



Green Leather Industry for the Environment (greenLIFE) is a project run by a group of industry partners in Italy's Arzignano tanning cluster. The project partners have committed to sharing their findings by publishing a series of papers in World Leather. The fourth of six greenLIFE articles comes from biotechnology firm Ilsa, one of the project's technical partners.

greenLIFE 4: By-products of the tanning cycle as fertilising components

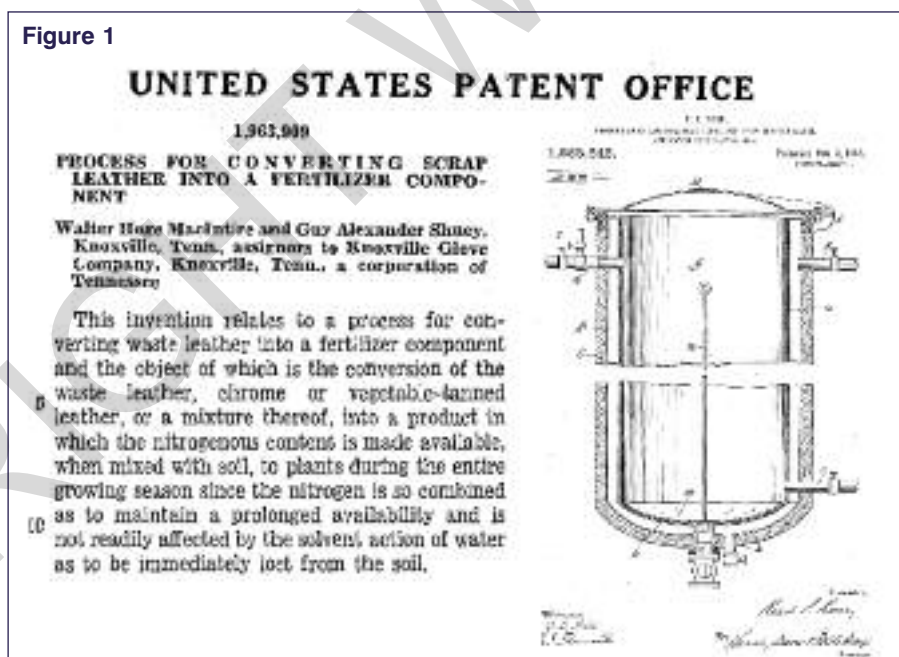
Franco Cavazza, Industrial Director of Ilsa SpA

It is well known that downstream of the tanning process several potentially valuable by-products are generated, typically characterised by an important organic protein substance content. Most of these by-products consist of fragments of animal skin or leather, produced both before and after the tanning process, typically containing trivalent chromium. In addition to these, there is the other solid waste generated during the liming steps, which is the flesh and hair, as well as other organic/protein substances of animal origin that are normally dissolved in the process wastewater.

For many decades, the possibility of recovering such a protein substance, especially in those areas where it is present in large quantities, has motivated the chemical industry to study the processes of doing so. The first patents appeared in the United States at the beginning of the 20th century (Reed P. Rose, 1918; *Figure 1*) when an initial chemical-thermal hydrolytic process was hypothesised for the production of nitrogenous organic fertilisers, proudly demonstrating a consequent, almost quantitative, transformation of the nitrogen contained in the hide in a form available for plant nutrition.

Animal hides mainly consist of nitrogenous organic matter but the protein nitrogen contained within, having stabilised the hide through the tanning process, is made practically rot-proof and no longer biodegradable. Therefore, the driving force of the early patents and industrial projects was to submit the residues and by-products of the processing of tanned hides to

Figure 1



hydrolytic processes capable of breaking the strong covalent bond between the tanning agent (usually trivalent chromium) and the polypeptide chains constituting the collagen.

Added impetus was given to this type of research by the continuous and growing attention to the sustainable development of agriculture, as well as by the increasing demand for organic substances in the soil and the need to find a smart and sustainable way of reusing the residues generated by the various industrial processes ('Circular Economy').

