Università degli studi di Napoli Federico II

Dipartimento di Ingegneria Chimica, dei Materiali e della produzione industriale

Dottorato di ricerca in Ingegneria dei Materiali e delle Strutture

XXVII Ciclo

Manipulation of dermis based collagen networks with engineered mechanical behaviour for applications in the tanning sector

Borsa di studio finanziata da P.O. CAMPANIA FSE 2007/2013 D.G.R.

Coordinatore
Prof. Giuseppe Mensitieri

Candidata
Ing. Maria Assunta Autiello

Tutor
Prof. Paolo Antonio Netti

Co-tutor
Prof. Maurizio Ventre

Tutor aziendale
Dr.Diodato De Maio

A mio padre

Contents

Abstract

In this thesis, technologies and processes of the biomaterial field, were implemented in the tanning sector, in order to obtain leather prototypes having engineered mechanical properties such as tear resistance and elastic recovery. The work is basically divided in two parts. First, the characterization of conventional leather material is reported and it is aimed at defining the benchmarks to which the chemical physical parameters of the engineered products need to be compared. The obtained results provided information on the structure-composition-functions relationship of animal skin and most importantly they provided clear hints on how to modify the substrate and in what step of the tanning process this is most convenient.

Second, the identification of materials and the chemical–physical strategies is disclosed. Materials and processes were studied in order to most effectively affect the macroscopic properties of the final product. Then, the chemical-physical characterization of the obtained engineered samples is reported. Finally, tanning process was performed in order to realize engineered prototypes.

Two manipulation strategies were implemented: crosslink and reinforcement. Crosslink involved the use of high temperature while reinforcement was carried out selecting polymeric materials to be delivered within dermis. In particular, polydimethylsiloxane and nylon were selected in order to increase tear resistance while elastomeric materials were selected in order to improve elastic recovery. Good prototypes were obtained using PDMS and Elastollan as elastomeric polymer. In particular increase of tear resistance were higher of 30% and improvement in the elastic recovery was found obtaining a decrease of 20% for the permanent strain values.

Preface

The present work, performed in collaboration with the DMD company of Solofra, is mainly aimed at exploring and developing, in the experimental stage, of technologies and processes of the biomaterial sector, which can be implemented in the tanning industries, in order to obtain leather prototypes having macroscopic mechanical properties engineered “ab initio”.

Indeed, the experimental part has been focused on the processing of raw materials to produce finished articles in leather with improved performance which can be obtained through the implementation of the properties such as elasticity, tear resistance, breathability, water resistance, antibacterial properties, etc. In the last years the use of nanotechnology based products has increased rapidly in different fields of applications, such as textile, constructions, biomedical , electronic, packaging etc. In particular, nanotechnology has emerged as an efficient and powerful strategy to upgrade the structural and functional properties of natural and synthetic polymers [1-9]. In a first approximation, nanotechnology based processes developed for textiles can be transduced into the leather making processes. Textiles, however, owing to their open porosity, high specific surface, along with the possibility of functionalizing individual yarns before weaving, make these much more susceptible to the nano-functionalization. Conversely, leather possesses a much more closed and heterogeneous structure.

Therefore, the scope of this work is to engineer raw material using chemical-physical manipulation strategies in order to modulate leather properties and to fabricate final products with improved performance (tear resistance and elasticity). The work has been divided in different steps, which have allowed to acquire knowledges about the chemical

and morphological properties of skin, manipulation of biopolymer networks, production and characterization of final leather. In particular, in the first part, I have focused my attention to the characterization of leather material in order to define the benchmarks to which the chemical physical parameters of the engineered products need to be compared. This step has been very important since the macroscopic characteristics of the animal skin are usually evaluated in a qualitative manner by defining empirical parameters such as "touch", "soft", "fullness" and no experimental measurements in order to quantify these parameters have been developed so far.

The obtained results provided information on the structure-composition -functions relationship of animal skin and most importantly they provided clear hints on how to modify the substrate and in what step of the tanning process this is most convenient.

The second part of thesis has focused on the identification of the chemical-physical processes, which can most effectively affect the macroscopic properties of the final product. Finally, an experimental campaign has been designed in order to extract the relevant physical parameters, which describe the macroscopic properties of the engineered samples.

Chapter 1-State of art

1.1 Introduction

The manipulation of the chemical and structural features of dermal based network, has the potential to engineer the mechanical response of the network itself to various extents. This can have direct applications in both the biomaterials and industrial sector. However, this requires a thorough understanding of the composition-structure-function relationship of the native tissue. It can be very useful to understand the nature of skin, in particular of dermis, in order to rationalize the structure-function, the structure-reactivity, and structure-property relationship. Moreover the knowledge of dermis structure facilitates the understanding on how the skin is processed in leather, how the structure can influence the properties of leather and above all how the modification of natural structure can affect the performance of leather. Therefore, before describing the leather making process, a detailed description of the skin and in particular of collagen is reported. Collagens represent the most abundant material in the skin. To exploit the unique properties of collagen, this is modified with various treatments [10]. For instance, the leaher making process represents the first process in which chemical modification of collagen was involved [11]. Another important sector where chemical physical modification of this biopolymer is often necessary is the biomedical field. Indeed manipulation strategies of collagen in order to modulate mechanical properties of dermal based collagen networks are widely used in tissue engineering [12,13] and some of them were reported in the following chapter [14,15]. Finally, a brief part of chapter sums up materials and

methods used to modify leather properties in order to obtain high performance.

1.2 Composition-Structure-Properties of the Skin

The skin is the body's largest organ; it covers the surface of the body and separates it from the outside. It is made up of different layers (epidermis, dermis, and hypodermis) and is composed of about 70% water, 25% proteins, and the remainder mainly includes glycosaminoglycans and proteoglycans, which are arranged in an amorphous phase reinforced by fibrous phase. The skin must withstand repeated reversible extensions and compressions with the movement of the body while simultaneously acting as an impermeable barrier [16-21]. The actual mechanical properties of the skin reside mainly in the collagen and elastic fibers of the dermis, whereas the barrier is confined to the uppermost layer of the epidermis, the stratum corneum. Thus skin can be described as a laminate composite material composed of three distinct layers [22]. The deepest one, which is the hypodermis composed of adipocytes, acts as an energy reserve, offers mechanical shock protection, insulates the body against external heat and cold.

The second layer is the dermis, a dense fibro-elastic connective tissue that is mainly composed of collagen, arranged in fiber bundles. It contains numerous cells, including fibroblasts and macrophages. The first ones are responsible for the production and assembly of the dermal ECM while macrophages contribute to eliminate foreign material and portions of damaged tissue. Collagen represents about 70% of dermal proteins and it is assembled in the form of fibers which are in turn packed in collagen bundles and are mainly responsible for skin elasticity (rigidity and strength). Indeed under normal conditions, skin

mechanical behavior is directly related to dermal behavior [23]. Another important component of the dermis is elastin, that forms a network which wraps collagen bundles around. The dermis can be roughly divided in two overlapped layers: the papillary layer and the reticular dermis. The first one contains the sweat glands and hair follicles and reticular dermis is predominantly composed of an entangled mass of collagen bundle [24]. Finally, the epidermis is the upper layer of the human skin; i.e. it is the final interface between the external environment and the human body [25]. It can be subdivided into several layers or strata, starting with the basal layer (or stratum basale) just above the dermis and proceeding upward through the spinous and granular layers to the top layer, the stratum corneum, as shown in fig. 1.1. It contains keratinocytes that synthesize an important structural protein: keratin. In addition to keratinocytes, also Langerhans cells, Merkel cells, melanocytes, and lymphocytes can be found in the epidermis as resident populations or in response to transient conditions: they migrate into the epidermis early in embryogenesis (melanocytes and Langherans cells) or differentiate in situ, probably from an ectodermal/keratinocyte progenitor cell (Merkel cell) [26, 27].

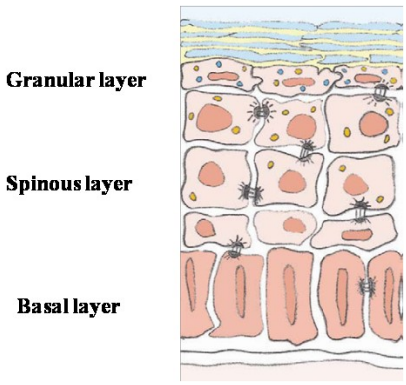

Fig.1.1 Epidermis layers

1.2.1 Collagen

The most abundant protein in mammalian tissues, is the main structural component of skin, leather, and also of several medical scaffolds [28, 29]. Its main function is mechanical reinforcement of the connective tissues of vertebrates [30, 31].

The polypeptide chains of collagen contain 20 different amino acids but different tissues include specific amino acid sequences. Indeed, collagen molecules are characterized by the sequential Gly-XY triplet structure that is a prerequisite for triple helix formation. At least 20 collagen types were characterized [32] and type I collagen is the predominant type being found as the main component of skin, tendon, and bone [33].

Because skin is composed of fiber forming collagen types, their structure is discussed in more details below. In particular, an amino acid sequence that is rich in glycine (Gly), proline (Pro) and hydroxyproline (Hyp) forms triple helical collagen [34], that generates a symmetrical pattern of three left-handed helical-chains. The presence of the cyclic amino acids, Pro and Hyp imparts rigidity and stability to the coil while Gly, the smallest amino acid, is in every third position in order to create the right-handed triple helix. Furthermore, the hydroxyl groups of Hyp residues are involved in inter-chain hydrogen bonding and are important for stabilizing the triple-helix structure. Two hydrogen bonds are formed: one can be noticed between the NH-group of a glycyl residue with the CO-group of the residue in the second position of the triplet in the adjacent chain, and one via the water molecule participating in the formation of additional hydrogen bonds with the help of the hydroxyl group of Hyp in the third position [35]. Such a 'water-bridged' model of the triple helix was confirmed by

physiochemical studies of the collagen molecule in solution and was supported by the observation that the thermal stability of the helix is dependent on the content of Hyp and not of Pro [36].

Aggregation of triple helices forms the microfibril [37-39], with a diameter range of 3.5 and 4.0 nm. About 1000 microfibrils can aggregate laterally and end-to-end into a fibril having a diameter of 80-100 nm [40-42] and then more fibrils (approximately 500) can bonds to form a collagen fiber characterized from a diameter of about 1-4 μm. Finally, aggregation of fibers became fiber bundles with a thickness between 10 and 100 μm. Therefore collagen show a hierarchy (fig.1.2) that is highly dependent on its function because it affects strongly its properties.

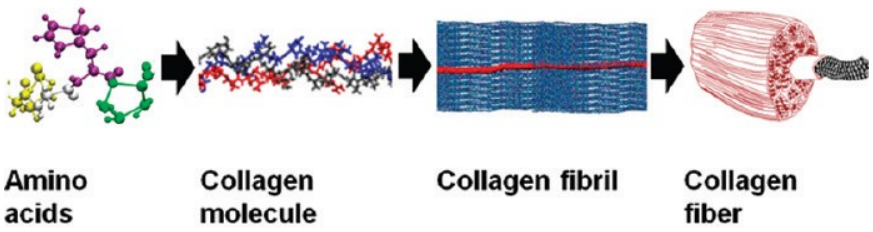

Fig.1.2 Hierarchy of collagen

For example, the fibril and fiber diameter of collagen in skin varies between 20 - 100 nm and 0.3 - 40 μm, respectively while fibril and fiber (fiber bundle) in tendons and ligaments have a diameter ranging in the 20 - 250 nm and 1 - 300 μm intervals, respectively [43].

In order to develop an extracellular network of collagen fibers, the cells involved in the biosynthetic process must first synthesize a precursor known as procollagen. This molecule possesses a long, non-interrupted triple helical region with N- and C-terminal globular extensions called

pro peptides. The molecule is later proteolytically trimmed of its pro peptide domains, giving rise to a tropocollagen molecule with short non-helical ends of 15 to 25 amino acid residues (N-and C-terminal telo peptides) that spontaneously assembles into fibers in the extracellular space [34].

Cross-linking makes these fibers mechanically and thermally stable and provides them with an adequate degree of tensile strength and visco-elasticity to perform their structural role. In particular the number and the degree of crosslinking can result very important to affect the properties of collagen network [44, 45].

1.3 **Manipulation strategies of biopolymer network**

The mechanical characteristics of collagen networks are very important in both medical and industrial fields. For example high strength is often required for collagen-based medical materials such as extracellular matrix scaffolds [46, 29] or processed pericardium for heart valve repair [47]. Also leather, which is mostly composed of collagen and is produced on a large scale for shoes, clothing, and upholstery [48], need high strength in order to be used for its high-value applications. Moreover for most applications, natural collagen needs to be modified to introduce stability against denaturation and degradation. Factors that can affect the strength and the stability of the collagen structure include the amount of collagen present, the molecular structure of the collagen, the nature and density of the crosslinking, collagen bundle size, and collagen orientation [49-54]. It was found that collagen molecules alone are not capable of providing the broad range of mechanical functionality required for physiological function of collagenous tissues. Rather, the existence of an array of deformation mechanisms, derived

from the hierarchical makeup of the material, is critical to the material's ability to confer important mechanical properties, specifically large extensibility, strain hardening, and toughness, despite the limitation that collagenous materials are formed from only few distinct amino acids.

Nevertheless, often collagen matrix constructs lack desired mechanical properties for vascular grafts [55, 56] or hydrated reconstituted collagen matrices are mechanically weak and stiff [44]. They thus need to be modified for high compliance and elongation as vessel substitutes and be strengthened to be ready for in vivo use. In order to increase collagen strength, two strategies are generally implemented: crosslinking [57, 58] and reinforcement [59-61].

Crosslinking involves the use of physical or chemical crosslinkers such as glutaraldehyde [62, 63], formaldehyde [64, 65], Nethyl-N0-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDAC) [66] and genipin [67], that form covalent bonds between characteristic chemical groups on biopolymers. Reinforcement involves the addition of mechanically stronger components to the collagen matrix. As regard the crosslinking method, one of the most widely used chemical methods for the crosslinking of collagen lattices is based on 1-ethyl-3-(3 dimethylaminopropyl) carbodiimide (EDC). EDC is a water-soluble crosslinking agent that mediates the amide crosslinks between the carboxyl groups of aspartic and glutamic acid side chains and the primary amines of lysine and hydroxylysine side chains of collagen [68]. As already stated, other types of natural cross-links in collagen are based on the aldehyde groups [69] but currently there is increasing pressure from industry to find alternatives to the aldehydes in order to meet the stricter environment and legislation requirements. Crosslinking by multi-functional epoxides may afford a new approach

for this problem. Epoxy monomers (some commonly synthesized into reactive, higher molecular weight prepolymers), also known as epoxy resins, have some distinct advantages over other reactants, including [70-72]:

-reactivity to a wide range of different functional groups under suitable reaction conditions;

-different molecular structures, which may be tailored for specific application methods;

-polymerization, via polyaddition and/or crosslinking through the formation of covalent bonds;

-lower toxicity.

Reinforcement, can be implemented for example by incorporation of synthetic polymers such as poly(ethylene terephthalate) (PET) fiber [73], adding biometallic nanopartcles [74] or also carbon nanotube, that are very attractive for use in fiber-reinforced composite materials in neural and orthopedic tissue engineering applications [61]. Moreover in the last years great attention was focused on the use of decellularized dermis, composed with 97% of collagen, as the base material in the tissue interface. Indeed it is important that tissue interface is compatible with soft and hard tissue; thus often it can be necessary to reinforce the decellularis dermis using polymeric materials in order to realize a decellularized tissue-polymer complex (fig.1.3) with better mechanical properties [75].

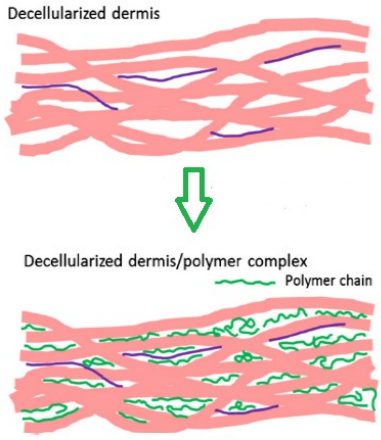

Fig.1.3 Decellularized dermis structure and dermis/polymer complex structure.

1.4 Leather making process

The manufacture of leather represents a chemical processing of biological dermis. Basically, leather industry transforms a by-product of the food industry in valuable artifacts [76, 77]. Leather industry market is strictly connected to the meat market because of the basic raw material of tanning industry is a by-product of the meat processing industry. The technology of leather making aims to retain the skin's natural properties, to stabilize its structure and at the same time to chemically process it so it will no longer be subject to putrefaction. Moreover this process makes the non uniform skin structure as uniform as possible in the final leather product. The most important component of hide in the leather making view is the collagen. The approximate composition of a bovine hide is 64% of water and 33% of structural and non-structural proteins. In particular, structural proteins are collagen (29%), keratin (2%), elastin (0.3%) while non-structural proteins are albumins, globulins (1%), mucins, mucoids (0.7%). As minor

components, fats (2%) and other substances (0.5% inorganic components, 0.5 % pigments, etc.) are contained in the skin

Leather making process requires several chemical and mechanical steps that can be classified into three groups: pre-tanning or beamhouse operations, which clean the hides or skins; tanning, which permanently stabilizes the skin or hide matrix; and post-tanning and finishing operations, where aesthetic value is added [78, 79].

Beamhouse operations include soaking, liming, deliming and bating and finally pickling [80]. In the first step the animal skins are soaked with water in the presence of additives in order to remove the excess of salt, dirt, dung, blood, and restore a suitable water content inside the skins, that helps opening up the fibres preparing the collagen to the subsequent liming step. It is generally carried out at pH values between 9 and 10 by addition of sodium carbonate or sodium sulphide, which leads to moderate swelling of the hides during rehydration.

In the second step (liming) the soaked skins are steeped in an alkali and sulphide solution that breaks down the structure of the hair at the root (its weakest point) by a reduction of an important constituent of keratin, cystine sulphur-sulphur linkages, in order to facilitate hair removal. Indeed it is a industrial practice to run unhairing and liming in one step; the first consist of removing the hair from the pelt by the chemical attack at the sulphur bridge of cystine (CySSCy). The hairless skin is immersed in a solution of alkali and sulphide to complete the removal of the hair and to alter the properties of the skin protein (collagen). The collagen becomes chemically modified and swells, leaving a more open structure. The typical reagents in the liming stage are Ca(OH)2, used to rise the pH in a controlled way, and Na2S, possibly combined with NaHS, as reductant. Ca(OH)2 is preferred as base for its low solubility

(1,3 g/L), that limits the rise of pH to 12.6; because if pH exceeds 13 a rapid hydrolytic damage of collagen takes place. It is commonly accepted that high values of the pH lead to four different effects:

1) alkaline hydrolysis of amide side chains (asparagines and glutamine), that results in a shift of the isoelectric point of collagen from native pH 7.4 to pH 5-6;

2) removal of residual non-collagenous components of the skin (albumins, globulins, dermatan sulphate, etc.). Indeed hydrolysis of peptide bonds is particularly effective in removing linear proteins, as they are progressively broken down into smaller and more soluble peptides while collagen having a hierarchical structure (polypeptide chain, tropocollagen, microfibrils, fibrils, fibres), is less affected by hydrolytic phenomena;

3) saponification and emulsification of fats;

4) swelling of the fibrous structure, due to an osmotic pressure effect.

The “osmotic swelling” effect allows the separation of the fibres and the fibrils from one another and an opening up of the whole structure that irreversibly determine the features of the final leather [81]. After the strong alkaline action, the skin structure is further opened up during the deliming and bating process. In particular the deliming step aims to solubilise residual lime and deflate the structure by lowering the pH down to 8.5-9.0, that represents a better PH value for the enzymatic digestion that occur during the bating step. The use of enzymes results important (usually proteases) to catalyze hydrolysis of non-structured proteins and in order to optimize the biochemical reactions, pH should range in the interval 8.0-9.0 and temperature set around 37 °C. This last step is achieved by treatment with acid salts (ammonium chloride or ammonium sulphate) or carbon dioxide until the desired pH is reached.

The last pre tanning operation is known as pickling and it consists of a acidification process, that allows a decrease of PH values in a range 2.8-3.5. The skins are agitated in a solution of salt and sulphuric acid until they are at or near equilibrium at a pH value of 3.0-3.5. In particular once the salt is dissolved, formic acid and, subsequently, sulphuric acid are added after previous dilution.

Tanning step represents the most chemically complex step that allow to transform the structurated collagen in the leather [82]. The transition to leather requires permanent incorporation of chemical species called "tanning agents" into the collagen network. Tanning agents can be inorganic and organic tannins such as chromium, aluminum, titanium, iron and zirconium basic salts as well as high molecular weight vegetable substances, aldehydes, oils and other substances [80, 83]. Tanning agents are used in order to prevent the leather from chemical and thermal degradation. The most common tanning agent is chromium sulfate. It enters the pores of the skin by a diffusion process to react with the collagen carboxyl groups and form intermolecular cross linking which results in physical, chemical and biological stability. This step is followed by a basification step using weak chemical bases which enhances the anionic character of the carboxylic collagen groups and hence increases the attraction towards the chromium cations Cr3+ which results in a final covalent bond. The whole tanning process takes about 5 to 6 hours [80, 84].

After tanning process, leather must be neutralized to remove unwanted acids, to prevent deterioration during the drying process, and to prepare the leather for the next steps: dyeing and fat liquoring. Neutralization is often carried out using mild alkalis which have some effect on the chromium complex. Dyeing is performed using commercial dyes. They

are essentially mixtures of chemical compounds which can be fixed to the material to be dyed and which have appreciable resonance within the molecule giving colour. Finally fat liquoring is the process in which 'tanned' fibres are treated with reactive oils, which attach themselves to the fibrous structure, and lubricate them so that they can move readily in relation to one another, producing a soft, supple leather.
All reactions are conducted in an aqueous medium, therefore at the end of processing water needs to be removed.

1.5 Optimization of leather properties

The physical properties of leather make it an ideal material for different manufacturing applications and in the last decades, great attention was devoted to the development of modification in leather in order to improve macroscopical properties of final products.
Already during prehistory leather was considered a useful material in the applications such as cushions, ornaments, book bindings and also for production of slings and shields, widely used during wars [85]. During industrial revolution new applications were introduced and today, it represents a significant agricultural resource, having the potential for developing materials with high performance. Even though a wide variety of leather like synthetic materials was developed, the synthetics materials were not able to replace leather in different products [86]. Therefore important techniques, already used in order to add value to technical textiles, were started to be used also in the leather field. One of them is “coating”, that is a process in which a polymeric layer is applied directly to one or both surfaces of the fabric and then the coated fabric is heated and the polymer is cured (that is, polymerized). The coating formulation with different textile grade

polymer like PVC, PU, acrylic, PTFE were hugely used to make a textile product with multipurpose way like-waterproof protective clothing [87, 88]. Thus polymeric coatings were investigated in order to increase the leather's scuff resistance and other important properties, such as the antimicrobial one [89-91]. Indeed an example of coating involved an alternative solution based on chitosan in order to develop antimicrobial leather [92]. Also acrylic and polyurethane resins were widely used to perform impregnation of the grain layer and to optimize its properties. The investigations, including both surface treatments and the incorporation of polymer beyond the grain layer, continued through the efforts of several researchers with aim to improve also macroscopical mechanical properties, such as the hardness.

Moreover, some works discuss the realization of interpenetrating polymer networks (IPNs) formed by polymerization of an epoxy resin within a leather being epoxy resins relatively brittle thermosets with good dimensional stability and commonly used as adhesives, coatings, and matrix materials in fiber-reinforced composites [93]. Furthermore, it was know that monomeric polemeryzable organic compounds in the form of emulsions or dispersions could be drummed into tanned or pre tanned leather and then to be polymerized within the leather in the presence of peroxidic catalyst or accelerators. The most important part of works was focus on impregnation through the grain-corium interface while few data report full impregnation of leathers or however impregnation of dry white samples through the flesh side was not discussed in literature.

The efficiency of the impregnation processes can be fairly low, being mainly governed by diffusion mechanisms driven by concentration gradients. Indeed the work aims at providing practical solutions to the

above mentioned problem by using as substrates dry collagen networks to improve diffusion within dermis through a new vehiculation mechanism based on the capillary suction. In more details, the goal of work is to obtain leather prototypes with engineered mechanical properties such as tear resistance and elastic recovery involving the use of high temperature and polymeric materials. In particular in order to increase tear resistance tough polymers were selected such as polydimethylsiloxane and nylon while to optimize elastic recovery of samples elastomeric polymers were chosen.

Chapter 2 - Experimental analysis: materials and methods

2.1 Introduction

The materials used to engineer the collagen network, are presented in the following chapter. Moreover, different manipulation strategies and methodologies are reported, along with a description on the chemical-physical analysis on raw, tanned and engineered materials.

2.2 Leather substrates

Substrates were provided by the DMD company (Solofra, Italy). The provided substrates to be subjected to treatments were DW and DB networks. In particular DW was obtained from several steps, that are reported in tab.2.1. The same protocol was used in order to realize DB samples but in this case a tanning step was performed after adding the formic acid and before the dehydratation process. Tanning step involved the use of small amount of a tanning agent, known as Chromitan B. Other two types of DW substrates were used: DW type for gloves and crossbreed type (tab.2.2). They were subjected to

different pre tanning operation that can allow them a higher relaxation of the protein structure and thus a better diffusion of materials whitin the network during the treatments.

% weight pickled		
100%	Water	
7%	Sodium chloride	Bè 7
1%	Baking soda	
+	Skins	pH=4.5/5
0.7%	Bating agent enzyme	
Draining, fleshing		
70%	Water	
7%	Sodium chloride	
1%	Deliming agent	
0.5/1%	Formic acid	pH=3.5
		Drain
		Dealing in prototype
150%	Acetone	
Draining		
150%	Acetone	
		Draining
150%+3%PEG400	Isopropanol	
		Drying

Tab.2.1 Steps performed in order to obtain DW samples

Samples DW	Characteristics of samples
DW	Dry white –goat type
DW-G	Dry white–goat type for gloves
DW-I	Dry white–crossbreed type

Tab.2.2 Different types of DW substrate

At last samples (B, C, D and E) that were tanned in conventional manner were characterized in order to define the benchmarks to which the engineered products need to be compared. The classification of

substrates along with their characteristics are summarized in tab 2.3. Representative images of the samples are depicted in fig. 2.1

Samples	Characteristics of samples
DW	dry white-goat type
DB	Dry blue - semi chrome tanned at 1%
B	Chrome tanned and finished with aniline
C-D-E	Chrome tanned and dyed

Tab.2.3 Characteristic of samples

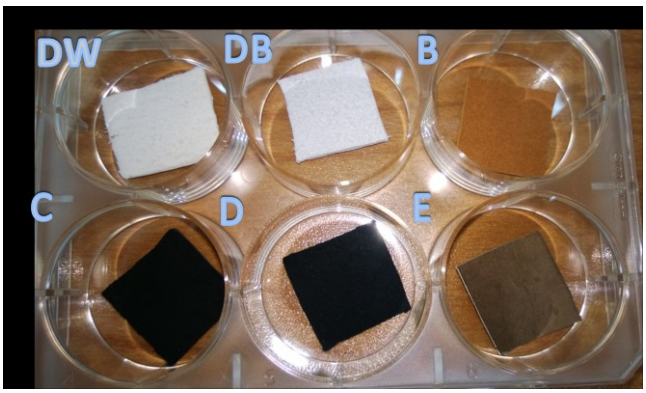

Fig.2.1 DW sample , DB sample , B sample tanned and finish with aniline, C-D-E samples chrome tanned and dyed.

