Modern theory of the tanning process

COLLAGEN is the protein of animal skin. Its structure is that of a right-handed triple propeller formed by three left-handed α-propellers. By the term TROPOCOLLAGEN is meant the fundamental unit. α-helix are characterized by the repetition of the triplet

Xaa-Yaa-Gly (1) COLLAGEN – α-HELIX

where the xaa residue is often a Pro residue while the YAA residue is often a Hyp. Pro and Hyp account for about 25% of the entire amino acid fraction, of which 14% is Hyp.

Hyp is a fundamental component. It is formed in the course of post-translational modifications by hydroxylation of a carbon in the γ position of the Ring of a Pro once it has been incorporated into polypeptide chains. It is, therefore, an imminoacid (nonessential amino acid) that, together with Hyl, directs the conformation of the propellers by virtue of the limits of rotation that it is able to confer to this type of structures. It also contributes to the stabilization of the structure of the triple helix forming many hydrogen bonds that, as we shall see, can be intra-chain, inter-chain, which is inside the same α-helix, and intramolecular, that is, between a triple helix, and another.

Figure 1 shows the schematization of a triple helix with a pro-Hyp-Gly triplet. The hydroxyls of the three amino acids always point outward both of the single helices and of the triple helix and the same applies to all the lateral substituents of the amino acids of the generic triplet reported in (1):

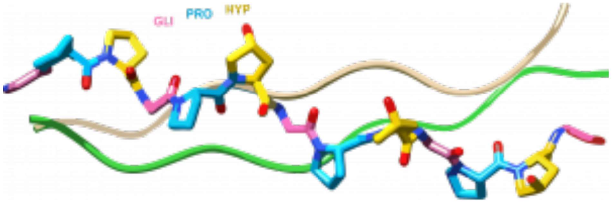
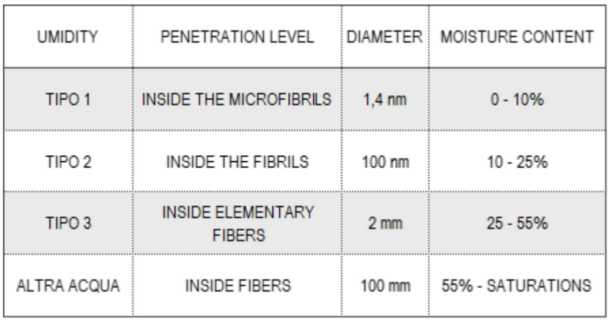


Figure 1: modern theory of the tanning process – representation of an alpha helix with Pro – Hyp-Gly triplet and triple helix

The stability of both the α-propellers and the super structure of the triple propellers is very high. Scholars have always searched for the causes of such stability often coming to erroneous conclusions. The definitive answer seems to have arrived a few years ago, the result of all the studies we are going to report. The understanding of what factors stabilize collagen has allowed the formulation of a modern theory capable of explaining the stabilization of collagen as a result of tanning processes.

In the early ‘900s, researchers W. D. Evans and C. L. Critchfield had demonstrated how moisture absorbed by Skins had favorable effects on their technological properties. The researchers compared samples of bovine hides tanned with chrome tanning and with vegetable tanning by performing tests of technological resistances at different degrees of relative humidity. The study clarified the importance of the moisture content in finished skins in defining its technological properties, recommending that the relative moisture content be fixed at values of 14-16%.

In the early 80s, Bienkievicz formulated a model to explain how the moisture absorbed by the Skins was distributed. He divided the moisture contained in them into three levels of penetration, defining each of them as Type 1, 2 and 3 Water (Table 1):



UMIDITY	PENETRATION LEVEL	DIAMETER	MOISTURE CONTENT
TIPO 1	INSIDE THE MICROFIBRILS	1,4 nm	0 - 10%
TIPO 2	INSIDE THE FIBRILS	100 nm	10 - 25%
TIPO 3	INSIDE ELEMENTARY FIBERS	2 mm	25 - 55%
ALTRA ACQUA	INSIDE FIBERS	100 mm	55% - SATURATIONS

Table 1: modern theory of the tanning process – moisture penetration levels in the bienkiewicz model

In Bienkievicz's model, type 1 water represents the moisture that is contained between tropocollagen molecules, and the structural level is that of microfibrils. The structural level of microfibrils is that of the fundamental unit, or tropocollagen, in which three α-propellers (average length 300 nm) are organized in a checkerboard manner until they form microfibrils. Bienkievicz had sensed how Type 1 water linked externally to the triple propeller and by means of hydrogen bridges between the side groups of each single propeller that forms its structure. As Table 1 shows, Type 1 water accounts for the first 8-10% of the moisture that is retained by the skins.

Type 2 water is the water retained within the structure of the fibrils, and the structural level is the space between one microfibrilla and another. It is bound by the lateral groups of microfibrils that are the same as those of tropocollagen. Compared to what is seen for Type 1 water, we are at a higher structural level. Type 2 water represents about 15% of the total moisture that the skin manages to retain and it is that contained within the range that we can estimate about 10-25%.

Type 3 water represents the moisture that is contained within the fibers and that is bound to the skins by means of capillary forces. Here too, compared to type 2 water, we are at a higher structural level, that is, the internal level of the fibers. Type 3 water can make up about 25-30% of the total moisture that the skin can retain and represents moisture within the range of about 25-55%.

Humidity values exceeding 55% consist of excess water.

Bienvievicz had guessed that type 1 moisture is important to guarantee the mechanical properties of the skins because this type of water helps the mutual mobility of the triple helices of tropocollagene and, in particular, favors the mutual flow:

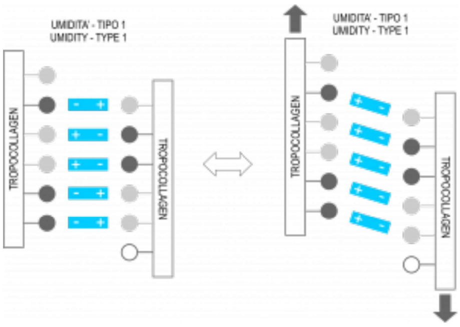


Figure 2: modern theory of the tanning process – type 1 moisture and tropocollagen triple helix mobility

According to Bienkievicz, in non-tanned skin, the loss of this moisture is irreversible because it approaches the propellers of tropocollagen that stick irretrievably and this causes the denaturation of the peptides. Tanned leathers, on the other hand, are able to recover excessive dehydration due to their stabilization, their network of transverse bonds. This is visible in Figure 3:

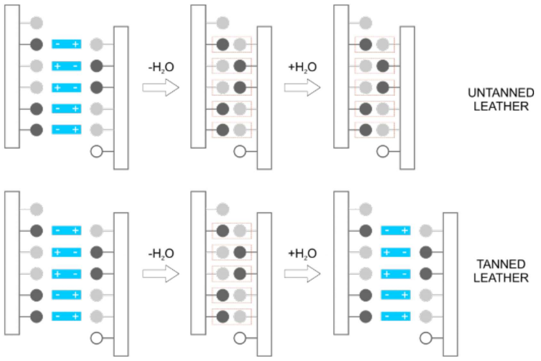


Figure 3: modern theory of the tanning process – type 1 moisture removal from tropocollagen propellers on tanned and non-tanned skins

Bienkievicz had therefore sensed the importance of the fraction of water present in the immediate vicinity of the tropocollagene unit and, with the means available at the time, he had not been able to formulate more in-depth explanations.

