Obtaining high-quality gelatines from untanned tannery wastes on a semi-industrial scale using an enzymatic pre-treatment

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Abstract

During the transformation of hides into leather, the tanning industry generates large quantities of wastes, among them untanned hide wastes. Current European policy has prompted the minimisation of industrial wastes and also the recycling and transformation of wastes into valuable co-products. Due to the fact that solid tannery wastes are a rich source of collagen, the extraction of gelatine from them is a suitable way for their valorisation.

In this sense, INESCOP is working on the LIFE microTAN project (LIFE12 ENV/ES/000568), which aims to demonstrate, on an semi-industrial scale, the technical, environmental and financial feasibility of the isolation of gelatine from untanned tannery wastes, in order to reuse it as a microencapsulating agent.

Tests carried out in a pilot plant showed that it is possible to recover tannery wastes using an enzymatic pre-treatment to obtain high-medium Bloom value gelatines as a function of processing conditions, with suitable properties for microencapsulation. It is worth noticing that the enzymatic pre-treatment alternative to the conventional alkaline pre-treatment process allows cost savings and a noticeable reduction in pre-treatment time and wastewater, thus minimising the environmental impact.

Keywords: microcapsules, collagen, hydrolysis, microencapsulation

1. INTRODUCTION

During the transformation of hides and skins into leather, the tanning industry generates large quantities of waste. Even though untanned hide and skin wastes are not considered to be dangerous and, hence, their management sometimes consists in their disposal into landfill sites, their organic nature makes them prone to suffer degradation by microorganisms. This fact can generate problems during waste transportation and handling.

Current European policies relative to the Environment not only encourage the reduction of industrial wastes, but also their recycling and transformation into added value by-products. In view of the fact that solid waste from the tanning industry has high collagen content, its use as a raw material in the production of gelatine is envisaged as a suitable way for the valorisation of this kind of waste.

Gelling properties of gelatine constitute the basis for its use in the food, cosmetic and pharmaceutical industries, photography, etc. In addition to these traditional uses, gelatine can also be used as a microencapsulating agent, that is, as a shell biopolymer for encasing active substances in microcapsules production. Microencapsulation is a technology that enhances active substances' stability against external factors, such as moisture, light and oxygen. Besides, this technique allows the controlled release of active substances (Benita, 1996; Pérez-Limiñana et al, 2014; Nakagawa and Nagao, 2012; Sánchez-Navarro et al, 2013; Schrieber and Gareis, 2007).

However, gelatine has a wide range of properties that determine its final use (Mariod and Adam, 2013; Nagarajan et al, 2012; Schrieber and Gareis, 2007; Zhang et al,

2006). One of the most important properties is gel strength or Bloom value. It determines gelatine quality as well as its potential application.

Gelatine properties are governed by extraction conditions, including temperature, pH, and time, among others. (Mariod and Adam, 2013; Nagarajan et al, 2012; Pérez-Limiñana et al, 2016; Schrieber and Gareis, 2007; Zhang et al, 2006). For this reason, it is necessary to optimise the process of transforming collagen into gelatine, so that medium-high quality gelatines can be produced, in order to ensure that they are suitable for their use in industrial applications such as microencapsulation (Pérez-Limiñana et al, 2016; Schrieber and Gareis, 2007).

The process of obtaining gelatine from hides, skins and/or bones requires that a pre-treatment stage is performed, prior to the extraction stage. The conditions of this pre-treatment stage depend on the raw material used. In the case of bovine hides being used as a raw material, an alkaline pre-treatment is preferred. This is a long process consisting of several treatment cycles with NaOH followed by washing with water, which generates high volumes of wastewater. The pre-treatment process aims at the cleavage of collagen crosslinkings, breaking the collagen structure in order to enable its further extraction. During extraction, non-collagenous proteins and other non-protein substances are also removed. Afterwards, the pre-treated raw material is washed in order to remove alkalis and be neutralised. Most salts produced in this process are removed by several washing processes (Schrieber and Gareis, 2007).

