RECOVERY OF TANNERY WASTES FOR FUNCTIONAL MICROENCAPSULATED PRODUCTS

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Leather is one of the most used materials in the footwear and leather goods industries, and is also employed in the manufacture of a variety of products in the clothing/garment industry as well as in furniture upholstery. Even though the tanning industry is considered to play an important environmental role as users of a by-product of the meat industry, the different stages involved in the transformation of hides and skins into leather generate a significant amount of wastes, both liquid and solid. In this sense, the advancement of European policy and legislation protecting the environment has prompted the transformation of tannery solid waste materials into valuable co-products that can be recycled or employed in other industries. The paper focuses on the recovery of collagen derivatives from untanned solid wastes, more specifically by isolating gelatine in order to use it as a natural microencapsulating agent in the production of active materials with functional properties. Gelatine was the first shell-forming material used in microencapsulation and, nowadays, it is still a promising material for the creation of natural and biodegradable microcapsules. In the footwear industry, microencapsulation can transform a traditional shoe into an "active shoe" that ensures the continuous care of our feet by the incorporation of microencapsulated products with therapeutic and/or antimicrobial properties. This work describes the project and the results obtained to date.

Keywords: gelatine, tannery wastes, microencapsulation.

INTRODUCTION

The tanning processes carried out during the different processing stages involved in the transformation of hides into leather generate significant amounts of wastes, both liquid (wastewater) and solid (tanned and untanned waste and sludge). Several approaches have been suggested for the minimisation, treatment and valorisation of effluents and solid wastes generated by the leather industry. In this sense, the advancement of European policies and legislation protecting the environment has prompted the transformation of tannery solid waste materials into valuable co-products that can be recycled or employed in other industries, for instance for the preparation of organic fertilisers, the production of biomaterials, gelatines or collagens, and the production of biofuel (Schrieber and Gareis, 2007).

Gelatine is a soluble protein compound obtained by the partial hydrolysis of collagen. The most abundant sources of gelatine are pig skins, bovine hides and pork and cattle bones. The gel-forming properties of gelatine are the basis of classical applications in food, photographic, cosmetic and pharmaceutical industries. Recently, new applications have arisen, such as its use as a shell-forming polymer for microencapsulation applications (Schrieber and Gareis, 2007, Perez-Liminana *et al.*, 2014; Sánchez-Navarro *et al.*, 2013).

Microencapsulation is a coating technology by which active substances are encapsulated in a polymeric shell, leading to core-shell particles called microcapsules (Figure 1). Microencapsulation is an effective method to protect reactive, sensitive or volatile chemicals from reaction with moisture, light and oxygen. Furthermore, this technology allows a controlled release of the active substance and enhances stability

against external factors. Indeed, microcapsules, when firmly anchored to a material can add new smart functionalities without affecting the look and feel of the material. Therefore, this technology holds great promise for the future of the footwear industry since it can transform a traditional shoe into an "active shoe" that ensures the continuous care of our feet by the incorporation of microencapsulated products with therapeutic and/or antimicrobial properties improving the comfort and welfare of the user (Morace, 2010).

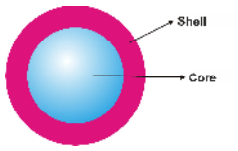


Figure 1. Microcapsule structure. Shell: polymeric cover; Core: encapsulated active chemical

One of the most widely used shell-forming materials is gelatine. Nevertheless, gelatine shows a wide range of properties such as gel strength, film-forming capability, and emulsion properties, among others, which determine its final application. Those properties are governed by extraction conditions (temperature, pH, time, etc). Therefore, the optimisation of the transformation process of collagen into gelatine is necessary in order to obtain specific properties suitable to their use in added value applications, for instance as a shell forming biopolymer for microencapsulation (Schrieber and Gareis, 2007).

The extraction process of gelatine from untanned wastes consists of several steps. As a first step, collagen must be acid or alkaline pre-treated since its hieratical fibrous structure makes it insoluble in water. Such pre-treatment breaks non-covalent bonds so in order to produce fibre swelling and collagen solubilisation, thus enabling its extraction. The alkaline pre-treatment is usually preferred when bovine hides are used as a raw material. During the treatment – a long process that takes several months – the non-protein substances such as mucopolysaccharides and sulphur-containing compounds, as well as non-collagenous proteins, especially albumin and globulin, which are always contained in the raw material, are reliably dissolved out. Subsequently, the treated material is washed free of alkali and neutralised by the addition of acid. Most of the neutral salts produced during this process are then removed by numerous washes (Schrieber and Gareis, 2007).

Because of the high water requirement and the high content of protein in wastewater, the gelatine industry views the improvement of the process along with savings in energy and water as being of high priority. The energy balance is similar in the case of the alkaline procedure using hides and in the acid procedure using pigskins. However, the water requirement increases to 400 L kg^{-1} gelatine for the alkaline procedure versus 150 L kg^{-1} gelatine for the acid procedure. The reason for this is the fact that water has to be replaced about 20 times during the conditioning and washing processes. So, this implies the treatment and management of large amounts of wastewater generated. Nowadays, the enzymatic pre-treatment as an alternative to the alkaline pre-treatment for gelatine manufacture is raising interest in order to save costs by reducing wastewater and time (Schrieber and Gareis, 2007; Zhang *et al.*, 2006).