In 1994 J. Bella and colleagues demonstrated how the ordered structure of tropocollagen forces water in the immediate vicinity of the triple helices to form, in turn, an ordered structure they called the OUTER WATER SHELL. The outer water shell is a super structure in turn formed by several sub-levels in which water molecules are bound by hydrogen bonds to the side groups of amino acids, especially the -C=O groups and the Hyp hydroxyls.

In this super structure, the water molecules bind to the different lateral groups through structures that are called WATER BRIDGES and are formed by 4-6 water molecules and the geometry present in the majority is the pentagonal one. They can consist exclusively of water molecules or they can exploit the lateral groups of the α-propellers as support vertices. Furthermore, they can be present as intra and inter molecular bridges, i.e. inside and between the single α-helices (Figure 4) or as intramolecular bridges, i.e. between the different triple helices:

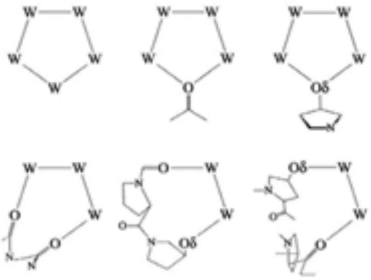


Figure 4: modern theory of the tanning process – closed water bridges observed in the structure of a crystalline peptide Gly-Ala (w=H2O)

In collagen peptides, the water bridges that are found very frequently are those that connect the C=O groups with other C=O groups of the same α-helices (α-bridges, Figure 5) or of different α-helices (β-bridges, Figure 6):

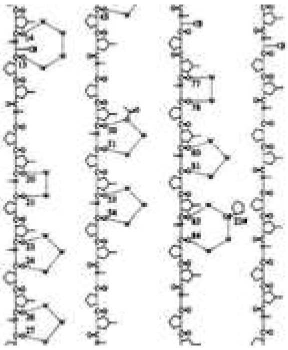


Figure 5: ponti-α

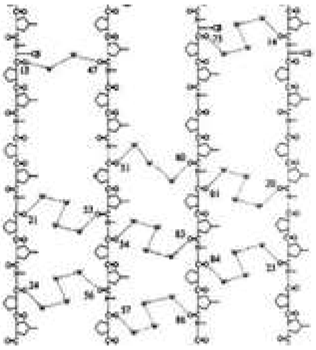


Figure 6: ponti-β

Much more frequent than the first are water bridges connecting Hyp-OH groups and carboxylic side groups:

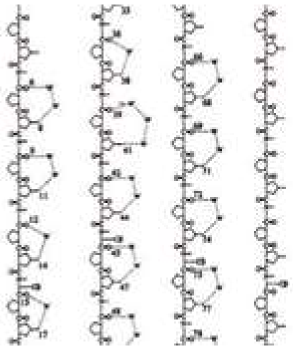


Figure 7: ponti-γ

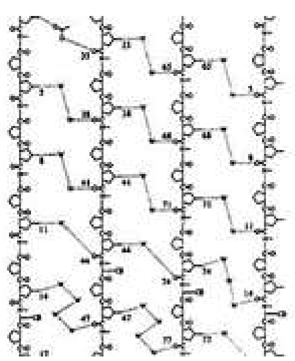


Figure 8: ponti-δ

As in the previous case, these can also connect side groups of the same chain (bridges-γ, Figure 7) or of different α-propellers (bridges-δ, Figure 8).

Water bridges between amino groups and carboxyl groups of peptides are also possible. These bridges are different from those seen earlier because they consist of four water molecules that sneak between the α-propellers to form what are called intersitalwater Bridges (Bridges-ζ, Figure 9).

These bridges are tetramerally coordinated to other water molecules and are able to nuclear additional water bridges that can connect other surrounding groups:

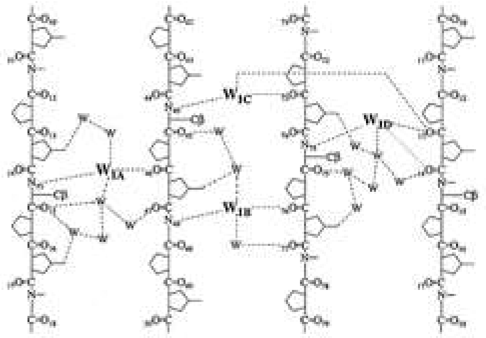


Figure 9: intersital water bridges (w= H2O)

The last category of water bridges is one that includes those bridges that connect the anchor points between the neighboring triple propellers (intrermolecular bridges), which are called ω-bridges.

These can be of three different types (Figure 10):

$$-C_y-O_\delta\cdots\cdots W_n\cdots\cdots O_\delta-C_y-$$

$$-C_y-O_\delta\cdots\cdots W_n\cdots\cdots O=C-$$

$$-C=O\cdots\cdots W_n\cdots\cdots O=C-$$

Figure 10: water bridges – ω-bridges (w= H2O)

The outer water shell is formed by the orderly combination of all these bridge structures and, as we have said, by overlapping three structural levels that are the 1st, 2nd and 3rd shells (Figure 11). Researchers have shown that water in the immediate vicinity of tropocollagen has a much higher level of organization than the rest of the water absorbed by the skins (which can be physiological water, environmental humidity or a processing bath). This water is nothing more than the type 1 moisture predicted by the Bienkievicz model.

