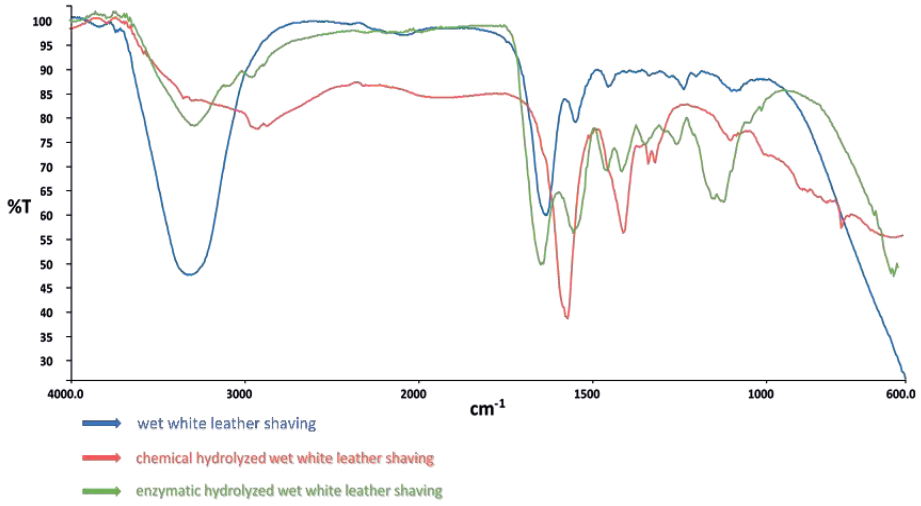


Fig.1: ATR-IR spectra overlap of differently treated and not treated samples

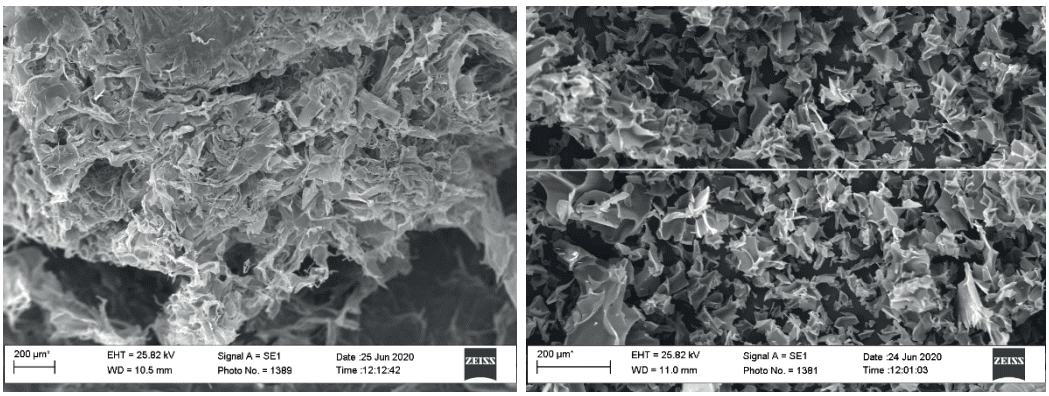


Fig.2: SEM images of native (a) and extracted (b) collagen

3.2 Cellulose film bio-synthesis and application for novel circular finishing

The biofilm, produced by the symbiotic systems described in the materials and methods section, was developed and floated in culture tanks; it was then raised and subjected to 3 successive washes in distilled water inside a beaker under magnetic stirring (each cycle 200 rpm x 10 min). After 20 minutes of partial drying in the oven at

40 ° C, the material was mechanically placed on the surface of the leather; then, subjected to compression by means of a weight of 5 kg for 72 h and conditioned at room temperature for a further 72 h. During the application phase, the film had a pH of 3.5. The reactivity and consequent ability to adhere to leather were correlated, on a chemical level, to the abundance of OH groups, as evidenced by the ATR-IR examination of the film (Figure 3), which also confirmed the cellulosic nature of the material; on a physical, chemical-physical level, it has been hypothesized that the removal of residual moisture from the film on the tendentially hygroscopic surface of the collagen fibers that make up the leather, in addition to the applied pressure, contributed to a successful application.