2.3 **Manipulation strategies and materials**

In order to engineer macroscopic properties of the collagen-base networks, two manipulation strategies were presented: crosslinking and reinforcing [57-61]. The former relies on the dehydrothermal crosslinking achived at high temperatures. The latter aims at modifying the network mechanical response by inserting a synthetic polymeric network which acts in parallel with the collagenic one. The choice of the specific polymer was based on :

a) mechanical characteristic of the polymer itself

b) process of polymer insertion into the collagen
c) intended mechanical properties of the engineered network

For example polymeric materials, characterized by a high tensile strength, were used in order to improve mechanical strength while elastomeric materials were chosen to promote the elastic recovery of leather. In particular in order to engineer the macroscopic properties of network the following polymeric materials were used: Nylon 6.10, PDMS, elastollan 685 e 2180 and ecoflex [94-99] and they were crosslinked or dissolved in specific solvents. The efficiency of the diffusion processes can be low because it is mainly governed by diffusion mechanisms of dermis that is a network characterized by low permeability. Therefore, in order to improve the diffusion of polymers and chemical deep within the network and to speed the diffusion process up, enzymatic treatments have been performed to open up collagen network [100-102].

2.3.1 Sampling positions

Leather, as well as skin, is an anisotropic material and its structure and properties vary over its area; therefore mechanical properties can be affected by the sampling position and by the direction of applied load [103]. The parts of whole hide or skin can be devided in “butt”, “belly” and “neck” (fig.2.2) that present some differences. The “butt” shows a tight fibre structure, the belly is the thinnest part characterized by a more open structure, making them relatively weak and finally the neck rapresent the thickest part. Therefore in order to make best comparison of leather mechanical properties and to evaluate the effect of treatments, it was necessary to consider all samples in the “Official Sample Position” (included in butt region) and to take away them from

perpendicular backbone direction because skin exhibits greater strength. Moreover another important thing in order to have a good comparison between treated and their controls was to take away them on the left side and right one respectively (or vice versa), because of the symmetry of the features about the backbone [104].

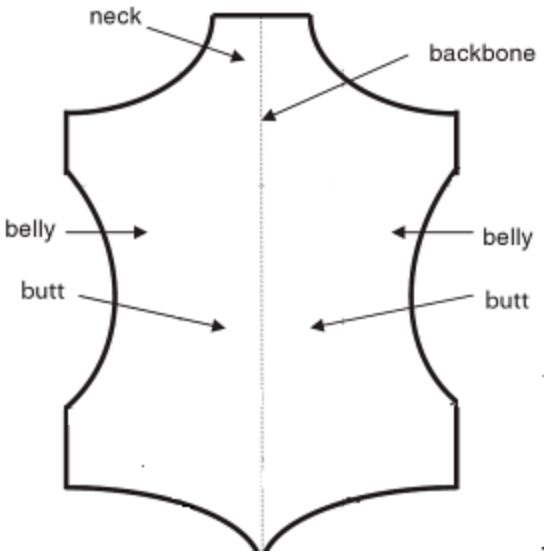

Fig.2.2 Parts of skin

2.3.2 Enzymatic treatments

Enzymatic treatments were performed on dry white samples using Basozyme C10, a pancreatic enzyme widely used in tannery industry as bating agent. The percentage of enzyme compared to the weight of dry sample is 5% . Enzyme and samples were put in a PBS solution (300% of the wet weight of skin) in the bottle of the rotavapor and this solution was stirred at 250 rpm at 30°C. Treatments were performed for 30 min., 1h and 3 h. After enzymatic treatment, the samples were rinsed in PBS and then dehydrated. The dehydration process was performed placing hydrated samples in acetone bath. Acetone was refreshed after 30 minutes. Subsequently, acetone solved samples were

placed in isopropanol bath for 20 minutes. Samples drying was carried out in a rotavapor under vacuum at 40°C for 1h (Fig.2.3) and then left overnight under the extraction hood.

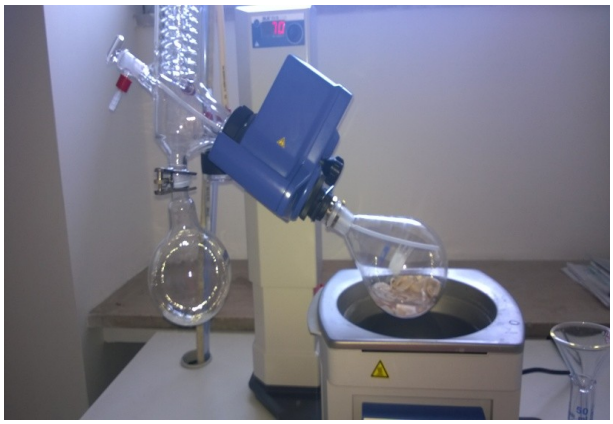

Fig.2.3 Rotavapor used for enzymatic treatment

2.3.3 Thermal treatments

Thermal treatments were performed on dry white samples using high temperatures in order to improve elastic recovery because heating can modify collagen structure and form physical crosslinks.

Thus this molecular change can induce a shortening of these tissues and gives them a rubber-like behavior [105]. These treatments were performed on dry white substrates and on split DW networks in order to treated flesh and grain side separately. In particular DW substrates were treated by leaving specimens in oven overnight at 90°C while split DW substrates were previously preconditioned before to be subjected to high temperatures. Some samples, both flesh and grain side, were stored 24 h in the conditioning room (98% humidity and 37°C) and others were stored 24h in oven under vacuum at 37°C. After the storing step all split specimens were left in oven at 140°C for 8 h.

2.3.4 Polymeric treatments

Preliminary tests were performed by treating both DW and DB with a selected number of polymers in order to assess the substrates capability of uptacking exogenous synthetic materials. The results provided by this campaign were employed to optimize the treatment conditions to modify mechanical properties of skin. In particular, a systematic study was performed to engineer mechanical properties of DW samples with a selected number of polymers having specific chemical physical characteristics.

Treatment with Nylon 6,10

Nylon was selected to improve ultimate tensile strength and tear resistance because it is characterized from high strength. It is a semi-crystalline polyamide and it is produced from hexamethylenediamine and 1,10-decandioic acid (sebacic acid). It is commonly used in the form of monofilamen in the textile field (swimwear, sportswear, bags) for its low cost, superior fiber forming ability, good mechanical strength, and strong chemical and thermal stabilities [106].

Nylon treatment was performed on DW and DB networks. Two solution were prepared : in the first one 3.2 g of hexamethylenediamine was dissolved in 200 mL of water (1:63 w/w) in a recipient while in a different recipient, 5.85 mL of sebacoyl chloride were dissolved in 250 mL of hexane (1:43 v/v). The solutions were thoroughly mixed. Then, either DW and DB samples were placed in the hexane-based solution for 5 minutes until a complete soaking. Subsequently, the samples were extracted and immersed in the water-based solution for additional 5 min to allow the polymerization reaction to occur in order to develop the growth of Nylon 6.10 inside collagen fibers. This step was repeated

three times in order to improve absorption of reactants and polymerization. Finally, the samples were washed with fresh hexane (once) for 10 min and isopropanol (twice), and dried under the extraction hood. The amount of two solutions were calculated in order to obtain a treatment with 17% of Nylon 6.10 compared to weight of dry susbtrate.

Treatment with PDMS

PDMS was selected to improve ultimate tensile strength and tear resistance because it is characterized from high strength but can confer softness properties to the leather. Indeed polydimethylsiloxane, containing silicon oxygen bonds, is widely used in the textile applications as finishing agents: softeners, antistatic, antisoil and anticrease [94]. Because of their inorganic-organic structure and the flexibility of the silicone bonds, it has some unique properties including low surface energy, excellent lubricity, heat stability, high compressibility and low surface tension. Treatments with PDMS were performed according three strategies:

1. treatment with a PDMS solution containing 25% of polymer compared to the weight of dry skin. PDMS was dissolved in hexane and dry skin :hexane ratio was of 1:24 (w/w). Sample was left in stirring in the PDMS solution for 3h at 60°C and then was left overnight at 37°C. This treatment was performed on DW and DB networks.
2. treatment was performed preparing a PDMS solution containing 25% of polymer compared to the weight of dry sample in 0,12mL/cm^{2}of hexane that was sprayed on sample . After that, the sample was left in oven at 40°C for 15-18 h.

The same treatment was performed also preparing a PDMS solution containing 30% of polymer compared to the weight of dry sample.

3. drop by drop treatment by using a solution containing 40% of PDMS compared to the weight of dry sample. In particular, the amount of solvent corresponds to the amount of solvent that the piece of skin can completely absorb (dry skin :hexane ratio was of 1:2.5 w/w). This last protocol were performed also by replacing the hexane with tetrahydrofuran.

Treatment with Elastollan 685 A e 2180

Two commercial thermoplastic polyurethanes elastomers (TPU), Elastollan 685 and Elastollan 2180, exhibiting high elastic properties, were selected in order to improve elastic recovery of leather. Elastollan is a polyether-based thermoplastic polyurethane used currently in several field such as coating, adhesive, etc [107]. It presents excellent mechanical properties, high tear resistance and abrasion resistance, toughness and good flexibility at low temperatures. Both elastollan, 685 A and 2180, were provided in a granular form (fig.2.4) and have similar physical-chemical properties as shown in tab.2.4

Fig.2.4 Granular form of Elastollan

Elastollan treatments were performed according to three protocols. All protocols involved the use of THF as solvent and need a preliminary step. This step consists of dissolving elastollan in solvent for about 3 h at 60°C and the amount of solvent was calculated considering the dry skin weight. In particular the dry skin/THF ratio was of 1:3 w/w.

1. Treatment drop by drop was performed using respectively 3%, 5%, 10%, 15% of Elastollan 685, compared to the weight of dry sample. Treatment using 15% of elastollan was performed also on DW-I and DW-G samples (tab.2.2). Moreover drop by drop treatment was performed on split samples without flesh side, using lower percentage of polymer .In particular these protocols involved the use of 3% elastollan 685, 3%elastollan 2180 and a polymeric blend containing 5%elastollan and 5%pdms;
2. treatment with rotavapor was performed by placing the sample in the polymeric solution prepared with 15% elastollan (dry skin :THF ratio was of 1:3 w/w) and the bottle conteining the solution and the samples was left rotating for about three hours at 25°C .
3. the treatment with the roll was performed by spreading the polymeric solution prepared with 15% elastollan (dry skin :THF ratio was of 1:3 w/w) on the sample using the roll.

Properties	Elastollan 685	Elastollan 2180	Comments
Density	**1.21g/cc**	**1.13g/cc**	**ISO 1183-1-A**
Hardness-shore A	**86**	**77**	**ISO 868**
Tensile strength	**50Mpa**	**45Mpa**	**ISO 37**
Elongation at break	**600%**	**450%**	**ISO 37**
Tear strength	**75Kn/m**	**40Kn/m**	**DIN ISO34-1/Bb**
Processing temperature	**175-220°C**	**175-220°C**	**Extrusion**

Tab.2.4 Elastollan 685 A and 2180 properties

Treatment with Ecoflex C1200

Ecoflex C1200 was selected in order to improve elastic recovery of collagen network because it shows high elastic properties and good flexibility.

It is a commercial elastomeric aliphatic-aromatic copolyester including 1,4-butanediol, adipic acid, terephthalic acid in polymer chain and it presents properties similar to the low-density polyethylene because of its high molecular weight and its iper-branched molecular structure. Its main applications are in packaging.

Absorption of ecoflex solution in THF was performed with drop by drop treatment using two different concentration of polymer: 10% and 20% compared to the weight of dry sample respectively. In particular polymer was dissolved in THF at 60°C (dry skin/THF ratio was of 1:3 w/w) and then polymeric solution was put drop by drop on samples.

Treatment with polymeric blend composed from Elastollan and Ecoflex C1200

Engineered samples with elastollan and ecoflex were realized dissolving both polymeric materials in THF (dry skin/THF ratio was of 1:4 w/w) at 60°C. In particular the amount of THF, calculated respect to the weight of dry sample, was divided equally in order to prepare the two polymeric solutions : one containing 10% of ecoflex compared to the weight of dry sample and the second one containing 10% of elastollan 685 compared to the weight of dry sample. Then the two obtained polymeric solutions were mixed and the final solution containing polymeric blend was put on samples drop by drop.

Treatment with polymeric blend composed from Elastollan and PDMS

Engineered samples with elastollan and pdms were produced by dissolving 5% of elastollan compared to the weight of dry sample in thf and putting 5% of PDMS (monomer and crosslink agent with ratio 10:1) compared to the weight of dry sample in a backer with THF. PDMS solution was stirred and then the two different solutions were mixed and final solution was put drop by drop on sample .The amount of THF used to prepare both solutions was calculated respect to the weight of dry sample and in particular it was of 1:4 (w/w) .

2.4 Experimental characterization of samples

2.4.1 Mechanical analysis

Dynamic Mechanical Analysis

Dynamical mechanical analysis were performed in order to evaluate viscoelastic properties of samples shown in tab.2.3 and engineered samples reported in tab. 2.5. Preliminary strain sweep tests were performed on the samples, at the oscillation frequency of 1Hz in order to identify the linear viscoelastic response range of the materials.

Viscoelastic properties of samples were evaluated on Dynamic Mechanical Analyser (TA Instruments DMA Q800). The tests were carried out at 25 °C by using a thermostatic bath. Samples of dimensions 4x0.5 cm were mounted on the tension clamp. The samples were subjected to periodic oscillation in a dynamic experiment to evaluate the dependence of the elastic and viscous moduli, E' and E'', upon the frequency. E' provides information about the elasticity or the energy stored in the material during deformation, whereas E" describes the viscous character or the energy dissipated as heat. Samples were subjected at a strain 0.1 and the frequency range investigated has been 0.1Hz-10 Hz. The tests were repeated at least three times on each samples. Values are reported as mean ±standard deviation, calculated with Microsoft excel software.

Samples	Sample name
Control /dry white +pdms	CTR DW+PDMS
Dry white +25%pdms	DW+PDMS
Control /dry blue +pdms	CTR DB+PDMS
Dry blue +25% pdms	DB+PDMS
Control/dry white+Nylon	CTR DW+Nylon
Dry white+Nylon	DW+Nylon
Control /dry blue+Nylon	CTR DB+Nylon
Dry blue +Nylon	DB+Nylon

Tab.2.5 Engineered samples with Nylon and PDMS

Uniaxial tensile tests

Samples reported in tab.2.3 and tab.2.5 were subjected to uniaxial traction tests in order to evaluate the ultimate tensile strength. The specimens were taken along the backbone direction and cut along the perpendicular backbone direction. Shape and dimensions of the specimens are shown in fig.2.5 and tab.2.6. All tests have been carried out using an Instron Dynamometer (mod. 2752). The crosshead speed of tests performed on samples in tab 2.3 was set at 1 mm/min and on samples in tab.2.5 at 5mm/min. Moreover PDMS engineered samples have been tanned and subjected to uniaxial traction test (crosshead speed at 5mm/mm) in order to evaluate how the interaction between polymeric materials and tanning agents influences mechanical properties. Values are reported as mean ±standard deviation, calculated with Microsoft excel software.

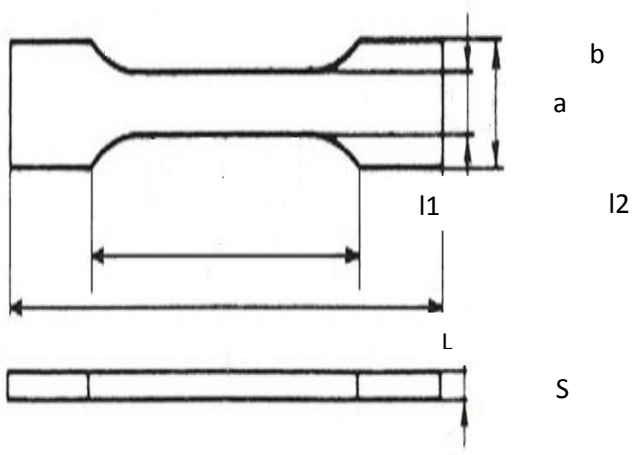

Fig.2.5 Shape samples subjected to uniaxial traction test and cyclic traction test

Samples	L [mm]	**l 1[mm]**	l 2[mm]	**a[mm]**	b[mm]
CTR DW+PDMS	38	22	8	5	15
DW+PDMS	38	22	8	5	15
CTR DB+PDMS	38	22	8	5	15
DB+PDMS	38	22	8	5	15
CTR DW+Nylon	38	22	8	5	15
DW+Nylon	38	22	8	5	15
CTR DB+Nylon	38	22	8	5	15
DB+Nylon	38	22	8	5	15

Tab.2.6 Dimension of samples reported in tab2.5

Tear resistance

Tear resistance materials were carried out using an Instron Dynamometer (mod.2752) and the test method provided by ASTM D 1938-02 “Standard test method for tear-propagation resistance (Trouser tear) of plastic film and thin sheeting by a single-tear method”. Tear tests were performed on samples (specimens in tab 2.5 and on PDMS engineered and tanned samples) characterized from a rectangular shape (fig.2.6) and dimension 75x25 mm. Measurements were done by

securing sleeve A (Fig. 2.6) in one grip and sleeve B in the other grip, using an initial grip separation of 50 mm and aligning the specimen so that its major axis coincides with an imaginary line joining the centers of the grips. Specimens were subjected to a grip-separation speed of 250 mm/min and the test have been continued until the tear has propagated through the entire 25-mm portion. Values are reported as mean ±standard deviation, calculated with Microsoft excel software.

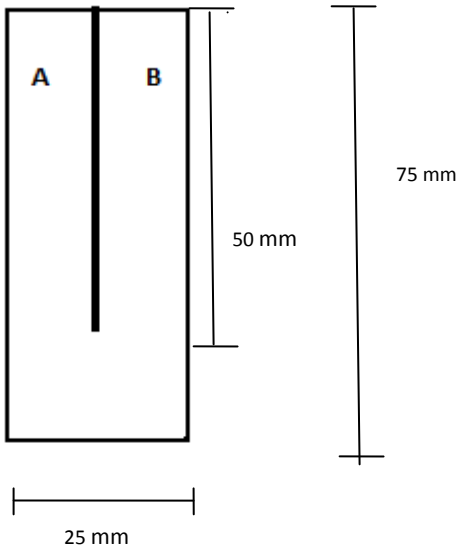

Fig.2.6 Specimen shape subjected to tear tests

Cyclic traction tests

Dynamic traction tests were performed on sample in tab.2.3 and on all engineered samples using an Instron Dynamometer (mod.2752) in order to evaluate hysteresis and elastic recovery of networks. Samples, characterized by a dog bone shape (fig.2.5), were subjected to 5 cycles of loading and unloading imposing respectively strain of 10%, 15% and 20% at constant speed of 22mm/min. Before performing the tests on all

engineered samples, dynamic traction tests were performed also on 40 samples (20 on left part and 20 on right one) that were cut from a whole piece of leather (DW) along and across backbone direction (fig.2.7) in order to evaluate if dynamic mechanical properties could be affected by sample location on the hide. Moreover, dynamic traction tests were carried out on PDMS (25%PDMS) and elastollan (15%Elastollan) engineered and tanned samples and also on split, engineered and tanned samples (3%Elastollan 685, 3% Elastollan 2180, 5%Elastollan 685+5%PDMS). Hysteresis results were normalized to the first cycle area. Values are reported as mean ±standard deviation, calculated with microsoft excel software.

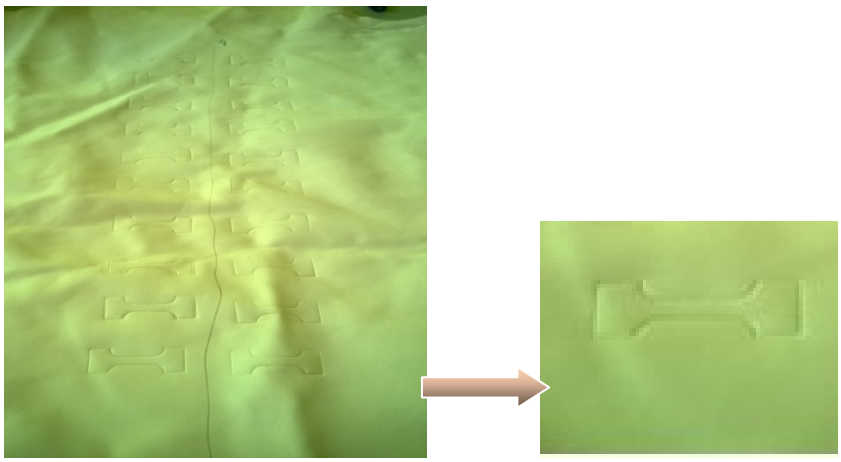

Fig.2.7 Dog bone shape samples taken from whole piece of leather along backbone direction

Nanoindentation tests

The nanoindentation technique has recently emerged as a powerful mean for the measurement of the mechanical properties (micro and nano scales) of tissues and other biomaterials [109,110]. Nanoindentations were performed on engineered tanned samples (samples engineered by using 15%elastollan, chrome tanned and then split) using a Nanoindenter G200 (Agilent Technologies) equipped

with a spherical tip with a 100 μm diameter. NanoSuite software was used to collect and analyze data in order to obtain load–displacement curves and Young's modulus. During measurements, the indenter moved towards the testing sample (loading) up to the imposed indentation depth and then moved back to its original position (unloading).

The samples were placed on a substrate such that movement cannot occur. On each sample 30 nanoindentation locations were selected manually with use of microscope of the Nanoindenter and parameters that have been setted are surface approach distance to 20000 nm, surface approach velocity to 100 nm/s and depth limit to 20000 nm.

Values are reported as mean ±standard deviation, calculated with microsoft excel software.

2.4.2 Morphological analysis

SEM

Electron micrographs of raw samples allow to know morphological details of collagen network while sem images of engineered samples are important in order to know if some treatments denature collagen fibers and how polymers join to the collagen fibers.

SEM examinations, performed at different magnification (100x-1 Kx) e with values of tension between 7 and 20 kV, were carried out using a Ultraplus Zeiss on dry samples that were gold sputtered prior to examination. In particular morphological analysis was done on both raw DW and DB and on engineered DW and DB samples.

Micro CT

A Bruker SkyScan 1172 microCT X-Ray tomography was used to scan engineered chrome tanned samples (15%elastollan and split

15%Elastollan samples) in order to evaluate if polymeric materials was penetrated in the collagen network. They were scanned at 37 kV source voltage and 234 μA source current, without filter and at a resolution of 3.2 μm. After the acquisition, images were reconstructed using the SkyScan NRecon software.

2.4.3 Absorption properties

Contact angle

Water contact angle measurements were performed in order to investigate the wettability of subtrates engineered with polymers. The measurements were carried out with an Attension optical tensiometer (mod. Theta, KSV instruments) at room temperature. Measurements were performed on the samples reported in tab.2.3, on both the flesh and the grain side. On engineered samples (elastollan and elastollan-pdms engineered samples), contact angle were evaluated on split sample without flesh side. In particular drops were put on side that was next to flesh side. At least three measurements were performed for each sample, by placing the liquid drops in different parts of the sample surface. The method used to test samples is called “fast+normal”, volume drop is 4 μl at a falling rate of 1 μl/s . This method allow to acquire 10 images every 512 ms and 25 images every 30s. Values are reported as mean ±standard deviation, calculated with Microsoft excel software.

2.4.4 Thermal analysis

TGA

Thermogravimetric analysis (TGA) is an analytical technique used to determine material's thermal stability and its fraction of volatile components by monitoring the weight change that occurs as a specimen is heated. Thermogravimetric analysis (TGA) were carried out in a TAInstruments Q5000 analyzer on samples reported in tab.2.3. The temperature program consisted of a ramp from 40°C to 750°C at a heating rate of 20° $Cmin^{-1}$. Initial weight of samples was lower than 15 mg and all tests were carried out in triplicate .

DSC

This investigation allow to know the shrinkage temperature of collagen network in every stage of the leather making: soaking, liming, deliming, pickling and tanning [108] and this temperature is the only parameter that allow to define a tanned sample.

Calorimetric analysis was performed with TA Instruments DSC Q20. The temperature program consisted in a ramp from 25°C to 100°C at a heating rate of 5°$Cmin^{-1}$. Each experiment was carried out in triplicate. DSC was performed on sample reported in tab.2.3, on engineered samples and finally on selected engineered and chrome tanned samples. In particular engineered samples subjected to DSC analysis were split specimens (grain side) subjected to thermal treatment, samples treated with 17%Nylon, 25%PDMS and 3%Elastollan 2180 (grain side) samples, while engineered and tanned samples selected were 15%Elastollan (grain side), 3%Elastollan 2180 (grain side) and 25%Pdms. All samples were rehydrated in water for 10 minutes prior examination.

Chapter 3 - Results

3.1 Introduction

The following chapter presents the results of the experimental analysis performed on raw materials and final products in order to define the benchmarks to which the chemical-physical parameters of the engineered products need to be compared. Moreover, in this chapter the results of an experimental campaign performed on engineered and engineered chrome tanned samples were reported. In particular mechanical, morphological, absorption and thermal properties of engineered samples were evaluated in order to extract the relevant physical parameters and to know how the treatments effectively affect the macroscopic properties of the leather. Finally, the results obtained for engineered chrome tanned samples were shown in order to evaluate how the interaction of the performed treatments and tanning agents can affect the properties of engineered networks.

3.2 Mechanical characterization

DMA

Dynamic mechanical analysis were performed on both raw and engineered materials in order to investigate viscoelastic properties of collagen network and to know how polymeric materials can affect either DW and DB substrates. Indeed the dependence of the elastic and viscous moduli upon the frequency were evaluated for samples in tab. 2.3 and for samples in tab.2.5. Viscoelastic properties were evaluated on both substrates, DW and DB and for two type of engineered samples, in order to evaluate how the different properties of substrates affect the penetration of polymers.

Plots of E’ and E’’ against frequency of samples shown in tab.2.3 were reported in figures 3.1 and 3.2 respectively. Collected data were summarized in tab. 3.1. Elastic moduli are higher than the viscous ones, all over the frequency range scanned, of about a factor ten.

E sample presents the highest viscoelastic moduli and D, that results to be the softest sample, displays the lowest viscoelastic moduli.