The production processes that began to be developed from the 1950s focused on the three main types of hydrolysis that were chemically and industrially applicable to this type of matrix:

- 1) **chemical hydrolysis:** based on the creation of an extremely acidic or alkaline reaction medium. This type of hydrolysis causes the random breakage of protein chains, with the production of racemic mixtures of dextrorotatory (D-) and laevorotatory (L-) forms of amino acids. This type of hydrolysis typically allows the obtaining of greater degrees of hydrolysis and higher content of free amino acids,
- 2) **thermal hydrolysis:** based on an increase in temperature and pressure of the reaction medium (steam/water). It is typically applied to trimmings and shavings for the production of solid fertilisers (of the "nitrogenous organic fertiliser" type).



3) **enzymatic hydrolysis**: based on the use of proteolytic enzymes that can, in appropriate conditions of temperature and pH, break protein chains into fragments of peptides, polypeptides and amino acids of varying length and molecular weight, with greater propensity to the production of amino acids and peptides in their laevorotatory (L-) form.

Using these hydrolysis techniques, individually or in combination with each other, different types of fertilisers are currently being manufactured, regulated and defined, for example, by the Italian National Legislation governing fertilisers. *Table 1* is a list of the main kind of fertilisers currently available and authorised, obtainable from by-products of animal origin resulting from the liming and tanning processes.

The development of these three different processes, individually but also frequently in synergy with one another, was also accompanied by a technological and plant development that made it possible to achieve greater control over the process. This made it possible to predetermine and maximise certain important agronomic aspects of the obtainable fertiliser product, such as its solubility; mineralisation speed in the soil; the release curve over time in certain soil and climatic situations; and even the degree of racemisation or the degree of hydrolysis obtainable with the hydrolytic process.

In the case of thermal hydrolysis, typically used on wet blue tanning leather trimmings and shavings, the use of spherical rotating autoclaves, the intimate mixing and contact between the matrix to be hydrolysed and the hydrolysing agent (steam usually at 6 bar pressure), together with the development of low temperature (<150°C) dehydration systems and the high level of control of process parameters, have allowed the obtaining of a nitrogenous organic matrix that is particularly efficient in the agronomic area. This is because of its controlled and modulated release capacity of organic nitrogen, together with an important contribution of organic matter to agricultural soil.

In parallel with these hydrolytic processes, aqueous processes for the extraction of the protein fraction have also been developed in the last 40 years, with the production of hydrolysed proteins in aqueous solution (and then

Table 1: Italian National Legislation governing fertilisers

Type designation	Preparation method and essential components	Minimum title in fertiliser elements (% and weight). Evaluation of the fertilising elements. Other necessary requirements
1. Leathers and horsehair (Leathers-meal or leather meal)	Hydrolysis treatment of complex complex organic substances constituting the hides. Leather processing residues	5% N Nitrogen evaluated as organic nitrogen
2. Integrated leather meal	Product obtained by mixing leather meal and stabilised protein sludge of the tanning cycle	4% N 20% C organic Nitrogen evaluated as organic nitrogen C/N ratio: maximum 8
3. Leather meals	Leather trimmings treated with sulphuric acid and dried	5% N Nitrogen evaluated as organic nitrogen
4. Roasted leather	Roasted leather trims	8% N Nitrogen evaluated as organic nitrogen
5. Hydrolysed animal epithelium	Animal epithelium residues from tanneries and slaughterhouses, hydrolysed with mineral acids	4% N Nitrogen expressed as nitric nitrogen of which at least 1% is soluble organic nitrogen 15% C organic C/N ratio: not more than 6
6. Hydrolysed leather and hides	Product obtained through pressurised hydrolysis from the processing waste of hides and leather and subsequent drying	10% N Nitrogen evaluated as organic nitrogen C/N ratio less than or equal to 4
7. Hydrolysed gelatine for agricultural use	Product obtained through the hydrolysis of hides previously treated in technical systems (Reg. EC/1774/2002).	Organic nitrogen (N) 10% Organic nitrogen (N) soluble in water 5% Organic carbon (C) 30% Extractable organic carbon/total organic carbon 90% pH in water < 6
8. Fluid flesh in suspension	Suspension of solubilised and partially hydrolysed meat processing residues	3% N Nitrogen expressed as organic nitrogen of which at least 90% is soluble organic nitrogen 10% C organic
9. Hydrolysed fluid animal epithelium	Product obtained from the enzymatic and/or chemical hydrolysis of animal epithelium	8% N Nitrogen expressed as total nitrogen of which at least 90% in organic form 20% C organic pH 4.5 – 6.5

