Introduction of the John Arthur Wilson Memorial Lecture

by

George Stockman

As we gather this morning to begin the technical program of the 106th Annual Convention of the American Leather Chemists Association, it is appropriate to take a moment to recognize the namesake of this featured lecture series, John Arthur Wilson. Born August 16, 1890 in Chicago, John Arthur Wilson packed a lot into his brief 52-year lifetime and is widely regarded as one of the giants in the advancement of the understanding of leather science and technology during the 20^{th} Century.

Meeting here in Wisconsin, I am reminded of one of the more obscure aspects of this remarkable leather scientist's legacy. I would not want his contribution to a local engineering icon to be lost to posterity. From 1920 to 1930, John Arthur Wilson was Research Director of the chemical laboratories of the Milwaukee Sewerage Commission, where he was engaged in the development of the huge Jones Island Treatment Plant. He was a pioneer in elucidating the mechanism of the activated sludge process. He applied the disciplines of the bourgeoning science of colloid chemistry, not only to help explain poorly understood aspects of leather processing, but also, to solve a serious problem with dewatering the Jones Island sludge. These improvements led to the development of the world famous process for producing Milorganite®, Milwaukee's fertilizer byproduct that has become a favorite green technology for gardeners and greens keepers throughout the United States. The Jones Island Wastewater Treatment Plant, located on the shores of Lake Michigan in the City of Milwaukee, is on the National Register of Historic Places. At this site, where Milorganite is made, a plaque designates this facility as a National Historic Civil Engineering Landmark by the American Society of Civil Engineers. Now you know the rest of the story.

Today's John Arthur Wilson Memorial Lecturer is Dr. José M. Adzet. Dr. Adzet comes to us from the AIICA—Asociacion de Investigacion de las Industrias del Curtido y Anexas, where he is the Research Manager. John Arthur Wilson once said, "The science of the tanner built up through centuries of practice is very different from the pure science of a modern university and in general tanners and professors live in widely separated spheres, each seeing the weaknesses but failing to appreciate the real achievements of the other." Professor Adzet is a rare individual that bridges this knowledge gap. Professor Adzet holds a Chemical Science degree from the Central University of Barcelona and Ph.D's in Science from the University of Lyon and the Central University of Barcelona. He holds a doctorate in Gemology from the Facultat de Geologia of the University of Barcelona.

Until 1997, Dr. Adzet was the Manager of the Engineering University School and the High Tannery School of Igualada, Barcelona, Spain. During his tenure at University School, Dr. Adzet coauthored a number of important books; some regarded as required reading for students of leather tanning in the Spanish-speaking world. These include:

- Leather Chemical Technology
- Tanning Technical Chemistry
- Environmental Course

Since 1994, Dr. Adzet has been Research Manager of AIICA in Igualada, Barcelona, where he has collaborated with other scientists to complete more than 75-research works.

Of particular note, he has contributed to numerous projects supported by the European Commission. Among the most notable are projects dealing with:

- Clean Technologies
- Quality Assurance
- Analysis of soaking and liming operations and its optimization for cowhide manufacturing
- Recycling treatment of Tannery Waste
- Non chrome or reduced chrome tanning
- Increased leather performance through the application of specific enzymes at early stages of processing
- Innovation of the waste water treatment processes and plants presently operating in tanning industry
- Tannery Net — A concerted action for the European Leather Industry
- Radical environmentally sustainable tannery operation by resource management
- Dermagenisis — Bio-engineering of leather: structure design, biosynthesis — Towards zero emission production

Dr. Adzet was granted the AQEIC Award for a lifetime of contributions to the industry (AQEIC is the Spanish Leather Chemists Association). He subsequently was the recipient of an honorific by the Joined Managerial of l'Anoia, as recognition of his dedication to teaching, research, and publications related to the development of modern leathermaking technologies.

If John Arthur Wilson were present here today, I believe he would be most pleased with our selection of this year's John Arthur Wilson Memorial Lecturer, who will speak to us on the subject of "Transformation of Lime Splits and Trimmings from Hides into Different Collagen Materials." Please meet Dr. José M. Adzet.

Transformation of Lime Split Trimmings into Different Collagen Materials

by

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Presented at the 106th Annual Meeting of the American Leather Chemists Association, June 10-13, 2010, Grand Geneva, Lake Geneva, Wisconsin.
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ABSTRACT

A review of the composition and transformation of the skin collagen into its derived materials is presented. The main application of hides and skins is to obtain leather. The untanned by-products coming from tanneries are used to obtain gelatin, sausage, casings, pet food, etc. A small amount of collagen material is applied for cosmetics, medicine and other industrial applications. Selling prices of base protein products are compared for potential market exploitations.

RESUMEN

Se revisa la composición y transformación del colágeno piel en diversos materiales. La principal aplicación de las pieles es la obtención de cuero. Los subproductos sin curtir procedentes de la tenería se utilizan para obtener gelatina, tripas para embutidos, comida para animales de compañia, etc. Una pequeña cantidad de material colagénico se utiliza en productos para cosmética, medicina y otras aplicaciones industriales. Se comparan los precios de venta de productos a base de proteínas para su potencial explotación comercial.

INTRODUCTION

The name collagen is derived from two Greek terms: Kolla glue and genno producer.

The aim of this lecturer is to give a global vision of today's applications of collagen and its future possibilities. I think a better knowledge of the fibrous collagen, a very complex subject, and its different derivatives can be useful to better understand the materials that we are using. You can find large amounts of collagen in hides, skins, bones cartilages, sinew and teeth of animals. The cattle, sheep, goats and pigs are slaughtered for the human consumption. The hides, skins and bones considered by-products of the slaughterhouses are recovered to obtain collagen. Fish and poultry skins contain collagen as well. The world production of collagen is higher than wool production, which is about 1.5 million tons per year.

The cost of the whole hides and skins (bovine, sheep and pigs) is too expensive to be used as raw materials to obtain collagen derivatives at a competitive price. In practice, byproducts from slaughterhouses, tanneries, as well as fishery and poultry sectors are used to obtain collagen materials. There are books and lectures[1-7] to which we refer in order to let readers obtain a data collection of potential tannery by-product applications.

Thirty years ago ALCA carried out a very interesting symposium on collagen applications. But, from that time until now, a lot of new publications have been edited. Last year Brown explained to us the stability of triple helix structure of native collagen molecule, and in 2004, COT introduced us to the genesis of collagen in mammalians and the recycling from chrome shavings. I will try to give you another perspective on hide collagen and its derivatives.

HIDES AND SKINS

The hide fibrous tissue is an interwoven network of fiber bundles and has its origin in the fibroblastos. This tissue is set up essentially by collagen fibers, some elastic fibers, in very small amounts, and some soluble proteins, which include proteoglycans. The fibrous proteins make up the structural morphology of the tissue and are referred to as structural proteins, which are insoluble in water.

The fibrous materia[33] is embedded in amorphous quasi-liquid matrix adhering on the surface of the fibrils like a gel.[8] In the fully hydrated hide of the living animal there are no air filled free spaces. The non-fibrous proteins from the matrix are usually involved in the vital processes of skin life. They are soluble in water or dilute salt solutions.

The skin of the young animals is thin and hydrated, but old animals' hides have a thicker and less hydrated reticular layer.

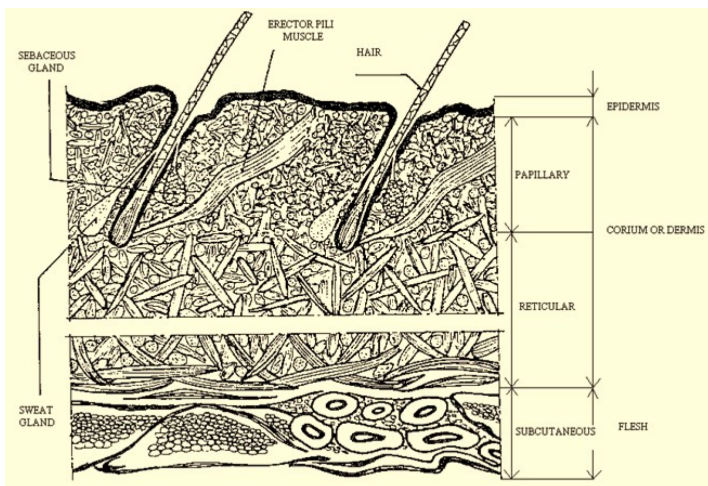


Figure 1. Schematic cross section drawing of fresh bovine hide

We can see three layers in a cross-section hide in Figure 1.[9,10]

Epidermis
External thin layer that comprises hair or wool. Hair or wool are considered to be epidermal productions, although its roots are inserted inside the dermis.