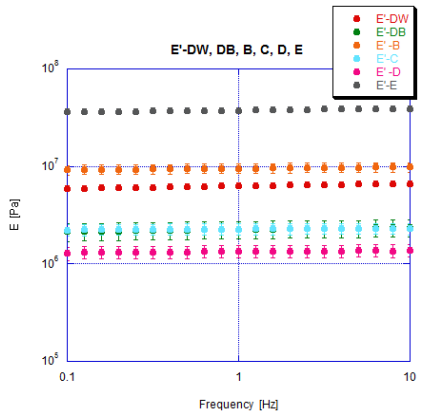

Fig.3.1 Comparison between E’ curves of samples in tab.2.3

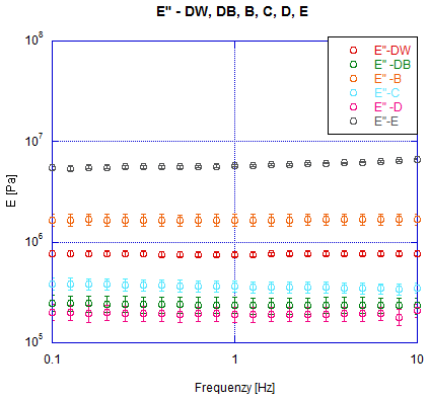

Fig.3.2 Comparison between E’’ curves of samples in tab.2.3

Samples	E' [Pa](1 hz)	E'' [Pa](1 hz)
DW	**$(6.33\pm0.25)*10^6$**	**$(7.59\pm0.35)*10^5$**
DB	**$(2.26\pm0.45)*10^6$**	**$(2.38\pm0.43)*10^5$**
B	**$(9.56\pm0.11)*10^6$**	**$(1.67\pm0.21)*10^6$**
C	**$(2.28\pm0.29)*10^6$**	**$(3.64\pm0.46)*10^5$**
D	**$(1.34\pm0.19)*10^6$**	**$(1.93\pm0.32)*10^5$**
E	**$(3.77\pm0.14)*10^7$**	**$(5.76\pm0.16)*10^6$**

Tab.3.1 E' ed E''values at 1 Hz for samples in tab.2.3

DMA -PDMS and Nylon engineered samples

Samples engineered in order to improve ultimate tensile strength and tear resistance were subjected to dynamic mechanical analysis in order to verify that viscoelastic moduli obtained after treatment with tough polymers don't increase a lot exceeding values range of final products.
Plots of E' and E'' against frequency of samples shown in tab.2.5 were reported in figures 3.3-3.6. Collected data were summarized in tab. 3.2. Elastic moduli are higher than the viscous ones, all over the frequency range scanned, of about a factor ten.
Comparison between E' and E''values are shown for engineered DW and DB samples in figures 3.7 and 3.8 respectively (E' and E'' values of control were reported in both figures). It can be noticed that PDMS engineered samples show lower viscoelastic moduli than their controls for both DW and DB samples, whereas Nylon engineered samples show higher viscoelastic moduli than their controls. The differences

between treated and untreated samples is more evident for DW substrates than DB ones. Moreover comparing Nylon and PDMS treatment it can be noticed that DW and DB Nylon engineered samples show higher E' and E''values than PDMS engineered DW and DB substrates

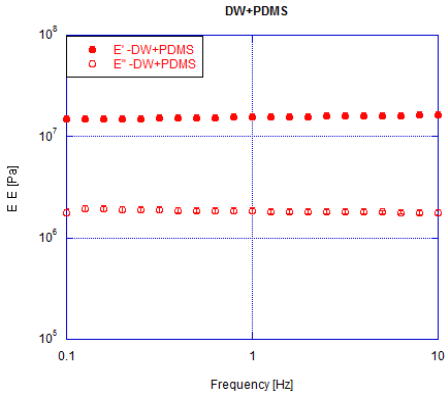

Fig.3.3 E'and E''curves of 25%PDMS engineered DW

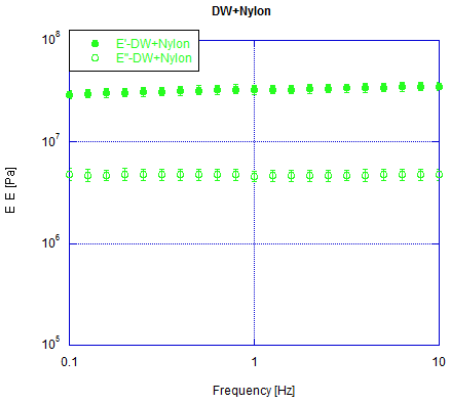

Fig. 3.4 E'and E''curves of Nylon engineered DW

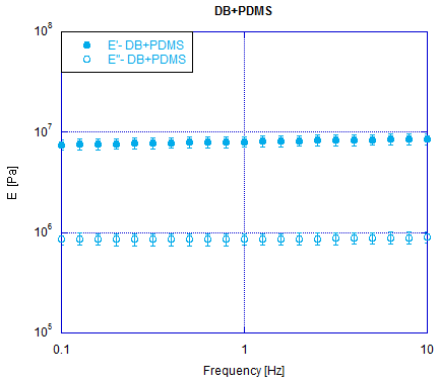

Fig.3.5 E'and E''curves of 25%PDMS engineered DB

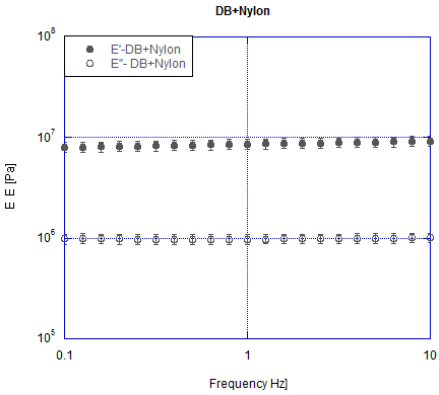

Fig.3.6 E'and E''curves of Nylon engineered DB

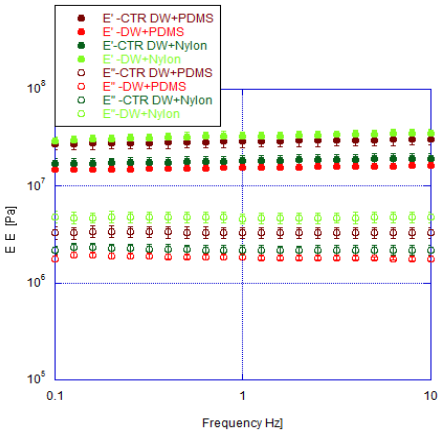

Fig.3.7 Comparison between E' and E''curves of PDMS and Nylon engineered DW

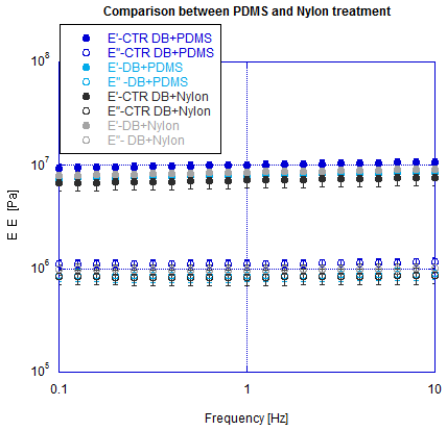

Fig.3.8 Comparison between E' and E''curves of PDMS and Nylon engineered DW

Samples	E' [Pa](1 hz)	E" [Pa](1 hz)
CTR DW+PDMS	$(2.91\pm0.39)*10^7$	$(3.32\pm0.43)*10^6$
DW+PDMS	$(1.56\pm0.001)*10^7$	$(1.84\pm0.09)*10^6$
CTR DB+PDMS	$(1.01\pm0.08)*10^7$	$(1.11\pm0.1)*10^6$
DB+PDMS	$(8.08\pm1.01)*10^6$	$(8.61\pm1.16)*10^5$
CTR DW+Nylon	$(1.83\pm0.23)*10^7$	$(2.2\pm0.23)*10^6$
DW+Nylon	$(3.27\pm0.31)*10^7$	$(4.63\pm0.55)*10^6$
CTR DB+Nylon	$(7.22\pm1.22)*10^6$	$(8.32\pm1.43)*10^5$
DB+Nylon	$(8.63\pm0.99)*10^6$	$(9.77\pm1.04)*10^5$

Tab.3.2 E' ed E'' values at 1 Hz for samples in tab.2.5

Uniaxial tensile traction

Uniaxial tensile traction tests were performed in order to evaluate ultimate tensile strength of raw materials and final leather. Typical stress strain curve of leather samples is shown in fig.3.9; it was obtained for DW sample. Figure 3.10 show a comparison between ultimate tensile strength values of samples in tab.2.3 and values were

summarized in tab.3.3. DW sample displays a higher ultimate tensile strength reaching 21.8 Mpa.

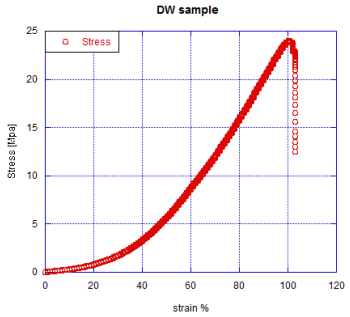

Fig.3.9 DW -Stress-strain curve

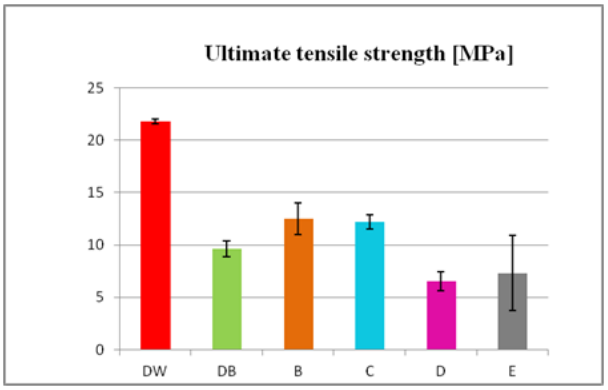

Fig.3.10 Ultimate tensile strength values of samples in tab.2.3

Samples	Ultimate tensile strength [Mpa]
DW	**21.8 ± 0.2**
DB	**9.6±0.7**
B	**12.5 ± 1.5**
C	**12.2 ± 0.7**
D	**6.5 ± 0.9**
E	**7.3 ± 3.6**

Tab.3.3 Ultimate tensile strength and elongation at break values for samples in tab.2.3

Table 3.4 reports ultimate tensile strength, starting and elastic modulus (the first one is the slope of the linear interpolation of the loading curve

in the 0-5% strain range and the second one the slope of the linear region) of samples shown in tab.2.2 and a comparison between ultimate tensile strength is shown in fig. 3.11. The comparison between DW substrates (reported in tab.2.2) shows that there are not any differences between DW and crossbreed DW samples, but ultimate tensile strength of DW-G is lower than DW and DW-C. Moreover starting and linear moduli of DW-G have lower values, as shown in tab.3.4.

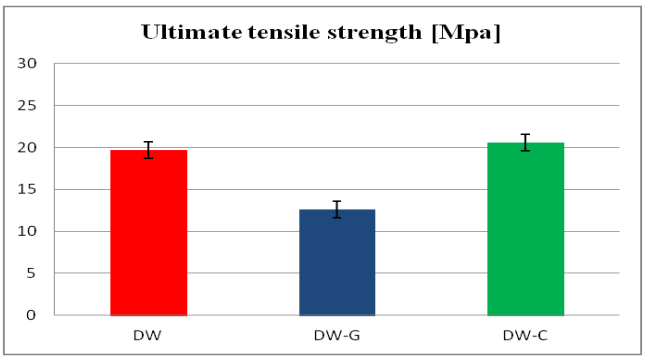

Fig.3.11 Ultimate tensile strength values for samples in tab.2.2

Samples	Ultimate tensile strength[Mpa]	E' –starting modulus	E'-elastic modulus
DW	**19.7±0.3**	**5.4±0.7**	**34.8±1.3**
DW-G	**12.5±1.4**	**4.9±0.5**	**24.5±4.4**
DW-C	**20.6±3.9**	**5±0.5**	**41.2±9.4**

Tab.3.4 Ultimate tensile strength and elastic modulus values for samples in tab.2.2

Uniaxial tensile traction-engineered samples

Uniaxial tensile traction tests were performed in order to evaluate improvement in the ultimate tensile strength after polymeric treatments. Thus these tests were carried out on substrates engineered with polymeric materials selected to improve tensile strength. Engineered substrates shown in tab.2.5 were subjected to uniaxial tensile tests and

values of ultimate tensile strength were summarized in tab.3.5. Moreover comparison of values are clearly displayed in fig.3.12. It can be noticed that engineered DW substrates present higher ultimate tensile strength than engineered DB ones. In particular DW engineered by using PDMS reaches higher values than DW engineered by using Nylon. Moreover in tab.3.6 % changes of the maximum tensile strength values between engineered samples and their controls are reported. Treatment with PDMS increases ultimate tensile strength, thus PDMS treated samples show ultimate tensile strength values always higher than control. Whereas a decrease in the ultimate tensile strength is evident in Nylon treated samples (both DW and DB substrates)

DW samples treated respectively with 25% and 30% of PDMS (spray methodology) were tanned and subjected to uniaxial tensile tests. Comparison between ultimate tensile strength values of control and treated samples are show in fig. 3.13 and % changes of the maximum tensile strength values compared to the controls are reported in tab.3.7. Results validate that treatment with PDMS increase ultimate tensile strength and it can be noticed that the interaction with tanning agents does not affect negatively the PDMS treatment. In particular 25% PDMS engineered sample reaches higher ultimate tensile strength values, as show in table 3.7.

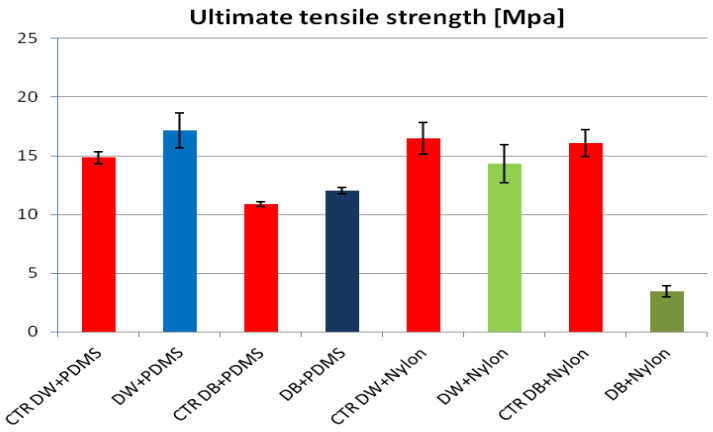

Fig.3.12 Ultimate tensile strength values for samples in tab.2.5

Samples	Ultimate tensile strength [Mpa]
CTR DW+PDMS	14.8 ± 0.5
DW+PDMS	17.2 ± 1.5
CTR DB+PDMS	10.9±0.2
DB+PDMS	12± 0.3
CTR DW+Nylon	16.5 ± 1.4
DW+Nylon	14.3±1.6
CTR DB+Nylon	16.1 ± 1.2
DB+Nylon	3.5± 0.5

Tab.3.5 Ultimate tensile strength values for samples in tab.2.5

Ctr vs engineered samples	% changes
DW+PDMS	16%
DB+PDMS	10%
DW+Nylon	-13%
DB+Nylon	-78%

Tab.3.6 %changes of the ultimate tensile strength values of samples in tab.2.5

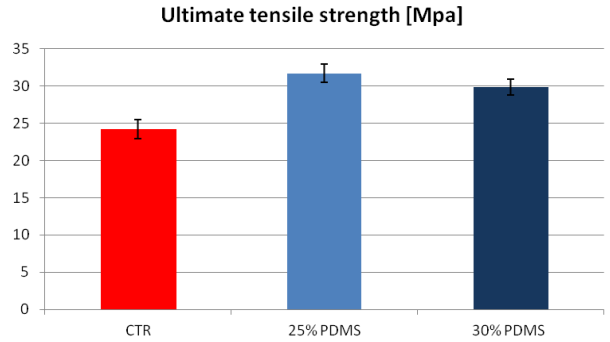

Fig.3.13 Ultimate tensile strength values for PDMS engineered and tanned samples

Engineered and tanned samples	
Ctr vs engineered samples	**% changes**
DW+25%PDMS	**33%**
DW+30%PDMS	**25%**

Tab.3.7 PDMS treated and untreated samples-%changes of the ultimate tensile strength values

Tear resistance - engineered samples

Tear resistance tests were performed in order to evaluate improvement of tear resistance after polymeric treatments. Thus these tests were carried out on substrates engineered with polymeric materials selected to improve this mechanical property.

Tear resistance comparison values of samples in tab.2.5 are shown in fig.3.14. Both treatments (PDMS and Nylon) increase tear resistance of DW and DB substrates. Tables 3.8 and 3.9 display tear resistance values and % changes between treated and untreated samples respectively. It can be noticed that DW+Nylon sample attains higher tear resistance value reaching 28 N and increasing of 154% than the control. DW samples treated respectively with 25% and 30% of PDMS (spray methodology) were tanned and subjected to tear resistance tests.

Comparison between tear resistance values of treated and untreated samples are show in fig. 3.15 and % changes of the tear resistance values compared to the controls are reported in tab.3.10. Results validate that treatment with PDMS increase tear resistance; moreover increasing % of PDMS higher values are obtained, as shown in tab. 3.10.

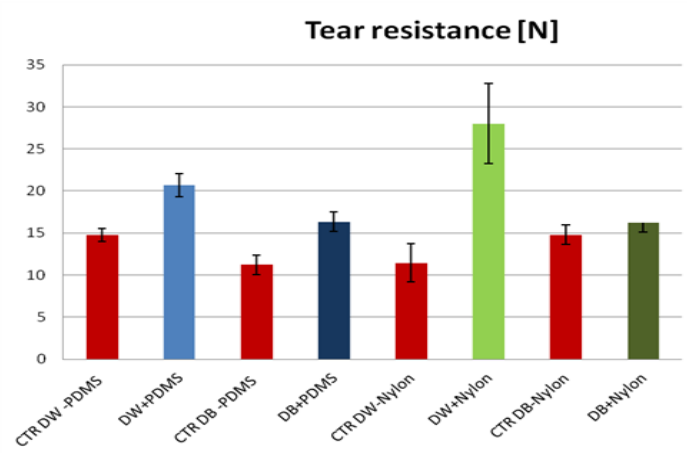

Fig 3.14 Maximum tear resistance for samples in tab.2.5

Samples	Tear resistance [N]
CTR /DW+PDMS	15±0.8
DW+25%PDMS	21±1.4
CTR /DB+PDMS	11±1.2
DB+25%PDMS	16±1.1
CTR /DW+Nylon	11±2.3
DW+Nylon	28±4.7
CTR/DB+Nylon	15±1.1
DB+Nylon	16±1.1

Tab.3.8 Maximum tear resistance values for samples in tab.2.5

CTR vs engineered samples	% changes
DW+PDMS	40%
DB+PDMS	45%
DW+Nylon	154%
DB+Nylon	7%

Tab.3.9 %Differences in the maximum tear resistance between treated and untreated samples

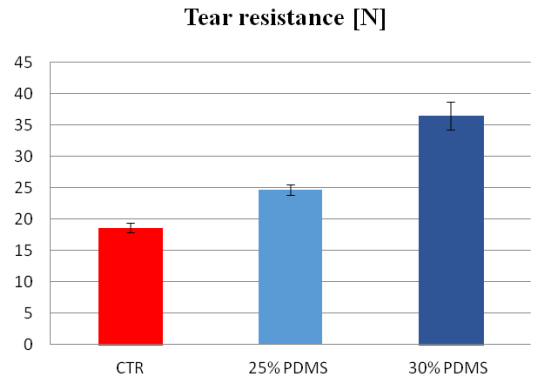

Fig 3.15 PDMS treated(blue) and untreated samples(red)- Maximum tear resistance values

Engineered and tanned samples	
Ctr vs trattato	**Variazione%**
DW+25%PDMS	**32%**
DW+30%PDMS	**89%**

Tab.3.10 %Differences in the maximum tear resistance between treated and untreated samples

Dynamic traction test

Dynamic traction test were carried out in order to evaluate improvement in the elastic recovery of engineered samples.

The evolution of the stress-strain curves under dynamic cyclic deformation was conveniently described by two parameters: permanent strain (ε_r) that is the strain at zero stress in the unloading curves (the strain at zero stress in the unloading curves of the second cycle) and the hysteresis that is the area included between loading and unloading curve (hysteresis area, obtained keeping out only the first cycle, was normalized to the first cycle area). Following figures show hysteresis (a) and permanent strain (b) values of treated and untreated samples; red color is used to indicate untreated samples.

Raw materials

Typical stress-strain response of dry white substrate is reported in fig.3.16. Results obtained from tests performed on 40 samples (20 on the left hand side and 20 on the right hand side) in order to evaluate how dynamical mechanical properties could be affected by sample location on the hide, are shown in fig.3.17 and 3.18. Fig.3.17 reports comparison between left and right hand side, for inner side (a) and outside (b) respectively and fig.3.18 displays comparison between inner and outside for left(a) and right (b) hand side respectively. It can be

noticed that there is not a evident variability and hysteresis values are 0.4-0.5 MJ/m^3. Diagrams shown in fig. 3.19 show respectively a comparison between hysteresis values and permanent set values obtained for substrates presented in tab.2.2. It can be noticed that no significant differences are observed between three DW substrates as regard to dissipate energy during deformation and elastic recovery.

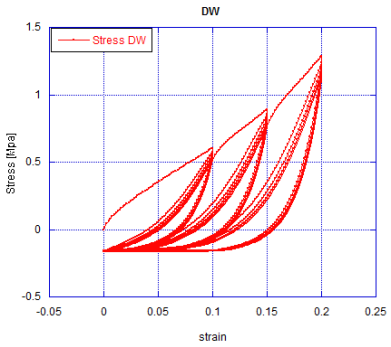

Fig 3.16 DW- stress-strain curve

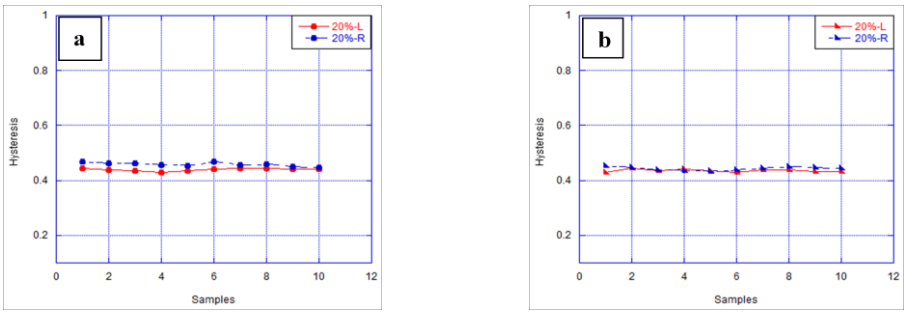

Fig 3.17 Comparison of hysteresis values (left hand side vs right hand side) between samples along backbone direction; (a)inner side ; (b) outside

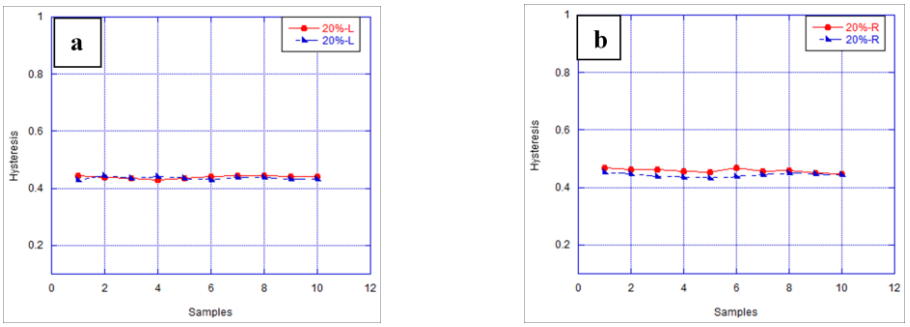

Fig 3.18 Comparison of hysteresis values (inner side vs out side) between samples along backbone direction; (a) left hand side; (b) right hand side

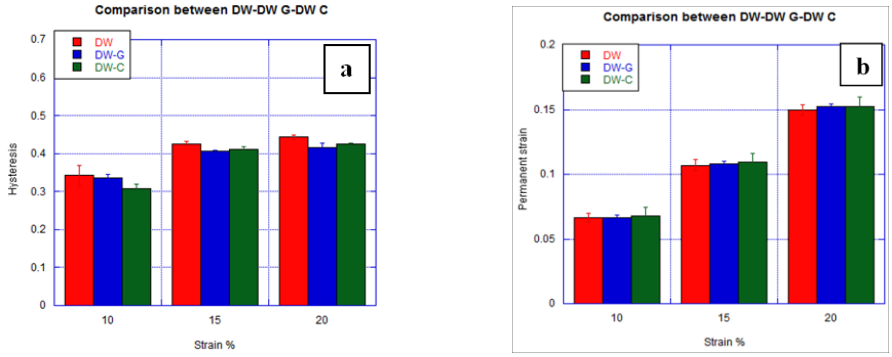

Fig 3.19 Hysteresis (a) and permanent strain (b) values obtained for substrates in tab.2.2

Enzymatic treatments

Enzymatic treatments should affect mechanical properties of DW samples conferring them an more open fiber structure. Thus enzymatic treatments were performed on DW substrates in order to evaluate how enzymatic treatment influence hysteresis and permanent strain. Results of dynamic traction tests performed on samples treated by using Basozyme enzyme, are reported in the following figures. In particular figures 3.20, 3.21 and 3.22. shown results obtained after 5%Basozyme treatment for 30 minutes, 1 h and 3 h respectively. It can be observed that there are not evident differences between control and treated samples, even if decreasing time of treatment (fig.3.20) treated samples seem to have a lower hysteresis, as shown at 10% imposed strain.

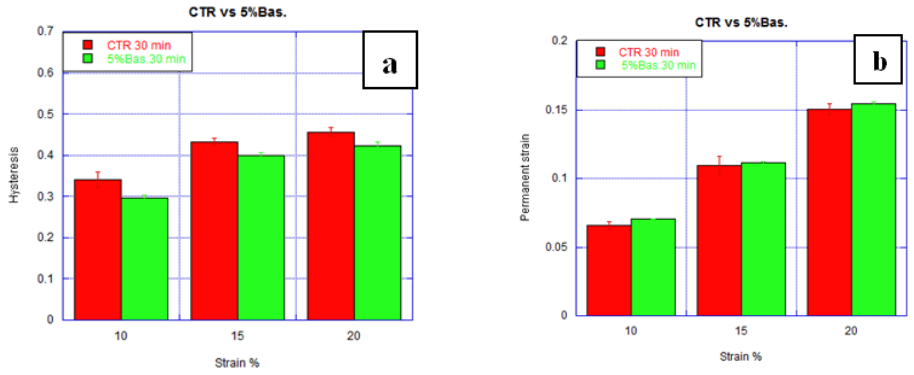

Fig 3.20 Hysteresis (a) and permanent strain (b) values obtained, at three imposed strain, before and after 5%Basozyme treatment for 30 min .