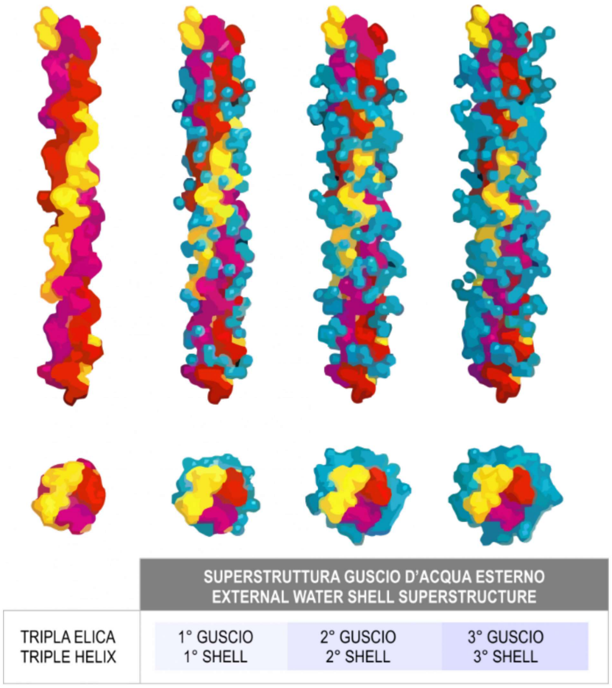


Figure 11: modern theory of the tanning process – structure of the outer water shell

The stabilization of the super structure of the water shell is due to the presence of Hyp, whose hydroxyl participates in most water bridges and acts as a pivot for the formation of the entire structure of the outer shell involving intra and inter chain bridges. The super structure thus formed is supported by a widespread hydrogen bond that accompanies the twisting of the triple helix.

To confirm their findings, and the contribution of Hyp, scholars reported the results of some experiments on the thermodynamic which showed how the denaturation of a peptide of the type Pro–Hyp–Gly in aqueous solution requires more energy than that required for the denaturation of the peptide Pro–Pro–Gly because the presence of Hyp confer increased stability of the peptide.

The conclusions that J. Bella and colleagues came to were that the role of the water shell was fundamental for the stabilization of the triple propellers, but these were partly incorrect conclusions that were corrected by studies carried out about 25 years later. We know that the mechanical properties of skins are affected by their moisture content and that this also affects their denaturation temperature. The denaturation temperature of collagen increases as its water content decreases.

In 1999, researchers Christopher A. Miles and Michael Ghelashvili introduced the polymer in a BOX model to clarify how the denaturation mechanisms of fibrous peptides such as collagen occur. Even if they used tendon collagen in the tests, the results of their study can be applied to any type of existing collagen, including that which makes up animal skin.

According to the POLYMER IN A BOX MODEL, an isolated tropocollagen chain has a lower thermal resistance of 25-30 °C than that of the same peptide found within a fibrous lattice. This happens because the confinement of the protein within a closed structure reduces its freedom of movement. In the polymer in a box model, the structure of the fibrous lattice is imagined as a cylindrical box inside which a single fiber is contained:

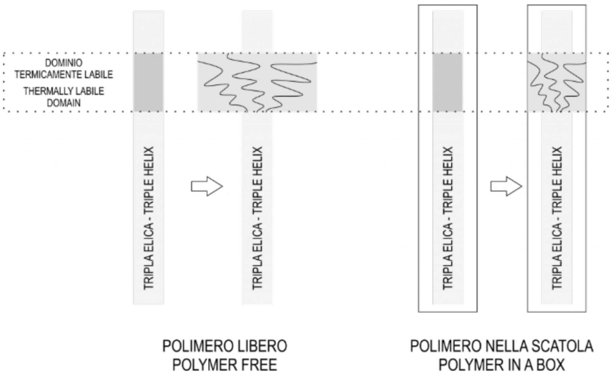


Figure 12: modern theory of the tanning process – polymer in a box model

The researchers demonstrated how the confinement of the peptide within a closed structure reduces the entropy of activation of denaturation reactions.

The denaturation of a collagen triple helix begins in a region that is called the THERMALLY LABILE DOMAIN which is a 65 amino acid trait in which Hyp is absent. Thermal denaturation begins in this precise area where, as a result of temperature, the α-helices begin to fray and then open and Flake the entire Triple Helix (Figure 12).

A fibrous lattice can be imagined as a box containing a triple helix whose walls are nothing more than the adjacent triple helices. In the model, the walls of the box hinder the opening of the thermally labile domain, keeping it compact in cases where the temperature tries to flake it. The number of possible configurations of the activated state of the thermally labile domain when it is confined within the box is limited by the presence of the walls. This is why entropy decreases while activation enthalpy increases. By transporting the model in a real-world situation, the immersion of a fibrous protein within a lattice increases its thermal stability because more energy is needed to degrade it.

Scholars explained the effects of confinement by increasing the cross-link network between the different collagen triple helices. The greater the closeness, the greater the possible interactions, and therefore the greater the possibility of forming cross-links. The higher the number of transverse bonds, the closer the triple helices are brought together, resulting in a synergistic effect that compacts the fibrous structure:

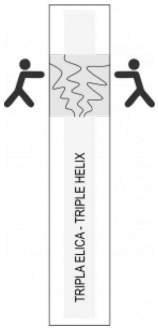
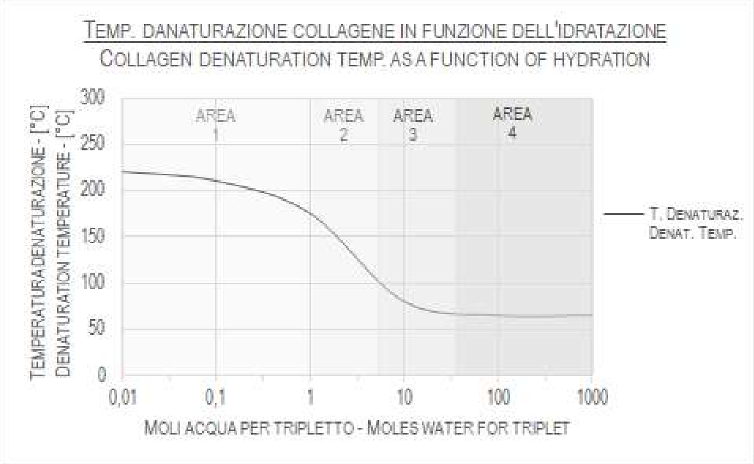


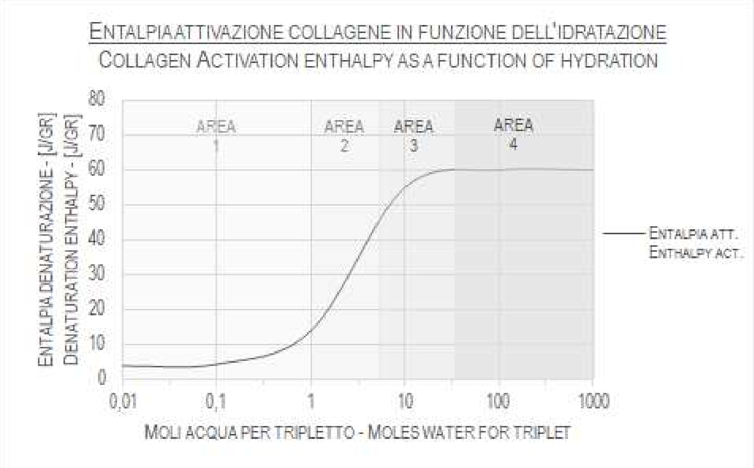
Figure 13: modern theory of the tanning process – effect of the confinement of fibers within a fibrous lattice – polymer in a box model

They demonstrated how cross-linking promotes the stability of the collagen structure because by means of it the free spaces that are present between a triple helix and another are reduced. Compared to free fibers, those that are confined within a closed space have lower entropy.