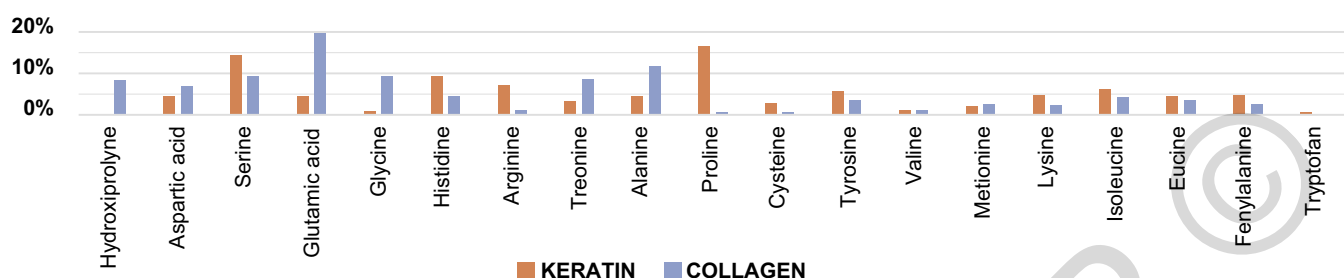
Table extracted from Legislative Decree no. 75, 29 April 2010, as amended



Thermal hydrolysis plant – Ilsa SpA, Arzignano (VI)



Figure 2: Amino acid profile of keratin (hair) protein hydrolysate and of collagen protein hydrolysate (from wet-blue shavings) compared



dehydrated if necessary by generating a water-soluble solid protein hydrolysate) in which there is also normally a simultaneous, almost total removal of the trivalent chromium salts present. These types of protein hydrolysates have found uses in many applications, most notably in fertilisers (organic nitrogen fertilisation and bio-stimulating activities), but also in other sectors such as textiles, plastics, and for reuse within certain phases of the processing of tanned hides. These extractive processes were applied and optimised starting from either the waste protein from the processing of hides before tanning (trimmings, fleshings, etc.), or after the tanning step with chromium salts (wet-blue shavings). In this type of hydrolysis-extractive process, normally applied are a chemical hydrolysis phase (that is in a strongly alkaline or acidic medium using strong acids or bases), or an enzymatic hydrolysis. A combination of the two is also possible and it is sometimes assisted by a prior or subsequent stage of thermal hydrolysis.

In all cases, the fingerprint of the amino acid profile determined by the initial matrix, animal collagen, is well defined. In fact, some amino acids typical of that matrix, such as hydroxyproline (Figure 2), glycine and proline, are always present, regardless of the type of hydrolysis applied. By changing the hydrolytic technique adopted and the process parameters, it is possible to obtain different results in terms of degree of hydrolysis, degree of racemisation, distribution of the molecular masses and the content of free amino acids in the final protein hydrolysate. It is these four parameters, together obviously with the maximisation of yield of the hydrolysis process and of the purity of the final product, that are normally considered the key quality parameters on which

process innovation has worked and is working nowadays to obtain ever improved and reproducible results. It aims to give an added quality to the product and, above all, to better satisfy the needs of agriculture, which is becoming increasingly specialised.