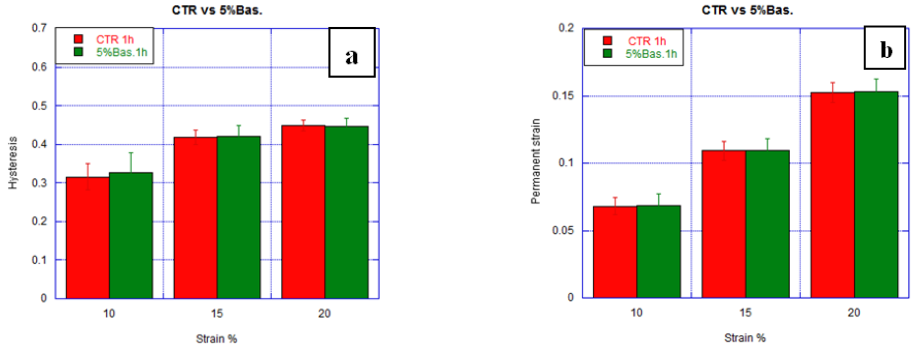

Fig.3.21 Hysteresis (a) and permanent strain (b) values obtained at three imposed strain before and after 5%Basozyme treatment for 1h

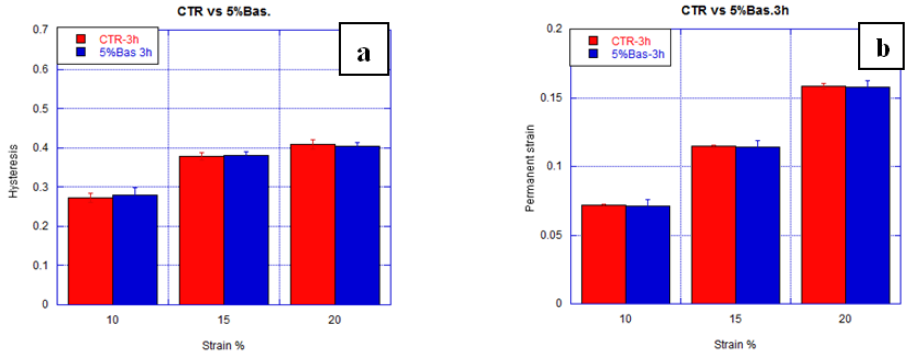

Fig 3.22 Hysteresis (a) and permanent strain (b) values obtained at three imposed strain before and after 5%Basozyme treatment for 3h

Thermal treatments

Because heating can induce molecular change and consequently the formation of crosslinks, thermal treatments were performed in order to improve elastic recovery. Hysteresis and permanent strain values of samples stored overnight in oven at 90°C are reported in figure 3.23. From comparisons with their controls it can be noticed that considered high temperature does not affect mechanical properties of DW substrates. Results of split samples, both flesh and grain side, that were subjected to higher temperature (8h at 140°C) are displayed in fig.3.24-3.27. In particular fig. 3.24 and 3.25 show dried samples results (samples and controls were stored 24 h in oven under vacuum at 37°C) respectively of flash and grain side and fig. 3.26 and 3.27 report incubated sample results (in conditioning room at 37°C and 98%umidity). High temperature has a higher effect on incubate samples, as clearly shown in table 3.11 that reports hysteresis and permanent strain % difference between controls and treated samples (at 15% imposed strain). Mechanical properties of incubated grain side samples are mainly affect from temperature; indeed hysteresis values increase of 21% and permanent strain values decrease of 22%.

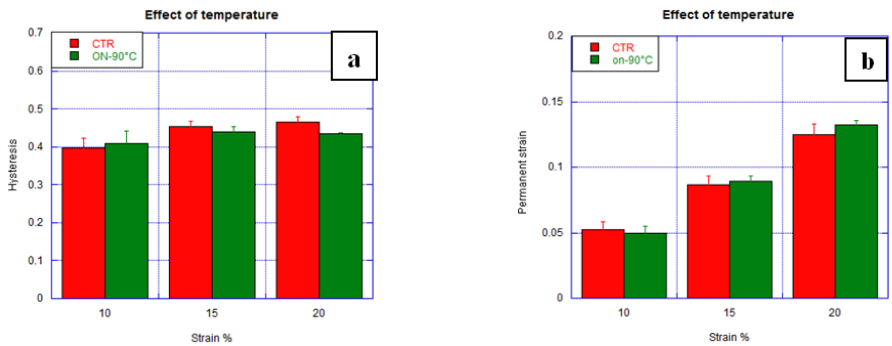

Fig.3.23 Hysteresis (a) and permanent strain (b) values obtained, at three imposed strain, before and after thermal treatment at 90°C

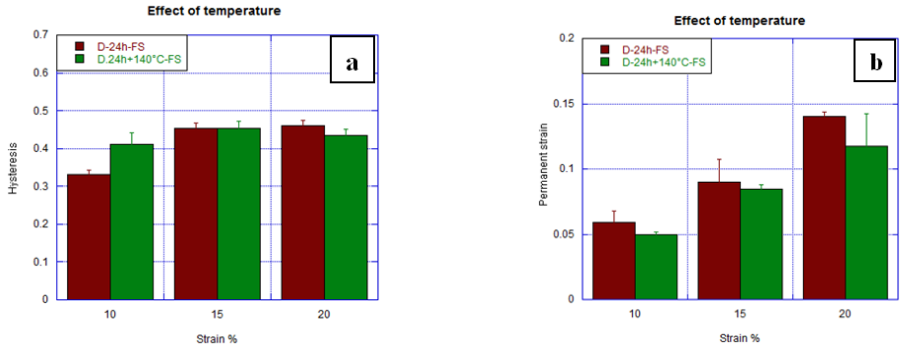

Fig.3.24 Hysteresis (a) and permanent strain (b) values obtained at three imposed strain for DW sample (flesh side) stored 24 h in dryer and subjected at 140°C for 8 h

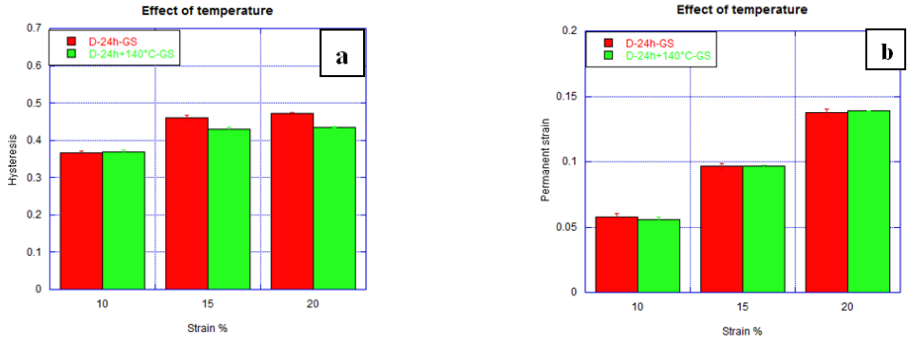

Fig.3.25 Hysteresis (a) and permanent strain (b) values obtained at three imposed strain for DW sample (grain side) stored 24 h in dryer and subjected at 140°C for 8 h

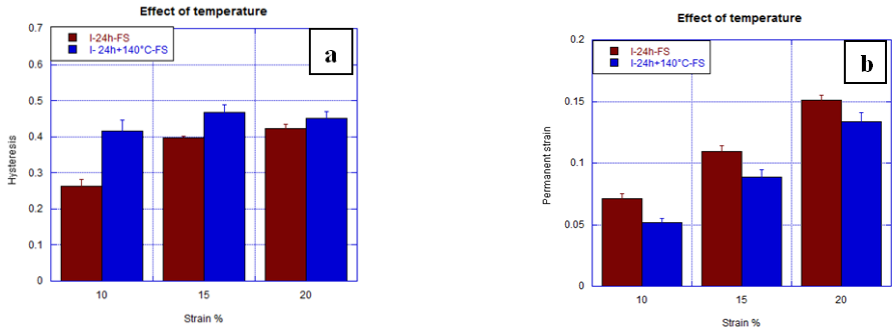

Fig.3.26 Hysteresis (a) and permanent strain (b)values obtained at three imposed strain for DW sample (grain side) stored 24 h in conditioning room and subjected at 140°C for 8 h

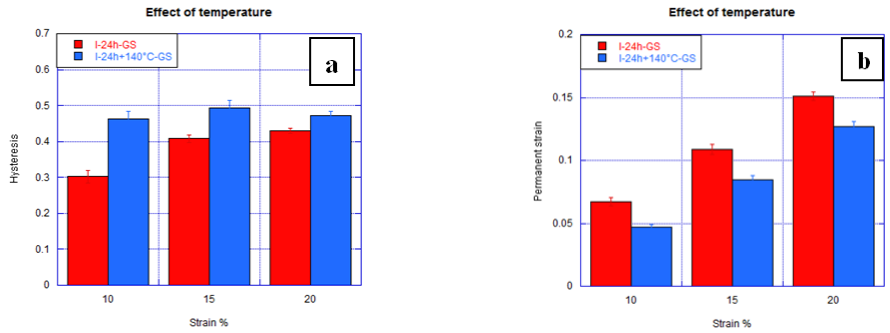

Fig.3.27 Hysteresis (a) and permanent strain (b) values obtained at three imposed strain for DW sample (grain side) stored 24 h in conditioning room and subjected at 140°C for 8 h

Samples	%hysteresis difference between controls and treated samples[15% strain]	% permanent strain difference between controls and treated samples[15%strain]
Dried flesh side	**0,3**	**-6**
Dried grain side	**-6**	**-0,02**
Incubated flesh side	**18**	**-19**
Incubated grain side	**21**	**-22**

Tab.3.11 % Changes in the hysteresis and permanent strain values between controls and treated samples

Polymeric treatments

Nylon treatments

Hysteresis and recovery strain values of Nylon engineered and untreated samples are shown respectively in fig.3.28 (a) and (b). From comparison it can be noticed Nylon does not affect hysteresis and elastic recovery of dry white substrates.

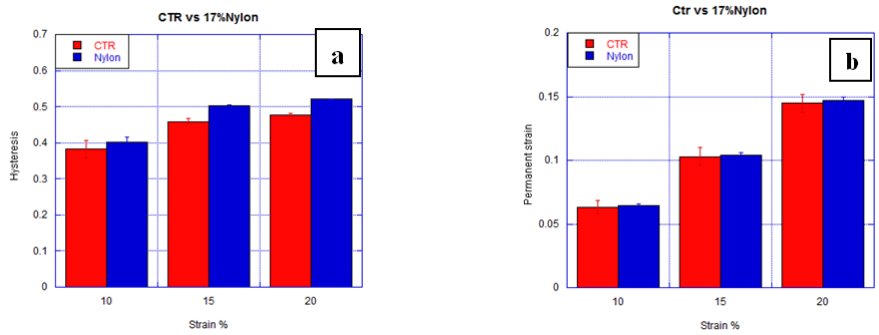

Fig.3.28 Hysteresis (a) and permanent strain (b) values obtained, at three imposed strain, before and after Nylon treatment

PDMS treatments

Hysteresis and recovery strain values of 40%PDMS engineered and untreated samples are shown in the following figures. Results obtained after PDMS treatment, performed by using hexane as solvent, are reported in fig.3.29 while figure 3.30 displays hysteresis and permanent strain values obtained after PDMS treatment, performed by using THF as solvent. As regards to hysteresis values, it can be noticed a slight increase after PDMS treatment in THF, that is significant only at 10% imposed strain. Both treatments have not a significant effect on elastic recovery of samples, as shown in comparison in fig. 3.31.

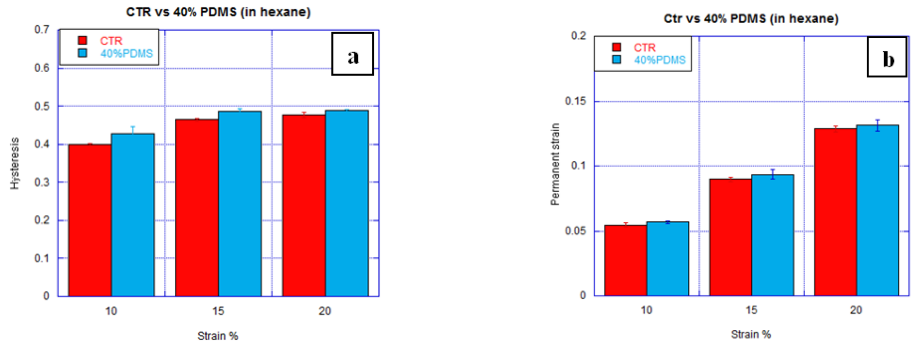

Fig.3.29 Hysteresis (a) and permanent strain (b) values obtained at three imposed strain before and after PDMS treatment by using hexane.

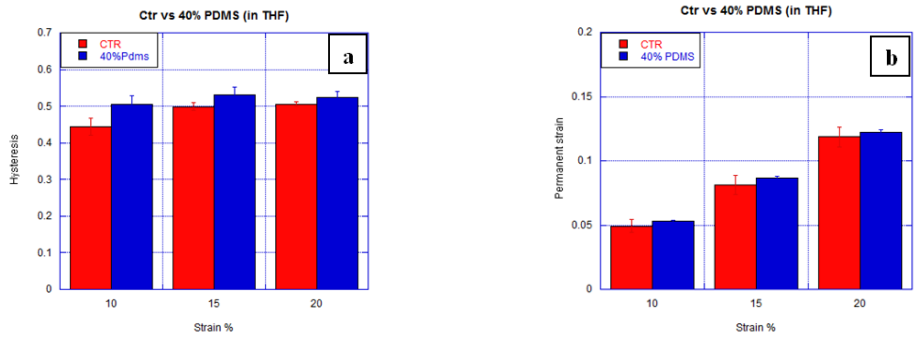

Fig.3.30 Hysteresis (a) and permanent strain (b) values obtained at three imposed strain before and after PDMS treatment by using THF .

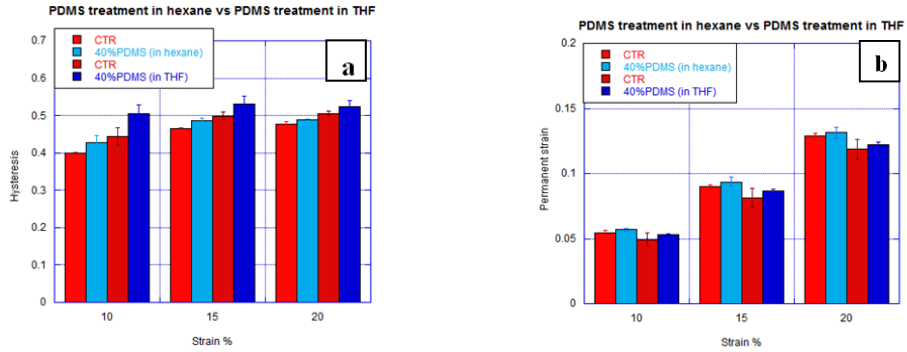

Fig.3.31 Comparison between hysteresis (a) and permanent strain (b) values for PDMS engineered samples by using different solvent.

Elastollan treatments

Results of elastollan treatments at different percentage (3%, 5%,10% and 15%), performed with the drop by drop methodology are shown in fig. 3.32-3.35. It can be noticed that increasing the amount of polymeric materials used in the treatments, the differences between treated and untreated samples become more evident. In particular it can be observed an increase of hysteresis and a decrease of permanent strain values.

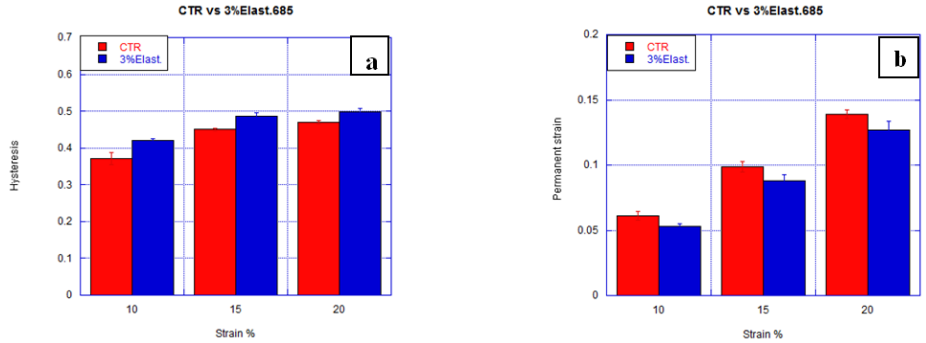

Fig.3.32 Hysteresis (a) and permanent strain (b) values obtained, at three imposed strain, before and after 3%elastollan treatment

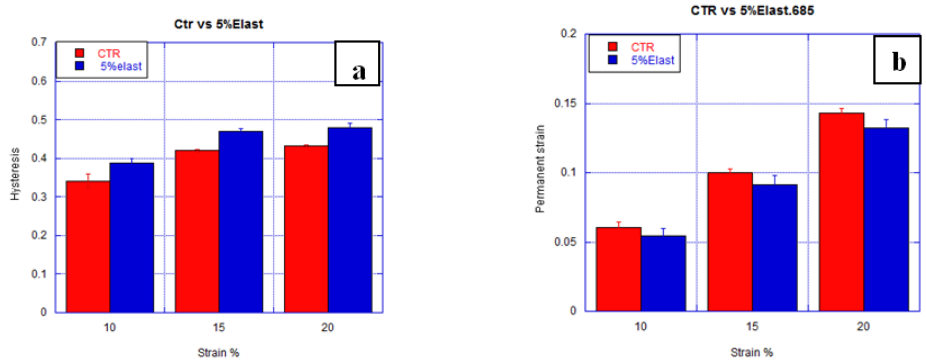

Fig.3.33 Hysteresis (a) and permanent strain (b) values obtained, at three imposed strain, before and after 5%elastollan treatment

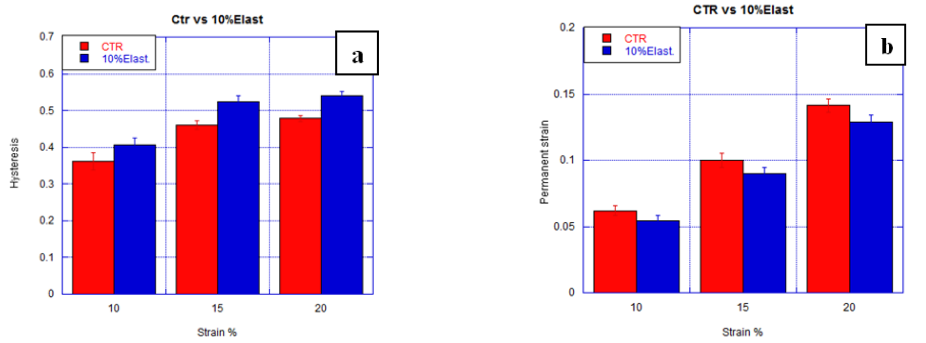

Fig.3.34 Hysteresis (a) and permanent strain (b) values obtained, at three imposed strain, before and after 5%elastollan treatment

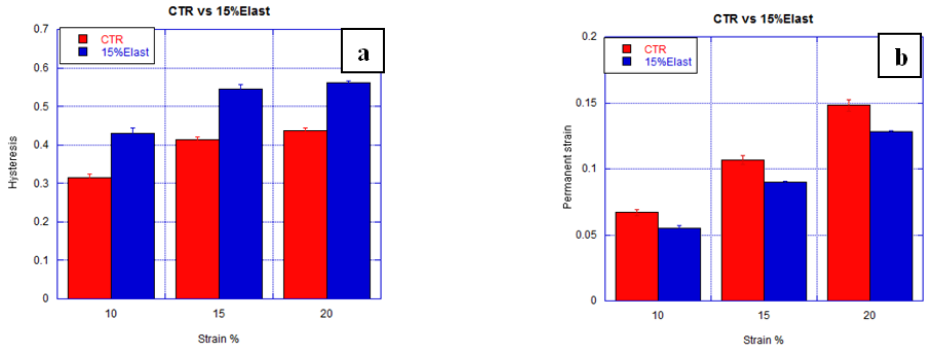

Fig.3.35 Hysteresis (a) and permanent strain (b) values obtained, at three imposed strain, before and after 15%elastollan treatment

The figures 3.36 and 3.37 report the results obtained after 15% elastollan treatment performed with two different methodologies: by using rotavapor and by using a roll. Hysteresis and permanent strain results of both methodologies validate the results obtained after drop by drop treatment. Collective data of elastollan treatments are listed in tab.3.12, that shows the % changes between engineered and controls samples.

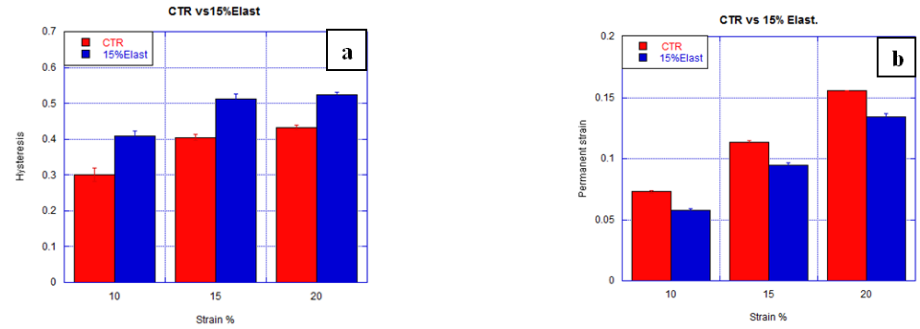

Fig.3.36 Hysteresis (a) and permanent strain (b) values obtained, at three imposed strain, before and after 15%elastollan treatment (by using rotavapor)

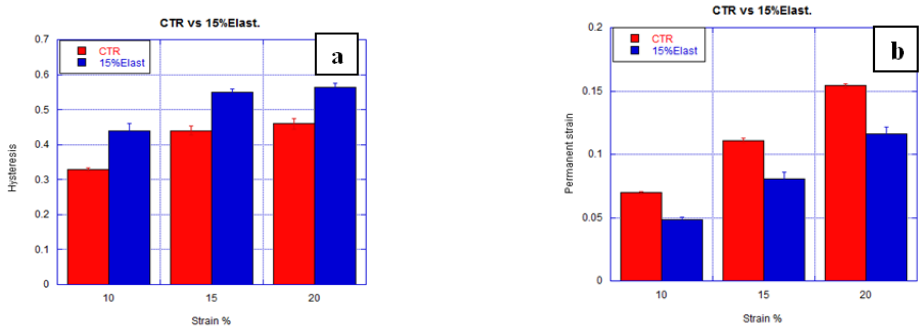

Fig.3.37 Hysteresis (a) and permanent strain (b) values obtained, at three imposed strain, before and after 15%elastollan treatment (by using roll)

Samples - Elastollan 685 treatments	%hysteresis difference between controls and treated samples [15%strain]	% permanent strain difference between controls and treated samples[15%strain]
3%elastollan	**7**	**-11**
5%elastollan	**12**	**-8**
10%elastollan	**14**	**-11**
15%elastollan	**32**	**-24**
15%elastollan (rotavapor)	**26**	**-17**
15%elastollan (roll)	**25**	**-27**

Tab.3.12 % Changes in the hysteresis and permanent strain values between controls and elastollan 685 treated samples

Drop by drop ecoflex treatments

Results obtained after 10% and 20% ecoflex treatments are shown in fig. 3.38 and 3.39 respectively. It can be noticed that there is a higher difference between untreated and treated samples increasing polymer concentration. In particular hysteresis values of treated samples increase, while permanent strain values decrease. Comparison between hysteresis and permanent strain values of two treatments (10% and 20% ecoflex) is reported in fig. 3.40 and it clearly displays a higher

difference between untreated and treated samples for 20% ecoflex treatment.

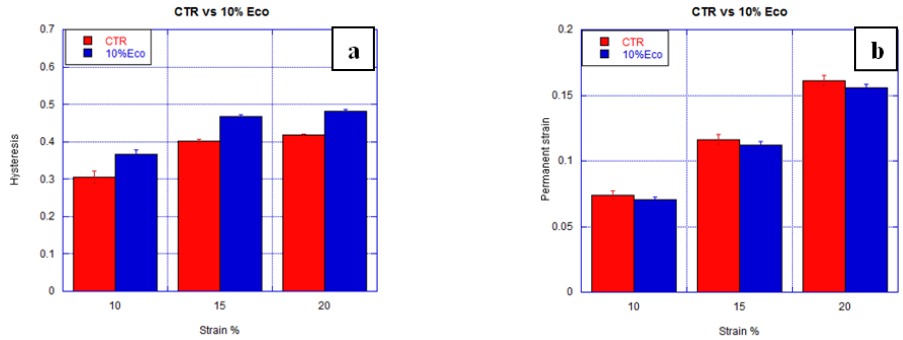

Fig.3.38 Hysteresis (a) and permanent strain (b) values obtained at three imposed strain before and after 10% Ecoflex treatment

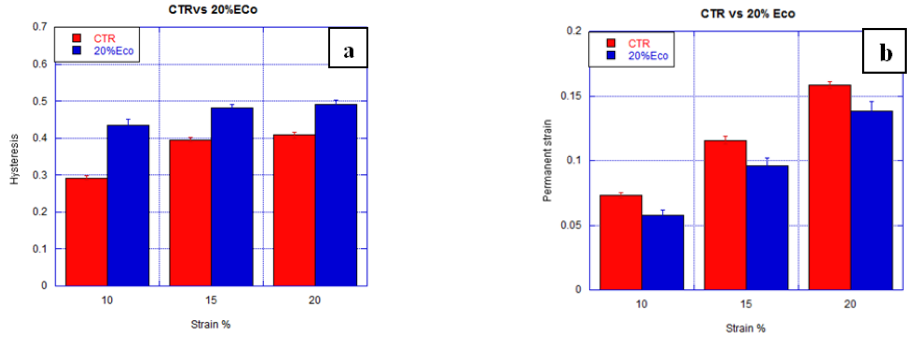

Fig.3.39 Hysteresis (a) and permanent strain (b) values obtained at three imposed strain before and after 20% Ecoflex treatment

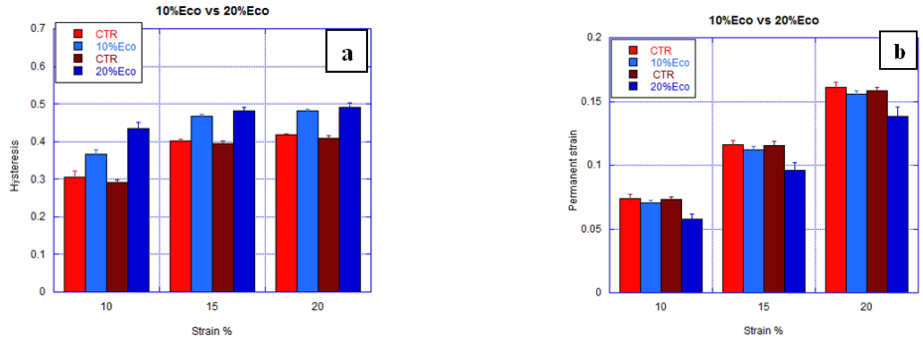

Fig. 3.40 Comparison between hysteresis (a) and permanent strain (b) values obtained at three imposed strain for 10% and 20% Ecoflex engineered samples.

Polymeric blend treatment with Elastollan 685 and Ecoflex

Figure 3.41 shows hysteresis and permanent strain results after blend polymeric treatment with 10% elastollan and 10% ecoflex. It can be noticed that treated samples show an increase of hysteresis values and a decrease of permanent strain; thus same results of above reported Elastollan and ecoflex treatments are obtained.

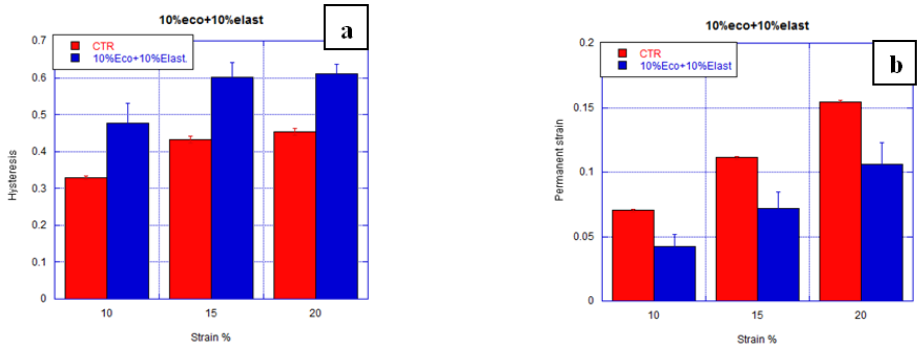

Fig3.41 Hysteresis (a) and permanent strain (b) values obtained at three imposed strain after polymeric blend treatment

Polymeric treatments: flesh side vs grain side

The outcome of the elastomeric treatments was evaluated separately for flesh and grain side in order to investigate how the different structure of two sides can influence absorption polymeric solutions. The figures 3.42 and 3.43 show results obtained by engineering with 15%elastollan respectively flesh and grain side and the comparison between flesh and grain side treatment is reported in fig. 3.44. As regard to hysteresis values a slight difference between treated and untreated samples is shown for both treatments, on flash and grain side, but a higher increase can be noticed for treatment performed on flesh side. As regard to the elastic recovery properties, 15%elastollan decrease permanent strain values of treated flesh side but the treatment

performed on grain side doesn't affect permanent strain values, as show in fig.3.43 and in the comparison reported in fig. 3.44.

