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A more efficient process to develop protein films derived from agro-industrial by-products

Tania Garrido [a], Miriam Peñalba [b], Koro de la Caba [a], Pedro Guerrero [a, *]

[a] BIOMAT Research Group, University of the Basque Country (UPV/EHU), Department of Chemical and Environmental Engineering, Engineering College of Gipuzkoa, Plaza de Europa 1, 20018 Donostia-San Sebastián, Spain

[b] BIOMAT Research Group, University of the Basque Country (UPV/EHU), Department of Applied Physics I, Engineering College of Gipuzkoa, Plaza de Europa 1, 20018 Donostia-San Sebastián, Spain

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ABSTRACT

Soy protein isolate (SPI) and hydrolyzed keratin (HK) were employed to develop compression molded films. In contrast to other studies, in which toxic chemicals or expensive methods are usually employed to hydrolyze feather keratin, in this research a novel approach was addressed. A solution of NaOH (1 M) was employed and the use of high temperatures was avoided during the process in order to obtain a simple, environmentally friendly and inexpensive method to hydrolyze feather keratin, which can be used for the development of thermoplastic films. In addition to the valorization of agro-industrial waste, the main aim of the incorporation of hydrolyzed feather keratin into film forming formulations was to increase the sulfur content and, thus, the ability to form disulphide bonds, improving the chemical and thermal stability of the resulting films.

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1. Introduction

The continuous growth in plastic use has brought an increasing environmental awareness from the perspective of waste management, emissions released during manufacture, and resources use (Clark, Farmer, Herrero-Davila, & Sherwood, 2016; Rasheed et al., 2016). Therefore, the need of reducing conventional non-biodegradable plastic materials has encouraged the development of innovative biodegradable materials from renewable resources (Gupta & Nayak, 2015; Sheldon, 2014). Biopolymers such as proteins have been regarded as alternative materials to petroleum-derived polymers since they are abundant, biodegradable, inexpensive and renewable (Shi & Dumont, 2014). Proteins have a complex molecular structure since each sequence may contain up to 20 different amino acids with side groups that differ in length, hydrophobicity, polarity and charge; thus, different combinations of amino acids result in the formation of various three-dimensional structures (Miravella, Castellani, & Sala, 2014).

Proteins can be extracted from residual biomass (Arshadi et al., 2016). Chicken feathers are worldwide obtained as a by-product from the poultry industry; some of them are pre-treated and used as animal feed with low nutritional value, but most of them are disposed as a waste in landfills, leading to loss of a valuable raw material. In fact, they are renewable, biodegradable and possess reliable quality, and supply. According to the Food and Agriculture Organization (FAO), the global production of poultry meat is forecasted to be 116.2 million tons in 2016 (FAO, 2016). Feathers represent about 5–7% of the total weight of a matured chicken. Hence, around 7 million tons of chicken feathers are generated annually world-wide. The major components of chicken feathers are proteins (91%), lipids (1%) and water (8%) and the protein fraction is mostly composed of keratin (Song, Lee, Al Mijan, & Song, 2014). This keratin is a structural protein and, together with collagen, represents the most important biopolymer in animals (Wang, Yang, McKittrick, & Meyers, 2016).

In recent years the number of investigations looking for new applications of this type of materials are raising due to economic and environmental concerns (Dou, Zhang, He, Yin, & Cui, 2016). Keratin found in feathers is mainly composed of β-keratin, also called hard keratin, and it is characterized by the presence of a range of non-covalent interactions, such as electrostatic forces, hydrogen bonds, hydrophobic forces, and covalent interactions like disulphide bonds, which are difficult to be disrupted. These

* Corresponding author.
E-mail address: pedromanuel.guerrero@ehu.eus (P. Guerrero).

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disulphide bonds are mostly due to the high content of cysteine residue in keratin, which distinguishes it from other proteins (Tanase & Spiridon, 2014). Cysteine is a sulphur containing amino acid which can be easily oxidized to form inter- and intra-molecular disulphide bonds and create a cross-linked network, improving strength as well as thermal and chemical properties of keratin materials. Moreover, this cross-linking by disulphide bonds, hydrogen bonds, and hydrophobic interactions makes keratin insoluble in many common solvents, such as water, most organic solvents, and dilute acid and alkaline solutions, preventing the use of keratin for many applications (Bragulla & Homberger, 2009).

In order to overcome the abovementioned drawback, a wide range of methods has been conducted. These methods involve the addition of reagents that break disulphide and hydrogen bonds and make keratin soluble due to its partial disruption. Physical, chemical and biological treatments to convert feathers into valuable products have been reported (Bohacz, 2017; Poole & Church, 2015). Physical treatments involve high temperature and/or high pressure steam to hydrolyze feathers. Chemical treatments utilize acid and alkaline chemicals to hydrolyze feathers through the modification of keratin structure by disrupting the disulphide and peptide bonds, so improving keratin solubility. Biological treatments involve the application of keratinolytic microorganisms and their enzymes (keratinase) for keratin hydrolysis, but due to the fact that it is a long-lasting process, its industrial feasibility is limited. The rate of enzymatic degradation of keratin is slow and requires 3-5 days for bacterial reaction and 2-4 weeks for fungal reaction.