Dermis or Corium
It is formed by the papillary and reticular layer. The papillary layer contains several elements such as fibroblasts, a tissue of thin collagen fibers, sweat and sebaceous glands, erector pili muscle, hair root, elastic fibers, and blood vessels. The

reticular layer is formed by a tissue of coarse collagen fibers with not too many other materials.

Subcutaneous Layer or Flesh

This layer is formed by tallow muscle tissue, blood vessels, etc. This is an internal layer between the animal carcass and the hide.

Hide composition varies with the age, race and origin of the animal.

Nevertheless, to give a very general idea of what hide contains we are providing the following data corresponding to a bovine hide of 31 Kilos fresh weight:

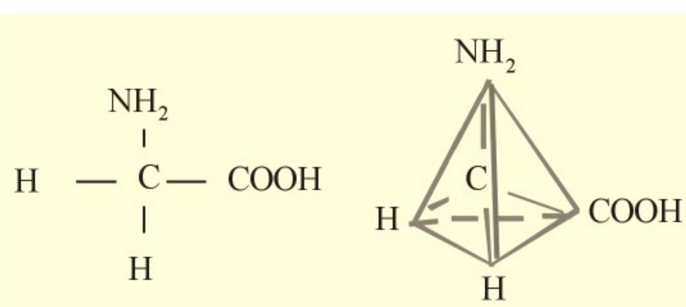
	Fresh Hide	Dried hide (without salt)	Fresh salted hide
Humidity	65,0	12,5	43,0
Fat	1,5	3,7	1,8
Minerals salts	0,5	1,3	20,5
Protein			
Keratin	3,0	7,5	3,5
Insoluble fibers	25,0	62,5	30,5
Soluble proteins	5,0	12,5	0,7
	100,0	**100,0**	**100,0**

Figure 2. Composition of bovine hides expressed in percentages

When a fresh hide is preserved with salt (sodium chloride), the common salt penetrates the inner part of the hide, acting as a bacterioestatic agent. Consequently, an important dehydration of the hide is produced. Part of the water together with salt and soluble protein goes out as dirty brine. As a result of the salting a decrease in weight of about 18% is obtained. This represents that 1000 Kilos of fresh hides produce 820 Kilos of salted hides. According to FAO 2004 statistics, the worldwide figure of slaughtered bovine animals was 333.4 millions, with a fresh hide salted weight of 6.02 million tons, which contains approximately two million tons of dry collagen from this origin.

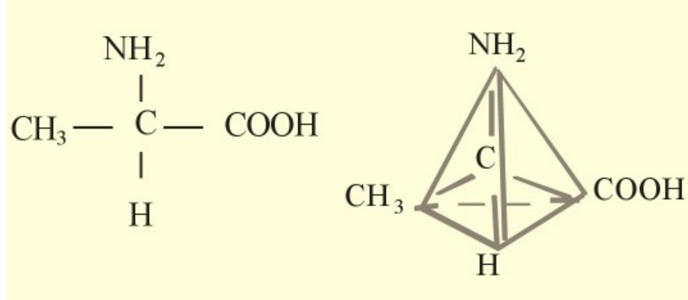
COLLAGEN

The total number of aminoacids forming proteins is twenty-one. The most simple aminoacid is glycine.



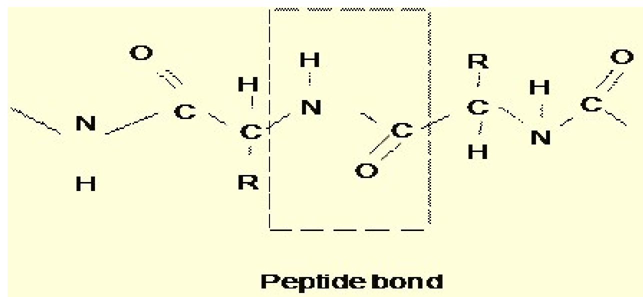
α – Amino acetic acid

followed by amino acid alanina



α – Amino propionic acid

Aminoacids have one or more asymmetric carbon atoms and are therefore able to exist in at least two optically active forms. The peptides are polymeric structures formed by the elimination of water between the carboxyl and amino groups of different α-aminoacid molecules to form long peptide chains and its gathering to form proteins.



The characteristic properties of proteins depend on:

1. The number and types of aminoacids compounding the chain, and the sequence in which they are arranged in the chain.

2. The type and amount of interaction between the chains and the number of chains per molecule of protein.

3. The spatial configuration of the chains, and the shapes of the protein molecules.

The first stages of collagen synthesis take place in the cells known as fibroblasts, after the collagen passes through the membrane cell to the extracellular matrix where the transition from soluble collagen to fiber protein takes place.

The collagen family of proteins is biomacromolecules formed by polypeptide chains twisted together in helix.

The side chains are placed outwards of the cylindrical helix. The side chains can finish with either non-polar or strongly basic or acidic groups and provide a charge profile along the chain, which spontaneously drives to the self-assembly of collagen molecules. One half of the total weight of the molecule is in the side chains.

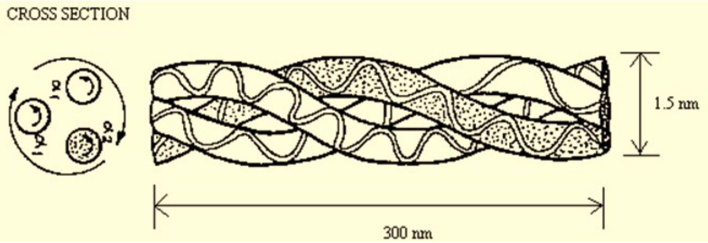


Figure 3. Triple helix of collagen molecule

The ability of nature to modify this structure is the key to understand that the collagen is extensively widespread in animal body. The collagen constitutes about one-third part of all animal proteins. More than 27 collagen types have been identified, but the main fibrous types are I, II and III; and the non-fibrous type IV of the basement membrane. They all own the basic triple helix based on multiple repeats of the peptide Gly-X-Y. However, the basic tripe helix can change in length to form different supramolecular assemblies to achieve optimum function for each type of tissue. The collagen molecules are stabilized by cross-linkings involving oxidation of specific lysine and hydroxilysine residues. Collagen shows considerable changes with age, which in turn affects its physical properties. The rod-like triple helix allows intermolecular cross-linkings either naturally or promoted by polyvalent reagents, that increase shrinkage temperature and resistance to enzymatic attack.

To assess the amount of protein in a material we must analyze the nitrogen contained by the Kjeldahl method and multiply the nitrogen value by a factor that can vary between 5.62 and 6.25 according to the protein type. If we have a mixture of proteins and we wish to know its amount of collagen, we must carry out the hydroxyproline contain.[11] The chemistry, biochemistry and biology of collagen are today considered a new science. The research in this field has produced a considerable body of published literature with numerous patents concerning the uses of collagen in the medical, pharmaceutical and cosmetic fields among others.[12] The different protein chains have been identified by SDS, gel electrophoresis and by antibody reactions. The table in Figure 4 indicates where to find some of these collagen types.

Figure 5, shows the triple helix domains represented by solid blocks, and non-helical regions by lines:

The table in Figure 6 shows the conventional designations of molecular chains from different collagen types, as well as their size and structure.

Figure 7 indicates the shape that the assemblies of collagen molecules acquire when they are organized in higher structures.

I	Skin, tendon, bone, muscles, aorta, placenta, nerves, liver
II	Cartilage, spinal discs
III	Skin, muscle, aorta, placenta, lung, liver
IV	Basement membrane, EHS tumor
V	Skin, tendon, muscle, liver, lung, kidney, placenta, cornea
VI	Skin, muscles, others
VII	Anchoring fibers between dermis and epidermis
VIII	Endothelial cells
IX	Fibril-associated collagen with interrupted triple helix, in cartilage
X	Transition between bone and cartilage
XI	Cartilage
XII	Fibril-associated collagen with interrupted triple helix, in skin, bone
XIII	Epidermis, muscle, cartilage
XIV	Fibril-associated collagen with interrupted triple helix, in skin, cartilage
XV	Cells of skin, uterus, lung, smooth muscle. Multiple collagen domains
XVI	Skin, bone
XVII	Skin. M-RNA only
XVIII	Skin, heart, brain, liver. M-RNA only. Multiple collagen domains.