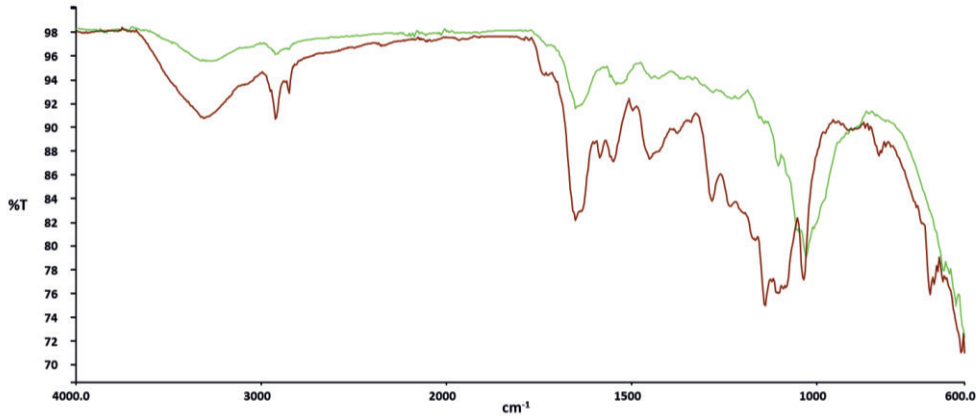


Fig.3: ATR-IR spectra overlap of treated (red) and not treated (green) samples

The film was then characterized by means of optical and electronic SEM microscopy with an X-ray probe, in order to:

- measure the finishing thickness;
- verify the product characteristics;
- verify the absence of metallic contaminants by means of microanalysis.

This investigation highlighted the material's ability to form a thin film, of the order of 20-25 µm, capable of carrying out a protective action, while leaving the original grain structure visible (Figure 4).

In order to verify the protective capacity of the material, against environmental agents, the water penetration time was determined, in accordance with the UNI EN ISO 5403-1: 2012 standard, which was found to be> 30 min, highlighting that the characteristics of water resistance meet the requirements for leathers for footwear and leather goods; the absorption dynamics of water micro-drops (4 µl) were also studied in detail, using the Pocket Goniometer, with the determination of the contact angle in dynamic mode, within 0 and 10 seconds from the impact of the water drops, for the evaluation of the characteristics of surface permeability and hydrophilicity. This test showed that the finishing biofilm, while exhibiting a certain surface hydrophilicity, as can be seen from a CA (Contact Angle) <90°, provides to leather surface a significant resistance to water permeability (Figure 5).

In order of verifying the solidity characteristics of the material, when used as a new finishing product, its resistance to dry and wet rubbing was also assessed, according to UNI EN ISO 5403-1: 2012; this test has shown that the new material satisfies the dry requirements for footwear and leather goods, while surface abrasions were found after wet rubbing, suggesting the necessity to plan improvements in this sense. In this direction, in-depth studies are underway concerning the use of basecoat from natural non- fossil sources, such as terpenes-based ones deriving from the agri-food sector waste, in order to improve the adhesiveness, as well as the use of natural oils, to be applied as plasticizers, to improve the mechanical properties of the film. The conjugated use of the novel cellulose films and collagen transformation products obtained from activity 3.1 is also under investigation.