The relationship that exists between the water content inside the peptite and the size of the fibrous lattices is easy to guess. The presence of water swells the fibers. Dehydration deflates them and brings them closer. It has been calculated that, in the event that tropocollagen is heavily dehydrated, it takes 30 minutes for a complete network of intermolecular hydrogen bonds to form. The graphs Graph 1 and Graph 2 show us how the denaturation temperature and the denaturation enthalpy of some collagen samples vary according to their degree of hydration.



Graphic 1: modern theory of the process of – temp. denaturation of a collagen peptide according to its degree of hydration



Graphic 2: modern theory of the tanning – enthalpy process of activation of a collagen peptide as a function of its degree of hydration

We can divide both graphs into four different areas that correspond to progressive hydration States.

Area 1 is the area where collagen is characterized by an extremely low degree of hydration, about 1 mole of water per triplet. In this region, the enthalpy of denaturation is almost constant, while the denaturation temperature is extremely high, around 200°C. Although the collagen in these conditions, thermal stability, outstanding, in this state of dehydration of the protein is unstable because it lacks a shell, external water (moisture Type 1). The removal of what Bienkievicz defined as Type 1 moisture reduces the denaturation enthalpy and the configurationsl entropy of the thermally labile domain. It is necessary to break fewer hydrogen bonds to separate the α-helices from each other.

Area 2 is a region where collagen has a low degree of hydration, from 1 to 6 moles of water per triplet. In this area, the activation enthalpy grows rapidly, and the denaturation temperature decreases just as sharply. When the degree of hydration is equal to 2 moles of water per triplet it means that half of the hydrogen bridges present have been broken and the super structure of the outer water shell consists of the first and perhaps the second shell.

Area 3 is the region where collagen has moderate hydration, from 6 to 30 moles of water per triplet. Here the increase in denaturation enthalpy and the decrease in denaturation temperature become constant. When the amount of water reaches six molecules per triplet and above, all possible water bridges that the super structure of the water shell requires are formed. The enthalpy of denaturation settles to constant values and is not affected by increases in hydration.

Area 4 is the area where collagen has normal (physiological) hydration or excess water (over 30 moles of water per triplet). This area has the maximum value of activation enthalpy and a "physiological" value of denaturation temperature, which is 50-55°C.

With progressive dehydration there is a continuous rearrangement in the packing of the fibers inside the fibrous Lattice and a progressive destruction of the hydrogen bonds inside and outside the triple helices.

The polymer in a box model also explains how, under equal conditions, swollen collagen has a lower denaturation temperature than that of non-swollen collagen and overturns the conclusions reached by J. Bella and colleagues. Although it increases its technological properties, water does not stabilize the collagen peptide but, in this sense, produces deleterious effects.

In 2005 Christopher A. Miles, Nicholas C. Avery, Victor V. Rodin, and Allen J. Bailey used the polymer in a box model to confirm that cross-linking collagen causes an increase in its denaturation temperature due to a decrease in the degree of hydration. In their study, the researchers stabilized rat tendon collagen through three different tanning agents with progressive molecular dimensions:

Malondialdehyde (MDA) three-carbon bivalent aliphatic aldehyde;

Glutaraldehyde (GT), five-carbon bivalent aliphatic aldehyde;

Hexamethylene dissocyanate (HMDC), eight-carbon bivalent isocyanate.

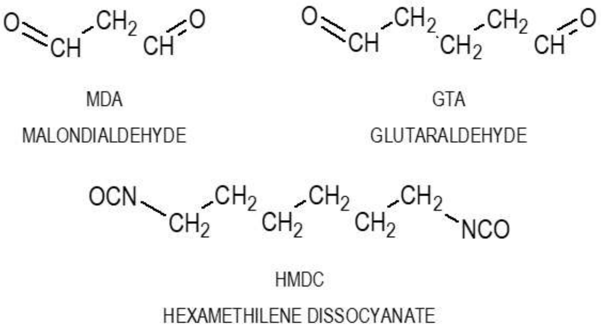
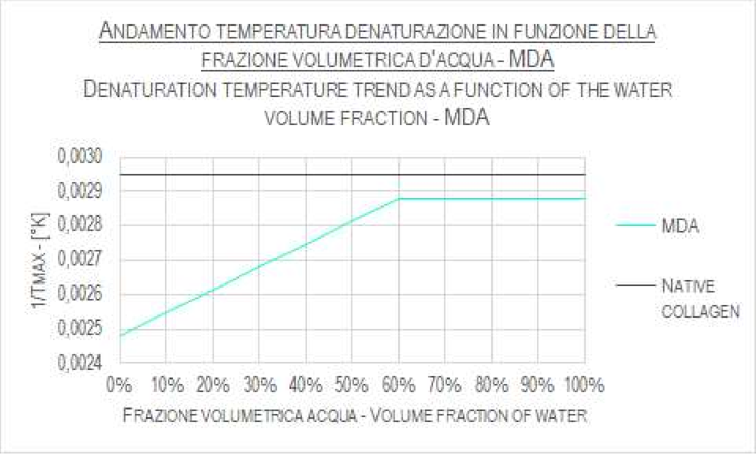
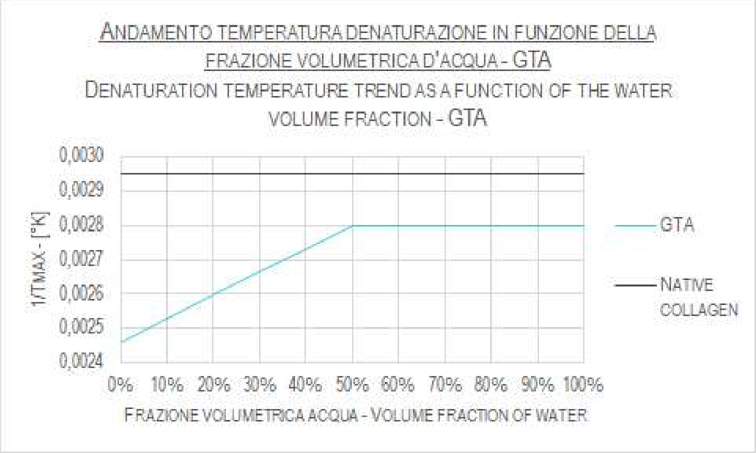