Figure 4. Table Source of Collagen types

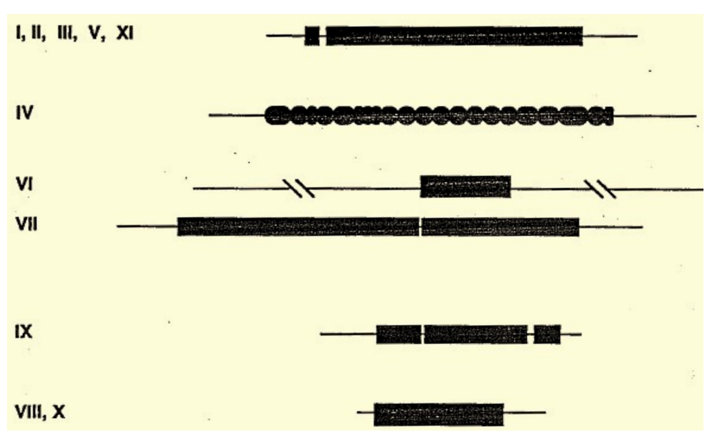


Figure 5. Graphic of triple helix domains

Type	Alfa-chains	Size	Structure type
I	$(1)_2$,2	rods, 300 nm	crosstriated fibrils
II	(1,) $_3$	rods, 300 nm	" "
III	(1) $_3$	rods, 300 nm	" "
IV	$(1)_3$, (2) $_3$, (1) $_2$- (2) $_1$	390 nm	net, web
V	$(1)_3$	390 nm	thin fibrils
VI	1-2-3	105 nm N-C-term.dom	microfibrils, crossstr.
VII	(1) $_3$	450 nm	dimer antiparallel
VIII	EC_1, EC_2, EC_3	450,375,300KD	uncertain network
IX	1-2-3	200nm, glob. domain	or surface of fibrils
X	(1) $_3$	150nm, glob. domain	fiber network
XI	1-2-3	fibrillar	rich in carbonhydrates

Figure 6. Collagen types, size and structure

Type I collagen is the main component of hide fibrous tissue. It has been the subject of many research studies. It contains associated collagen types II, III and V. Type V was detected late due to the fact that only small amounts were present in the tissues and it is considered a minor collagen. This type V is associated with one or another of the main collagens. The minor collagens IX y XI can be found with collagen type II in cartilage, where form fibers of small diameter, oriented randomly in a viscous matrix of proteoglicanes.

Collagen type III forms the fibril of the blood vessels. Collagen type IV forms planar molecular tissues that set up the basal membranes, which are located at the boundary line between different tissues. The main source of collagen type IV is found in the hide, between dermis and epidermis. It is a meager source because of the small volume of the membrane. To identify the different types of collagen some antibodies were developed some years ago, and now these antibodies are available in biochemical supply houses.[13]

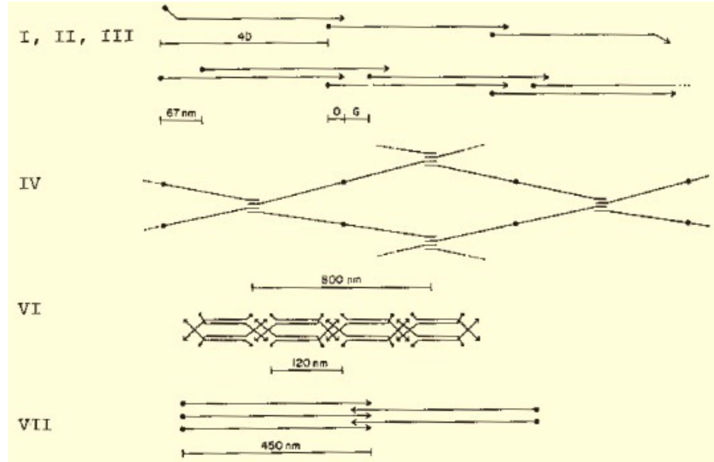


Figure 7. Organization of collagen molecules in higher structures

Amino acid	α1 (I)	α2 (I)	α1 (II)	α1 (III)	α1 (IV)	α2 (V)	α1 (V)	α3 (V)	1α	2α
Alanine	115	102	103	96	37	54	39	49	54	49
Arginine	50	50	50	46	26	48	40	42	45	48
Aspartic acid	42	44	43	42	51	50	49	42	46	50
Gal-hydroxylysine	1	1	4	-----	2	3	5	7	-----	-----
Glc-gal-hydrosylysine	1	2	12	-----	30	5	29	17	28	34
Glutamic acid	73	68	89	71	79	89	100	97	107	98
Glycine	333	338	333	350	328	331	332	330	334	327
Half-cystine	0	0	0	2	0-1	0	0	1	0	0
Histidine	3	12	2	6	6	10	6	14	6	11
3-Hydroxyproline	1	1	2	0	7	3	5	1	-----	-----
4-Hydroxyproline	108	93	97	125	133	106	110	91	98	93
Isoleucine	6	14	9	13	29	15	17	20	15	16
Leucine	19	30	26	22	52	37	36	56	35	39
Lysine	26	18	15	30	9	13	14	15	19	15
Methionine	7	5	10	8	13	11	9	8	10	9
Phenylalanine	12	12	13	8	29	11	12	9	11	11
Proline	124	113	120	107	65	107	130	98	109	119
Serine	34	30	25	39	37	34	23	34	25	28
Threonine	16	19	23	13	20	29	21	19	17	25
Tyrosine	1	4	2	3	2	2	4	2	2	3
Valine	21	35	18	14	28	27	17	29	28	18

Residues per 1000 total residues
From M. E. Nimmi, Senin. Arthritis Rheum, 8 (1) 1993

Figure 8. Aminoacid composition of different collagen types of humans

Analytical tests based on antibodies are very sensitive and specific.

Collagen type I molecule is the same in different tissues of the same species, for example in tendons and bones. Small differences between them can be introduced after the production of molecular chains in the fibroblast. These small differences take place with the oxidation of lysine or hydroxylysine residues, which may trigger the formation of crosslinks.

The sequence of amino acids is called the primary structure of a protein. This sequence determines the way in which the protein is folded either into a helix, beta sheet, beta turn or collagen triple helix. Amino acid sequences from collagen became known for the first time seventeen years ago. “Alpha” denotes a collagen protein chain which is a single gene product whereas “beta” is formed by two chains covalently linked. Each polypeptide chain of collagen contains about 1000 amino acids residues linked together by peptide bonds. The crosslink formed by the ring structure of amino acids proline and hydroxyproline stabilizes strongly the global structure of the three alpha chains of the triple helix. By means of chemical or enzymatic treatments it is possible to break down the collagen molecules into several bits. Each one is a peptide of different size, but the lower ones can be single aminoacids. The solution of these materials is known as hydrolyzed collagen. The table below indicates the composition of amino acids from different collagen types.

The collagen molecule is characterized by high quantity of glycine, alanine, proline and hydroxyproline and low contents of aromatic aminoacids.

The human body cannot synthesize some aminoacids, called essential aminoacids. For this reason it is necessary to provide them in a balanced diet, which will enable the body to grow healthier. Figure 9 shows essential aminoacids

Amino Acid	Bovine Hide	Bovine Meat	Filet Fish	Milk	Egg
Histidin	—	3,8	2,0	2,6	2,2
Isoleusin	1,9	5,2	6,0	7,2	7,1
Leucin	3,7	8,2	8,4	10,2	8,4
Lysine	4,0	9,3	8,8	8,1	6,8
Methionine	1,0	2,9	4,0	4,3	3,3
Phenylalanine	2,3	4,5	3,9	5,3	5,4
Threonine	2,3	4,2	4,6	4,4	5,5
Tryptophane	—	1,1	1,0	1,6	1,9
Valine	2,5	5,0	6,0	7,6	8,1

Figure 9. Essential amino acids of several proteins

In the collagen, the aminoacid tryptophan is missing, while these are found in milk and eggs proteins, among others.