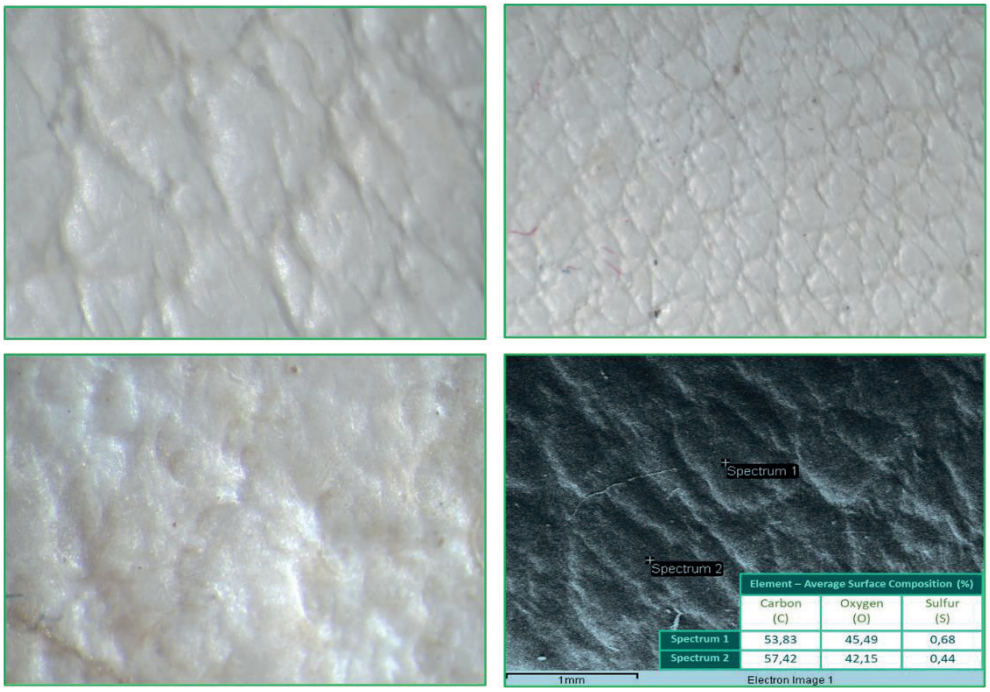


Fig.4: Optical and electronic SEM microscopy analysis of treated samples

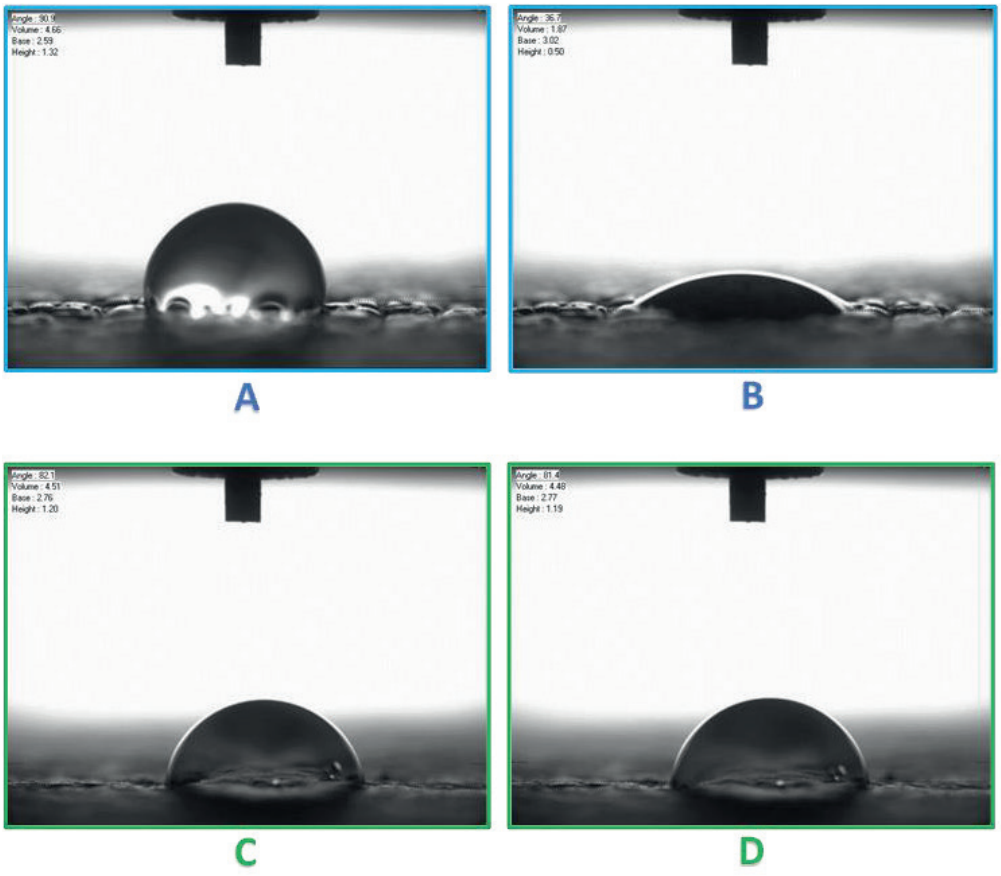


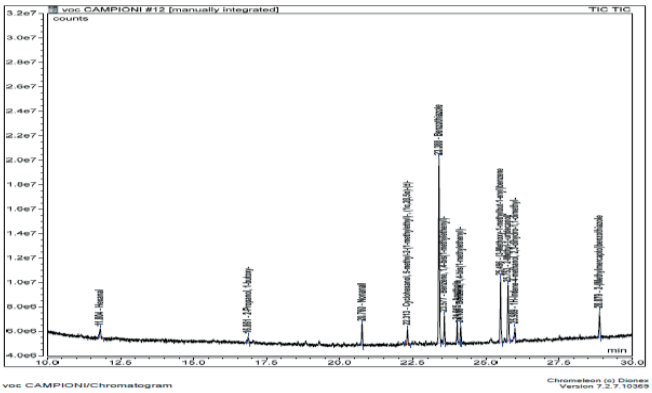
Fig.5: CA value at t0 (impact time) and at t10 (after 10 seconds) of a no treated sample surface (A-B) CA value at t0 (impact time) and at t10 (after 10 seconds) of a treated sample surface (C-D)