Proteolytic enzymes (proteases) are commonly used in the leather industry for dehairing, bating and soaking processes, as well as in the detergent industry for

breaking down proteinaceous matter caused by body secretions, food stuff, and blood. As in the pre-tanning process, the use of enzymes during the conditioning of hides prior to gelatine extraction opens up a new alternative to the alkaline pre-treatment to reduce time and wastewater (Kanagaraj, 2009).

The selection of the pre-treatment procedure (acid, alkaline or enzymatic) and also the extraction conditions (temperature, pH, time) will influence the final properties of the gelatine and, therefore, its final application. For technical applications such as microencapsulation, gel strength (Bloom strength, related to gelatine's average molecular weight), film-forming or emulsifying properties of gelatine affect to some extent the quality of the microcapsules and, therefore, the microencapsulation process (Schrieber and Gareis, 2007).

Currently, INESCOP is working on the LIFE microTAN project (LIFE12 ENV/ES/000568) which focuses on the recovery of collagen derivatives from untanned solid wastes, more specifically by isolating gelatine in order to use it as a natural microencapsulating agent in the production of active materials with functional properties. This work proposes the enzymatic pre-treatment as an alternative to the current alkaline pre-treatment process in order to save costs and reduce time and, finally, the use of gelatine for microencapsulation applications. This paper describes some results obtained to date.

MATERIALS AND EXPERIMENTAL TECHNIQUES

Bovine Hides

Bovine hides were supplied by a local tannery as a by-product (INCUSA, Valencia, Spain). Limed bovine pelt wastes were used, which were in the previous condition to the tanning process. The bovine hides were frozen for their conservation up to their use. Previously, the samples were cut into pieces of approx 0.5x0.5 cm.

Gelatine Extraction Process

Prior to gelatine extraction, the hide-waste pieces were conditioned by applying an alkaline pre-treatment using a sodium hydroxide solution (1%) or by applying an enzymatic pre-treatment. After conditioning, the gelatine was extracted under different extraction conditions (pH, time, temperature). The extraction was carried out in a three-neck, 3-L Pyrex glass flask to which a condenser (vertical position), a thermometer and a mechanical stirrer were fitted. The flask was placed in a water bath at a determined temperature. The gelatine extracts were filtered under vacuum. Next, it was concentrated by evaporation in a water bath and kept at a constant temperature using a rotavapor device. Finally, the concentrated gelatine solution was placed in a PTFE mould and left to dry overnight in a furnace at T=45ºC to obtain the gelatine films for further characterisation.

In addition, type B commercial gelatine G9382 supplied by Sigma-Aldrich was used as a reference of suitable properties for microencapsulation applications.

The properties of the gelatine films were evaluated by Thermogravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC), Infrared Spectroscopy (FTIR) and SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The microencapsulation capability of the extracted gelatines was also evaluated.

Assessment of Microencapsulation Capability

The assessment of the microencapsulation capability was carried out by the complex coacervation method. The complex coacervation process is based on the phase separation that takes place spontaneously when in an aqueous phase, two or more colloids of opposite charges (a polycation and a polyanion) are mixed in the presence of an active substance dispersed (oil phase). In this work almond oil was chosen as the oil phase. The extracted gelatines (polycation) and sodium carboxymethylcelulose (CMC) (polyanion) were used as biodegradable shell-forming polymers.

The morphology of the microcapsules were analysed by Scanning Electronic Microscopy (SEM) and optical microscopy. The thermal properties of the microcapsules were evaluated by Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC), and their chemical composition was determined by Infrared Spectroscopy (FTIR).

RESULTS AND DISCUSSION

The degree of conversion of collagen into gelatine depends on the processing conditions. The collagen conversion was reduced when no pre-treatment was carried out and a high amount of solid wastes was not transformed. The hide pre-treatment (enzymatic or alkaline) prior to extraction doubled the yield of the extracted gelatine due to the collagen swelling, which enabled its solubilisation and made the extraction easier. Furthermore, the yield increased as temperature raised and the pH decreased. The enzymatic treatment of the bovine hide wastes produced higher or similar gelatine yields than the alkaline pre-treatment.

The results obtained from SDS-PAGE assays showed that the different extraction conditions greatly affected the molecular weight distribution (Mw) of gelatine. An increase in temperature or a decrease in pH decreased the Mw of gelatine. The enzymatic treatment produced gelatine with lower molecular weights than the alkaline treatment.