Figure 14: modern theory of tanning process – bivalent stabilizing agents-tendon collagen

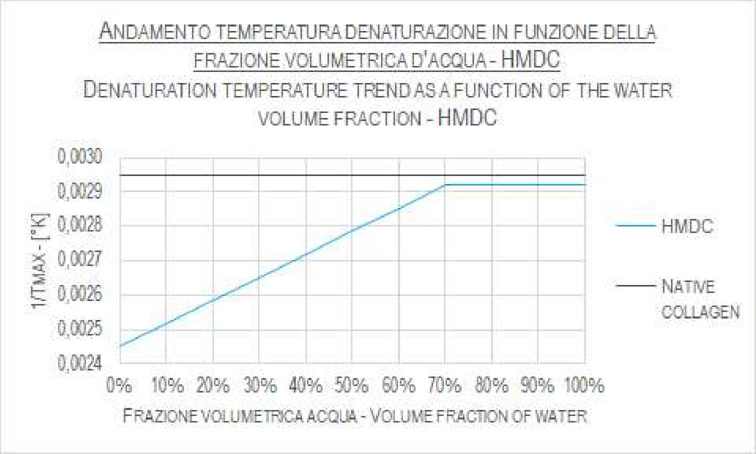
They then determined the value of the denaturation temperature of the collagens stabilized at different values of the water content present in them. The results of their study are summarized in graphs Graph 3, Graph 4 and Graph 5:



Graphic 3: modern theory of the tanning process-collagen denaturation temperature as a function of the volumetric fraction of water – MDA



Graphic 4: modern theory of the tanning process-collagen denaturation temperature as a function of the volumetric fraction of water -GTA



Graphic 5: modern theory of the tanning process-collagen denaturation temperature as a function of the volumetric fraction of water – HDMC

In the graphs, denaturation temperature values are reported as ratio [1/Tmax] while water contents as volumetric percentage fraction. In the case of all three stabilizing agents there was confirmation of the linear relationship between the term [1/Tmax] and the volume fraction of the water present within the collagen. This means that increasing the amount of water inside the protein reduces its thermal stability. The value of grows linearly until it reaches the maximum value at the conditions of maximum water absorption, which are different in the different tanners used. Once maximum hydration is reached, the term [1/Tmax] becomes constant and the collagens are in the conditions of minimum thermal stability.

This test allowed scholars to confirm one very important thing. The thermal stability (S_t) of collagen peptides that are confined within fibrous lattices depends on the sum of two terms:

The intrinsic thermal stability of the peptide fundamental units without any stabilizing interaction (S_i);

Thermal stability resulting from the confinement of peptides within fibrous tissue (S_c). The volumetric fraction of water determines the size of the lattice, that is, the size of the box of the polymer model in a box, because water fills the inter-fibrillar spaces and bulges them, expanding or narrowing the lattice. Therefore the presence of water influences this second term. The greater the amount of water in the protein, and the less will be the contribution of S_c

St = Si + Sc (2) COLLAGEN TERMICAL STABILITY

The results of this study confirmed that cross-linking is indirectly responsible for collagen stabilization. It is not the transverse bonds that stabilize the peptides but it is the tension generated by them that narrows the size of the fibrous lattices, reducing the ability of collagen to be inflated by the action of water.

The work of these scholars has been limited to the study of collagen denaturation temperatures, without paying attention to how the mechanical properties of the protein vary as the degree of hydration varies.

A further confirmation of the fact that the presence of water does not contribute to stabilization of the collagen was provided in 2005 by the work of the researcher Ronald T. Raines which has demonstrated how it is true that the stability of the triple helix of collagen depend on the presence of Hyp but not for his contribution in the stabilization of the structure of the shell of water outside, but through the stereoelectronic effects that the imminoacido transfers on each of the α-helices.

In his study, the researcher replaced a triplet Hyp (xaa-Hyp-Gly) with an Flp by evaluating the stability of the propeller produced. He later did the same by inserting an flp in the Xaa position.

Flp and flp are Hyp in which the lateral –OH group is replaced by a fluorine atom, much more electronegative. Flp and flp are diastereoisomers of the same molecule:

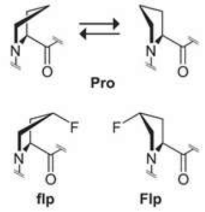


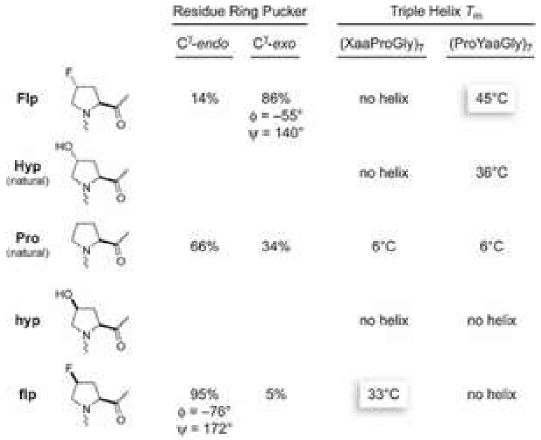
Figure 15: modern theory of the tanning process – Flp and flp diastereoisomers

The reasons why the researcher replaced the hydroxyl group with a fluorine atom were two:

The first because fluorine has greater electronegativity than that of hydroxyl. Its introduction served the researcher to exasperate the inductive effect on α-propellers;

The second because organic fluorine is a bad acceptor of hydrogen bonds. This served the wanted to eliminate the interference of water in the stabilization of the peptide.

Table 2 summarizes the results of what was observed:



	Residue Ring Pucker		Triple Helix T_m	
	C^{γ}-endo	C^{γ}-exo	$(XaaProGly)_7$	$(ProYaaGly)_7$
Flp	14%	86% $\phi = -55°$ $\psi = 140°$	no helix	45°C
Hyp (natural)			no helix	36°C
Pro (natural)	66%	34%	6°C	6°C
hyp			no helix	no helix
flp	95% $\phi = -76°$ $\psi = 172°$	5%	33°C	no helix

Table 2: modern theory of the tanning process – stability of the triple helix as a function of the composition of the X_{aa}-Y_{aa}-Gly triplet

The researcher noticed how Flp is able to stabilize α-propellers more than Hyp. As we see, the denaturation temperature was about 10°C higher. Since it is not possible formation of hydrogen bonds with the water of the outer shell of the triple helix, the only explanation for the results was to call in due to the increase of the electronegativity of the substituent and its inductive effect on the α-helices. Since the formation of hydrogen bonds was excluded, the conclusion reached by the scholar was that the α-propellers are not stabilized by the presence of the water shell but are stabilized by the electronic stereo effects that the electronegative substituent induces in both single α-propellers and triple propellers.