The sustainability content of the novel formulation was also highlighted compared to traditional finishing products; the latter, in fact, are essentially made up of polymers in aqueous dispersion, where the use of solvents, diluents, dispersing agents is often required in the formulations, which can sometimes have eco-toxic characteristics.

On the other hand, it can be seen, from the comparative analysis of the VOCs (Volatile Organic Compounds) of a sample treated with the bio-film and of an untreated sample, how the biofilm does not produce further volatile emissions, but how, on the contrary, it manages to partially contain the emissions of volatile substances deriving from the leather (Figure 6).

A

Area %	time (min)	Substance	Cas
34.045,592	11.80	Hexanal	66-25-1
20.412,002	16.86	2-Propanol, 1-butoxy-	5131-66-8
58.247,991	20.76	Nonanal	124-19-6
33.063,538	22.31	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1	15356-70-4
515.504,644	23.39	Benzothiazole	95-16-9
66.946,009	23.58	Benzene, 1,4-bis(1-methylethenyl)-	1605-18-1
43.700,529	24.02	Anethole	4180-23-8
38.026,528	24.14	Benzene, 1,4-bis(1-methylethenyl)-	1605-18-1
154.762,890	25.50	(3-Methoxy-1-methylbut-1-enyl)benzene	0
167.540,964	25.75	2-Methyl-1-undecanol	10522-26-6
23.880,736	25.99	1H-Indene-4-methanol, 2,3-dihydro-1,1-dimet	55591-09-8
73.836,817	28.88	2-(Methylmercapto)benzothiazole	615-22-5



B

Area %	time (min)	Substance	Cas
76.586,716	11.81	Hexanal	66-25-1
99.684,604	16.86	2-Propanol, 1-butoxy-	5131-66-8
64.830,259	20.76	Nonanal	124-19-6
141.571,770	22.31	Cyclohexanol, 5-methyl-2-(1-methylethyl)-	15356-70-4
188.938,465	23.39	Benzothiazole	95-16-9
131.087,786	24.02	Anethole	4180-23-8
121.929,707	25.50	1H-Indene-4-methanol, 2,3-dihydro-1,1-dimethyl-	55591-09-8

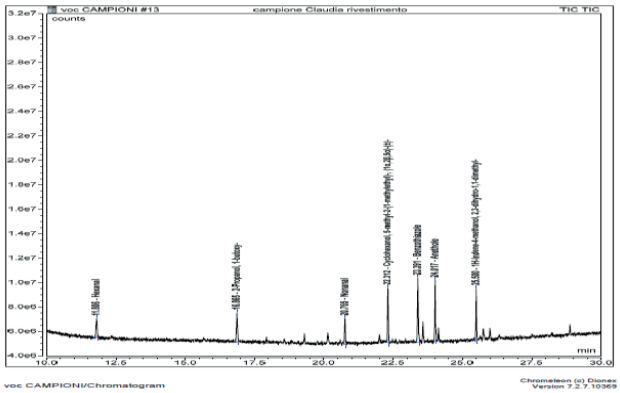


Fig.6: Comparative VOC analysis of a not treated (A) and of a treated (B) sample

4. Conclusion

The enzymatic approaches used for the treatment of tanning waste have been refined for the main categories of shavings treated; categories of enzymes were selected and sequences of enzymatic processes with chemical, chemical and physical pretreatment were identified, depending on the categories treated (organic / vegetable tanning shavings and mineral / chromium tanning shavings); in any case, the processes developed were found to be highly sustainable, allowing the use of traditional chemicals to be minimized up to about 10 times; the treatments were found to be effective in obtaining collagen hydrolysates with technical and structural characteristics to allow their subsequent use in the retanning and finishing phases.