It is precisely by using these techniques that it was decided to test the production of a fluid protein hydrolysate obtained by reprocessing a solid waste from the liming process (i.e. hair extracted in the early stages of liming). As an alternative to its complete dissolution in the wastewaters of the liming itself, the hair was continuously removed during the first phase of liming, that is immediately after its detachment from the animal skin. The hair is characterised by a high keratin content, a valuable protein that is particularly rich in sulphur because of the high content of cysteine, one of the main amino acids of which it is formed.

The hydrolysis mechanism chosen was of a mixed thermal-enzymatic type: after initial homogenisation and grinding of the sample in an aqueous medium, the mixture was placed in an autoclave at a pressure of 2 bar for approximately 2 hours. This was followed by a further phase of enzymatic hydrolysis through the use of stereoselective proteolytic enzymes, using the traditional mechanism of enzymatic biocatalysis. The reaction mixture obtained, properly clarified and concentrated, resulted in an excellent fluid protein hydrolysate (keratin hydrolysate) characterised by a high content of cysteine.

The product obtained is compatible in terms of its chemical and physical properties with the keratinic hydrolysis typically present on the international market (technical and cosmetic sector) and is also compatible with its use in agriculture as a fertiliser.

Parallel to this line of research, within

the greenLIFE project a method of coagulation of the organic-protein fraction contained in liming wastewater was also evaluated, both of a traditional (lime-sulphide) and oxidative type liming phase.

For this purpose, several techniques of precipitation of that organic fraction were tested, giving priority to one based on adding phosphoric acid down to pH 5-5.5. This precipitation, to be carried out immediately after liming water production in order to prevent and anticipate the inevitable processes of self-hydrolysis and degradation of the protein substance, results in a solid sludge that, once dried, contains important concentrations of organic nitrogen, carbon and phosphorus, essential nutrients in the fertiliser industry.

After these pre-industrial studies aimed at the production of new prototypes of products/fertilisers, (see analytical profiles of the two NP fertilizer produced and tested on next page) which were produced in quantities both useful and sufficient to perform the first agronomic testing, the second



Freshly washed sample of bovine hair obtained by mechanical separation from the first stages of liming



experimental phase of the project was the preliminary assessment of the prototypes fertilising properties, with an approach called “soil/plant”. These kinds of studies evaluate, with multidisciplinary approaches, the effect on the soil, the effect on plants (taking out any interference of the soil), and the overall effect on the entire soil and plant system.

In particular, for the various systems under investigation:

“Soil” approach: involves studying the mineralisation curves of the nitrogen content, and measuring the transformation kinetics of the nitrogen in its various forms, including organic, ammoniacal and nitric nitrogen (the final form which is typically metabolised by plants through the root system).

“Soil/plant” approach: during agronomic tests in greenhouses and in the field, the main effects of the prototypes tested on plants are studied, assessing the main indicators (like leaving ground cover (LGC), dried and fresh biomass, etc.) and their possible interaction with soil and climatic conditions.

“Plant” approach: during tests in a climatic chamber, various bioassays are performed on different inert substrates (or in hydroponic cultivations) with model plants, typically those with a short and rapid life-cycle, in order to test plant reactions in the early vegetative phases. The typical parameters analysed are the root and leaf development, the weight of the fresh or dried biomass, etc.