As for the individual α-helices, the Gauche effect on the pyrrolidine ring first intervenes, which fixes at an angle of 60°C the electronegative substituents that are bound to two adjacent carbon atoms. The two substituents may form a hydrogen bond (difficult in the case of fluorine), while the orbital σ of the substituents electropositive and σ* of the substituents electronegative have the possibility of overlap (and this is possible), giving rise to the first important contribution to the stability of the peptide.

The appearance of the Gauche effect deforms the pyrrolidine ring on the Cy and fixes it in the preferential conformation Cy-exo, which was present in 86% of the residues:

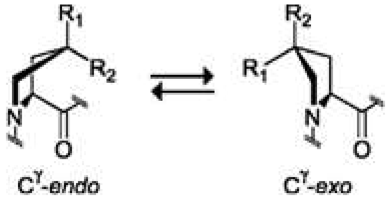


Figure 16: modern theory of the tanning process – endo and exo conformations of the amino acid proline

The Cy–exo conformation of the pyrrolidine ring influences the entire geometry of the α-helices and organizes the torsion angles of the main chains (φ, ψ and ω) to values with great stability, also favoring the trans configurations of peptide bonds.

Flp stabilizes the peptide when placed in the X_{aa} position less than Flp does in the Yaa position and this is due to the inductive effect that forces 95% of the peptide bonds in the cis position, less stable:

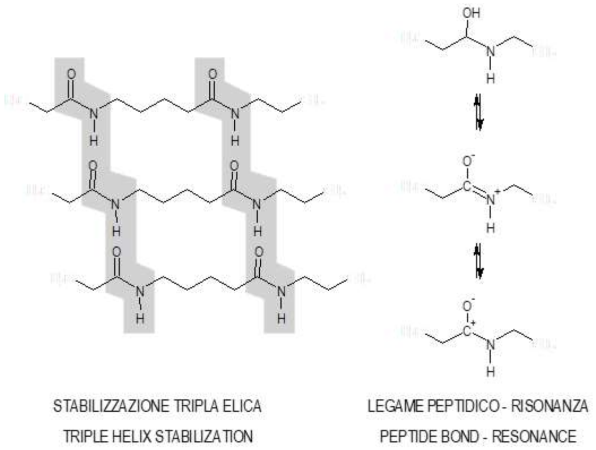


Figure 17: modern theory of the process of tanning – stabilization of peptide bonds of a triple helix by effect of resonance induced by electronegative substituents of the hyp

By virtue of these results, it was definitively confirmed that the water Shell has no role in the stabilization of collagen. The studies mentioned above, however, confirm that water has an unfavorable effect because of the swelling it induces on the fibrous lattice. Of course, we always remember that we are talking only about the stability of the skin protein, and not about its technological characteristics.

What centers all this with tanning?

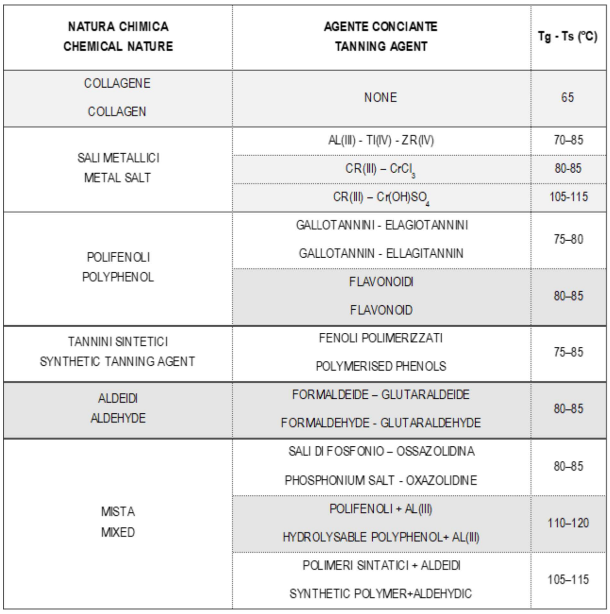
In his book on tanning technology, Anthony D. Covington used the results of these studies to propose a modern theory of the tanning process that would justify the tanning action of different chemical species. The aforementioned studies, in fact, state that cross-bonds are an indirect cause of collagen stabilization. Covington wondered, then, according to which mechanism tanning agents stabilize the skin demonstrating, first of all, how the theory of cross-linking by cross-linking was actually incorrect.

The first thing Covington did was to divide the main tanning agents into two categories according to the thermal stability given to the skins, that is, as a function of the contraction temperature reached:

Tanners that give a moderate increase in T_c – i.e. poorly stabilizing;

Tanning agents that give a high increase in T_c – that is, strongly stabilizing.

With reference to tanning technology, the contraction temperature is the temperature at which the thermal denaturation of the peptide begins:



NATURA CHIMICA CHEMICAL NATURE	AGENTE CONCIANTE TANNING AGENT	Tg - Ts (°C)
COLLAGENE COLLAGEN	NONE	65
SALI METALLICI METAL SALT	AL(III) - TI(IV) - ZR(IV)	70–85
	CR(III) – $CrCl_3$	80-85
	CR(III) – $Cr(OH)SO_4$	105-115
POLIFENOLI POLYPHENOL	GALLOTANNINI - ELAGIOTANNINI GALLOTANNIN - ELLAGITANNIN	75–80
	FLAVONOIDI FLAVONOID	80–85
TANNINI SINTETICI SYNTHETIC TANNING AGENT	FENOLI POLIMERIZZATI POLYMERISED PHENOLS	75–85
ALDEIDI ALDEHYDE	FORMALDEIDE – GLUTARALDEIDE FORMALDEHYDE - GLUTARALDEHYDE	80–85
MISTA MIXED	SALI DI FOSFONIO – OSSAZOLIDINA PHOSPHONIUM SALT - OXAZOLIDINE	80–85
	POLIFENOLI + AL(III) HYDROLYSABLE POLYPHENOL+ AL(III)	110–120
	POLIMERI SINTATICI + ALDEIDI SYNTHETIC POLYMER+ALDEHYDIC	105–115

Table 3: modern theory of the tanning process – stabilizing capabilities of different tanning agents

The first question raised by Covington focused on the fact that the conventional view of the mechanism of tanning, in which the thermal stability reached by the skin is related to the degree of crosslinking achieved, it was not sufficient to explain why the majority of tanning agents to be able to give the skins for only a modest thermal stability, in spite of the resistance to the action of enzymes. If so, all tanning agents capable of binding to the skins through covalent bonds should make it possible to obtain a highly stabilized leather. The same should happen with vegetable tanners, which must be offered in very high quantities (percentages of 25-40% by weight) and which should ensure the creation of a huge network of stabilizing bonds.