Collagen Shrinkage

The denature of a protein as collagen consists in breaking down the triple helix structure to a randomly coiled structure.[14] The denature is an irreversible process. The native protein collagen with triple helix fibers shows a stability which is necessary for biological activity. By increasing the temperature in an aqueous medium the triple helix is stable up to a certain temperature. If this temperature is exceeded, the triple helix gets disorganized to a random coil.

The bond forces stabilizing the triple helix of the collagen molecules are:

- Hydrogen bonds
- Hydrophobic bonds
- Van der Waals forces
- Electrostatic forces

All these bonds are non-covalent and they break down at a certain temperature. This temperature is influenced by several factors such as pH, addition of organic solvent, and surfactants to the aqueous medium. The temperature at which the collagen tissue shrinks is called shrinkage temperature (Ts).

To assess the shrinkage temperature of collagen tissue we must immerse it in water and increase the temperature a few degrees each minute, until we reach the temperature where the tissue shrinks. The shrinkage temperature in pure water is a measurement of the hydrothermal stability of collagen structure. The native mammalian skin has a Ts of 58–63ºC and the Ts of limed hides from bovine is 45ºC. Vegetable tanned skin reaches a Ts of 70–80ºC. In chrome-tanned hides Ts can reach 100–110ºC. Ts of cold water fish skin in native state is between 37–42ºC and its hydroxyproline content is 8.0% in average, whereas for warm water fish skin Ts is 50–57ºC and its average content of hydroxyproline is 10.7%.[15]

The sharp denature temperature of collagen gives to the triple helix molecule a certain crystalline character. The variation of shrinkage temperature between species depends on the concentration of hydroxyproline. A hydroxyproline deficiency in the chain forms a thermally low stable domain where the melting process starts, allowing the triple helix to fold along its length.[16] The isoelectric point of native collagen is between 7.0–8.0, and the one of limed collagen is 5.2.

When the temperature of the collagen tissue, at pH 5.2 in aqueous medium, increases until shrinkage, the operation lasts only some minutes and the material retains its fibrous structure. When the acidified shrunk collagen fibrous suspension is placed in sheets and dried at 30ºC, it forms a thin fibrous film. When it is subjected to 100ºC a strong shrinkage takes place, forming a gummy new material without fibers. The completely dry untanned collagen fibers have a Ts under 200ºC; and start to break down with temperatures above 250ºC.

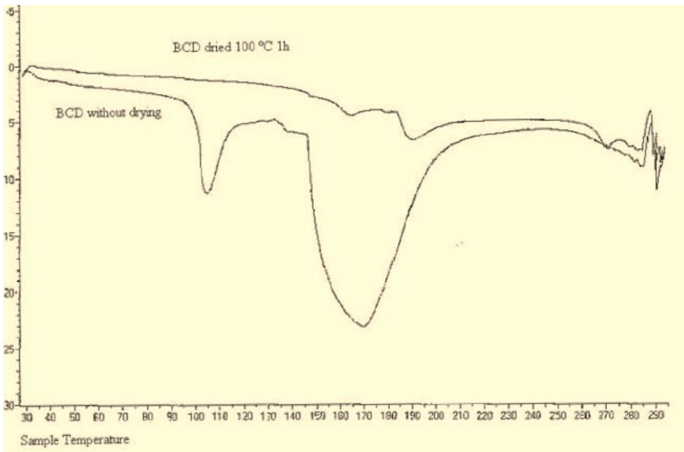


Figure 10. Differential Scanning Calorimeter(DSC) of BCD, without drying and dried.

When the untanned collagen tissue at pH 5.2, in aqueous medium, is held at 60ºC for several hours, a hydrothermical hydrolysis takes place and the collagen is transformed into gelatin, which is a non-fibrous material.

Collagen soluble

Skins and hides contain both soluble and insoluble collagen. The soluble collagen can be extracted from the skins and hides by a treatment with neutral buffers or with very diluted acids solutions. The collagen has the feature to be soluble in cold neutral buffer with an ionic strength between 0.1–0.4. By heating it at room temperature or higher it is transformed again into native fibrils that form a gelatinous networking, which fills all the space of the initial solution. This gel has a certain stiffness that allows handling it and provides it with a sticky feel. The amount of soluble collagen of a skin decreases when animal age increases. From a young calf skin treated with diluted acids, we can extract 15–20% of soluble collagen, while from a bull hide we can only extract between 2–5%.[17,18]

FROM RAW HIDES TO LEATHER

When the hides are treated with brine they are cleaned from external dirt (blood, urine, manure) and brine containing soluble proteins is removed. This process starts the collagen purification treatment before the hides get to the tannery. During the manufacturing process from salted hides to pelts, which is performed in the tanneries, the hides are submitted to beamhouse operations that consist of:

- Soaking
- Fleshing
- Unhairing
- Splitting
- Liming
- Deliming/Bating

In the soaking, common salt and a very significant part of the globular protein and glycoprotein's are removed. During the unhairing and liming operation the hair and the epidermis are eliminated, and the deamination of asparagine and glutamine take place.

In the mechanical operation of fleshing the layer of subcutaneous tissue, formed by tallow's, meat and collagen fibers is removed. In the operation of pelt splitting, the grain layer and the under layer know as lime split are obtained. The grain layer and the part corresponding to the butt of the lime split are used for chrome tanning. The trimmings of lime split have been used for many years to obtain gelatin and casing for sausages. The best parts of the hide to obtain collagen compounds are the limed splits since they have greater collagen concentration and smaller amounts of non-collagen materials. The pelt has a pH of about 12.0 and the isoelectric point of the limed collagen has changed to 5.0–5.2.

In the tannery after liming the deliming operation is performed. Its objective is to decrease the pH of the hide towards values 8.0–9.0. Next, the bating operation uses enzymes to eliminate remaining globular proteins. The objective of the operations from soaking to bating is to remove most of the impurities in the collagen fabric. The structure of the hide is a non-uniform material. The neck tissue is spongier and easier to shred than the butt tissue, where the fibers are more compact.

To transform the pelts into finish leather we must carry out the following operations:

Pickle	Sammying in wet-blue
Tannage	Shaving in wet-blue
Post-tannage	Sammying and setting in wet crust
Drying	Mechanical operations before finishing
Finishing	

During the manufacturing process from raw hide to crust chrome leather numerous solid byproducts are obtained. Their name and weight are indicated in Figure 11.

Wastes	Kilos wastes	Collagen percentage	Kilos dry collagen
Common salt	24,6	-----	-----
Grain salted trimming	24,6	30,5	7,5
Grain fleshing	123,0	15,0	18,5
Wet hair	123,0	-----	-----
Lime fleshing	95,6	8,0	7,6
Graine lime trimming	5,0	22,0	1,1
Split lime trimming	316,0	23,0	72,7
Shavings	64,0	30,0	19,2
Dry crust trimming	5,0	60,0	3,0
			129,6
Leather			
Grain crust (1,2 mm)	111,0		
Split crust	63,0		
	174,0	60,0	104,4
			234,0

Figure 11. Solid byproducts in the manufacture of 820 Kg of salted hide

For the thickness indicated the quantity of collagen in the byproducts is higher than the collagen in the leather, including the split.

The price of any material changes according to the market, that is to say the offer and demand of the particular product, as well as the economic situation. Nevertheless, we offer an indication of the prices of different materials at the beginning of the year 2010. Tanners must pay to take away the fleshings and the solid tanned by-products as well as those which are in crust or finished condition. The tanner sells the trimmings of lime split in the market for about $0.25/Kg and they contain 23% of collagen. Consequently, a kilo of dry collagen costs $1.10/Kg and green lime trimmings are sold at half this price, because they are more contaminated.

The pure collagen standardized, known as "Hide powder", used for analytical purposes in tannin determination, is sold at $360/Kg.

The finished chrome leather sold by surface in square feet, which corresponds to a square of 30.48 x 30.48 = 929.03 cm2 for a thickness of 1.2 mm. weighs 104.17g., is sold for $3.00 per sq. feet on average. Taking into account that the leather contains an average of 60% collagen we deduce that a kilo of collagen costs $48.00 as finished chrome leather. The split is sold cheaper, the velour type quotes between $1.60–2.00/ft.^{2} and the thermoplastic split between $2.10–2.90/ft.^{2}. Doing an average it is possible to calculate that the kilo of dry collagen as split is sold at $33.00. The heavy leather vegetable tanned is sold at $9.00/Kg and contains 52% of collagen. Consequently, the selling price for one kilo of dry collagen is about $17.30.