By way of example, the chemical composition of the extracted gelatines as a function of the type of treatment was determined by FTIR spectroscopy (Figure 2). The infrared spectra of the extracted gelatines were similar to those of commercial gelatine. The gelatine spectra showed vibration bands at 3400-3100 cm^{-1} for N–H stretching (amide A and B), 3100-2800 cm^{-1} for alkenyl C–H stretching, 1635 cm^{-1} (Amide I) for C=O stretching, a band at 1550 cm^{-1} (Amide II) for out of phase combination of the N–H in plane bend and the CN stretching vibration, 1480-1300 cm^{-1} for CH2 bending, 1249 cm^{-1} (Amide III) for in phase combination of the NH bending and CN stretching vibration (Nagarajan *et al.*, 2012; Barth and Zscherp, 2002). Additionally, the gelatine prepared using the enzymatic pre-treatment showed typical bands of fatty acids due to the presence of the C=O stretching band characteristic of ester groups and also an important increase in C-H stretching bands. In contrast, the alkaline pre-treatment used was more effective than the enzymatic one for the removal of fat from hides.

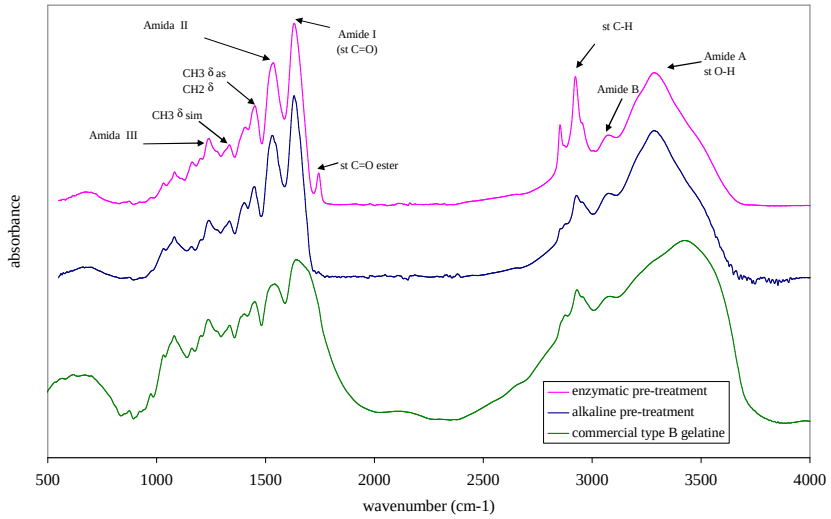
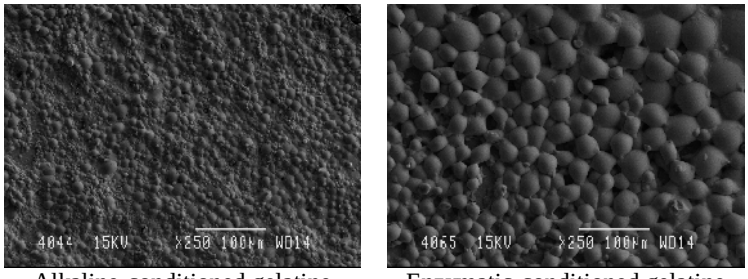


Figure 2. Infrared spectra of enzymatic or alkaline pre-treated gelatines compared with commercial gelatine

Microencapsulation

The microencapsulation capability of the extracted gelatines was evaluated by the complex coacervation process. Microcapsules containing an oil phase as a core material were successfully prepared using both alkaline and enzymatic pre-treated gelatines. Optical microscopy showed the typical elongated-shaped shell around the oil of the microcapsules obtained by complex coacervation. SEM images of the different microcapsules obtained are shown in Figure 3. The particle size of the microcapsules obtained from the enzymatic-treated gelatine was higher than that of the microcapsules obtained from the alkaline-treated gelatine. The particle size was affected by gelatine properties, acting as an emulsifier and as a shell-forming polymer. The emulsion properties of gelatine depend on its chemical composition (based on the presence of hydrophilic/hydrophobic amino acids) as well as the gelling power, both parameters being governed by manufacturing conditions.



Alkaline-conditioned gelatine Enzymatic-conditioned gelatine

Figure 3. SEM images of microcapsules containing almond oil using the extracted gelatines

CONCLUSIONS

Gelatine from bovine untanned wastes can be successfully extracted as valuable co-products using alkaline or enzymatic pre-treatments prior to extraction. It shows suitable properties as a shell-forming polymer allowing the microencapsulation of oils by complex coacervation.

The degree of conversion of collagen into gelatine depends on the processing conditions (pre-treatment, T, t, pH). The pre-treatment of bovine hides is a fundamental step during gelatine manufacture since it allows the swelling of collagen and enables its solubilisation, thus increasing the yield. Moreover, an increase in temperature or a decrease in pH also increases the conversion of collagen into gelatine yield.

The enzymatic pre-treatment is an efficient procedure to open the collagen fibres and remove non-collagenous proteins and other substances. However, some fats from original bovine hides are present in the extracted gelatine, which can be successfully removed when the alkaline pre-treatment is carried out. This indicates that the enzymatic procedure needs to be optimised in order to improve the removal of impurities.

Finally, the particle size of the microcapsules is influenced by gelatine properties (molecular weight, conformation, and chemical composition), which depend on extraction conditions.

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