Yet, in both cases, this does not happen, the simple cross-linking of the triple propellers was not enough to justify the tanning effects.

Subsequently, Covington reported the results of the isometric tension (HIT) tests performed on samples of tanned leather with the tanning agents shown in Table 3:

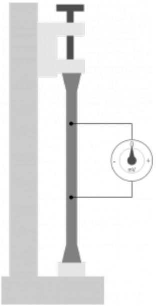
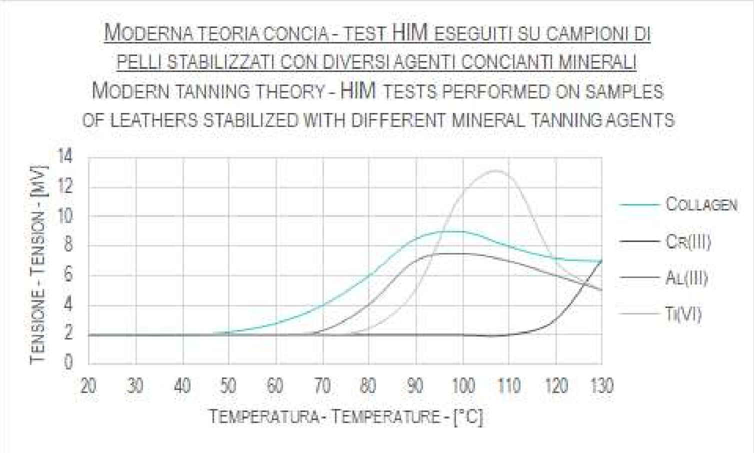


Figure 18: modern theory of the tanning process-schematization of a hit isometric voltage test

This type of test is performed on muscle fibers to measure their contractions. Muscle fibers are very special fibers because they are able to contract even without changing their length due to the effect of electrical impulses. To measure this type of phenomena, samples of muscle fibers are locked between two clamps, and contractions are measured not in terms of strength but in terms of electrical voltage.

The researcher did the same using samples of tanned leather, measuring its electrical contractions according to the different temperatures at which they were immersed.

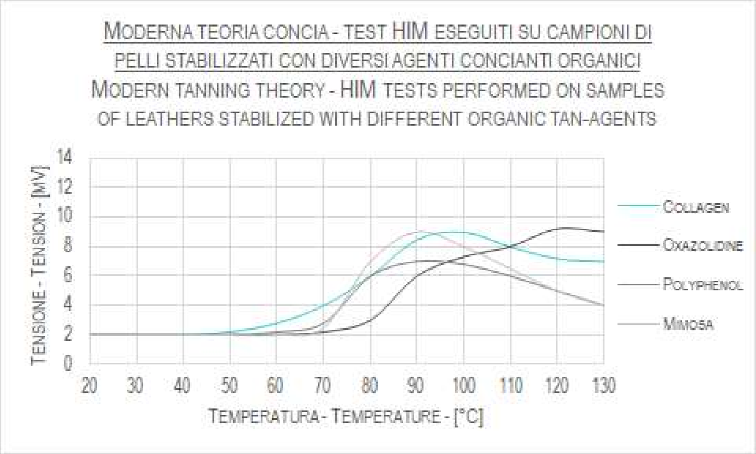
The graphs obtained from this type of test show a first phase in which there is no increase in voltage and in which the curves have constant trends. At a certain temperature value, different depending on the different tanners used, the samples begin to contract, a sign that a thermal denaturation is beginning. With the increase in temperature also increases the recorded tension because the fibers oppose more and more resistance and also increase the slopes of the curves. The resistance offered by the fibers depends on the toughness of the transverse bonds that they form with the Tanners. The curves reach a maximum beyond which the slope reverses, a sign that the fibrous structure is now compromised and is falling apart. The slopes of the downhill sections define the ease with which the fibrous tissues relax, that is, the stability of the cross-links:



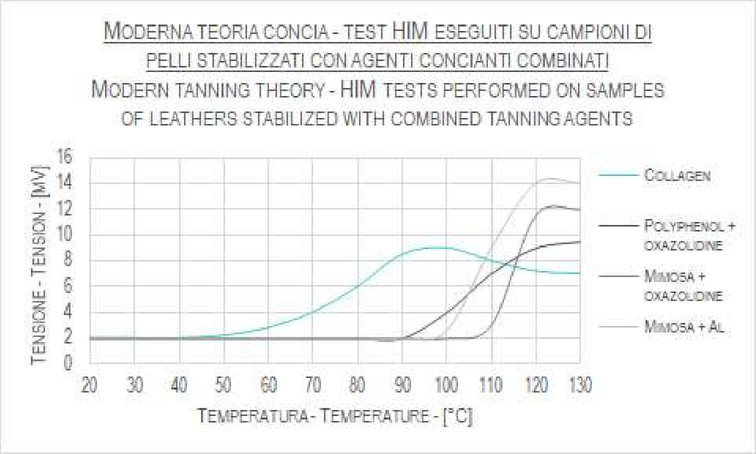
Graphic 6: modern theory of the tanning process – him analysis of samples of tanned leather with different mineral tanning agents

In the case of the three mineral tanners, Cr (III), Al(III) and Ti(IV) the initial resistance curves have the same slopes as the curve of unstabilized collagen. The difference is in the temperature at which the contraction of the samples begins, which is a function of the tanning agent used. Covington’s conclusion was that cross-linking of skin fibers alone is not sufficient to justify chemical stabilization of tanning agents.

Graph 7 shows the trends of the curves of the HIM tests for organic tanners which confirm that, due to the large quantities of vegetable tanners used, high T_c is not achieved. There is something that does not square in the thermal stability obtained and in the cross-linking that should have been produced by similar amounts of tanning agents:



Graphic 7: modern theory of the tanning process – him analysis of samples of tanned leather with different organic tanning agents



Graphic 8: modern theory of the tanning process – him analysis of samples of tanned leather with different tanning agents-mixed tanning

Finally, Figure 8 shows the results of the HIM tests in the case of mixed tannins, which are significantly higher than those achieved in the case of tannins seen earlier.

Based on the conclusions reached in the studies concerning the thermal stability of collagen and on the fact that cross-linking is indirectly responsible for the thermal stability achieved by the skins, Covington wonders why the tanning action is manifested.

According to the scholar, the tanning action of any chemical species is manifested by the creation of a CHEMICAL MATRIX formed by the four elements that intervene during the tanning process, which are:

- Collagen;
- Water;
- The tanning agent or the main tanning agent;
- The counterion or secondary tanning agent.