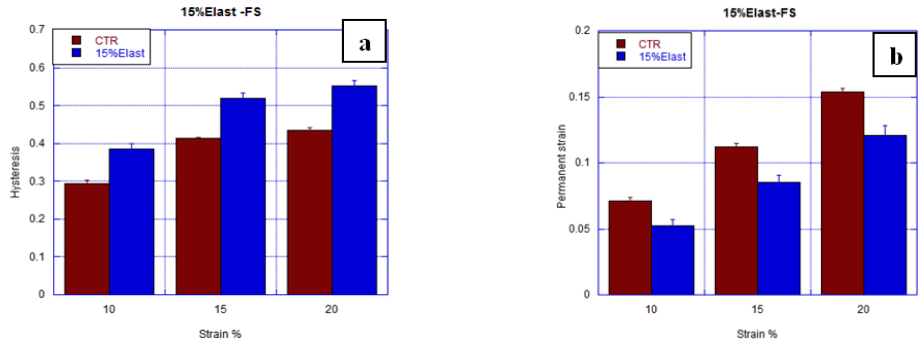

Fig.3.42 Hysteresis (a) and permanent strain (b) values obtained at three imposed strain before and after 15% Elastollan treatment (flesh side)

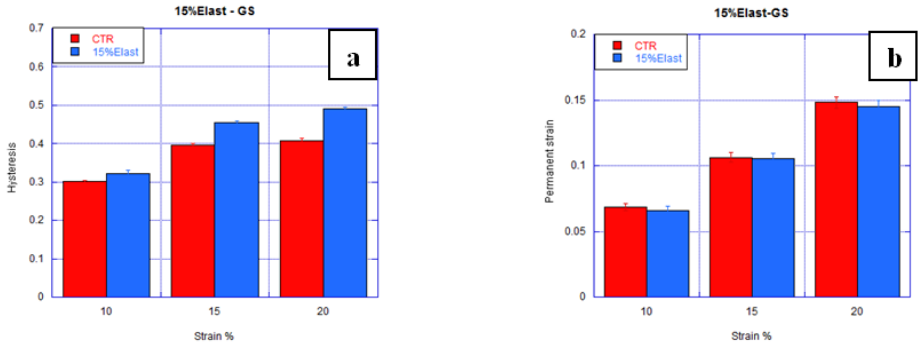

Fig.3.43 Hysteresis (a) and permanent strain (b) values obtained at three imposed strain before and after 15% Elastollan treatment (grain side)

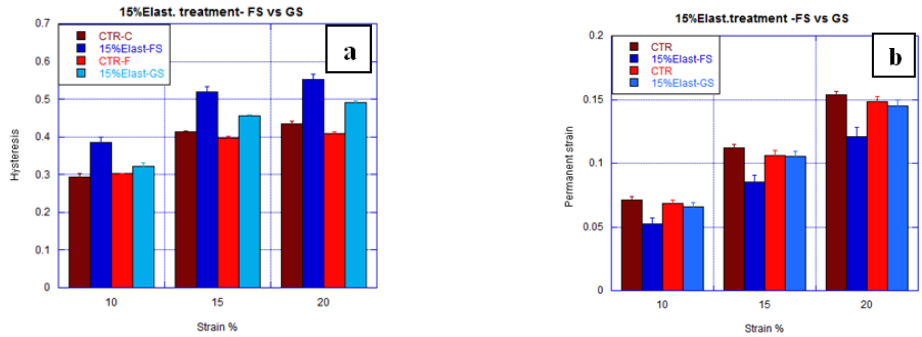

Fig.3.44 Comparison between flesh and grain side- hysteresis (a) and permanent strain (b) values obtained at three imposed strain after 15% elastollan treatment.

Polymeric treatments on split samples

Selected treatments (3%elastollan 685, 3%elastollan 2180 and polymeric blend with 5%elast685+5%pdms) were performed on grain side by using lower percentage of polymeric materials and results are reported in the following figures. Once again it can be observed a slight increase of hysteresis values and a decrease of permanent strain values after polymeric treatments.

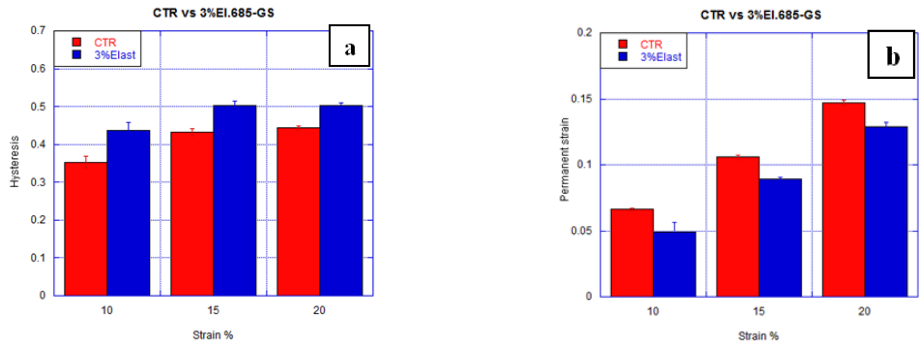

Fig.3.45 Hysteresis (a) and permanent strain (b) values obtained at three imposed strain before and after 3% Elastollan 685 engineered samples (grain side)

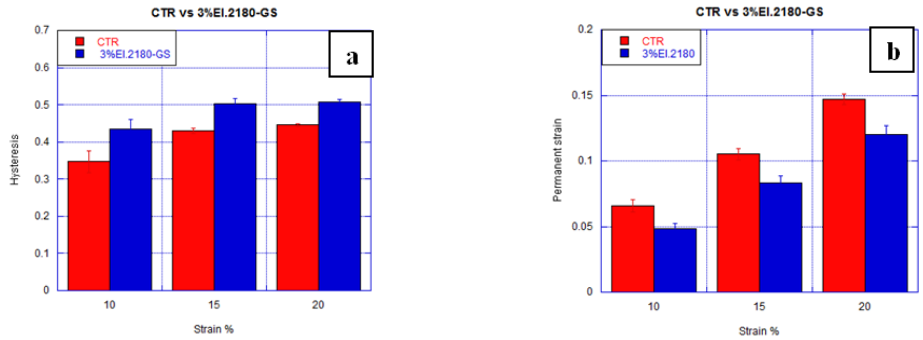

Fig.3.46 Hysteresis (a) and permanent strain (b) values obtained at three imposed strain before and after 3% Elastollan 2180 engineered samples (grain side)

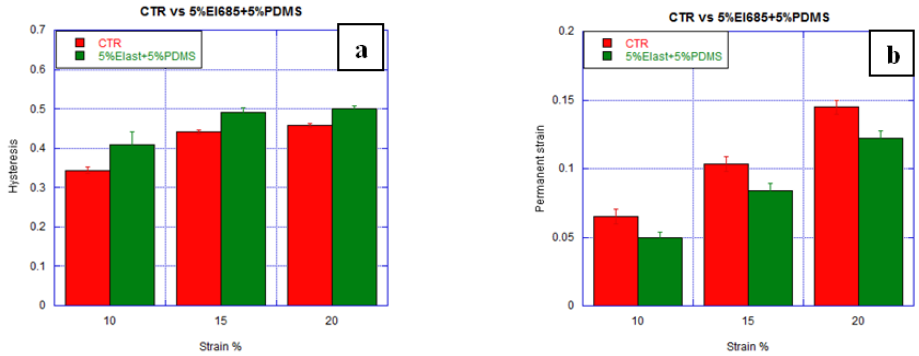

Fig.3.47 Hysteresis (a) and permanent strain (b) values obtained at three imposed strain before and after polymeric bland (PDMS+elastollan 685) treatment (grain side)

Polymeric treatments on different DW substrates

In order to evaluate if DW-G, that should have a more open structure, could amplify the effect of polymeric treatment, the best elastollan treatment was performed also on this substrate. Figure 3.48 shows obtained results of DW-G samples after treatment with 15%elastollan. Once again it can be noticed that treated DW-G substrate show an increase of hysteresis values and a decrease of permanent strain values, but the comparison between treatment performed on DW and DW-G (fig. 3.49) shows a more evident difference between treated and untreated DW than treated and untreated DW-G. It can be observed in % change values between treated and untreated samples, reported in tab. 3.13.

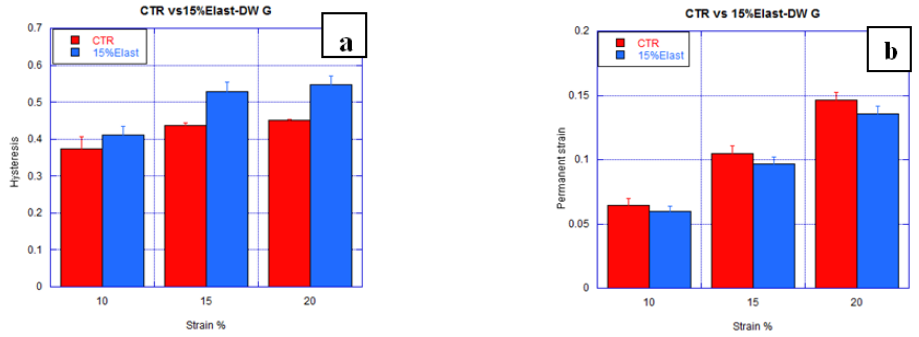

Fig. 3.48 Hysteresis (a) and permanent strain (b) values obtained at three imposed strain before and after 15% elastollan engineered samples (DW-G)

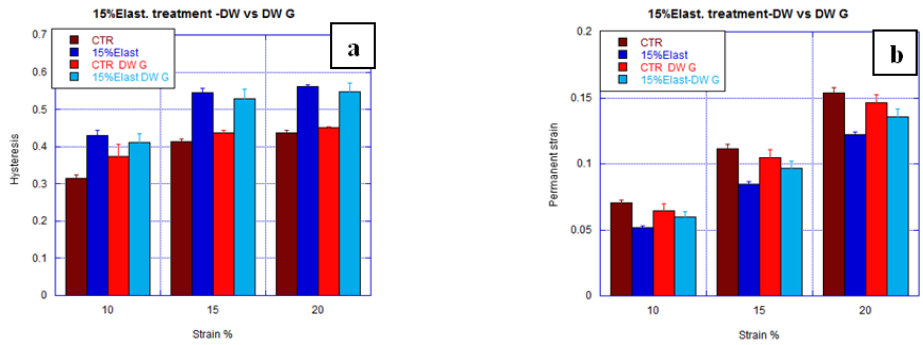

Fig. 3.49 Comparison between hysteresis (a) and permanent strain (b) values obtained at three imposed strain for 15% elastollan engineered samples (DW vs DW-G) .

Samples - Elastollan 685 treatments	%hysteresis difference between controls and treated samples [15% strain]	% permanent strain difference between controls and treated samples[15%strain]
15%elastollan -DW	**32**	**-24**
15%elastollan –DW-G	**21**	**-8**

Tab.3.13 DW vs DW-G- %Changes in the hysteresis and permanent strain values between controls and elastollan 685 treated samples

Engineered leather samples

Selected engineered samples were chrome tanned, dyed and subjected to the finishing operations before to be characterized by dynamical

traction tests in order to evaluate if interaction between polymeric materials and tanning agents has a positive or negative effect on obtained results. Following figures show hysteresis and permanent strain results.

PDMS engineered leather samples

The figures 3.50 and 3.51 report hysteresis and permanent strain values obtained by performing dynamical traction test on PDMS engineered tanned samples. In particular fig.3.50 reports results of 25% PDMS treatment performed with immersion methodology while fig.3.51 shows results of 25% PDMS treatment performed with spray methodology. Both results show that PDMS treatments have not an evident effect on hysteresis and elastic recovery. Moreover a comparison between two treatments is reported in fig. 3.52 and it can be noticed that only after PDMS treatment with spray methodology permanent strain values increase; therefore samples present a lower elastic recovery.

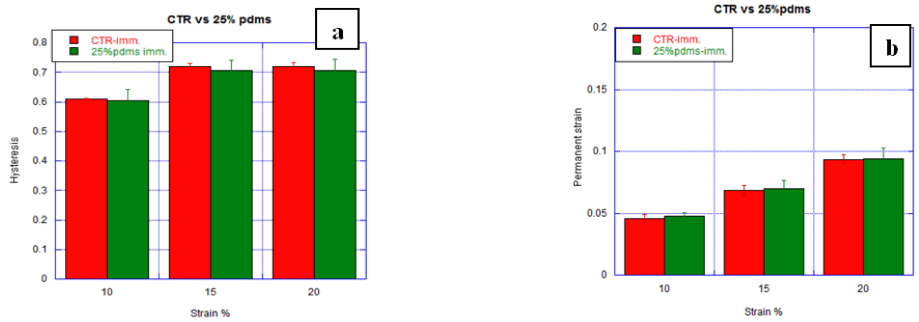

Fig.3.50 Hysteresis (a) and permanent strain (b) values obtained at three imposed strain before and after 25% PDMS treatment (immersion methodology)

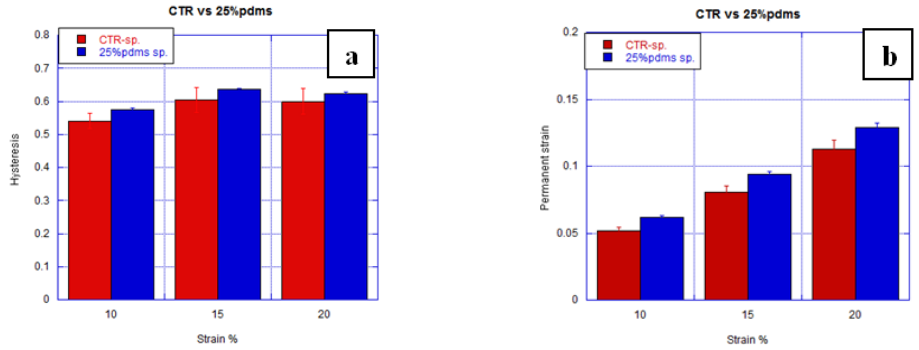

Fig.3.51 Hysteresis (a) and permanent strain (b) values obtained at three imposed strain before and after 25% PDMS treatment (spray methodology)

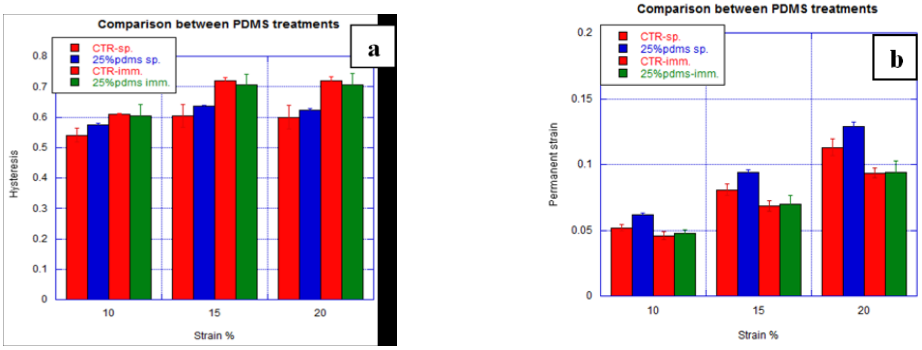

Fig.3.52 Comparison between hysteresis (a) and permanent strain (b) values obtained at three imposed strain for 25% PDMS engineered samples (immersion vs spray methodology)

Elastollan engineered leather samples :flesh vs grain side

Elastollan samples, realized by drop by drop methodology, were chrome tanned and split in order to perform dynamic traction tests separately on flesh and grain side. Hysteresis and permanent strain results of flesh and grain side are shown respectively in fig.3.53 and 3.54 and the comparison between flesh and grain side is reported in fig.3.55. Results show that hysteresis values change only on grain side; they increase after treatment, while permanent strain values changes for both sides. In particular after treatment both, flash and grain side, show

a lower permanent strain but the difference between treated and untreated sample is more evident for flesh side.

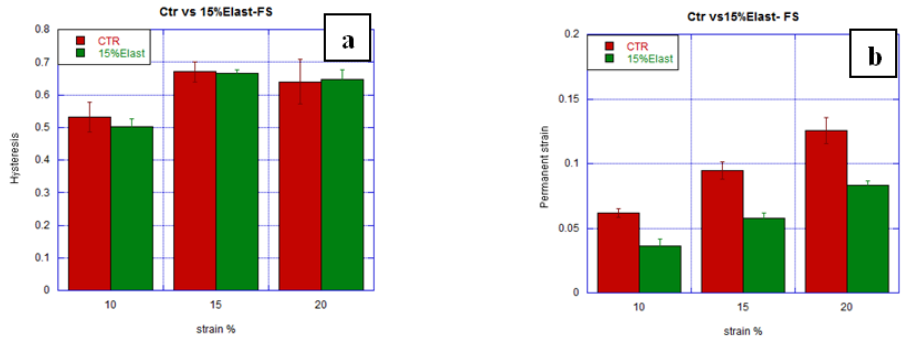

Fig 3.53 Hysteresis (a) and permanent strain (b) values obtained at three imposed strain before and after 15% elastollan treatment (flesh side)

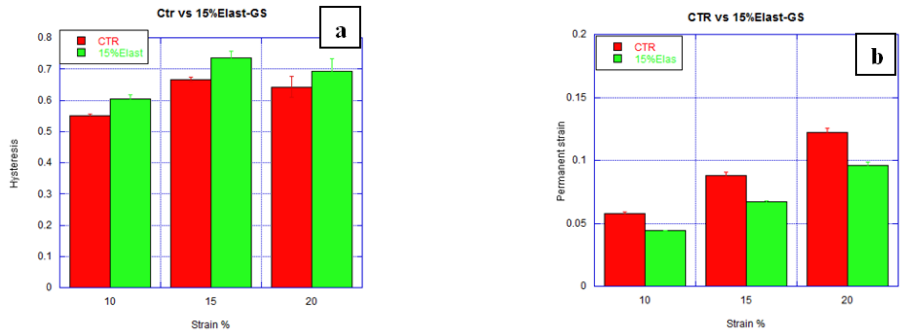

Fig.3.54 Hysteresis (a) and permanent strain (b) values obtained at three imposed strain before and after 15% elastollan treatment (grain side)

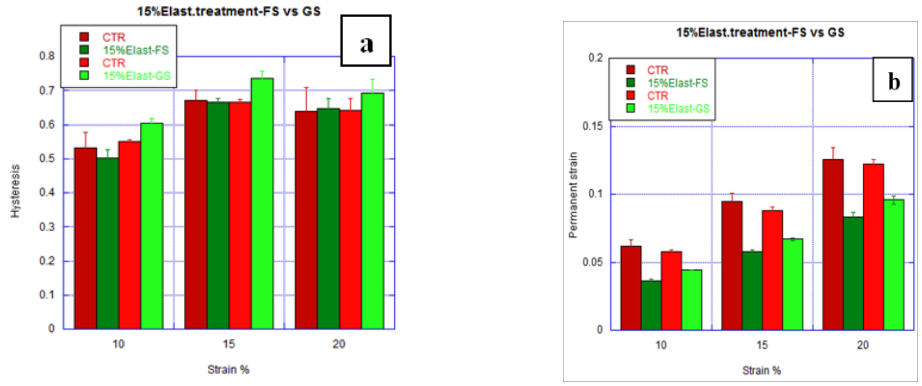

Fig.3.55 Comparison between hysteresis (a) and permanent strain (b) values obtained at three imposed strain for 15% Elastollan engineered samples (flesh vs grain side)

Engineered split leather samples

Select treatments were performed directly on grain side by using lower percentage of polymeric materials and obtained results show again an increase of hysteresis values and a decrease of permanent strain values. The 3%elastollan 2180 engineered sample displays the higher decrease of permanent strain values; therefore it shows an evident improvement of elastic recovery .

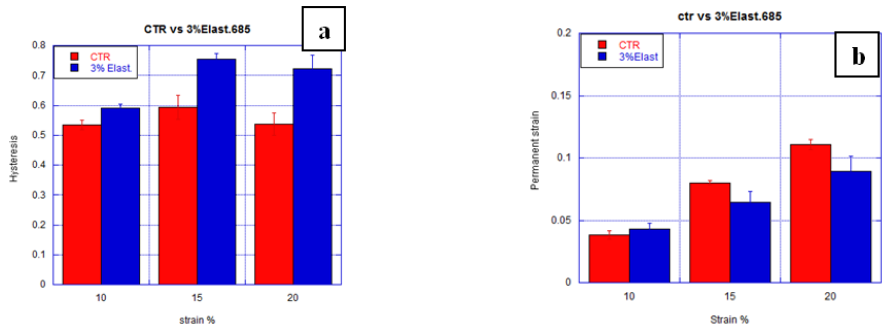

Fig.3.56 Hysteresis (a) and permanent strain (b) values obtained at three imposed strain before and after 3% elastollan 685 treatment (grain side)

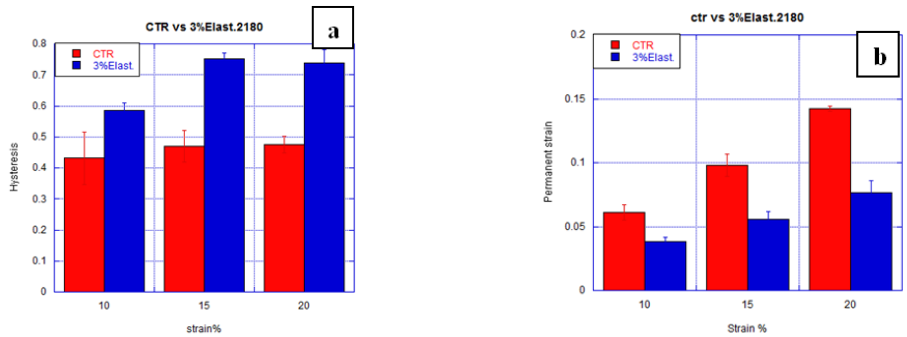

Fig.3.57 Hysteresis (a) and permanent strain (b) values obtained at three imposed strain before and after 3% elastollan 2180 treatment (grain side)

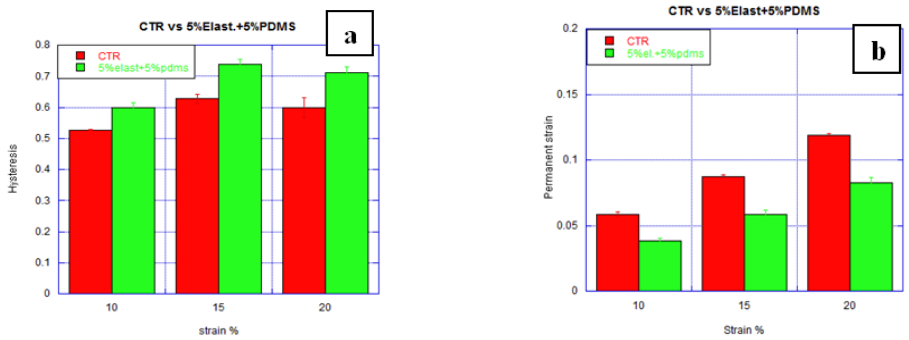

Fig.3.58 Hysteresis (a) and permanent strain (b) values obtained at three imposed strain before and after polymeric blend treatment with 5%elast.685 and 5%pdms (grain side)

Nanoindentation results

It can be very difficult quantify the effective amount of polymeric solution that crosses flesh side in order to reach the grain one and effects its properties. Thus, in order to evaluate the presence and effect of the polymer on grain side after treatment on DW substrate, 15% elastollan engineered leather sample were deprived of flesh side and in particular of polymeric layer and subjected to nanoindentation tests, that allow to investigate local mechanical properties.

Typical load-displacement curve obtained from nanoindentation tests is reported in fig.3.59, it was obtained by testing 15%elastollan chrome tanned sample on grain side (sample was engineered, chrome tanned and split in order to remove the polymeric layer). Permanent strain values and difference % between controls and treated samples are reported in tab.3.14.

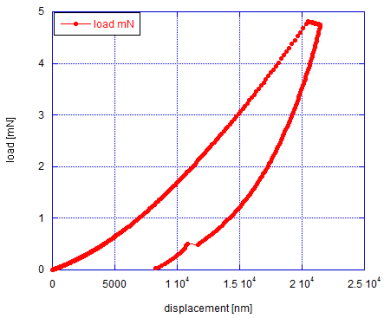

Fig.3.59 15%Elastollan (grain side): load –displacement curve

Samples –grain side	Permanent strain values [nm]	%Difference
CTR -15%el.685	$12.5*10^3 \pm 0.4*10^3$	17%
15%el.685	$10.3*10^3 \pm 1.4*10^3$	

Tab.3.14 Permanent strain values and % changes between controls and 15%elastollan 685 tanned samples

3.3 **Morphological characterization**

DW and DB micrographs are reported in figures 3.60 (a) and (b) respectively. Electron micrographs show a dense collagen network, with fibers characterized by a random arrangement. Both figures show the porous dense fiber network but in the first one the presence of salts, widely used in the leather processes [111], is more evident. Figure 3.61 shows micrographs of split samples subjected to the thermal treatment (stored 24 h in incubator and kept in oven 8h at 140°C); they show that high temperatures do not degrade dry white network.

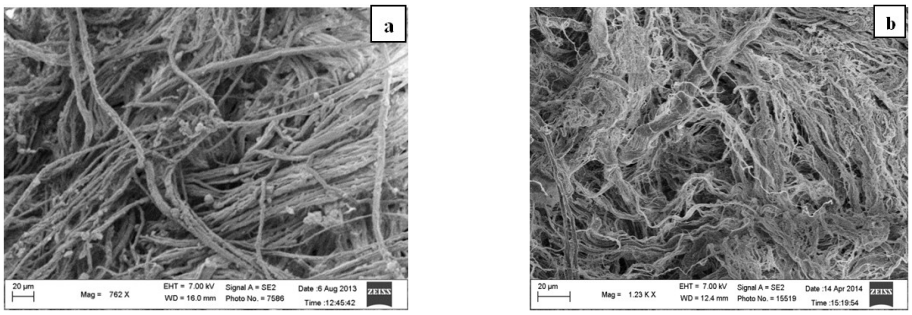

Fig.3.60 SE micrograph DW sample (a) and DB sample (b)

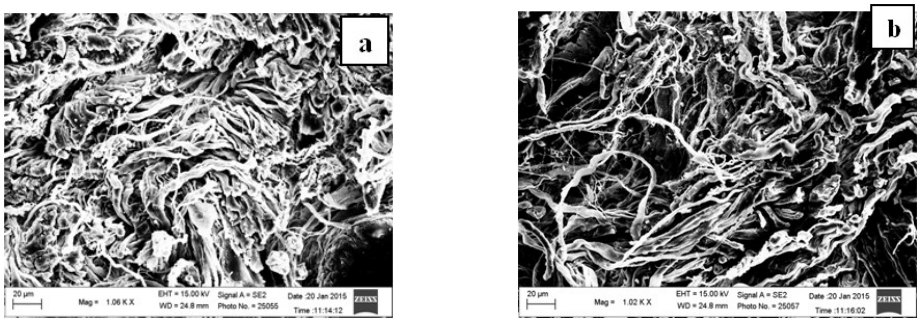

Fig 3.61 SE micrograph DW sample stored 24h in incubator and kept in oven 8h at 140°C

Figures 3.62 and 3.63 report Nylon engineered DW and DB samples respectively. DW flesh side shows a more homogenous structure than DB samples, even though its cross section (3.62b) shows more filaments than DB one. However from both cross-section electron micrographs it can be noticed clearly that the collagen network cross section possesses structural heterogeneities.