Due to the high water requirements and high protein content in wastewater, the gelatine industry considers that an improvement in the process is necessary in terms of power and water savings. Energy balance is similar when comparing the alkaline process for bovine hides and the acid process for pig skins. However, water requirements highly increase in the alkaline process (400 L per kg of produced gelatine) with respect to the acid one (150 L per kg of produced gelatine). The reason is that water (or alkaline solution) must be replaced some 20 times during conditioning and washing steps. Thus, large quantities of wastewater must be managed and treated. Therefore, there is an increasing interest in the enzymatic pre-treatment as an alternative to the alkaline treatment in gelatine production. This allows cost savings due to a reduction in processing time and wastewater (Nakagawa and Nagao, 2012; Schrieber and Gareis, 2007).

For this reason, INESCOP is working on the LIFE microTAN project (LIFE12 ENV/ES/000568), the objective of which is the demonstration, on a semi-industrial scale, of the technical, economic and environmental viability of producing gelatine from untanned tannery wastes and its use as a microencapsulating agent. With this aim in mind, an enzymatic pre-treatment is proposed, which produces high-medium quality gelatines suitable for the intended use, as well as for use in other industrial sectors.

More precisely, the objective of this work is to optimise enzymatic pre-treatment conditions, since preliminary laboratory results demonstrated that these variables highly affect final gelatine properties. To this end, a design of experiments (DOE) was created to assess the influence of several variables (factors) in the desired gelatine properties (responses). Pre-treatment conditions that are optimised by this procedure will be then used in the production of gelatine on a pilot plant scale.

2. EXPERIMENTAL

2.1. Materials

Untanned bovine hide wastes were provided by a local tannery (INCUSA, Valencia). When carrying out the DOE, waste samples were cut into 0.5 x 0.5 cm pieces.

Protease and lipase enzymes (Spain Enzymes, Valencia) were used in the pre-treatment stage.

2.2. Design of experiments (DOE) and statistical analysis

In order to optimise the process variables for gelatine production, a preliminary study was made to assess the influence of the enzymatic pre-treatment conditions in gelatine properties. This would allow the best conditions to be selected for semi-industrial scaling up (pilot plant). The said study was carried out using a 10 L reactor.

Consequently, a 2-level full factorial design for three factors (temperature, time and protease concentration) and two levels was created,

with two replicates (Sample A and Sample B). The design of experiments allows the prediction of the most suitable pre-treatment conditions that lead to high-medium quality gelatines with high yield values. Gelatine is then to be produced on a semi-industrial scale under the established conditions.

This 2^3 design can be depicted as a cube with eight factorial combinations. Tables 1 and 2 summarise the main features of this full factorial DOE.

In every experiment, the pre-treatment process was performed in the presence of a lipase (lipase concentration = 0.2%, referred to waste weight) and at pH=10 (for optimal enzymatic activity). The further extraction process was carried out at fixed conditions: 75ºC, 5.5 h, and pH=6.5.

After having carried out the experiments, two responses were analysed: Gel strength (measured as Bloom value according to EN ISO 9665 (see Figure 1) and yield.

Figure 1. *Gel strength (Bloom value) measurement in a texture analyser.*

Table 1. 2^3 factorial design. Factor names and levels

			Levels	
Factor	**Name**	**Units**	**-**	**+**
T	Temperature	ºC	40	55
t	Time	h	0.5	1.5
C	Protease concentration	%	0.02	0.5

Table 2. DOE matrix

Experiment	Replicate	Run	T(ºC)	t (h)	[protease] (%)
1	A B	1 9	40	0.5	0.02
2	A B	7 15	40	1.5	0.5
3	A B	3 11	40	1.5	0.02
4	A B	5 13	40	0.5	0.5
5	A B	2 10	55	0.5	0.02
6	A B	4 12	55	1.5	0.02
7	A B	6 14	55	0.5	0.5
8	A B	8 16	55	1.5	0.5

For yield calculation, the extracted gelatine was dried and weighed, and the moisture content was determined by thermogravimetric analysis (TGA). Yield was calculated based on dry weight of untanned waste, according to the following equation:

$$Yield\ (\%) = \frac{weight\ of\ \ gelatine - moisture}{weight\ of\ \ dry\ waste} * 100$$

Data were analysed by a factorial regression model using Minitab® 17 Statistical Software. A significance level $\alpha = 0.05$ was applied.