Leather and waste		Other proteins	
Finished light leather	48	**Gelatin**	5.0
Velour split leather	33	**Glue**	3.4
Heavy leather	17	**Casein**	9.0
Shreads limed splits	1.1	**Albumin**	10.0

Figure 12. Sale price in $ USA of a Kilo of dry protein according to the material

The food gelatin is sold at $4.50/Kg and the industrial glue at $3.00/Kg. Its concentration in collagen is about 85%. A kilo of dry collagen will cost respectively $5.00 and $3.40. The price of other similar proteins that are commercialized in the market such as the casein recovered from milk and the albumen from hen eggs is very variable. The casein price depends on its origin and its quality. Nowadays it costs between $6.40 and $11.40/Kg; which amounts to $8.90/Kg on average. The liquid egg albumin pasteurized at a concentration of 11% in dry matter costs in the market $1.00/liter. The cost of a kilo of dry albumin is $9.10 apart from the price of the drying operation. The cost of such noble proteins such as casein and albumen is higher than the price of gelatin.

In order to commercialize a product, it must have either a competitive price, better quality or enhance the characteristics of the final product.

The best option to add value to the collagen fabric for large scale production nowadays continues to be its transformation into leather for manufacturing leather goods, due to the prestige of this material for its strength and nobility. In the pharmaceutical, medical and cosmetic market the collagen can be sold at higher prices. However, in these niches the quantity used is very small compared with the current global productions of collagen.

The possibility exists to use directly the collagen protein in human nourishment. To achieve this aim a series of sanitary problems should be solved and one should have to hope for the acceptance of the new product on the part of the consumers. The gelatin already exists in the market at a very low price, having the corresponding regulations and being a product known by the consumer for a long time now. In order to use collagen directly for human nourishment it should have a selling price lower than that of gelatin, in order to be competitive, which is feasible. It is not necessary to forget that hides are by-products of the meat industry, and that their production depends on the meat industry. Consequently production is limited and applications as human nourishment could be applied only up to a certain extent.

Problems Of Tannery Collagen Byproducts

By-products produced in chrome tanned leather tanneries can be classified as:

- Byproducts without tanning (in fresh, salted or lime),
- Tanned by-products (treated with tannins),
- Crust and finished wastes (tanned, retanned, dyed and fatliquored).

By-Products Without Tanning

The residues in fresh state contain principally collagen proteins, keratins, globular proteins and also fats. The salty residues have lost part of the globular proteins and important quantities of sodium chloride have been added. The pelt contains mainly collagen and chemical products of the type sodium chloride, sodium sulfide and calcium hydroxide, which can be separated from the collagen relatively easily. Nevertheless, it can also contain biocides, amines, enzymes, surfactants, among others; depending on the manufacturing process that has been applied to the hide. Residues without tanning include fresh hide, salty hide and lime hide trimmings, green and lime fleshings and lime split trimmings.

Lime hide trimmings have no salt and no hair, except the roots. They contain the same chemicals than lime split trimmings. Lime fleshings from bovine hides, separated in the mechanical operation of the fleshing, contain essentially tallow and meat. Average values are indicated as follows:

Water	75%
Grease	12%
Protein	8%
Chemicals	5%
Total	100%

The greasy part of lime fleshings is formed by triglycerides of fatty acids and is solid at room temperature. The protein part of the fleshings is heterogeneous enough, since it contains collagen, elastin, and meat fibers, besides many chemicals. Normally fleshings are used for recovering the tallow by means of an acid hydrolysis. This tallow finds industrial applications in the manufacture of soaps or for animal feeding. The parts of the hide that contain fewer impurities are lime split trimmings. But it is necessary not to forget that many hides, for example in the States, are split in wet blue, which will reduce lime trimmings and will in turn increase wet blue trimmings.

Problems stemming from BSE have been solved by using only the hides of the animals that do not suffer from this disease.

Type of Residue	Quantity Dry/Collagen in KT	Recovered Products
Without Tanning		
Green and lime fleshings	63	Fat, protein, biogas
Salted limed trimmings	184	Glue
Lime split trimmings	533	Collagen, casings, gelatin (food)
Total	780	
Tanned		
Shavings and wet blue trimmings	141	Leather board, chrome salt, hydrolyzed collagen
Total	141	
Crust and Finish		
Crusted and finished trimmings	—	Conglomerates
Buffing Powder	22	Incineration
Total	22	
Grand Total	943	

Figure 13. World production calculated of solid tannery by-products that contain collagen.

Thorough controls are carried out in slaughterhouses, where animals are declared suitable, or not, for human consumption.

What Products Can Be Manufactured From Tannery Collagen Byproducts?

Depending on subsequent uses, it is preferable to use one or another type of byproduct, as the collagen has different grades of purity. In the preparation of fertilizers we can either use the proteins produced from lime fleshings or the proteins manufactured from the wet-blue.

For industrials applications we can use the hydrolyzed protein recovered from chrome shavings. When the application of the protein is for other uses such as human nutrition, pharmacy, health and cosmetics we then need the purest collagen possible. In this case we must utilize fresh hides or lime splits, which contain the least impurities.

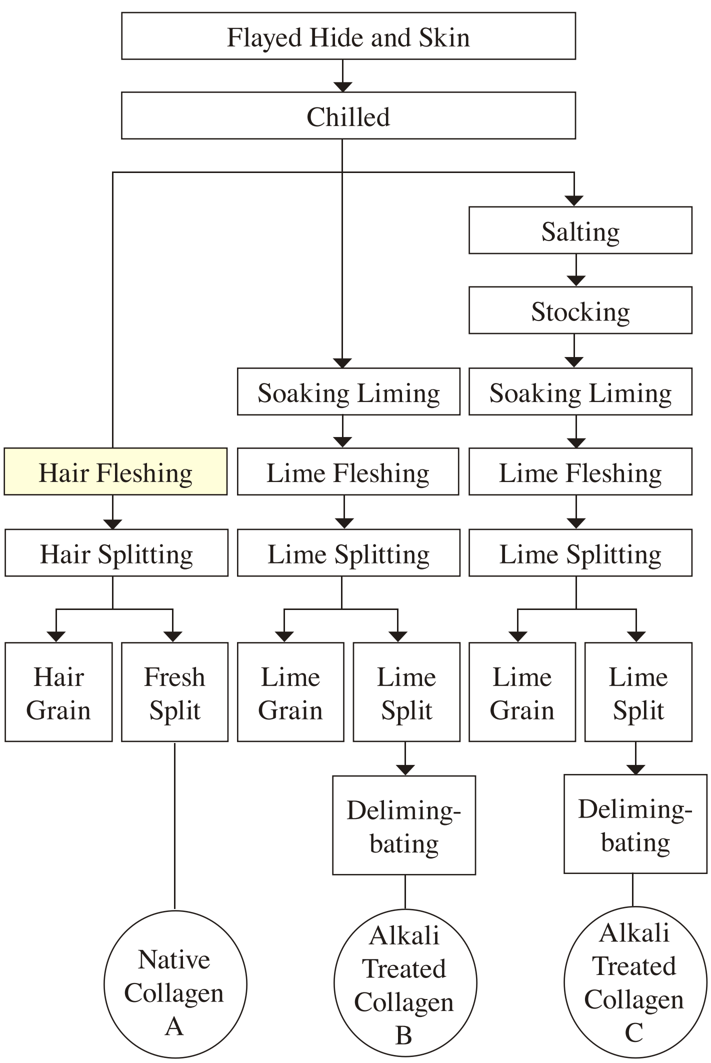


Figure 14. Three different beamhouse processes

For many years, the hides that did not meet the standards to make leather and the trimmings of lime splits have been used for the manufacturing of food gelatin and casings for sausages without major problems. Obviously, the companies that produce these products remove the chemicals from lime splits. The fiber network of the hide, which is a natural product, should be exploited in this form whenever possible. The highest values are attained by ultra-pure collagen for analytical, medical, tissue engineering and cosmetic purposes. The purification level must be measured as the financial yield per unit of collagen. In spite of the apparently low quantities of collagen obtained from these productions, economic returns may be quite substantial.