The letter biotechnological approach employed allowed to obtain cellulose films from symbiotic microbiological systems, capable of metabolizing sugars; the obtained films were applied to sheep and goat skins, in order to produce a wider range of products for bio-based finishing; the catheterization of the materials produced made it possible to identify good surface adhesion, protection and impermeability capabilities, and high added value in terms of sustainability of the novel films obtained.

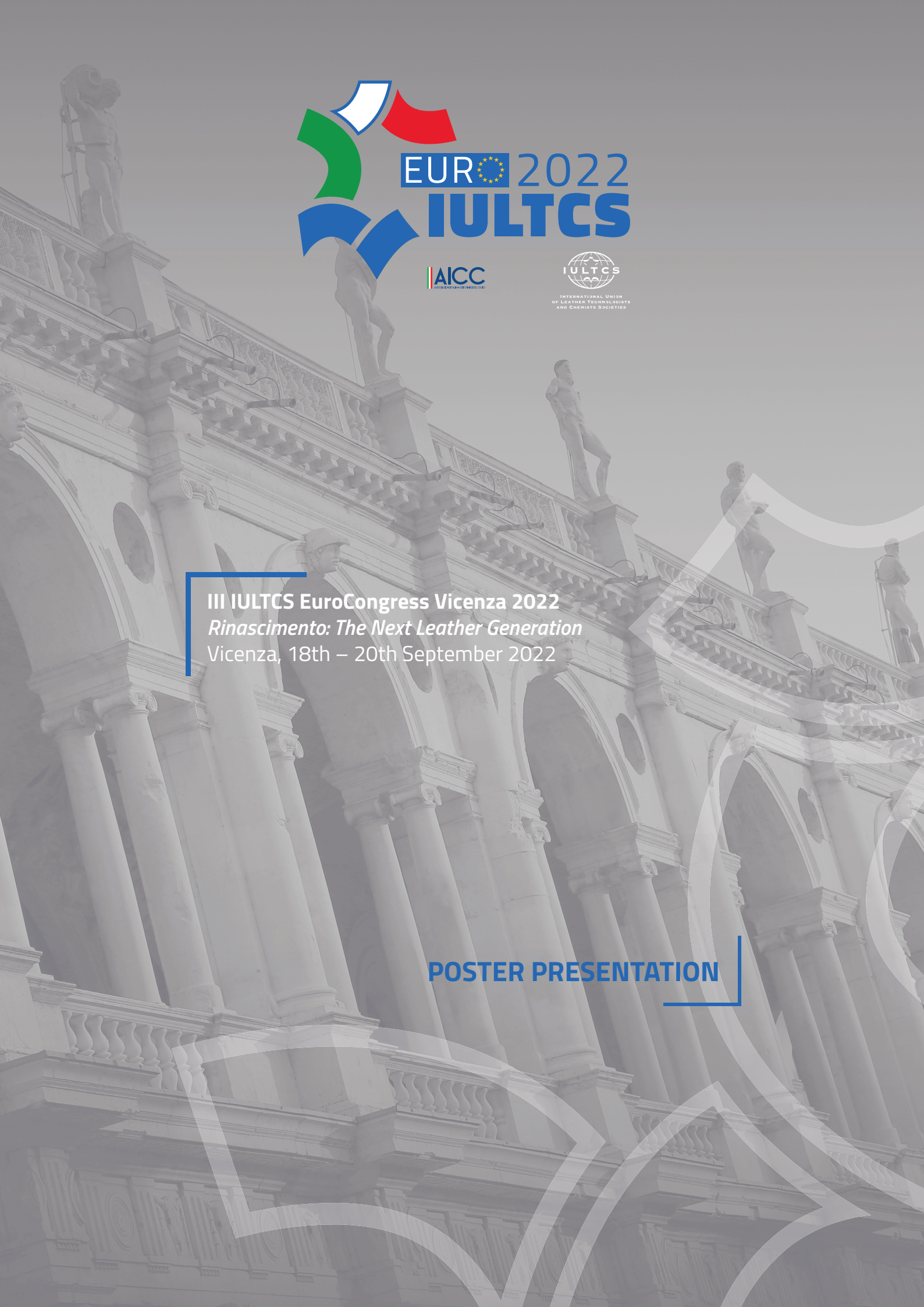
The joint use in finishing of the materials produced by the two approaches will allow to improve the overall quality features of the leather, for the development of novel generations of circular materials with high performance and sustainability.

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Note





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