Optimal conditions deduced from this analysis were selected for gelatine extraction on a pilot scale.

2.3. Gelatine production on a semi-industrial scale (pilot plant)

The pilot plant (see Figure 2) consists of a stainless steel 100 L vessel, equipped with an overhead stirring system (inclined-blades type). It is also equipped with pH and temperature probes for measuring these properties during the process. The plant is also outfitted with acid/alkalis dosing pumps which are controlled by an automated system that allows accurate pH and temperature settings.

Figure 2. *Pilot plant for gelatine extraction on a semi-industrial scale.*

Untanned bovine hide wastes (400 cm^2 size, approximately) were enzymatically pre-treated. Pre-treatment conditions were those optimised by the design of experiments analysis (see section 3.1).

Afterwards, gelatine was extracted from pre-treated wastes with warm water, at 75ºC for 5.5 h. pH was automatically corrected in order to maintain it at a value of 6.5±0.5.

After extraction, the gelatine solution was filtered with a double filter system (100 and 50 μm). Finally, an aliquot of this gelatine solution was dried at 50ºC in an oven, and was characterised by measuring the Bloom value (see section 2.2).

The gelatine solution is to be used for active substances microencapsulation by means of the complex coacervation method.

3. RESULTS AND DISCUSSION

3.1. Pre-treatment stage optimisation. DOE results

For each response (yield and Bloom value), the analysis assessed the significance degree of the three main effects: temperature (T), time (t) and concentration (C); the three 2-way interactions: temperature*time (T*t), time*concentration (t*C) and temperature*concentration (T*C); and one 3-way interaction: temperature*time*concentration (T*t*C).

Below, the results of the factorial regression model analysis for each response ('Yield' and 'Bloom value'), as generated by the statistical software, are shown.

3.1.1. Yield optimisation

Figure 3 shows the cube plots created for both the data means (measured values) and the fitted means (according to the factorial regression model) of the Yield response. Values predicted by the proposed model fitted quite well the experimental ones.

A reduced regression model was used, where non-statistically significant effects (those for which p-value was higher than 0.05) were removed. Namely, the effects that were removed were the 3-way T*t*C interaction and the 2-way t*C interaction.