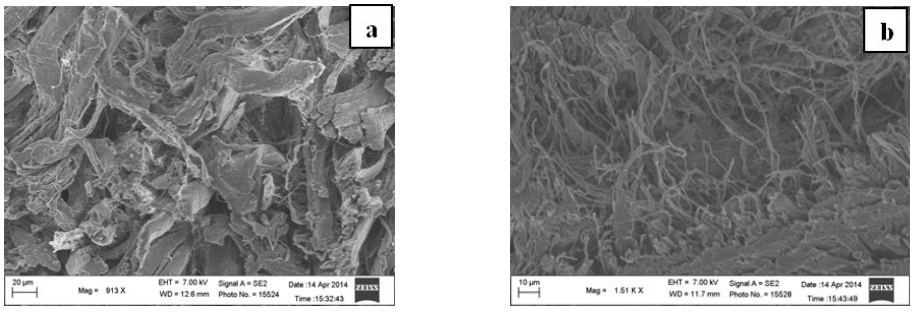

Fig 3.62 SE micrograph DW+Nylon sample (a) and cross section (b)

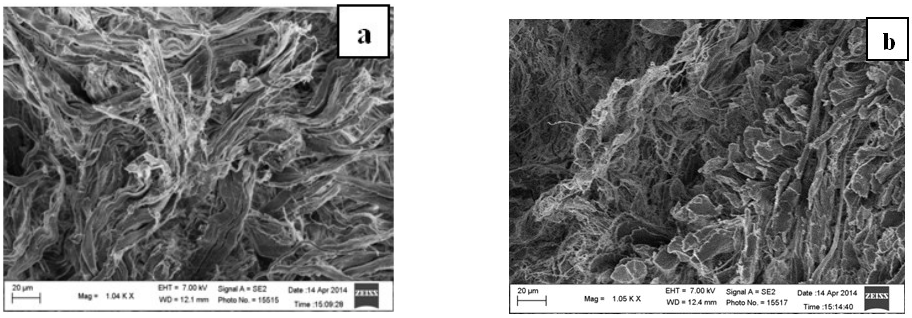

Fig 3.63 SE micrograph DB+Nylon sample (a) and cross section (b)

Engineered samples with 15% Elastollan are reported in images 3.64-3.66. In particular figures 3.65 and 3.66 display split samples micrographs; the first one reports flesh side and the second one shows grain side. Finally, in the last electron micrograph, sample engineered by using a polymeric blend (10%Elastollan and 10%Ecolflex) is shown. Electron micrographs 3.64 (a) and 3.67 (a) do not show a porous dense fiber network because a homogenous polymeric coating covers completely collagen fibers, occluding pores. In engineered elastollan sample (3.64 b) polymer layer thickness is about 40 µm, while on split samples (fig. 3.65 b and 3.66 b) and on specimen obtained by using polymeric blend (10% elastollan and 10%ecoflex) the layer seems thinner, as shown in cross section 3.67b.

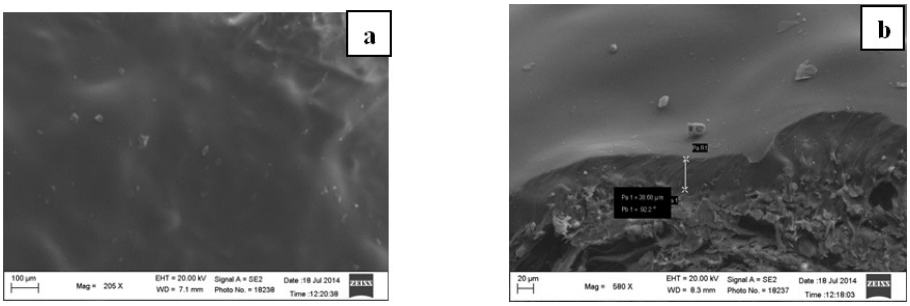

Fig 3.64 SE micrograph DW+15%Elast.685 sample (a) and cross section (b)

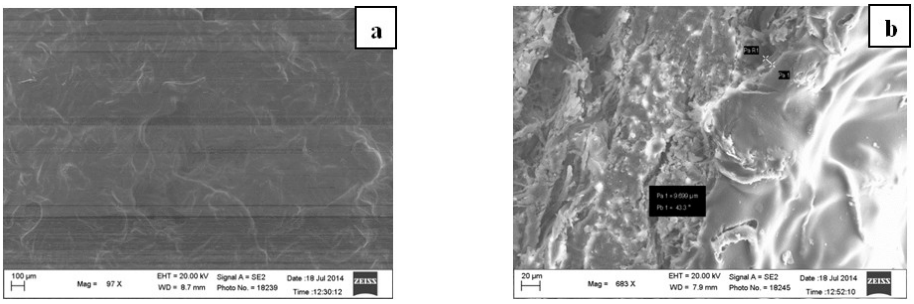

Fig 3.65 SE micrograph of flesh side-DW+15%Elast.685 sample (a) and cross section (b)

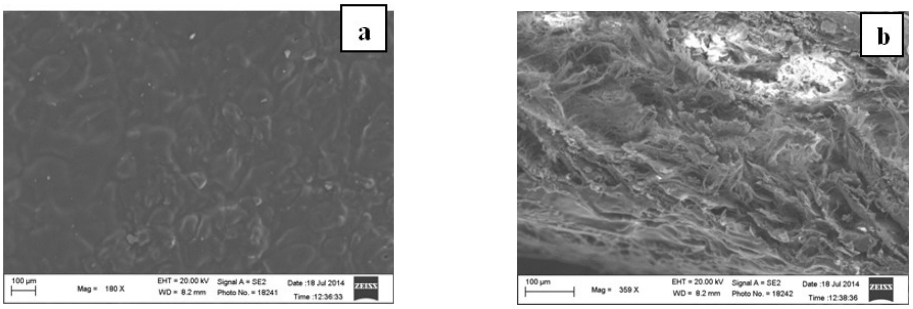

Fig.3.66.SE micrograph of grain side-DW+15%Elast.685 sample (a) and cross section (b)

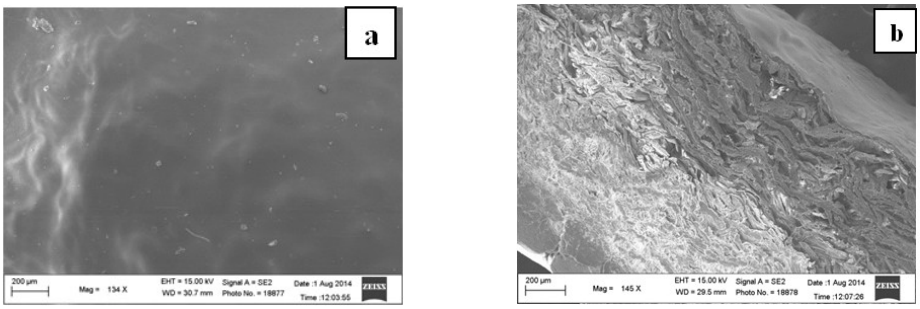

Fig 3.67 SE micrograph DW+10%Elast.685 and 10%Ecoflex sample (a) and cross section (b)

A more accurate morphological analysis (micro tac) was carried out on 15% elastollan engineered leather samples in order to investigate if elastollan placed by drop by drop methodologies on flesh side crosses flesh side in order to reach the grain one. Moreover micro tac, was performed also on 15% elastollan engineered tanned samples after removing flesh side with polymer layer in order to evaluate the

presence of elastollan only in the grain side. Therefore the following μCT images show 15% elastollan engineered tanned samples ((a) figures show controls and (b) figures indicate treated samples). In particular figure 3.68 and 3.69 show 15%elastollan tanned samples and its cross section respectively. Fig.3.70 shows μCT image of 15%elastollan, chrome tanned and split sample. It displays the grain side section close to the flesh side. Comparison between (a) and (b) μCT displays that treated samples show a more closely packed structure and less interfibrilar space, probably due to the polymeric material interposed between fibers.

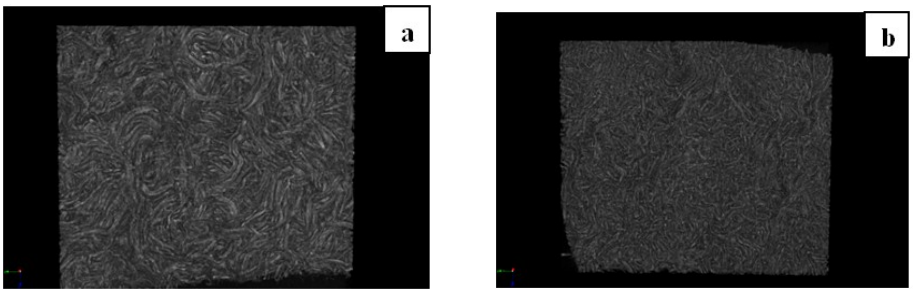

Fig 3.68 μCT chrome tanned DW+15%Elast.685 sample: ctr (a) and treated sample (b)

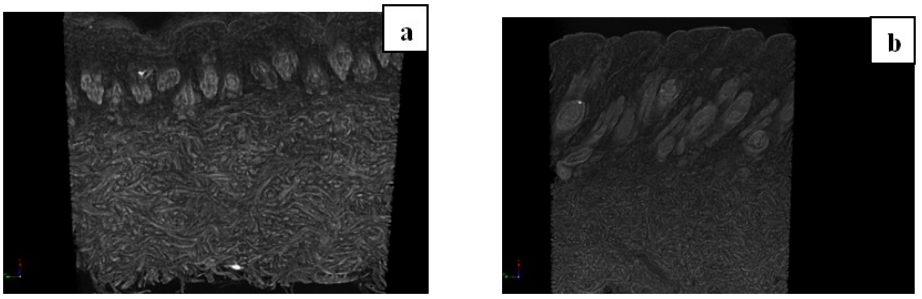

Fig 3.69 μCT chrome tanned DW+15%Elast.685cross section: ctr (a) and treated sample (b)

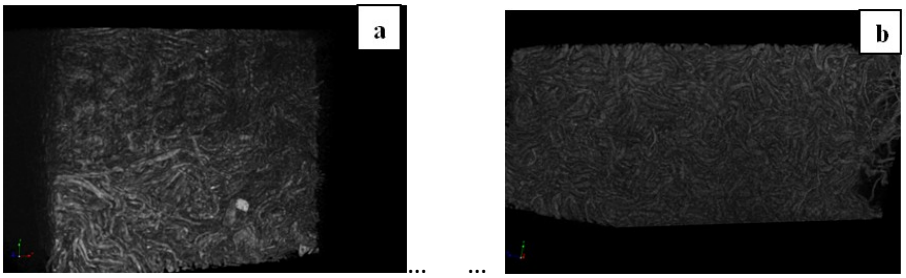

Fig 3.70 µCT chrome tanned DW+15%Elast.685 split sample: ctr (a) and treated sample (b)

3.4 **Absorption properties**

Wettabily properties : flesh vs grain side

In order to evaluate wettability properties of leather and to know how polymeric treatments can affect this property, contact angle measurements were carried out.

Contact angle values obtained for samples shown in tab.2.3, on both, flash and grain side, are reported in tab 3.15. It was not possible to obtain three values in order to calculate an average value of DW and DB samples. Comparison between contact angles obtained on flesh side and on grain side for C, D and E samples show that values obtained on grain side are lower than ones obtained on flesh one. Sample B displays similar contact angles on both side. Contact angle values obtained for selected engineered samples are shown in tab.3.16. In particular contact angles were evaluated on grain side (close to the flesh side) for 15%elastollan engineered and split samples and for split samples treated with lower % of polymers (3% elastollan 685, 3%elastollan 2180 and polymeric blend with 5%elast.685+5%pdms). It can be noticed that polymeric treatment increases contact angle values, decreasing the wettability of surfaces. Finally contact angles were evaluated on both grain and flesh side for 15%elastollan tanned and

split samples and results are reported in tab.3.17. After tanning process it can be noticed again that flash side shows a higher contact angle values but it can not possible to evaluate the effect of polymer treatment that is probably masked from agents using during leather making process.

Samples	Contact angle [1.536s]-FS	Contact angle [1.536s]-GS
DW	48°	17°
DB	-	-
B	117°±4.7°	116°±10
C	125°±1.9°	105°±4.1
D	128°±4.6°	87°±2.2°
E	127±10°	95°±5.6°

Tab.3.15 Contact angle values at 1.536 s evaluated on flesh and grain side for samples in tab.2.3

Samples	Contact angle [1.536s]-GS
Ctr-15%El.685	-
15%El.685	126°±1.2°
Ctr-thf	104°±22°
3%El.685	133°±5.5°
3%El.2180	138°±0.4°
5%El.685+5%PDMS	136±2.3°

Tab.3.16 Contact angle values at 1.536 s for selected engineered samples.

Samples	Contact angle [1.536s]-FS	Contact angle [1.536s]-GS
Ctr-15%El.685	120°±2.5°	116°±7.7°
15%El.685	122°±1.4°	111°±2.3°

Tab.3.17 Contact angle values at 1.536 s for 15%Elastollan tanned samples

3.5 **Thermal characterization**

Thermogravimetric analysis

Figures 3.71-3.76 show TGA and DTG curves obtained for the samples reported in tab.2.3. TGA curves plot weight % as function of temperature. DTG curves show first derivative of the original TGA curve; they were calculated from experimental data in order to obtain more clear results because it can be difficult to evaluate T_{onset} for the gradual transition occurring in the materials. Three informations can be extracted: residual mass, the temperature at which the maximum of the weight loss rate (dm/dTmax) occurs and the weight loss onset temperature (T_{onset}). Collected data were summarized in tab.3.18. TGA curves show that leather degrades through a single step mechanism and maximum weight loss occurs at about 300 °C. It can be attributed to the main component of leather, that is the collagen [112]. Degradation seems to occur through two main steps of degradation but the first one is due to the loss of water in the networks. Thus raw material and final leather do not show evident differences being mass loss attributed to the main component of leather; that is the collagen. Therefore it was more opportune to evaluate thermal properties of treated samples performing DSC analysis.

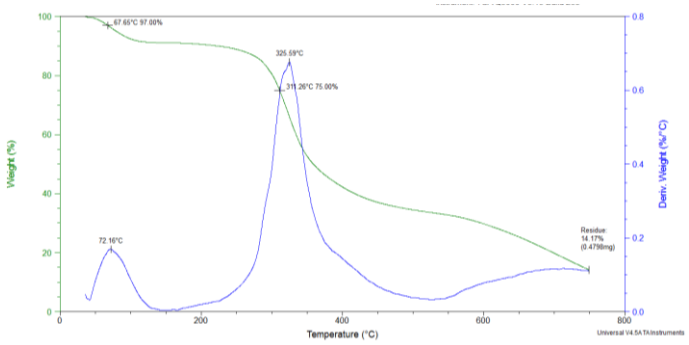

Fig.3.71 DW sample-TGA (green curve) and DTG (blue curve) curves

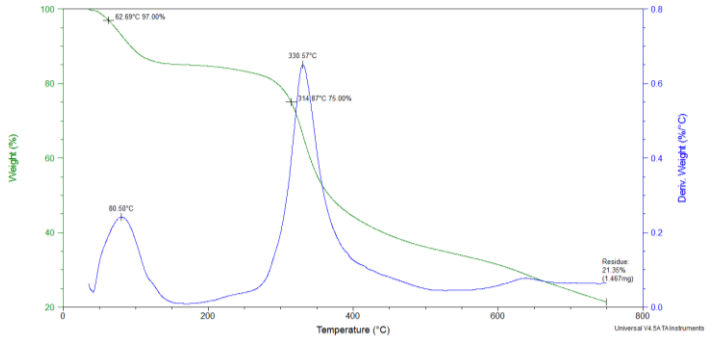

Fig.3.72 DB sample-TGA (green curve) and DTG (blue curve) curves

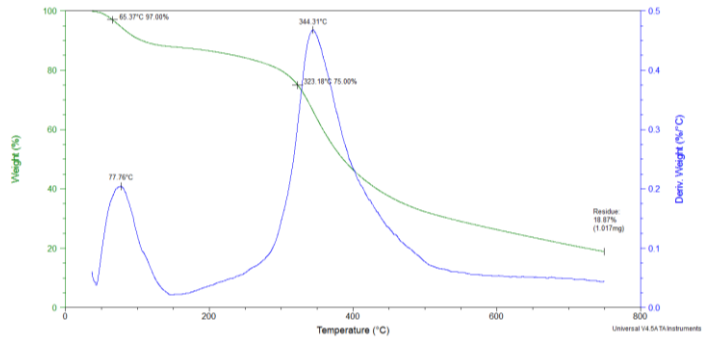

Fig.3.73 B sample-TGA (green curve) and DTG (blue curve) curves

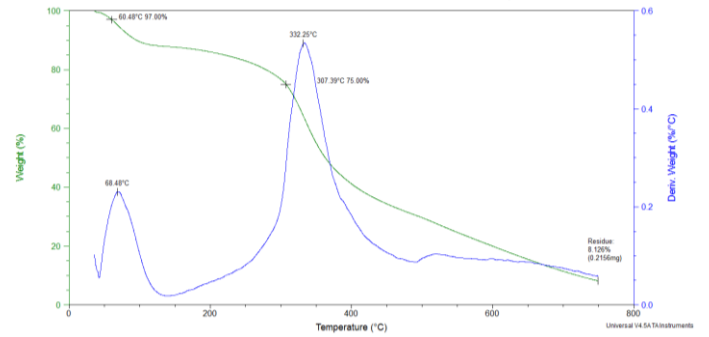

Fig.3.74 C sample-TGA (green curve) and DTG (blue curve) curves

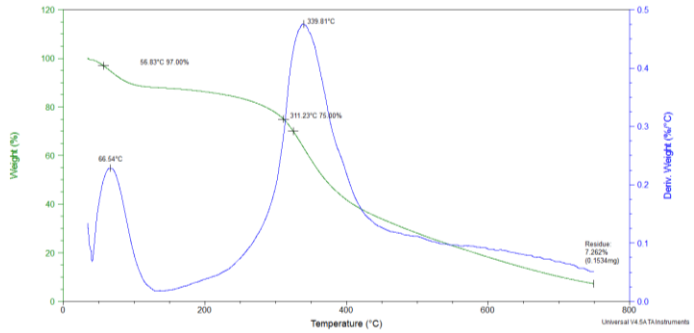

Fig.3.75 D sample-TGA (green curve) and DTG (blue curve) curves

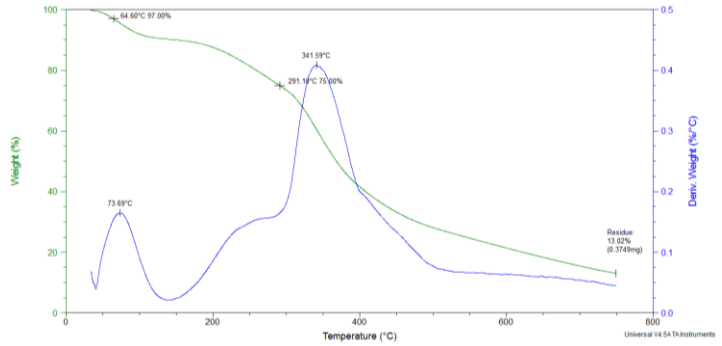

Fig.3.76 E sample-TGA (green curve) and DTG (blue curve) curves

Sample	Residue	Temp. onset °C		Tmax	
		1°Degrad. (3%wt)	2°Degrad. (25%wt)	1°Degrad. (3%wt)	2°Degrad. (25%wt)
DW	**14.17%**	**67.65**	**311.26**	**72.16**	**325.59**
DB	**21.35%**	**62.69°**	**314.87**	**80.50**	**330.57**
B	**18.87%**	**65.37**	**323.18**	**77.76**	**344.31**
C	**8.126%**	**60.48**	**307.39**	**68.48**	**332.25**
D	**7.262%**	**56.83**	**311.23**	**66.54**	**339.81**
E	**13.02%**	**64.60**	**291.10**	**73.69**	**341.59**

Tab.3.18 % residual mass at 750°C ,T_{onset} and T_{max} at weight loss of 3% and 25%

Differential scanning calorimetry measurements

Comparison of thermograms obtained for samples reported in tab.2.3 is shown in fig.3.77. DW sample has a peak temperature (T_c) lower than 100 °C and it is indicative of collagen thermal denaturation. DB sample

and tanned samples (B,C,D,E) present peak temperature higher than 100°C. Selected thermograms of engineered split samples are shown in fig.3.78 and 3.79 (respectively 3% elastollan 2180 sample and 3% elastollan chrome tanned sample). Peak temperatures values obtained for engineered and engineered tanned samples are reported in tab.3.19. Engineered samples show T_c between 48 and 63 °C and engineered tanned samples display peak temperature lower than 100°C. Therefore they show a slight decrease of thermal stability compared to Tc obtained for final leather (B,C,D and E samples).

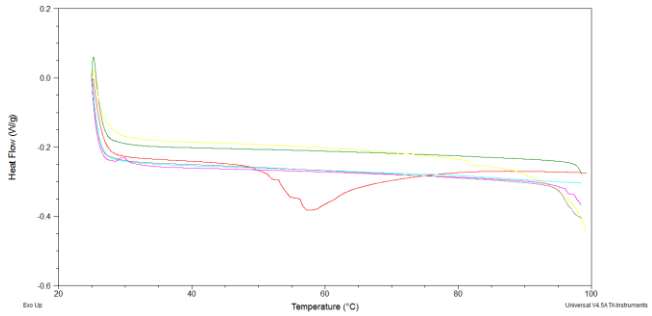

Fig.3.77 Comparison between DSC curves of samples in tab.2.1

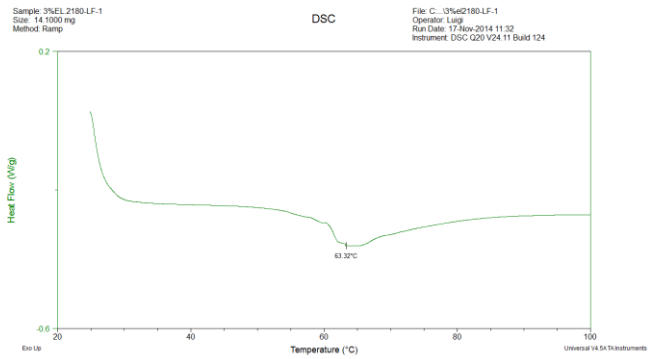

Fig.3.78 DSC curves of 3% elast.2180 engineered sample (GS)

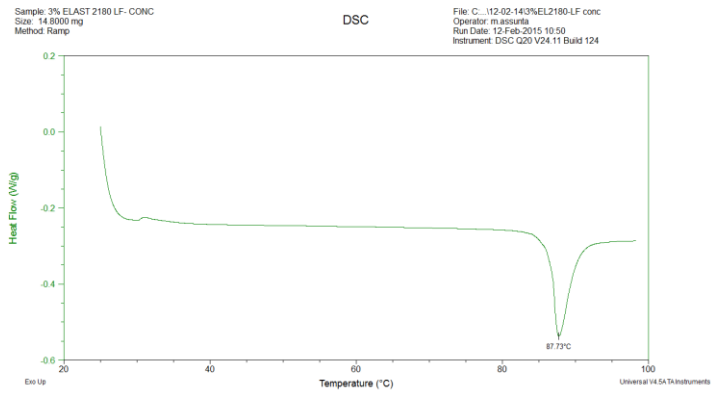

Fig.3.79 DSC curve of 3% el.2180 engineered tanned sample (GS)

Samples	Tc [°C]
DW	<100
DB, B,C,D,E	>100
Engineered samples	
DW(GS) 8h at 140°C	49±2.3
Ctr/3%Elast.2180	66±2.2
DW+3%Elast.2180	63±0.6
Ctr/17%Nylon	59±5.7
DW+17%Nylon	48±4.2
Ctr/25%Pdms	54±4.1
DW+25%Pdms	49±1.1
Engineered and chrome tanned samples	
Ctr/15%Elast.685 (GS)	88±0.1
15%Elast.685(GS)	88±0.6
3%Elast.2180(GS)	88±0.8
Ctr/15%Elast.685	90±0.5
15%Elast.685	88±1.1
Ctr/25%Pdms	91±1.9
DW+25%Pdms	89±2.9

Tab.3.19 Tc values

Chapter 4- Discussion

The chemical physical treatments we performed on leather samples can have different influence on morphological, thermal and mechanical properties of the collagen network. The manipulation strategies were carried out in order to obtain final products with superior mechanical behavior, such as tear resistance and elastic recovery. However it was also necessary to assess the properties other than mechanical ones. for two reasons. First, the characteristic appeal and "hand" of leather cannot be univocally defined with a single parameter. But it is rather the combination of several chemical physical parameter that defines the overall behavior. Second, any treatment for the collagen network functionalization should not alter significantly selected features as thermal stability of leather.

Discussion

Leather, as well as skin, is an anisotropic material and its structure and properties vary over its area; therefore mechanical properties can be affected by the sampling position and by the direction of applied load [103]. Anisotropic behavior arises from the local orientation of collagen fibres. It is therefore useful to know how fibres are distributed all over the skin in order to predict the anisotropic mechanical response [113]. Fig.4.1 shows how the collagen fibres are typically distributed in a ovine skin.

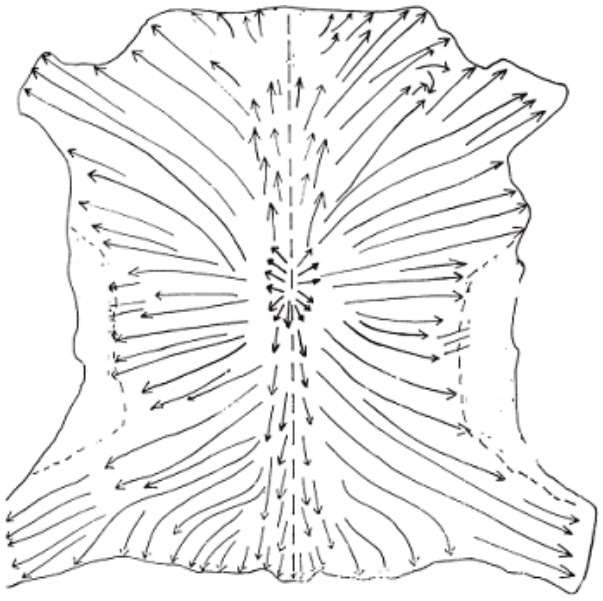

Fig.4.1 Anisotropy of sample

Another critical point is related to the inhomogeneities of skin structure. Indeed skin animals can vary for many reasons: breed , sex, age , curing, diet, etc ; furthermore, as already stated in first chapter, the parts of whole hide or skin can be defined in terms of “butt”, “belly” and “neck” (fig.4.2) and they present some differences. The “butt” makes the skin relatively firm and stiff because it shows a tight fibre structure,: it is thick compared to the belly, but thin compared to the neck. Generally the “bellies” of skin are the thinnest part characterized by a more open structure, making them relatively weak. Therefore in order to make best comparison of leather mechanical properties and to evaluate the effect of treatments, first of all it was necessary to consider all samples in the “Official Sample Position” (included in the butt region). Samples, whose longitudinal direction was cut perpendicular to the backbone, were isolated from the butt. Moreover another important thing in order to have a good comparison

between treated and untreated specimens was to take away them on the left side and right one respectively (or vice versa), because of the symmetry of the features about the backbone [104]. Finally the goodness of a treatment must be validated statistically with a large number of tests.