The tanner that will produce high-grade pure collagen must adapt the technology employed for leather manufacturing from soaking until bating. Pelts must thus be fleshed and thoroughly cleaned, and the splitting of the pelt must be carried out in lime condition taking into account hygiene regulations. Special attention must be paid to prevent any kind of pollution to the collagen tissue from the chemicals used in the manufacturing process.

When native hides are salted for preservation, a modification of this natural material starts to take place. By treatment with alkalis during the liming operation, the glutamine and asparagine aminoacids of the collagen molecule are transformed into acidic compounds.

The flayed hides and skins are easily spoilt materials that are found at animal body temperature and must therefore be chilled before preservation. The chilled hides and skins with hair are fleshed and split in order to obtain hair grain and fresh split. The fresh grain with hair can be manufactured as leather and the fresh split can be washed to get native collagen A. The chilled hides and skins are soaked in fresh state, limed, fleshed and split in order to obtain a lime grain and a lime split, which can be cut in butts and trimmings. The latter can be delimed, bated and washed to get alkali-treated collagen B.

Normally the hides and skins are salted, and stocked. When they get to the tannery, hides and skins are soaked, limed, fleshed, and split. We obtain then lime grain and lime split, which we cut in butts and trimmings. The lime split trimmings are delimed and bated and we obtain alkali treated collagen C. If the collagen molecule is subjected to heat, when a certain temperature is reached, the molecule shrinks, changing its structure, and many bonds are broken as a result. By maintaining the heat treatment during several hours the collagen can be transformed into gelatin or hydrolyzed protein.

By treating collagen with heat and chemicals we can obtain peptides of different size and aminoacids. Also, it is essential to control the temperature and conditions of the final drying, which can modify the hydrolyzed grade.

Slaugtherhouse		
Native collagen (natural product)	Fresh skin (raw skin fibers)	Without salting and liming
Tannery		
Limed collagen	Pelt Fibers	(Salted and limed) deamidation → acids
Heat Treatment		
Shrunken collagen	Pelt Fibers	Insoluble in hot water (Short time, minutes)
Gelatin	Gelatin Filaments	Soluble in hot water (Long time, hours)
Protein Hydrolysates		
Hydrolyzed	Chemical	Soluble in cold water
	Hydrotropic agents	
	Acid	
	Alkali	
	Enzyme	
	Non fibers	

Figure 15. Different types of collagen

The following graphic Figure 15 is an attempt to represent these ideas.

The different materials that you can obtain from pelts collagen can be graded as indicated in Figure 16.

TRIPLE HELIX FIBERS

Collagen Tissues

The main application of the hides and skins is to be processed as leather usually tanned with chrome salts, vegetable extracts and aldehydes, now this kind of tanning are questioned and hide is tanned with co-polimer material.[32] Leather is a strong durable material and has a warm feel. Leather is used for manufacturing shoes, leather goods, garments and upholstery, but has also other applications. Among tannery byproducts we find lime split trimmings, which are not suitable to obtain

Triple Helix (collagen molecules)

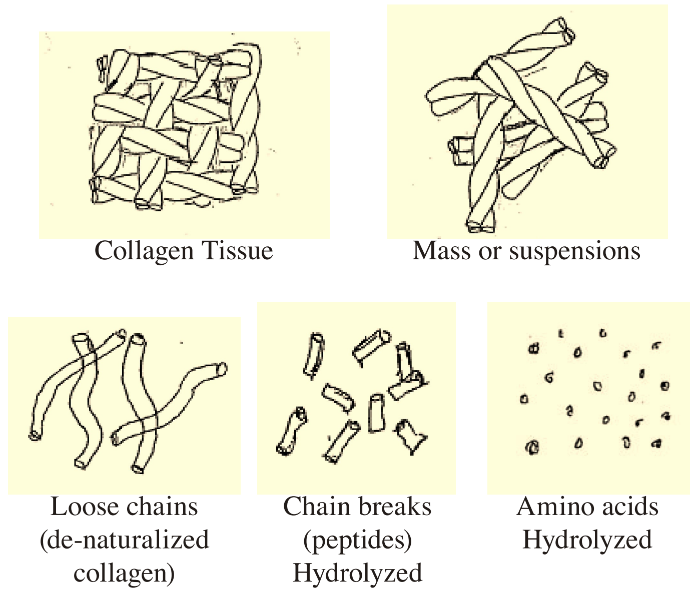


Figure 16. Different bits of collagen molecule

leather. However, their price is extremely reasonable, they are produced in big amounts, and they are relatively pure collagen. These lime split trimmings can be directed to food (gelatin), or as technical, medical or analytical products.

Lime split trimmings have a pH of 12.0–12.5 and, apart from water and collagen, contain some elastic fibers and chemicals such as sodium sulfide and calcium hydroxyl. Their composition is as follows:

Water	73%
Protein	23%
Chemicals	3%
Grease	1%
Total	100 %

In order to use lime splits as collagen it is necessary to remove the chemicals, the grease and the other proteins. To this end, the lime split trimmings must be well fleshed, delimed, bated and washed with pure water. Pure collagen fiber tissue will then be obtained. The fleshing operation's aim is to remove grease and meat. Deliming consists in neutralizing the alkalinity of the splits to reach a pH of 8.5–9.0, which is necessary for the bating. This is achieved using proteolytic enzymes to hydrolyze the globular protein. The washings with pure water remove the neutral salts and the hydrolyzed proteins.

The collagen tissue obtained is an easily spoiled material. Consequently, it must either be handled immediately or preserved by freezing or dehydrated with acetone. Dehydration can be performed in a drum, treating the washed, delimed-bated splits with pure acetone. The residual bath mixture of acetone and water is taken away and the operation is repeated three times. Finally the acetone wetting the splits is evaporated. The resulting dehydrated collagen tissue is white, opaque, flexible and porous. Very absorbent, similar to a sponge, it can be stocked during several years without being spoiled.

Pet Products

In 1980 in the United States of America approximately 22,500 tons of lime splits were consumed for the manufacture of chewable toys for dogs. A third of this amount came from the States and two thirds were imported.[19] In the sale of these materials it is necessary to satisfy the client, who, in this case, is not the one who eats.

Pet Toys

This type of product is manufactured from pieces of thick lime split of reasonable size, which are then transformed into articles with the shape of a bone. The pieces of lime split must be subjected to the following operations until their ultimate transformation in toys for pets:

- Washing
- Neutralizing
- Bleaching
- Dying
- Sammying
- Splitting
- Cutting
- Final material Formation
- Drying
- Packing

Chewable toys have a sanitary function in the life of the animals, namely to help keep their teeth clean, as the toys make the jaws work and avoid bad breath.

Pet Food

The small pieces can be crushed for homogenization and mixed with bindings, adding aromas and colors to make sticks and cookies more appealing. The market is limited by the cost and the availability of raw material. Other products include the food obtained mixing vegetable and animal proteins, together with starches, fats, fibers, flavors, and antioxidants. A possibility is to agglomerate the flour and meat using sodium caseine. A variety of chewable toys contains:

Soya protein	100 divisions
Gelatin	20 divisions
Glycerin	5 divisions
Fiber	10 divisions
Water	80 divisions

Furthermore, vitamins, minerals, flavors and hygienic additives can be added to take care of the health of the animal.

Hide Powder

Hide powder is produced from non-salted heavy, fresh bull hides that are washed intensively, cut in pieces, fleshed and split with hair. The middle split is of 1.5 mm each, washed with distilled water, slightly chrome tanned (or just not tanned) and dried at gentle temperatures. Finally it is cut in pieces, coarsely disintegrated and finally grinded to a fine fibrous powder, analyzed and standardized in respect to its absorption characteristics through appropriate blending.

This material is produced to assess the tannin concentration of vegetable extracts used by the tanners. The analytical solution of vegetable extracts is filtered through a column of hide powder. With this treatment the vegetable extract solution is untanned. The difference in mass between the initial and the untanned solution is referred to as tannins. The material which has not been absorbed is referred as non-tannins and contains neutral salts and organic substances of low molecular weight. Hide powder is a well standardized product. In supply houses “white hide powder” can be found. This material is untanned and is used to assess the tannins by the shaking technique. The weak “chrome-tanned hide powder” is used to assess tannin concentration by the filter bell technique. The grinding of the hide until reaching a very light powder without remaining coarse particles or knots is a difficult but important task. It is indeed not easy but it is a desirable aim to produce such a fine material for medical and cosmetic applications.