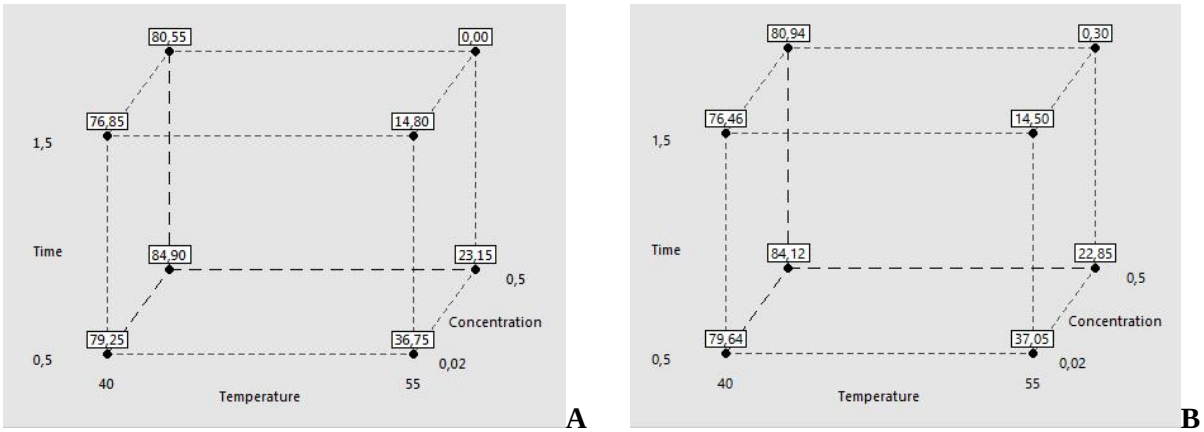

Figure 3. *Cube plots showing the relationship between the three factors and the Yield response.* ***A****: created with measured data means;* ***B****: created with fitted means, according to the reduced model.*

When comparing the standard error and the coefficient of determination of both the full and the reduced model, the latter proved to fit better. Besides, the reduced model produced no changes in the effects significance with respect to the full model.

The relative magnitude of the effect of each factor or interaction on the 'Yield' response evolved as follows:

$$T >> t > T*t \approx T*C$$

3.1.2. Bloom value optimisation

Figure 4 shows the cube plots created for both the data means (measured values) and the fitted means (according to the factorial regression model) of the Bloom value response.

The Bloom value for the 55ºC, 1.5h factor level combination could not be measured because wastes were completely hydrolysed and no gelatine could be extracted. For this reason, the full regression model, considering the missed value, could not estimate the 3-factor interaction. As in the analysis for the yield response, this first regression model was reduced by eliminating non-statistically significant effects (p-value > 0.05). More precisely, the effect of the 2-way t*C interaction was removed. The reduced model fitted better than the full model (lower standard error for coefficients) but changes in the effects significance happened. According to the reduced model, the three main effects (C, t and T) were significant, as well as the 2-way T*t and T*C interactions. However, for certain level combinations, within the range of study, the reduced model predicted negative Bloom values, with no physical sense, and for this reason it was considered to be unsuitable.

Then, an alternative analysis was made. In this analysis, a Bloom value equal to zero was considered for the 55ºC, 1.5h, 0.5% factor level combination (at which wastes were fully hydrolysed). The new model did allow the assessment of the effect of the 3-way interaction, which was found to be statistically significant. In addition, fitted values predicted by the model were all within the range of suitable values.

According to the alternative model, the relative magnitude of the effect of each factor or interaction on the 'Yield' response evolved as follows:

$$C >> t*C \approx T*t*C$$

3.1.3. Selection of optimal conditions

According to the results of the statistical analysis, both yield and Bloom value were highly influenced by the enzymatic pre-treatment conditions.

Table 3 summarises the main conclusions drawn from the statistical analysis of the results obtained in the design of experiments for the pre-treatment stage of the proposed process for producing gelatine from untanned hides.