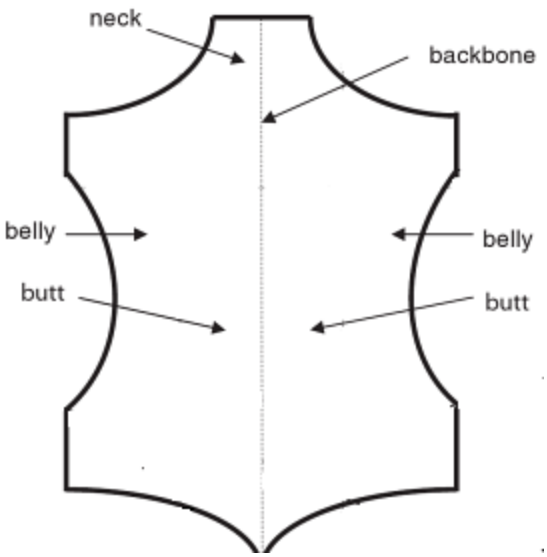

Fig.4.2 Parts of skin

Mechanical behavior is widely discussed, including viscoelastic properties, ultimate tensile strength, tear resistance and above all properties of elastic recovery. As regard to the choice of materials to engineer skin, selected polymers were chosen in order to improve specific mechanical characteristics. In particular PDMS and Nylon were selected to improve ultimate tensile strength and tear resistance because both show high tensile strength and were widely used also in the textile field [94]. PDMS was chosen also because it could improve mechanical properties, whilst conferring a softer “touch” to the skin without stiffening it. Furthermore, two commercial thermoplastic polyurethanes (TPU) elastomers, Elastollan 685 and Elastollan 2180

and Ecoflex, a commercial elastomeric aliphatic-aromatic copolyester such as poly (butylene adipate-co-terephthalate) were used with different methodologies in order to improve elastic recovery of final leather.

As regard the viscoelastic properties E' and E" plots against frequency of all tested samples (both raw and engineered materials) suggest that leather possesses viscoelastic properties closely related to polymeric gels where E" is lower than E' as reported in tab.3.5 and 3.6 and either moduli are roughly constant with frequency. In particular after PDMS treatments it can be noticed an decrease of elastic and viscous modulus compared to the controls. The lower values of viscoelastic moduli most probably could indicate that a complete crosslinking was not achieved [114] and the uncrosslinked PDMS that was interposed between the fibers carrying out a lubricant act that confer the softening properties. At the molecular level, the softening properties of siloxanes are believed to be derived from the flexibility of the siloxane backbone, that results from the freedom of rotations about the Si O Si linkages and the low interaction energies of the methyl groups [94,115]. Nylon treatments increase both E' and E''. Nylon samples seems somehow stiffer than their controls, acting the polymer as reinforcement of the structure and this confirms the qualitative evaluation obtained touching samples after treatment. Also macroscopic characteristic changes: the color becomes lighter and the skin becomes slightly more rigid to bending, especially in the case of DW sample. The higher affect on DW samples can be attributed to the structural differences between DW and DB networks; indeed DB substrates were subjected at a semi tanning process, thus they possess more crosslinks that may impair the penetration of polymeric materials. Therefore, polymeric treatments

can have a limited effect on them. Dynamical mechanical tests usually involve small deformations. Leather is generally subjected to high strain. Therefore, mechanical tests performed at high stains are important to determine mechanical parameters that are of interest for practical applications.

Typical stress strain curves, obtained from uniaxial tensile tests, are reported in figure 4.3; in particular figure shows a comparison between three different dry white substrates (tab.2.2). Stress strain curves presents a first region, called “toe region”, characterized from a shallow slope, followed by a linear region where all fibres are fully stretched and oriented towards the applied load. As the load increases, a transition from low to high stiffness occurs and it is known as the strain stiffening effect where fibres become over stretched and begin to rupture until failure occurs [116].

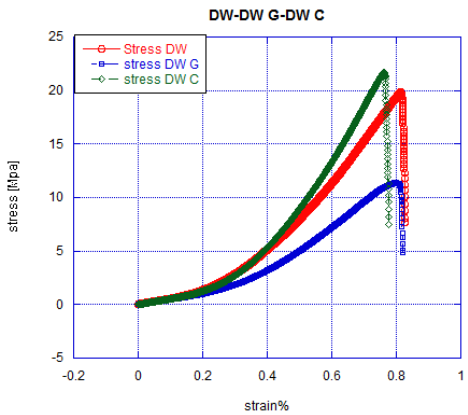

Fig.4.3 Comparison stress strain curves for samples in tab.2.2

As already shown in tab.3.8 dry white substrate, used for gloves, reaches lower ultimate tensile strength; it can be attributed to the different pre tanning operation. Indeed DW-G is subjected to a stronger liming process that aims to hydrolyze part of the polypeptide chains in order to allow a relaxation of the protein structure. For the same region

starting modulus obtained for DW-G is lower than DW and DW-C. DW shows higher ultimate tensile strength values compared to DW-G and similar values to the DW-C, therefore it was not necessary to perform traction tests on different DW substrates.

PDMS and Nylon treatments, performed in order to improve mechanical properties; proved to be effective in altering both tensile strength and tear resistance. Graphs 4.4 and 4.5 that show % increase of tensile strength and tear resistance respectively, for treated samples compared to their controls. Indeed, PDMS treated samples show a higher tensile strength and tear resistance but treated DB reaches lower ultimate tensile strength compared to PDMS DW substrates. PDMS treatments involves in situ polymerization between monomer and crosslink agents but the latter can produce crosslinking also with collagen fibers increasing the cohesion between synthetic and natural polymers. It explains the higher increase of ultimate tensile strength and the better result obtained for DW substrates than DB ones, that presents a lower reactivity as collagen fibres are coated with tanning agents. Nylon treatment enhances tear resistance of DW samples, but exert little effects on ultimate tensile strength. The latter however is strongly reduced for DB samples. Nylon filaments interposed among collagen fibers might in principle slow down tear propagation. This seems to occur in DW samples but not in DB ones, possibly owing to an interaction between the polymer and the metal tanning agent. Additionally, Nylon acts as a filler rather than a proper reinforcement since ultimate tensile strength does not improve upon Nylon treatment. Thus actually decreases suggesting a substantial hydrolysis of collagen fibres due to the acid conditions that develops during the in situ polymerization.

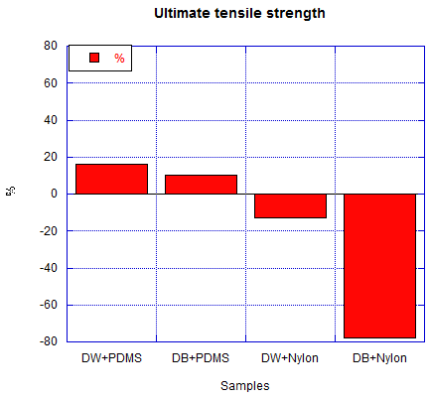

Fig.4.4 DW and DB samples: %improvement in ultimate tensile strength after PDMS and Nylon treatments

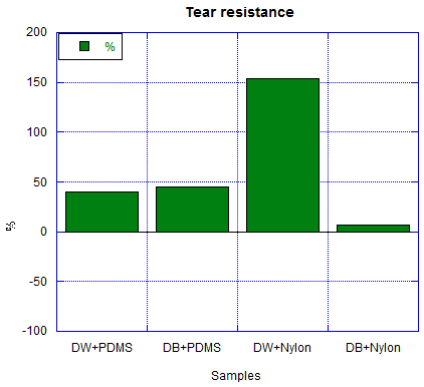

Fig.4.5 DW and DB samples: %improvement in ultimate tensile strength after PDMS and Nylon treatments

DW samples engineered with PDMS and Nylon were subjected to tanning process in order to obtain prototypes that could be compared with final products. After tanning process macroscopic characteristics of Nylon treated samples were unsatisfactory as well as their appear good and dyeability properties. For this reason only PDMS tanned samples were considered to be compared with final lather product (see fig.3.41 and 3.43). Fortunately, interactions between PDMS and tanning agents did not affect negatively ultimate tensile strength and tear resistance, increasing both values more than 30%, as reported in tables 3.11 and 3.14. Increasing amount of PDMS resulted in an

improved effect on tear resistance and not on the ultimate tensile strength value. Probably, increasing PDMS concentration higher amount of PDMS did not crosslink completely and remains in surface lowering tear resistance.

In addition to above mentioned mechanical tests, important results were obtained from dynamic traction tests because they allowed to know hysteresis and permanent strain values in order to provide useful information on elastic recovery of engineered samples. Typical strass strain curves obtained for leather (DW-G) are reported in fig.4.6. It is interesting to consider that the hysteresis shows a dramatic drop after the first cycle and then it reaches a constant value in the other four cycles; it can be noticed for all imposed strain: 10%, 15% and 20% and for all samples, treated and untreated. Thus skin exhibits preconditioning when subjected to cyclic deformations that is due to its viscoelastic property and it represents the gradual adaptation of the collagen network to the applied load. For this reason hysteresis results were normalized to the first cycle, in order to delete preconditioning effect and small difference due to sample position. As regards to permanent strain values, they remains constant throughout all loading cycle for each imposed strain.

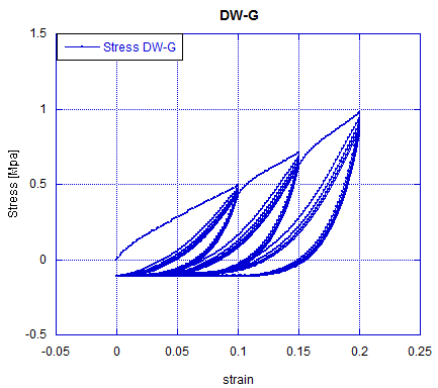

Fig. 4.6 DW-G sample :strass strain curve

No significant differences ($p>0.05$) can be observed after Nylon and PDMS treatments, as shown in fig.4.7 that reports the comparison between the most significant polymeric treatments. In particular comparison between hysteresis (a) and permanent strain (b) values are reported in fig.4.7.

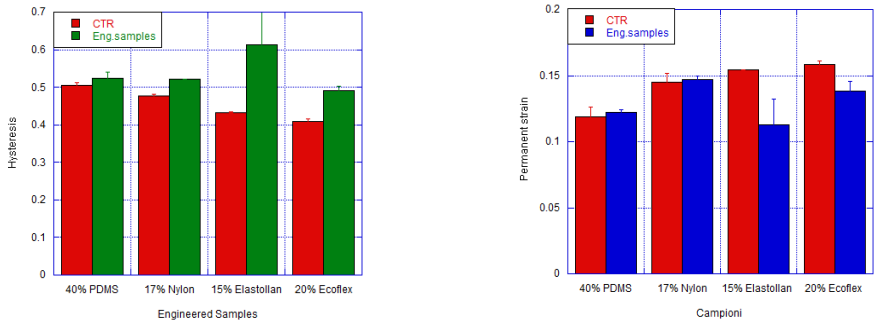

Fig.4.7 Hysteresis (a) and permanent strain (b) values obtained at strain of 15% before and after polymeric treatments (PDMS, Nylon, Elastollan and Ecoflex treatments)

Considerable modifications of elastic recovery are obtained using selected elastomeric materials. For both elastollan and ecoflex, low concentration of polymers did not produce a significant effect on elastic recoil. However, increasing concentration of polymers caused the treating solution to be so viscous to not be absorbed by the collagenic network. Therefore, after a set of experiments, we found that the 15% Elastollan was the optimal concentration, in terms of soaking ability and final outcome, as reported in fig.4.8. It shows the % gain of elastic recovery obtained at different concentrations of synthetic polymer, while the Ecoflex concentration was 20%, as reported in the chapter 3.

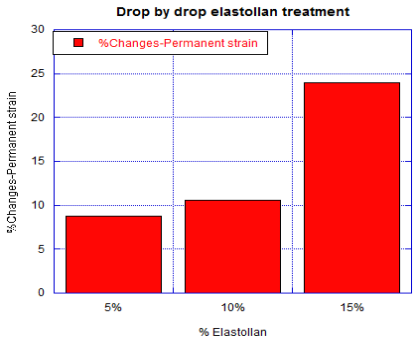

Fig.4.8 Permanent strain values obtained at strain of 15% after elastollan treatment at three different concentrations: 5%,10% and 15%

The optimal elastollan treatment was performed also on DW-G owing to it motive mobility of the collagenic fibres, which could better accommodate macroscopic deformations. Unfortunately, despite of a more mobile structure, a greater permanent strain difference between treated and untreated samples can be observed for DW samples. DW after 15%elastollan drop by drop treatment decreases the permanent strain of 24%, a higher value compared to 8% reached after treatment on DW-G. Probably, a better synergic effect of elastollan and collagen network is obtained in a more closely packed structure.

Along these lines enzymatic treatments performed in order to open up collagen structure proved to not enhance elastic recovery upon polymeric treatment and were therefore abandoned.

Moreover, a comparison between different methodologies, displayed in fig.4.9, was evaluated for 15% elastollan treatments. An higher decrease of permanent strain (in graph positive permanent strain values were reported in order to see clearly the gain that improves the elastic recovery) was reached placing solution by a roll (as already show in tab.3.16), because roll imparts an additional mechanical pressure that can facilitates a deeper penetration of the polymers.

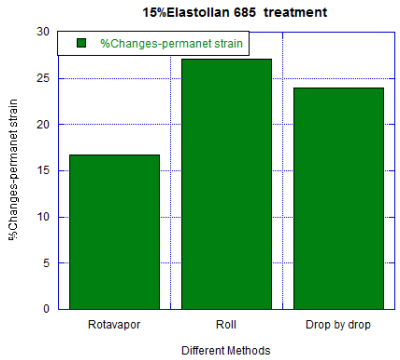

Fig.4.9 Permanent strain values obtained at strain of 15% after elastollan treatment with three different methodology

Furthermore the outcome of the elastomeric treatments was evaluated also separately for flesh and grain side, showing a better result for the flesh side (fig.3.44). It is due at different structure of two sides; in particular grain side shows a more closely packed collagen fibers and absorbs with more difficult polymer solution, despite water angle contact results show a better wettability of grain side compared to the flesh side. Indeed surface roughness, displayed on flesh side, decrease the wettability (tab.3.3) but can help the penetration of functional macromolecules having a more open structure.

Although treatments performed on splitted samples show that flesh side structure allows a better sorption compared to the grain side, during treatments on DW flesh side could act as filter preventing the polymer to reach the grain side. However, it is very difficult quantify the effective amount of polymer solution that crosses flesh side in order to reach the grain one. This causes the functionalization to not occur homogeneously trough the dermal thickness. Thus, in order to confirm the presence and effect of the polymer after treatment on DW, samples engineered with 15%elastollan were deprived of flesh side and in particular of the polymeric layer. They were tanned and tested carrying

out micro tac and nanoindentation, that allow to investigate morphology within the network and not just on the surface and local mechanical properties respectively. Both analysis confirmed the presence of polymer; in particular microtac shows that engineered samples display a closely packed fiber structure, due to the polymer interspersed among the fibers while nanoindation results showed a improvement of 17% of the elastic recovery after treatment. These results validate that penetration of polymer materials occurs and reach grain side.

As already stated grain side is the most interesting part in the tanning sector, therefore lower amount of elastomeric polymers were used in order to engineer directly this side whilst not altering significantly its appeal. Obtained hysteresis and permanent strain values display significant differences ($p>0.05$) after all performed treatments (3%elastollan 685, 3%elastollan 2180 and polymeric blend with 5%elastollan and pdms), as reported in fig.4.10. In particular it can be observed a slight increase of hysteresis values and a decrease of permanent strain, for all the samples treated with elastollan.

Results were confirmed also on the engineered tanned samples, reported in fig.4.11. In particular it can be noticed a higher difference between controls and treated samples after tanning process. Therefore, engineered tanned samples show, as reported in fig.4.10 and 4.11 a higher increase of hysteresis and a better elastic recovery, validated from a greater decrease of permanent strain values, that reaches 48% of variation for splitted sample engineered with 3% elastollan 2180. Probably the presence of tanning agents and the agitation of the drums during the tanning process promotes the formation of an interpenetrating network in the collagen one .

Also DW engineered Ecoflex were tanned, but they were not dyed well showing a yellow color in areas where polymer was mostly present. For this reason only elastollan samples were subjected to dynamic traction tests in order to evaluate hysteresis and permanent strain and to know how interaction between tanning agent and selected polymer can effect elastic recovery.

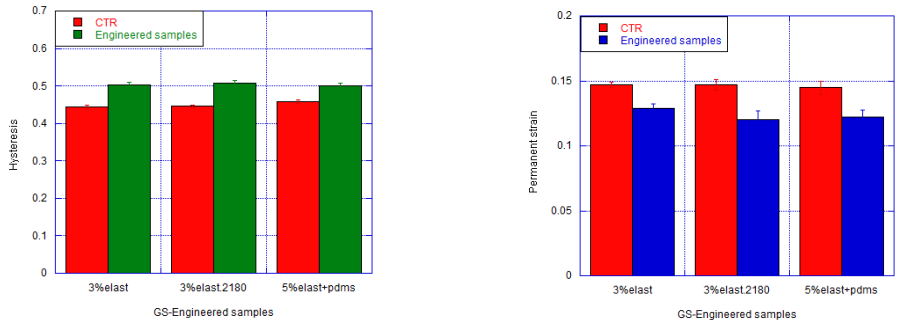

Fig.4.10 Hysteresis (a) and permanent strain (b) values obtained at strain of 15% before and after polymeric treatments (3% elast685, 3%elast2180, 5%elast685+5%pdms treatments)

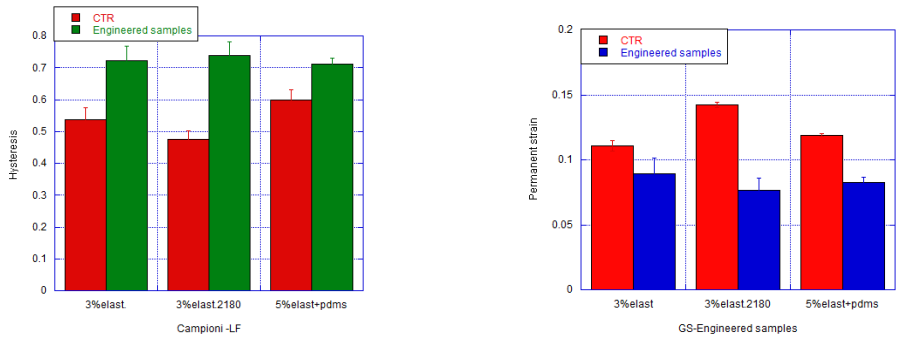

Fig.4.11 Hysteresis (a) and permanent strain (b) values obtained at strain of 15% before and after polymeric and tanning treatments (3% elast685, 3%elast2180, 5%elast685+5%pdms treatments)

Finally, polymer-less treatments to improve elastic recovery were performed by thermal treatments. No good results are obtained on dried substrates, for both flesh and grain side while a considerable difference

of hysteresis and permanent strain values reaching % differences of 21% and - 22% respectively, are reported for grain side after that they were stored in incubation for 24 h; thus heating have a higher effect on rehydrated fibers.

In order to gain a better insight into the mechanical-morphological-thermal relationship selected samples were subjected to morphological and thermal analysis.

Electron micrographs, for both DW and DB network, showed the dense collagen network with fibers characterized by a random arrangement while a graded structure was evident in the cross-section.

Concerning the engineered samples, it can be noticed that high temperatures do not affect the morphology as show in fig.3.3 although the heat transformation of collagen is assumed to denature the collagen triple helical structure into random coils and can be accompanied by a change in physical properties [117]. It is more likely that thermal energy promotes the formation of intermolecular crosslinks that improve the mechanical properties. Apparently, moisture enhances this phenomenon but does not allow the collagen denaturation to occur.

Polymeric materials exert different influence on morphological aspect, as show in fig.4.2. The figure reports three electron micrographs that display Nylon (a), PDMS (b) and elastollan (c) treated samples respectively. Nylon treated sample shows more filaments and a more inhomogeneous structure due probably to the Nylon presence (fig.4.2 a) The scanning electron micrograph PDMS treated DW displays a clear deposition of silicone on the treated fibers and the surface of treated fibers appears smoother than the untreated one [94], thus PDMS samples appear softer than their controls. Finally after a 15% Elastollan treatment (fig.4.2 c), as well as after ecoflex treatment, it is not possible

to observe the collagen network since both synthetic polymers makes a surface coating on the collagen network that reaches 40 μm in thickness. Elastollan and ecoflex cannot penetrate completely within the dermis and they form a synthetic polymer coating on collagen network, that seems to occlude the pores. Collagen network and polymer layer appear as coupled structures. Both treatments make collagen network stiffer. The differences in morphological properties of samples can help to explain the macroscopic characteristic changes of engineered samples.

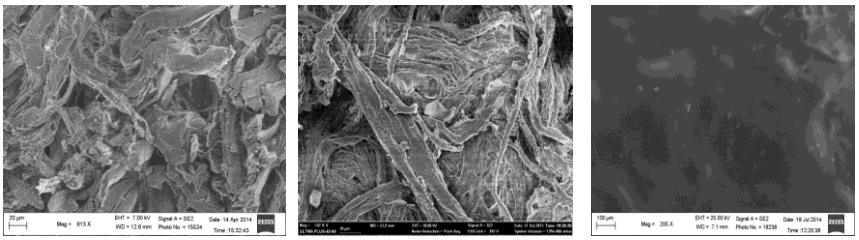

Fig.4.12 Engineered samples: Nylon DW (a), PDMS DW(b), Elastollan DW (c)

Type of polymer, solvent, manipulation strategy and the different interposition of polymer materials between collagen network can affect thermal properties as well. As regards the thermal stability, thermo gravimetric analysis confirms that leather degrades through a single step mechanism, whose maximum mass loss occurs in between 300 and 700 °C with a temperature of maximum degradation (Tmax1) of 330 °C. Such mass loss can be attributed to the main component of leather (for both raw and final product), namely collagen . The residue formed at T_{max}, evolves towards other carbonaceous species under heating and, at approximately 500 °C, a thermally stable char (up to 700 °C) is generate [112]. Raw material and final leather don't show evident differences being mass loss attributed to the main component of

leather; therefore it was more opportune to evaluate mechanical-thermal relationship of treated samples performing DSC analysis. Differential scanning calorimetry was used to study the thermal stability of collagen network and in particular calorimetric analysis provides useful information concerning the amount and the nature of crosslinks, thus DSC results can give information about crosslinks that arise after thermal and polymeric treatments. Collagen thermal denaturation of rehydratated dry white samples occurs around 50 -60 °C while final leather display peak temperature higher than 100°C. After polymeric treatments, DW samples show peak temperature between 48 and 63°C while control samples reach higher shrinkage temperatures. The factors that can be considered potentially important to influence denaturation temperature are loss of chain entropy brought about by the reduced number of molecular configurations and the dehydration state. Indeed cross-links can dehydrate the fibre by closer binding of the molecules and dehydration, caused by subjecting the fibre to a low water activity environment, has been shown to result in very substantial increases in the temperature stability of collagen fibres [118]. It can explain the increase of denaturation temperature values after tanning process.

Miles et all explained the mechanism of collagen thermal denaturation and how the hydratation can influence the thermal stability, according to the “polymer-in-a-box” mechanism of stabilization.

Briefly in this mechanism, the rate of unfolding is depressed by the proximity of the surrounding collagen molecules in the fibre. Collagen molecules can act like the walls of a box reducing possible molecular configurations and therefore the entropy of the unfolding molecule and the rate of unfolding. Consequently the Gibbs free energy of the

activated state is increased while the probability of activation and the rate of collagen denaturation are reduced. Cross-linking therefore stabilizes the collagen molecules in a fibre by reducing the separation of the molecules, i.e. by reducing the lateral dimensions of the box [119].

Nevertheless the stability of the triple helix is governed by many factors in addition to the cross-linking such as pH or the presence of salts, because of ions affect the denaturation temperature of collagen in solution and in fibres [120, 121]. Thus, a particular pH value could have a stabilizing or a destabilizing effect on the molecule itself, because it can swell or shrink collagen fibre. It was envisaged that adding a cross-link could change the charge and disrupt the hydration network around the molecule, as well as allowing a tension to develop within the cross-link itself by distortion of the unstressed molecular conformation. Even though, all crosslinks will not necessarily sustain a tension., but it could occur that a synthetic cross-link is in compression and this would tend to rehydrate the fibre [122]. Therefore the obtained crosslinking or the presence of another interposed polymeric material in the collagen network can affect in different way thermal and mechanical properties. This can explain the difference between thermal stability of treated and untreated samples and in particular the decrease of peak temperature after polymeric treatments. Effect on thermal stability of polymer treatments can be noticed also after tanning process. Indeed treated and tanned samples do not exceed values of 100°C (tab.3.2), that are conventionally reached after performing tanning process on dry white samples. However, treated sample thermal stability was affected by the use of solvents during polymeric treatments because also their controls after tanning process don’t reach

100°C. It is due to the effect of solvent on PH values and on hydratation of fibers because solvent can have direct influence on the hydrogen bonding, which is one of the primary forces for the stability of collagen triple helix . Thermal stability decreases also after thermal treatments, as shown from DSC results of samples after thermal treatment, suggesting a partial denaturation of the molecules deep within the fibres, as the network morphology remains intact.

Even though it can be noticed a decrease of peak temperature in engineered samples, these values can be considered still acceptable for a good final leather.

According of the results obtained and considering that in the tanning sector macroscopic characteristic of the leather are usually evaluated in a qualitative matter by defining empirical parameters such as “touch”, “soft”, “fullness” and no experimental measurement in order to quantify these parameters have been developed so far, it seems opportune to report a conclusive table summarizing the quality of final product, showing the goodness of the engineered samples. In particular symbols such as “++” or “.- -“ are used to indicate good or unsatisfactory results respectively. The table 4.1 is based on a qualitative scale, assessed by professionals of the tanning sector.

Engineered and tanned samples	Dyeability	Softness
25% PDMS	++	++
17%Nylon	--	-
15%Elastollan 685	++	+
20%Ecoflex	--	-
3%Elastollan 685-splitted	++	++
3%Elastollan 2180-spitted	+	++
5%Elastollan 685+5%PDMS-spitted	++	++

Tab.4.1 Final samples that show better improvement in the mechanical properties: red colour is used for prototypes with higher tear strength, blue colour for samples characterized from a improve elastic recovery.