Mass Fibers

Sausage Casings

Up to the present time significant quantities of well cleaned gut from pig or other animals has been stuffed with chopped meat mixtures in order to produce sausages. These guts are formed by a natural collagen tissue that is preserved by salting and refrigeration. The synthetic casings of collagen fibers are used to manufacture different types of products:

- Mixtures of fresh meat that the user will cook. It is preserved by refrigeration.
- Products already cooked by the manufacturer and that are intended for subsequently consumption either cold or heated. They are preserved refrigerated.
- Dried air products that do not need compulsory refrigeration for their preservation.

Especially in the last case it is desirable that the gut casing has pores to permit water evaporation and entrance of air to allow natural fermentations during the drying operation. The limitations of natural gut are lying in the desired caliber and length. In the industry synthetic guts are used. That is to say, casings of cylindrical form made with collagen fibers, cellulose or based in plastic. These are not porous. The synthetic casings from collagen fibers are the most similar to natural guts. They are obtained from the collagen tissue from lime split that must be processed with the following operations:

- Fleshed
- Delimed and bated
- Acidified
- Digested into a pasty viscous mass
- Extruded in cylindrical form
- Added tanning products
- Air dried at gentle temperature
- Packing

The purified tissue is swollen with lyotropic acids and is disintegrated to form a smooth mass of collagen fibers. This is extruded using a press and a cylindrical tube with thin walls, which can be from different calibers, is obtained. By spraying we can add products to add more flexibility and stabilization, which provides significant strength. Finally the film is dried with mild temperature air and packed, folded and left flat for its shipment to the customer. Advantages of synthetic casings include caliber regularity, the fact that it is possible to obtain the desired length to facilitate the automation of stuffing, easier preservation in dry conditions, and its sterility. Shape resistance while cooking and frying is for some kinds of sausage casings very important. During 2006 more than a billion meters of casings were produced. Also, food grade colors are added in different concentrations to the collagen casings to make them more appealing.

Color concentration has an influence on the casings. In this sense, the higher the color concentration, the stronger the tearing force of dry casings. Whereas in the case of wet casings, the higher the color concentration the weaker the tearing force.[20]

Fiber suspensions

Neutral fiber collagen suspensions are used for surgical purposes. Collagen fiber suspensions can be like transparent jellies or high viscous solutions. Acid soluble collagen can be extracted from collagen fibers in a few days in the cold, 4 ºC at pH values 3.4–4.2 ,using citric or acetic acid. A method to increase the yield of soluble collagen is carrying out a pepsin digestion of the collagen fibers at 10–15ºC. Telopeptides are cleaved off and the crosslinking is interrupted. At this temperature, even under acidic conditions, the triple helix is stable and cannot be broken by the enzyme. After pepsine digestion the 50–70%, telopeptides are removed and the solution is purified and precipitated to a fine stable suspension. This pepsin-treated collagen is 80% monomeric, 13% dimeric or higher, and the remaining 7% consists of shorter fragments.

Collagen foams

The basis of collagen foam is a fiber suspension. It is produced from masses similar to those used in the manufacturing of sausage casings, but with a much lower collagen concentration. The mass is frozen and dried in thicker sheets. Its appearance is white and light in weight, like polystyrene foam. These sheets show certain stability when being handled in dry and wet conditions, which is necessary for cosmetic and surgical applications. Swelling is extremely sensitive to the salt content of the water used for dilution. Time and different mechanical disintegrating procedures are also very important.

FILIMENTS

Gelatin

Gelatin has had a long and successful history. In ancient times, 8000 years ago, it was used as a “biological adhesive” and in the course of time it progressed to industrial manufacture and multiple applications. It is a protein mixture of different molecular weight that is obtained by thermal hydrolysis of the collagen, which is a natural product and by drying off forms a glassy, hard, not fibrous mass. Gelatin has a neutral flavor and doesn’t contain any fat, cholesterol, or purines; not even carbohydrates. Consumers are increasingly requesting more proteins with these characteristics. The manufacturing process starting with bovine lime split is as follows:[21-25]

Conditioning
Crushing
Neutralization
Washing
Extraction (several times with hot water 60–90ºC)
Filtration and clarification
Deionization
Concentration (vacuum evaporation)
Sterilization
Drying Process for granulated

Figure 17. Manufacture of gelatin from lime split trimming

In an article by Schrieber the whole process is described in detail: from the slaughterhouse through the processing treatment, to the final product, which is then ready for pharmaceutical or edible purposes. The gelatin is a hydrocolloid, which has the capacity to swell up by water absorption and forms colloidal solutions by heating. Pectin, carrageenan, and gum arabic are also hydrocoloide. All are used in the food industry but the gelatin is much more multifunctional than the others.

The most important properties of gelatin are:

Properties associated with gelling	Surface effects:
Gel formation	Emulsion formation and stabilization
Texturizing	Protective colloid function
Thickening	Foam stabilization
Water binding	Adhesion

The analytical measure of gelling power known as Bloom value is one of the most important. This value is the weight in grams required for a specific half inch plunger to depress the surface of a standard thermostatic gel to a depth of 4 mm under standard conditions. The gel must contain 6.67% gelatin and is aged for 17h at precisely 10°C prior to measurement.

Bloom values of commercial gelatin types are within the 50–300 g. range. The stronger the gelling power, the smaller the amounts of gelatin required to bring about the desired gel firmness in the finish product. The range 200 to 300g is designated as high-Bloom, that of 100 to 200g as medium Bloom, and that of 50 to 100g as low-Bloom.

	Gelatine Type B	Gelatine Type A
Alanine	117	112
Arginine	48	49
Asparagine	0	16
Aspartic acid	46	29
Glutamic acid	72	48
Glutamine	0	25
Glycine	335	330
Histidine	4,2	4
Hydroxyproline	93	91
Hydroxy lysine	4,3	6,4
Isoleucine	11	10
Leucine	24,3	24
Lysine	28	27
Methionine	3,9	3,6
Phenylalanine	14	14
Proline	124	132
Serine	33	35
Threonine	18	18
Tyrosine	1,2	2,6
Valine	22	26
Total	998,9	1.002,6

Source: Rose, P.I. Gelatin in Encyclopedia of Polymer Science and Engineering. Volume 3, Wiley & Sons (1987), p. 488–513.

Figure 18. Amino acid composition of two gelatin types expressed in residues per 1000 residues

Viscosity is the second key property of gelatin. High viscosities are required to stabilize food. Standard viscosity is measured using a calibrated pipette, where the run-out time for 100 ml of a 6.67% gelatin solution at 60ºC is determined (results expressed as mPas)Clarity can be determined with a nephelometer using a 6.67% solution at a wavelength of 620 nm.Color can be determined in a spectrophotometer at 450 nm using a 6.67% solution.

A study carried out by Zhang shows that circular dichroism spectra from collagen have two peaks, a positive peak around

221 nm and a negative peak around 192 nm, characteristics of triple helix. Gelatin and collagen hydrolyzed lacked any positive peaks around 220 nm, suggesting random coils.[12]

In Figure 18 the amino acid composition of two gelatin types is provided.

Acid and alkaline gelatins can easily be tested with the aid of SDS gradient PAGE (gel electrophoresis). The acid gelatin has proteins with a molecular weight below 100 KD. The gelatin manufactured by the alkaline process has the chains intact. Gelatin A has a very similar composition to collagen I. Gelatin B does not contain asparagine and glutamine and the tyrosine percentage is reduced by a half. Nevertheless, it is necessary to indicate that the essential amino acid tryptophan is not found.

The dry gelatin contains:

Protein	84–90%
Water	8–12%
Mineral salts	2–4%

According to the association of European Manufacturers of Gelatin, in the year 2005 305 thousand tons of this material was produced worldwide. Its origins are as follows:

Pig skin	44.9%
Bovine split	27.9%
Bones	27.2%

Today the industry consists of 90 production plants in 30 countries.