The Matrix acts as a single chemical compound, and its stability depends on the nature and interactions of all these four components.

The tanning agent forms the network of transverse bonds that join the α-helices and the triple helices. The crosslinking it produces contracts the fibrous tissue and reduces the size of the internal spaces, according to the typical mechanisms of each tanning agent. The type of bonds that are established between the tanning agent and collagen is one of the factors that determines the thermal stability achieved by tanning. Among the mineral tannins, the chrome one is the only one that gives the skins a high thermal stabilization. The low shrinkage temperature that are obtained by using aluminum or titanium salt are due to the fact that the complexes of these metals form much more labile bonds than chromium complexes. Moreover, unlike the latter, their polymers are not stabilized by oxolation reactions in which replacements of dative bonds with covalent ones further contract the size of the transverse bonds. In aldehydes, despite the formation of covalent bonds, the contribution of the counterion to the chemical matrix is lacking. Finally, vegetable tanning gives the skins modest thermal stability due to the labile tanning interactions that occur through the formation of hydrogen bonds between the phenolic groups of tannins and the peptide groups of the protein.

The mixed tanning allows us to understand the importance of the secondary tanning agent which serves to enhance the cross-linking capabilities of the secondary agent. In the case of aluminium-mimosa tanning (Graph 8) the high tension achieved by the fibres also depends on the contribution of the sulphate counter-ion.

The counter-ion of the tanning agent is very important and can be considered as a secondary tanning agent because, even if it does not participate in the formation of cross-bonds, it is able to interact with the chemical Matrix.

This is shown to us with the Cr (III). Tanning with basic chromium sulfate, whose counter-ion is the sulfate anion, produces a leather with a much higher thermal stability than that of chromium chloride, whose counter-ion is the chloride anion. The reason is to be found in the ability of the counter-ion to interact with the water molecules of the outer shell through the formation of hydrogen bonds, a capacity that is precluded to the Cl^- anion.

It has been shown that, in the case of chromium sulfate, the sulfate group is present within the tanning complexes in solution but is not present within the polymers forming the cross-bonds, despite being present within the chemical xatrix.

This was explained by the fact that the anion exits the complexes that are in the immediate vicinity of collagen to enter the matrix structure forming an outer layer to the water shell in which each sulfate group forms four hydrogen bonds with as many water molecules. It can be postulated that, despite the unfavorable effects on thermal stability, in the Matrix modified by tanning chemistry should remain a part of this structural water.

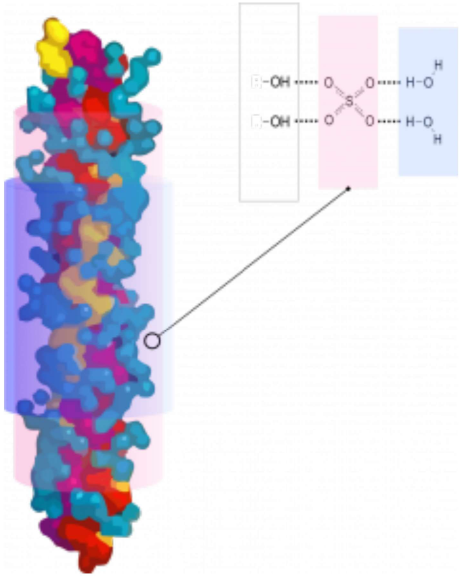


Figure 19: modern theory of sulfate tanning-counterion process in chemical matrix

The counterion should be considered as a secondary tanner and the overall synergistic nature of the combined reactions depends on the operation of the counter ion as a producer of structures in water.

The role of the chemical matrix within the fibrous structure of the skin is to reduce the mutual mobility of the fibers, reducing the enthalpy configuration of thermally labile domains. In accordance with the model of the polymer in a box, the reduction of mobility increases the thermal resistances.

Although we talked about the fact that the creation of cross-links reduces the hydration of the collagen, it is not possible to justify the effects tanning with dehydration because, under normal conditions of use of the skin, the moisture within them, than the values of the humidity of Type 1. It is also shown that the water associated with the collagen structure is practically independent of tanning chemistry and is equal to about 23%, whether it is stabilized with Cr(III), whether it is stabilized with formaldehyde or with hydrolysable or condensed vegetable tannins.

Thermal degradation of collagen must, as a first thing, displace or damage the chemical Matrix Inside it. If the matrix is water, as in unstabilized collagen, the removal of the matrix is simple and the T_c is low. If the Matrix consists of a chemical network, its removal requires as much energy as the more stable the network is.

Covington's conclusions were that, in order for tanning processes to be able to give leathers high thermal stability, it is necessary that the chemical Matrix become a compound with great stability. For these reasons, it is difficult to achieve high stability using single tanning agents and the best results, in terms of contraction temperatures achieved, are those that provide combined tanning.

I hope you enjoyed this article and found it useful!

For any comments and suggestions, do not hesitate to contact me!

Daniele Pistorio

Partial biography

- The Effects Of Atmospheric Moisture On The Physical Properties Of Vegetable And Chrome Tanned Calf Leathers – W. D. Evans And C. L. Critchfield, U.S. Department Of Commerce, Bureau Of Standards Research Paper Rp583, Part Of Bureau Of Standards Journal Of Research, Vol. 11, July 1933;
- Hydration Structure Of A Collagen Peptide – Jordi Bella, Helen M. Berman, Department Of Chemistry, Rutgers University, Piscataway, Nj 08855, Usa – Barbara Brodsky, Umdnj-Robert Wood Johnson Medical School, Piscataway, Nj 08854, Usa;
- Polymer-In-A-Box Mechanism For The Thermal Stabilization Of Collagen Molecules In Fibers – Christopher A. Miles And Michael Ghelashvili – Collagen Research Group, Department Of Clinical Veterinary Science, University Of Bristol, Langford, Bristol Bs40 5du, England;
- The Increase In Denaturation Temperature Following Cross-Linking Of Collagen Is Caused By Dehydration Of The Fibres – Christopher A. Miles, Nicholas C. Avery, Allen J. Bailey, Collagen Research Group, University Of Bristol, Langford Bristol Bs40 5du, Uk – Victor V. Rodin, Mati Russian State, Technological University 3, Orshanskaya Str, Moscow 121552, Russian Federation;
- Ronald T. Raines, Departments Of Biochemistry And Chemistry, University Of Wisconsin—Madison, Madison, Wisconsin 53706, Usa;
- Tanning Chemstry:, The Science Of Leather – Antony D. Covington – Chapter 19 – Theory Of Tanning: Concept Of Link-Lock
- Moisture In Skins And Its Influence On The Technological Properties Of Finished Skins – Daniele Pistorio
- Chrome tanning – Daniele Pistorio