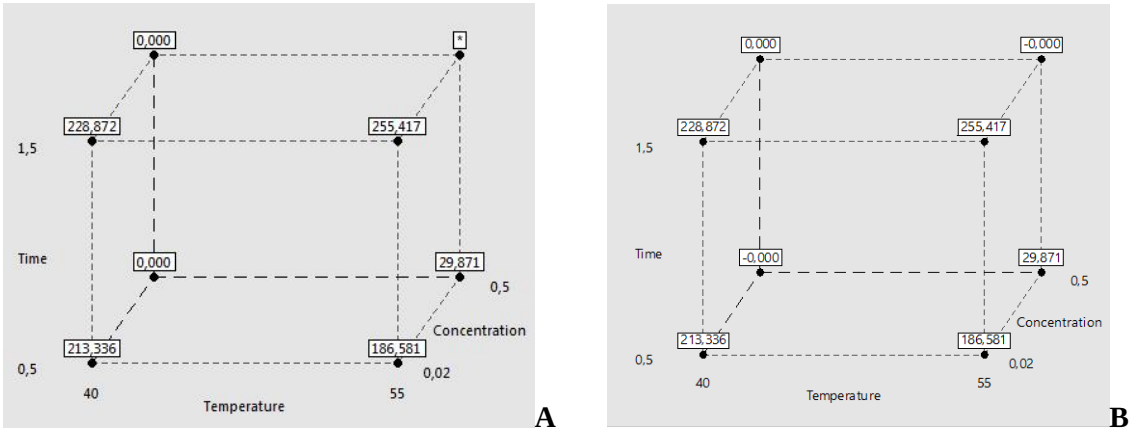

Figure 4. *Cube plots for Bloom value.* ***A:*** *created with measured data means;* ***B****: created with fitted means, according to the alternative model. *No gelatine was extracted with this factor level combination; therefore, the Bloom value could not be measured.*

Table 3. Summary of results of the statistical data analysis.

Factor/ Interaction	Yield		Bloom value	
	Statistically significant	Level/combination producing best results	Statistically significant	Level/combination producing best results
T	**Yes**	**40ºC**	No	55ºC or 40ºC
t	**Yes**	**0.5 h**	No	1.5 h
C	No	0.02 %	**Yes**	**0.02%**
T*t	**Yes**	**40ºC** **0.5 h or 1.5 h**	No	55ºC or 40ºC 1.5 h
T*C	**Yes**	**40ºC** **0.5% (0.02%)**	No	40ºC or 55ºC 0.02%
t*C	No	-	**Yes**	**1.5 h** **0.02%**
T*t*C	No	-	**Yes**	

According to these results, it can be concluded that, on a medium-size laboratory scale, the optimal pre-treatment conditions that may produce high yield and Bloom values are:

Temperature: 40ºC
Time=0.5h – 1.5h
Protease concentration=0.02%

3.2. Gelatine production on a pilot scale

Taking into account DOE results, pre-treatment conditions 40ºC, 1.5 h, 0.02% were selected as the most convenient for gelatine extraction in the pilot plant.

Figure 5 shows the hide waste after each stage during the gelatine extraction process in the pilot plant.

Table 4 compares experimental yield and Bloom values of gelatine produced under the selected conditions, with values predicted by the DOE models for that factor level combination.

Experimental yield and Bloom values of gelatine obtained in the pilot plant were lower than those predicted by the models obtained from laboratory scale data (10 L reactor). Therefore, variables had to be adjusted in order to maximise both yield and gelatine quality on a semi-industrial scale. Anyway, the produced gelatine had a Bloom value of 120 g, which was considered to be suitable for its use as a shell biopolymer for microencapsulation (see Figure 6).


A


B

Figure 5. *Gelatine extraction from tannery wastes in a pilot plant.* ***A:*** *hide waste after enzymatic pre-treatment;* ***B:*** *after extraction stage.*

Table 4. Properties of gelatine produced in the pilot-plant.

	Experimental value	**Fitted value (DOE models)**
Yield (%)	51%	76.46%
Bloom (g)	120±12	228.872

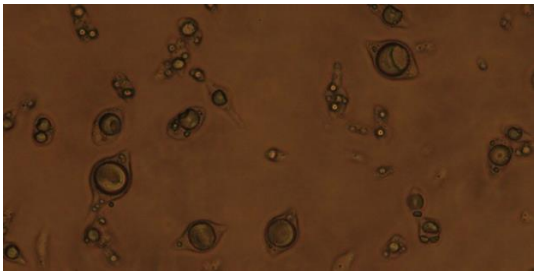

Figure 6. *Gelatine microcapsules produced by means of the complex coacervation method.*

4. CONCLUSIONS

The tests carried out in both the laboratory and the pilot plant showed that, depending on the process conditions, it is possible to valorise untanned tannery wastes by means of an enzymatic pre-treatment in order to produce high-medium Bloom values, with suitable properties for their use in microencapsulation.

It is worth pointing out that the enzymatic pre-treatment, which is an alternative to the conventional alkaline process, allows a substantial reduction of both pre-treatment time and generated wastewater, which reduces the environmental impact of the process.

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