Fears to bovine spongiform encephalopathy (BSE) and the fact that Muslim countries do not consume pork due to purity concerns have led gelatin manufacturers to explore new materials. These include fish skins and scales, as well as poultry skins, which do not present these problems.

Gelatin is food, not an additive, which is in use in different sectors of the food industry with the aim of thickening, gelling, emulsifying and stabilizing preparations.

- Prepared meats
- Sweetened gelatin
- Bakery products
- Ice cream, nougats
- Milk products
- Jams
- Sauces
- Animal Nutrition

The Gelatin Handbook Theory and Industrial Practice present many different applications as foodstuffs. In the industry gelatins are used as an adhesive for example in the manufacture of matches, abrasive papers stuck to wood and molding, in the manufacture of shoes brakes, as well as emulsifiers of fats. Gelatin is also applied as an assistant in the clarification of the wine and in the manufacture of photographic paper.

The amino acids contained by gelatin exert a positive effect on the animal body, especially on the bones, tendons and cartilages, strengthening the connective tissue, and providing sheen to the hair and strengthening the teeth. For this reason the pharmaceutical industry uses gelatin in some medicines or in capsules to be ingested. The cosmetic sector uses gelatin or its hydrolyzed form in creams for external use with the same purpose.

Low quality glues can be used in the agriculture sector as fertilizers, since they contain a high content of amino acids. Gelatin hydrolyzed can be obtained treating the gelatin with different enzymes such as pepsin, neutrase, alkalize, bromelain or papain. Essentially, the selection of enzyme and hydrolysis conditions determines the sensory properties of the final product. They have wide applications and are used in the food industry because their taste is neutral, and no bitter peptides are generated.

Hydrolyzed Collagen

There are cold water soluble peptides or aminoacid mixtures that can be obtained from collagen or from gelatin. The manufacturing process involves treating the collagen with heat in an aqueous medium, and adding lyotropic substances, acids, alkalis or proteolitic enzymes. The hydrolyzed collagen obtained is of higher purity when we are using the enzymes.

In alkali treatments aminoacids are racemizated and the amides glutamine and asparagine are transformed into the alkali salt of glutamic and aspartic acids with detachment of ammonium. The basic solution can be neutralized with carbon dioxide and if it is necessary the neutral salts can be removed using membranes that retain the aminoacids and peptides. The resulting solutions have a bitter taste and a honey color, and when dried produce a hard film. The acid hydrolysis preserves better the peptide and the aminoacids but its neutralization is also necessary. In this case, this can be carried out with calcium carbonate and after the soluble salts can be removed by means of membranes. The resulting solutions have a bitter taste and are dark brown in color, and when dried produce sticky films.

Enzymatic hydrolysis has the advantage that the resulting solutions are salt free and the size of the peptides is more homogenous.[26] Enzymatic proteolysis needs collagen denaturation, because the native collagen fibers only are attacked by specific collagenases. Gelatin is attacked by pepsine, neutrase, alkalase, bromelain or papain. The selection of enzyme and hydrolysis conditions determines the properties of final product.

Finally, it is necessary to destroy the enzyme, which can be done by acidification at pH 3 or lower or by heat. The resulting solution has a light beige color and the films obtained by drying are very hard and without taste. The solution is concentrated in vacuum and spray dried to yield a white or slightly yellow creamy powder. Hydrolyzed collagen of high purity grade is used for additions in food, cosmetics (creams, shampoos, etc) or washing powder formulations. It has great buffer capacity and can stabilize technical solutions, avoiding settling of dispersions and therefore stabilizing the foam. During 2006 200 MT of hydrolyzed collagen were produced. Most of it originated from chrome shavings.

OTHER APPLICATIONS

Medical

Takeda[27] has demonstrated that native collagen, even when injected, is not toxic. However, massive doses can produce antigenic reactions, which can be eliminated removing the telopeptids of the collagen or by reticulation of the protein chains. Purified collagen is well tolerated by the human body. Nowadays collagen materials at its structure present no major problems in applications such as artificial skin, films for surgical operations, pipes, sponges, powder, threads, supports for enzymes, bandages, etc.

In 2008 the "Forschungsinstitut für Leder und Kunststoffbahnen" 4th Freiberg Collagen Symposium was held.[29] Several presentations were delivered on the application of collagen materials to solve problems of the bones, cartilages, and skin. Technologies have been developed for manufacturing collagen scaffolds that facilitate the differentiation between epidermis and dermis with the sufficient mechanical stability to use them in skin transplants. Sheets were commercialized with the brand INTEGRA. When a template is placed on a wound where burned skin has been removed, it provides the needed framework for the blood vessels and dermal skin cells to grow again in the new skin layer. The silicone outer layer temporarily closes the wound to ward off injection and control fluid and heat loss. MATHIDERM is another registered brand for a collagen-elastin scaffold to grow keratonocytes and preadipocytes. Catrix is a brand from the company Lescarden (USA) for wound dressing. This is a powder derived from bovine cartilage for the management of chronic lesions and burns.

A porous support based on bovine collagen type I with transverse links is the principal part of one equivalent complete skin, developed for tests with animals. The dermal fibroblasts and the keratinocytes were obtained in biopsies of human skin and were sewn inside and out of the above mentioned support. In the course of five weeks of cultivation an epidermal fabric develops with all the layers and characteristics separated from the healthy human skin. Bel and his collaborators[28] introduce the native protein collagen for the growth of the keratinocytes in a process of textile engineering of the conjunctive tissue. It is used in treatments for the regeneration of bones. For degenerative diseases of the joints gelatins and collagen of marine origin have been used. Scaffolds of collagen base are in use for cultivating chondrocytes in the textile engineering of the cartilages. [*sic*- Micro structured of the supports applying technologies based on the effect 2-photon (1) Kaiser W, Garret CGB Rhy S. Rev. Lett. 7 (6) 229-231, 1961. Two-photon excitation in Ca: F2:Eu2 +.]

The possibility of making threads of collagen by means of different technologies has been widely tested for quite a few years. Possible applications include its use as materials of suture in surgical operations and the manufacture of bio-three-dimensional fabrics. However, no practical results have been obtained so far. Osteoarthritis is characterized by the onset of breakdown of the elastic buffering material of the joints (cartilage). The main components of cartilage are the collagen fibers that provide its structural framework and stability and the proteoglycans, which surround these fibers, furnishing them with elasticity and support. Today, there is no known cure for osteoarthritis and treatment focuses only on relieving pain and inflammation. Dr. Oesser's results indicate cartilage tissue developed more extensively following the addition of gelatin. Osteoporosis is a condition whereby the bone substance degenerates to such a degree that the bone density decreases drastically. The bone mass is thus reduced and its structure becomes porous. By administering hydrolyzed collagen, the building blocks required for renewing bone collagen are provided, and the body then uses them for this purpose.

Cosmetics

The statement "People are as old as their connective tissue" uttered by Bogomoletz in 1946 has not lost its meaning and it could well be a sort of credo for modern cosmeticians. With age, collagen takes increasingly longer to be formed. Consequently, the loss becomes greater than the quantity renewed.

In the market numerous formulations containing collagen can be found, though sometimes it is difficult to know exactly in what form. Collagen is used to stabilize, to provide delicacy or viscosity to various products including gels, creams, lotions, lip pencils, shaving creams, and shampoos, among others.[30,31] Their action is to provide these characteristics to the products but it is difficult to find out whether they actually improve the health of the human skin. In spite of the fact that there is no evidence of clear effectiveness, these products are nowadays embraced by consumers and can therefore be considered a profitable fashion. Hair care products containing hydrolyzed gelatin have proven to be of help in the fight against hair stress and hair dryness.

Conclusions

- The collagen tissue of raw hides is a natural material; so it is the best option to use it in any processes without previous treatment in tannery, that is, directly from slaughterhouse

- When the trimmings of lime split, not useful for tanning, are applied as derivatives, the tanner must be very careful in manufacturing them avoiding the addition of chemicals (biocides, surfactants, etc.) in the beamhouse, because they may affect the health of end-users.

- The collagen used by medical, pharmaceutical and cosmetic sectors is a product of high purity and, consequently, of high cost; but the total consumption for this kind of products represents only a very small amount of the total collagen production

- In the future, it is foreseen that collagen tissue will be used as human nourishment, because of the good quality and low cost of this kind of protein.

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