Chapter 5 - Conclusions

The work was aimed at developing of processes of the biomaterial field in the tanning sector in order to obtain leather having engineered mechanical properties, in particular characterized from higher performance in tear resistance and elastic recovery. The first experimental set was designed to define benchmarks to which the engineered prototypes were compared. The obtained results were important since no experimental measurement were developed so far in order to evaluate macroscopic characteristics of the animal skin, described usually in a qualitative manner by defining empirical parameters such as “touch”, “soft” and “fullness”. Moreover this step provided clear hints on how modify collagen networks and in what step of the tanning process this is most convenient. A second experimental campaign was designed in order to characterize engineered substrates

realized with selected polymers and specific chemical physical manipulation strategies. In particular PDMS and Nylon were selected to reinforce collagen network in order to increase tear resistance and good results were obtained from both treatments performed on DW substrates, reaching increase of 45% in PDMS engineered samples and 150% in Nylon engineered samples. Improvement of elastic recovery were obtained using high temperature and elastomeric polymers reaching decrease of more than 20% in the permanent strain values.

Finally DW samples engineered samples were subjected to leather making process in order to obtain prototypes that could be compared with final products. After tanning process macroscopic characteristic of specimens subjected to high temperature and of Nylon and Ecoflex samples were unsatisfactory as well as their appear good; moreover the samples engineered with Nylon and Ecoflex showed a very low dyeability. For this reason only PDMS and Elastollan tanned samples were considered to be compared with final lather product. The interaction between PDMS and tanning agents don't affect negatively ultimate tensile strength and tear resistance, increasing both values more than 30%. As regard the elastollan samples, in order to confirm the presence and effect of the polymer after treatment on DW, samples engineered with 15%elastollan were deprived of flesh side and in particular of the polymeric layer. They were tanned and tested carrying out micro tac and nanoindentation, that allow to investigate morphology within the network and local mechanical properties respectively. Both analysis confirmed the presence of polymer and validated that penetration of polymer materials occurs and reach grain side, the most interesting part in the tanning sector. Therefore lower amount of elastomeric polymers were used in order to engineer directly

this side whilst not altering significantly its appeal. Obtained hysteresis and permanent strain values display significant differences ($p>0.05$) after all performed treatments, showing a improvement of elastic recoil. Results were confirmed also on the engineered tanned samples. In particular after tanning process it can be noticed a better elastic recovery of treated samples, validated from a greater decrease of permanent strain values, that reaches 48% of variation for splitted sample engineered with 3% elastollan 2180. Therefore according to all obtained results it can be concluded that it is better to modify DW substrate in order to optimize the results of the treatment because of DW networks show a more open collagen structure not containing any tanning agents. Thus it facilities the penetration within the dermis. Polymeric treatments affect strongly mechanical properties but it is very important to clarify the aim of the treatment and the performance to be obtained before approaching to the selection of polymer and manipulation strategy. Indeed it can be noticed a better tear resistance selecting PDMS as polymeric materials that is tough and confers properties of softness while in order to improve a different mechanical characteristic, such as elastic recoil, it was necessary to involve different polymeric materials, such as the elastomeric ones. In particular prototypes characterized from improved elastic recovery were realized modulating Elastollan concentration on both grain and flesh side.

Chapter 6 -References

[1] Gupta, Ajay Kumar, and Mona Gupta. "Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications." Biomaterials 26.18 (2005): 3995-4021

[2] Lin, Jiang-Jen, et al. "The cellular responses and antibacterial activities of silver nanoparticles stabilized by different polymers." Nanotechnology 23.6 (2012): 065102.

[3] Zhang, Run-xin, et al. "Mechanical properties of composites filled with SMA particles and short fibers." Composite Structures 79.1 (2007): 90-96.

[4] Yang, F., et al. "Electrospinning of nano/micro scale poly (L-lactic acid) aligned fibers and their potential in neural tissue engineering." *Biomaterials* 26.15 (2005): 2603-2610.

[5] Karami, Zeinab, et al. "Preparation and performance evaluations of electrospun poly (ε-caprolactone), poly (lactic acid), and their hybrid (50/50) nanofibrous mats containing thymol as an herbal drug for effective wound healing." *Journal of Applied Polymer Science* 129.2 (2013): 756-766.

[6] Duncan, Timothy V. "Applications of nanotechnology in food packaging and food safety: barrier materials, antimicrobials and sensors." *Journal of colloid and interface science* 363.1 (2011): 1-24.

[7] Chung, Wan-Ho, et al. "In situ monitoring of a flash light sintering process using silver nano-ink for producing flexible electronics." *Nanotechnology* 24.3 (2013): 035202.

[8] Yousefi, Azam, Ali Allahverdi, and Parisa Hejazi. "Effective dispersion of nano-TiO 2 powder for enhancement of photocatalytic properties in cement mixes."*Construction and Building Materials* 41 (2013): 224-230.

[9] Musso, Simone, et al. "Influence of carbon nanotubes structure on the mechanical behavior of cement composites." *Composites Science and Technology* 69.11 (2009): 1985-1990.

[10] Di, Y., and R. J. Heath. "Collagen stabilization and modification using a polyepoxide, triglycidyl isocyanurate." *Polymer Degradation and Stability* 94.10 (2009): 1684-1692.

[11] Covington, A. D. "Atkin memorial lecture. Theory and mechanism of tanning: present thinking and future implications for industry." *Journal of the society of leather technologists and chemists* 85.1 (2001): 24.

[12] Freed, Lisa E., et al. "Advanced material strategies for tissue engineering scaffolds." *Advanced materials* 21.32-33 (2009): 3410-3418.

[13] Achilli Matteo, and Diego Mantovani. "Tailoring mechanical properties of collagen-based scaffolds for vascular tissue engineering: the effects of pH, temperature and ionic strength on gelation." Polymers 2.4 (2010): 664-680.

[14] Jorge-Herrero, E., et al. "Influence of different chemical cross-linking treatments on the properties of bovine pericardium and collagen." *Biomaterials* 20.6 (1999): 539-545.

[15] P.B. van Wachem et al. “Characterization and biocompatibility of epoxy-crosslinked dermal sheep collagens” *Journal Biomedical Material Reserch* ;47 (1999):270–277

[16] Marks R. Mechanical properties of the skin. In: Goldsmith LA, editor. Physiology, biochemistry and molecular biology of the skin. 2nd ed. New York: Oxford University Press; 1991. pp. 602–621.

[17]. Marks R. “The stratum corneum barrier: the final frontier” *The Journal of Nutrition* 134, (suppl 8) (2004): S2017–S2021.
[18] Madison, Kathi C. "Barrier function of the skin:“la raison d'etre” of the epidermis." *Journal of Investigative Dermatology* 121.2 (2003): 231-241.

[19] Brandner JM, Proksch E. Epidermal barrier function: role of tight junctions. In: Elias PM, Feingold KR, editors. Skin barrier. New York: Taylor and Francis; 2006. p. 191-210

[20] Wickett, R. Randall, and Marty O. Visscher. "Structure and function of the epidermal barrier." *American journal of infection control* 34.10 (2006): S98-S110.

[21] Breitkreutz, Dirk, Nicolae Mirancea, and Roswitha Nischt. "Basement membranes in skin: unique matrix structures with diverse functions?."*Histochemistry and cell biology* 132.1 (2009): 1-10.

[22] Agache, Pierre G., and Philippe Humbert. *Measuring the skin: Non-invasive investigations, physiology, normal constants*. Springer, 2004.

[23] Silver FH, Siperko LM, Seehra GP. “Mechanobiology of force transduction in dermal tissue” *Skin Res Technol* 9 (2003): 3–23.

[24] Santin, Matteo, ed. *Strategies in Regenerative Medicine*. Springer New York, 2009.

[25] Proksch, Ehrhardt, Johanna M. Brandner, and Jens-Michael Jensen. "The skin: an indispensable barrier." *Experimental dermatology* 17.12 (2008): 1063-1072.

[26].Cumberbatch, Marie, et al. "Epidermal Langerhans cell migration and sensitisation to chemical allergens." *Apmis* 111.7-8 (2003): 797-804.

[27] Pieri L, Domenici L, Romagnoli P. “Langerhans cells differentiation: a three-act play” *Italian Journal of Anatomy and Embryology* 106 (2001):47-69.
[28] Fratzl, Peter. *Collagen: structure and mechanics*. Springer Science & Business Media, 2008.

[29] Floden, Evan W., et al. "Biophysical characterization of ovine forestomach extracellular matrix biomaterials." *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 96.1 (2011): 67-75.

[30] Fratzl, P., et al. "Structure and mechanical quality of the collagen–mineral nano-composite in bone." *Journal of materials chemistry* 14.14 (2004): 2115-2123.

[31] Sasaki, Naoki, and Shingo Odajima. "Elongation mechanism of collagen fibrils and force-strain relations of tendon at each level of structural hierarchy."*Journal of biomechanics* 29.9 (1996): 1131-1136.

[32] Kielty, Cay M., et al. "Microfibrillar assemblies of foetal bovine skin: developmental expression and relative abundance of type VI collagen and fibrillin." *Matrix* 13.2 (1993): 103-112.

[33] Wang, Yan, et al. "The predominant role of collagen in the nucleation, growth, structure and orientation of bone apatite." *Nature materials* 11.8 (2012): 724-733.

[34] W.D. Comper, "Extracellular matrix Vol. 2: Molecular components and interactions", Amsterdam, NL: Overseas Publishers Association, B.V.(1996).

[35] K. Kadler, "The structure of collagens", *Protein Profile*, II (1995) :508-523

[36] Miles, Christopher A., Thengiz V. Burjanadze, and Allen J. Bailey. "The kinetics of the thermal denaturation of collagen in unrestrained rat tail tendon determined by differential scanning calorimetry" *Journal of molecular biology*245.4 (1995): 437-446.

[37] Hulmes, D. J., et al. "Radial packing, order, and disorder in collagen fibrils."*Biophysical Journal* 68.5 (1995): 1661.
[38] Brodsky, Barbara, and Anton V. Persikov. "Molecular structure of the collagen triple helix." *Advances in protein chemistry* 70 (2005): 301-339.

[39] Moroder, Luis. "Studies of protein folding and structure with model peptides."*Journal of Peptide Science* 11.5 (2005): 258-261.

[40] Persikov, Anton V., et al. "Amino acid propensities for the collagen triple-helix."*Biochemistry* 39.48 (2000): 14960-14967.

[41] Persikov, Anton V., John AM Ramshaw, and Barbara Brodsky. "Collagen model peptides: Sequence dependence of triple-helix stability." *Peptide Science*55.6 (2000): 436-450.

[42] Persikov, Anton V., John AM Ramshaw, and Barbara Brodsky. "Prediction of collagen stability from amino acid sequence." *Journal of Biological Chemistry*280.19 (2005): 19343-19349.

[43] F.H. Silver, G.D. Pins, M.-C. Wang, and D. Christiansen, "Collagenous biomaterials as models of tissue inducing implants", in "*Encyclopedic handbook of biomaterials and bioengineering*", Ed. by D.L. Wise, *et al.*, Marcel Dekker, Inc., New York. p. 1245-1266 (1995).

[44] Daamen, W. F., et al. "Preparation and evaluation of molecularly-defined collagen–elastin–glycosaminoglycan scaffolds for tissue engineering."*Biomaterials* 24.22 (2003): 4001-4009.

[45] Simmons, D. M., and J. N. Kearney. "Evaluation of collagen cross-linking techniques for the stabilization of tissue matrices." *Biotechnology and applied biochemistry* 17.1 (1993): 23-29.

[46] Rich, Harvey, et al. "Effects of photochemical riboflavin-mediated crosslinks on the physical properties of collagen constructs

and fibrils." *Journal of Materials Science: Materials in Medicine* 25.1 (2014): 11-21.

[47] Jöbsis, Paul D., et al. "The visceral pericardium: macromolecular structure and contribution to passive mechanical properties of the left ventricle." *American Journal of Physiology-Heart and Circulatory Physiology* 293.6 (2007): H3379-H3387.

[48]Commodities and Trade Division, FAO, United Nations. World Statistical Compendium for Raw Hides and Skins, Leather and Leather Footwear 1990−2009; Rome, Italy (2010).

[49] Chan, Yue, et al. "Mechanical model for a collagen fibril pair in extracellular matrix." *European Biophysics Journal* 38.4 (2009): 487-493.

[50] Schofield, A. L., et al. "The use of reflection anisotropy spectroscopy to assess the alignment of collagen." *Journal of Physics D: Applied Physics* 44.33 (2011): 335302.

[51] Friedrichs, Jens, et al. "Cellular remodelling of individual collagen fibrils visualized by time-lapse AFM." *Journal of molecular biology* 372.3 (2007): 594-607.

[52] Billiar, K. L., and M. S. Sacks. "A method to quantify the fiber kinematics of planar tissues under biaxial stretch." *Journal of biomechanics* 30.7 (1997): 753-756.

[53] Jor, Jessica WY, et al. "Modelling collagen fibre orientation in porcine skin based upon confocal laser scanning microscopy." *Skin Research and Technology* 17.2 (2011): 149-159.

[54] Falgayrac, Guillaume, et al. "New method for Raman investigation of the orientation of collagen fibrils and crystallites in the Haversian system of bone."*Applied spectroscopy* 64.7 (2010): 775-780.

[55] Chapuis, J. F., and P. Agache. "A new technique to study the mechanical properties of collagen lattices." *Journal of biomechanics* 25.1 (1992): 115-120.

[56] Roeder, Blayne A., et al. "Tensile mechanical properties of three-dimensional type I collagen extracellular matrices with varied microstructure." *Journal of biomechanical engineering* 124.2 (2002): 214-222.

[57] Powell, Heather M., and Steven T. Boyce. "EDC cross-linking improves skin substitute strength and stability." *Biomaterials* 27.34 (2006): 5821-5827.

[58] Lee, C. R., A. J. Grodzinsky, and M. Spector. "The effects of cross-linking of collagen-glycosaminoglycan scaffolds on compressive stiffness, chondrocyte-mediated contraction, proliferation and biosynthesis." *Biomaterials* 22.23 (2001): 3145-3154.

[59] Tan, Wei, Raj Krishnaraj, and Tejal A. Desai. "Evaluation of nanostructured composite collagen-chitosan matrices for tissue engineering." *Tissue engineering* 7.2 (2001): 203-210.

[60] Cummings, Christopher L., et al. "Properties of engineered vascular constructs made from collagen, fibrin, and collagen–fibrin mixtures." *Biomaterials* 25.17 (2004): 3699-3706.

[61] MacDonald, Rebecca A., et al. "Collagen–carbon nanotube composite materials as scaffolds in tissue engineering." *Journal of Biomedical Materials Research Part A* 74.3 (2005): 489-496.

[62] Gough, Julie E., Colin A. Scotchford, and Sandra Downes. "Cytotoxicity of glutaraldehyde crosslinked collagen/poly (vinyl alcohol) films is by the mechanism of apoptosis." *Journal of biomedical materials research* 61.1 (2002): 121-130.

[63] Kiernan, John A. "Formaldehyde, formalin, paraformaldehyde and glutaraldehyde: what they are and what they do." *Microscopy Today* 1.5 (2000).

[64] Abe, Masashi, et al. "The changes in crosslink contents in tissues after formalin fixation." *Analytical biochemistry* 318.1 (2003): 118-123.

[65] Singh, A., et al. "External stimuli response on a novel chitosan hydrogel crosslinked with formaldehyde." *Bulletin of Materials Science* 29.3 (2006): 233-238.

[66] Damink, LHH Olde, et al. "Cross-linking of dermal sheep collagen using a water-soluble carbodiimide." *Biomaterials* 17.8 (1996): 765-773.

[67] Chiono, Valeria, et al. "Genipin-crosslinked chitosan/gelatin blends for biomedical applications." *Journal of Materials Science: Materials in Medicine*19.2 (2008): 889-898.

[68] Koob, Thomas J. "Collagen fixation." *Encyclopedia of biomaterials and biomedical engineering* 1.1 (2004): 335-347.

[69] Lee, J. Michael, Christopher A. Pereira, and Lincoln WK Kan. "Effect of molecular structure of poly (glycidyl ether) reagents on crosslinking and mechanical properties of bovine pericardial xenograft materials." *Journal of biomedical materials research* 28.9 (1994): 981-992.

[70] Sung, Hsing-Wen, et al. "Cross-linking characteristics of biological tissues fixed with monofunctional or multifunctional epoxy compounds" *Biomaterials* 17.14 (1996): 1405-1410.

[71] Cai, Zaisheng, Guochuan Jiang, and Yiping Qiu. "Chemical modification of Bombyx mori silk with epoxide EPSIB." *Journal of applied polymer science* 91.6 (2004): 3579-3586.

[72] Ellis B. “Chemistry and technology of epoxy resins” Glasgow: Blackie Academic & Professional (1994)

[73] Takamoto, Tomoaki, Yosuke Hiraoka, and Yasuhiko Tabata. "Enhanced proliferation and osteogenic differentiation of rat mesenchymal stem cells in collagen sponge reinforced with different poly (ethylene terephthalate) fibers."*Journal of Biomaterials Science, Polymer Edition* 18.7 (2007): 865-881.

[74] Y. Cao, Y. M. Zhou, Y. Shan, H. X. Ju, X. J. Xue “TripleHelix Scaffolds of Grafted Collagen Reinforced by Al O –ZrO Nanoparticles” Advanced materials Vol. 18, Issue 14 July, (2006): 1838–1841

[75] Matsushima, Rie, et al. "Decellularized dermis–polymer complex provides a platform for soft-to-hard tissue interfaces." *Materials Science and Engineering: C* 35 (2014): 354-362.

[76] Thanikaivelan, Palanisamy, et al. "Recent trends in leather making: processes, problems, and pathways." *Critical Reviews in Environmental Science and Technology* 35.1 (2005): 37-79.

[77] Thanikaivelan, Palanisamy, et al. "Progress and recent trends in biotechnological methods for leather processing." *TRENDS in Biotechnology*22.4 (2004): 181-188.

[78] Santos, Letícia M., and Mariliz Gutterres. "Reusing of a hide waste for leather fatliquoring." *Journal of Cleaner Production* 15.1 (2007): 12-16

[79] Hekal, Samia A. "Histological Study of the Skin and Leather Characteristics in Two Types of Arabian Camels (Camelus dromedarius)." *Journal of American Science* 10.9 (2014).

[80] Cassano, A., et al. "Treatment of aqueous effluents of the leather industry by membrane processes: a review." *Journal of Membrane Science* 181.1 (2001): 111-126.

[81] Ricka, J., and Toyoichi Tanaka. "Swelling of ionic gels: quantitative performance of the Donnan theory " *Macromolecules* 17.12 (1984): 2916-2921.

[82] Nashy, Elshahat HA, et al. "Molecular spectroscopic study for suggested mechanism of chrome tanned leather." *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 88 (2012): 171-176.

[83] Krishnamoorthy, G., et al. "Green chemistry approaches to leather tanning process for making chrome-free leather by unnatural amino acids." *Journal of hazardous materials* 215 (2012): 173-182.

[84] Sivakumar, Venkatasubramanian, and Paruchuri Gangadhar Rao. "Application of power ultrasound in leather processing: an eco-friendly approach." *Journal of Cleaner Production* 9.1 (2001): 25-33.

[85] Bailey, D. G. In Encyclopedia of Polymer Science and Engineering, suppl. vol., 2nd ed.; Wiley: New York, 1990.

[86] Hioki, Katsumi. "Leather-Like Materials." *Kirk-Othmer Encyclopedia of Chemical Technology* (2000).

[87] Singha, Kunal. "A Review on Coating & Lamination in Textiles: Processes and Applications." *American Journal of Polymer Science* 2.3 (2012): 39-49.

[88] Mohamed, O. A., et al. "Styrene and butyl methacrylate copolymers and their application in leather finishing." *Journal of Applied Polymer Science* 111.3 (2009): 1488-1495.

[89] Hatton, Harold L. "Leather and method for producing it." U.S. Patent No. 3,103,447. 10 Sep. 1963.

[90] Haeberle, Karl, et al. "Coating for leather." U.S. Patent No. 5,135,963. 4 Aug. 1992.

[91] Worley, James Brice, et al. "Coated articles having enhanced reversible thermal properties and exhibiting improved flexibility,

softness, air permeability, or water vapor transport properties." U.S. Patent No. 7,135,424. 14 Nov. 2006.

[92] Fernandes, Isabel P., et al. "Development of chitosan-based antimicrobial leather coatings." *Carbohydrate polymers* 98.1 (2013): 1229-1235.

[93] Zeng, J., J. R. Schlup, and L. T. Fan. "Synthesis and mechanical properties of leather–epoxy interpenetrating polymer networks." *Journal of applied polymer science* 78.6 (2000): 1224-1232.

[94] Mohamed, Amina L., Meriem Er-Rafik, and Martin Moller. "Suitability of Confocal Raman microscopy for monitoring the penetration of PDMS compounds into cotton fibres." *Carbohydrate polymers* 96.1 (2013): 305-313.

[95] Perelshtein, Ilana, et al. "Sonochemical coating of silver nanoparticles on textile fabrics (nylon, polyester and cotton) and their antibacterial activity."*Nanotechnology* 19.24 (2008): 245705.

[96] Witt, Uwe, Rolf-Joachim Müller, and Wolf-Dieter Deckwer. "New biodegradable polyester-copolymers from commodity chemicals with favorable use properties."*Journal of environmental polymer degradation* 3.4 (1995): 215-223.

[97] Witt, Uwe, Rolf-Joachim Müller, and Wolf-Dieter Deckwer. "Biodegradation behavior and material properties of aliphatic/aromatic polyesters of commercial importance." *Journal of environmental polymer degradation* 5.2 (1997): 81-89.

[98] Shi, X. Q., H. Ito, and T. Kikutani. "Characterization on mixed-crystal structure and properties of poly (butylene adipate-co-terephthalate) biodegradable fibers." *Polymer* 46.25 (2005): 11442-11450.

[99] Alves, P., et al. "Surface grafting of carboxylic groups onto thermoplastic polyurethanes to reduce cell adhesion." *Applied Surface Science* 283 (2013): 744-750.

[100] Kanth, Swarna V., et al. "Cleaner tanning practices for tannery pollution abatement: role of enzymes in eco-friendly vegetable tanning." *Journal of Cleaner Production* 17.5 (2009): 507-515.

[101] Thanikaivelan, P., et al. "Integrated hair removal and fiber opening process using mixed enzymes." *Clean Technologies and Environmental Policy* 9.1 (2007): 61-68.

[102] Kanth, Swarna Vinodh, et al. "Kinetics of leather dyeing pretreated with enzymes: Role of acid protease." *Bioresource technology* 100.8 (2009): 2430-2435.

[103] Basil-Jones, M. M., et al. "Collagen Fibril Orientation and Tear Strength across Ovine Skins." *Journal of agricultural and food chemistry* 61.50 (2013): 12327-12332.

[104] Covington, Anthony D., and Tony Covington. *Tanning chemistry: the science of leather*. Royal Society of Chemistry, 2009.

[105] Lepetit, Jacques. "Collagen contribution to meat toughness: Theoretical aspects." *Meat Science* 80.4 (2008): 960-967.

[106] Pant, Hem Raj, et al. "Electrospun nylon-6 spider-net like nanofiber mat containing TiO 2 nanoparticles: a multifunctional nanocomposite textile material." *Journal of hazardous materials* 185.1 (2011): 124-130.

[107] Alves, Patrícia, et al. "Surface modification of a thermoplastic polyurethane by low-pressure plasma treatment to improve hydrophilicity." *Journal of Applied Polymer Science* 122.4 (2011): 2302-2308.

[108] Fathima, N. Nishad, et al. "A DSC investigation on the changes in pore structure of skin during leather processing." *Thermochimica Acta* 501.1 (2010): 98-102.

[109] Geerligs, Marion, et al. "In vitro indentation to determine the mechanical properties of epidermis." *Journal of biomechanics* 44.6 (2011): 1176-1181.

[110] Grant, Colin A., Peter C. Twigg, and Desmond J. Tobin. "Static and dynamic nanomechanical properties of human skin tissue using atomic force microscopy: Effect of scarring in the upper dermis." *Acta biomaterialia* 8.11 (2012): 4123-4129.

[111] Sundar, Victor John, Chellappa Muralidharan, and Asit Baran Mandal. "A novel chrome tanning process for minimization of total dissolved solids and chromium in effluents." *Journal of Cleaner Production* 59 (2013): 239-244.

[112] Sanchez-Olivares, G., et al. "Sodium montmorillonite effect on the morphology, thermal, flame retardant and mechanical properties of semi-finished leather."*Applied Clay Science* 102 (2014): 254-260.

[113] Groves, Rachel B., et al. "An anisotropic, hyperelastic model for skin: experimental measurements, finite element modelling and identification of parameters for human and murine skin." *Journal of the mechanical behavior of biomedical materials* 18 (2013): 167-180.

[114] Damiron, Denis, et al. "Crosslinked PDMS elastomers and coatings from the thermal curing of vinyl-functionalized PDMS and a diazide aliphatic crosslinker."*Journal of Polymer Science Part A: Polymer Chemistry* 50.1 (2012): 98-107.

[115] Skinner, Michael W., et al. "Fundamental aspects of aminoalkyl siloxane softeners by molecular modeling and experimental methods." *Textile Research Journal* 69.12 (1999): 935-943.

[116] Edsberg, Laura E., et al. "Mechanical characteristics of human skin subjected to static versus cyclic normal pressures." *Journal of rehabilitation research and development* 36.2 (1999).

[117] Li, Yang, et al. "Comparison of dynamic denaturation temperature of collagen with its static denaturation temperature and the configuration characteristics in collagen denaturation processes." *Thermochimica Acta* 469.1 (2008): 71-76.

[118] Fathima, N. Nishad, et al. "Structure and dynamics of water in native and tanned collagen fibers: Effect of crosslinking." *International journal of biological macromolecules* 47.5 (2010): 590-596.

[119] Miles, Christopher A., and Michael Ghelashvili. "Polymer-in-a-box mechanism for the thermal stabilization of collagen molecules in fibers." *Biophysical Journal*76.6 (1999): 3243-3252.

[120] Lim, Jong Jin. "Transition temperature and enthalpy change dependence on stabilizing and destabilizing ions in the helix–coil transition in native tendon collagen." *Biopolymers* 15.12 (1976): 2371-2383.

[121] Komsa-Penkova, Regina, et al. "Thermal stability of calf skin collagen type I in salt solutions." *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology* 1297.2 (1996): 171-181.

[122] Miles, Christopher A., et al. "The increase in denaturation temperature following cross-linking of collagen is caused by dehydration of the fibres." *Journal of molecular biology* 346.2 (2005): 551-556.

Acknowledgments

First of all, I would like to sincerely thank professor Paolo Netti for giving me a chance to work into his research group. I am extremely grateful for the opportunity to participate in such interesting and rewarding science activities. I would like to express my special appreciation and thanks to my advisor professor Maurizio Ventre, for encouraging my research activity and for allowing me to grow as a research scientist. He helped me with his great expertise in the field, providing very useful and precious advices during my work thesis.

I am grateful to my colleagues Dr. Barbara Ziaco and Dr. Francisco Ortega, that often gave me some practical suggestions during my experimental work. I would like to thank everyone I met in the DMD Industry of Solofra, in particular Mr. Michele De Maio and Dr. Diodato De Maio, for their great kindness. Moreover a particular thanks goes to Dr. Giovanna Botta for her support during my work in the DMD Lab.

Finally I can't forget to thank my family and Pierluigi for everything they have done for me and for giving me an unconditional support.