Conclusions

The results of these cycles of testing, which took place between 2016 and 2017, have been generally encouraging, especially in terms of non-toxicity to plants of the products tested, and have provided useful information in order to continue with the trial. They also allowed for the clear conclusion that samples of untreated by-products (dried hair and sludges with no modification) have very low fertilising properties, with extremely long nitrogen release kinetics and with possible phytotoxicity effects, probably due to the high salinity level of the same. Some of the prototypes obtained following the processes of separation, chemical-physical modification and thermal-enzymatic hydrolysis have compared favourably with high-end

SAMPLE 1 LIME VAT

	TQ LIME VAT	NP FERTILISER
pH		5.2
Salinità (ds/m)		0.65
N tot (%)	4.3	6.6
C tot (%)	31.5	29.7
S(SO ₃) tot (%)	4.6	3.4
N amm (%)		<0.1
N org (%)		6.6
P(P ₂ O ₅) (%)		20.9
Cl ⁻ (%)		0.6
Ca (CaO) (%)		15.6
Na (Na ₂ O) (%)		0.71



SAMPLE 2 LIME VAT

	TQ LIME VAT	NP FERTILISER
pH		4.9
Salinità (ds/m)		1.3
N tot (%)	2.5	8.2
C tot (%)	25.3	39.6
S(SO ₃) tot (%)	1	1.7
N amm (%)		<0.1
N org (%)		8.2
P(P ₂ O ₅) (%)		7.8
Cl ⁻ (%)		2.2
Ca (CaO) (%)		6.5
Na (Na ₂ O) (%)		2.1



Analytical profile and granulated sample of NP mineral organic fertiliser obtained from liming sludge



“Soil” approach



“Soil/plant” approach



“Plant” approach



“Plant” approach



commercial products with proven effectiveness. In the coming agricultural seasons, further studies will be completed to confirm the possibility of the use of these types of products in agriculture.

It is now important to better define and analyse certain other aspects related to the concrete application of the proposed innovations on an industrial scale:

- 1) Authorisation framework of a possible industrial manufacturing plant, both in terms of Animal By-products Regulation (Reg. 1069/2009) and in terms of environmental impact due to possible odorous and air emissions that the transformation process can generate.
- 2) Optimisation of the process with particular attention to aspects of economic sustainability both for the tannery company that provides the waste and for the processing company, which needs to offer the products to market at a competitive cost/benefit ratio.
- 3) Aspects related to the assessment of the product's environmental footprint, also assessing experimentally if negative interferences on the ecosystem may be present (improbable).
- 4) Repetition of agronomic tests on several cycles and different crop species to assess even more widely the

potential of these products and to identify the best conditions of use in terms of dosage, crops, vegetative stage, climatic and soil conditions, etc. 🌍

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Fleshing machine
PESCARNA



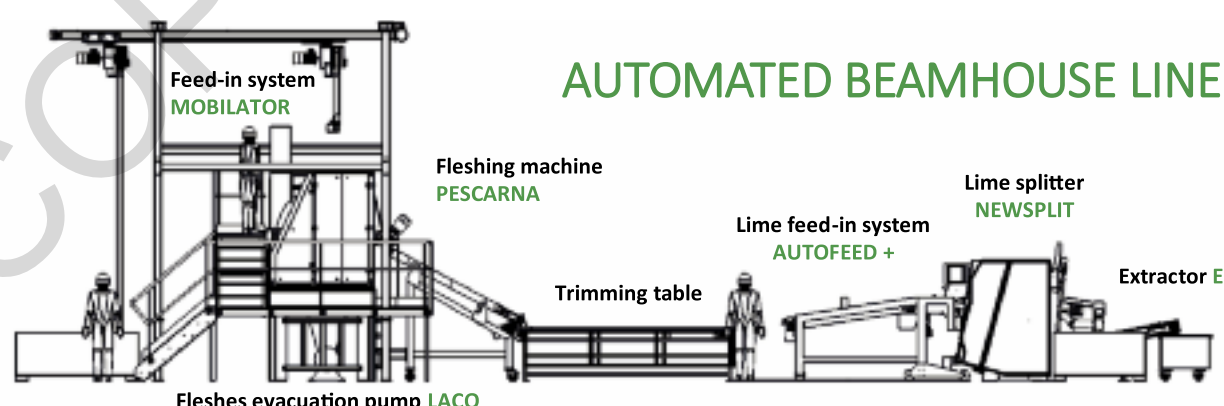
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