Among those methods, reduction and oxidation processes have been widely used since they have been proved as effective ways to cleave disulphide bonds, preserving the covalent bonding of the protein backbone (Ayuttaya, Tanpichai, & Wootthikanokkhan, 2015; Zhou, Yang, Yang, Cao, & Zhou, 2014). However, the chemical agents used in these reactions are often toxic and non-environmentally friendly. In order to overcome this problem, some authors have attempted to dissolve keratin using ionic liquids, but this method does not provide a sufficient amount of extracted protein (Idris et al., 2013). Chemical and enzymatic hydrolyses have also been employed. Stiborova et al. (2016) have recently compared the hydrolysis of non-treated feathers using whole cell microbial digestion, enzymatic and chemical cleavage and they concluded that the highest transformation of feathers into soluble peptides and amino acids was achieved by alkaline hydrolysis. Also, Tsuda and Nomura (2014) used the alkaline hydrolysis to disrupt disulphide bonds in the protein, thus, enhancing the protein solubility.

In this research, alkaline hydrolysis was selected as the method to obtain free thiol groups from feather keratin. These groups can be oxidized during the film forming process, promoting new disulphide cross-linking and providing chemical and thermal resistance to the films developed (Schrooyen, Dijkstra, Oberthür, Bantjes, & Feijen, 2001). When alkaline hydrolysis is carried out, heating is often used to ensure high yield, but increases the destruction of amino acids (Sinkiewicz, Sliwinska, Staroszczyk, & Kolodziejska, 2017). Therefore, in this study, alkali hydrolysis was carried out at room temperature in order to develop a simple, environmentally sustainable and low cost process to obtain hydrolyzed keratin, which was used as a source of sulfur for protein-based films (Reddy et al., 2013).

In previous works, soy protein isolate (SPI) was employed to develop sustainable materials with good physicochemical properties (Garrido, Etxabide, Guerrero, & de la Caba, 2016; Guerrero, Garrido, Leceta, & de la Caba, 2013). However, it is well known that the application of SPI is limited due to its low strength and high moisture absorption. Therefore, adding plasticizers and other biodegradable polymers to soy protein can be an effective way to overcome these drawbacks (Su, Huang, Wang, & Li, 2010). In this case, hydrolyzed keratin (HK) was employed as an additive into film forming formulations in order to increase the sulfur content of soy protein materials and, thus, S-S cross-linking, improving the physicochemical properties of the resulting films.

In this context, the aim of this study was to obtain a simple, environmentally friendly and effective method to hydrolyze keratin from chicken feathers in order to increase thiol groups, able to interact with each other and improve the functional properties of the films developed. Furthermore, SPI/HK films were processed by compression molding and the chemical, thermal and morphological properties of these films were analyzed by attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy, differential scanning calorimetry (DSC), thermo-gravimetric analysis (TGA), dynamic mechanical analysis (DMA), scanning electron microscopy (SEM) and atomic force microscopy (AFM).

2. Materials and methods

2.1. Materials

Soy protein isolate (SPI), with a minimum of 90% protein content on dry basis, was obtained from ADM Protein Specialties Division, Netherlands. SPI contains a maximum of 5% moisture, 4% fat, 5% ash and its isoelectric point is 4.6. Chicken feathers were kindly supplied from Lumagorri S.L. and used as a source of sulfur in the film forming formulations. Glycerol, supplied by Panreac, was used as a plasticizer.

2.2. Preparation and characterization of hydrolyzed keratin (HK)

Chicken feathers were washed with water and dried at room temperature. Then, the quills and the barbs were cut into small pieces and grinded. Afterwards, the grinded feathers were treated in a Soxhlet device with methanol during 3 h and, finally, they were dried and stored at room temperature in a closed container before use. These pre-treated chicken feathers (CF) were mixed with NaOH (1 M) in a CF/NaOH ratio of 1:20 (w/v) and the mixture was stirred during 8 h at room temperature (Lavall, Assis, & Campana-Filho, 2007). After that, 100 mL of distilled water were added and the solution was freeze-dried using Alpha 1-4 LD freeze dryer (Martin Christ, Germany). Finally, the freeze-dried powder was analyzed to determine the content of sulfur and amino acids.

The analysis of protein fractions with different molecular weights was carried out by using a sodium dodecyl sulfate-polyacrylamide gel electrophoresis technique (SDS-PAGE), according to the method of Laemmli (Laemmli, 1970). Different amounts of the hydrolyzed keratin samples were dissolved in a buffer containing SDS, tris-HCl buffer (pH 6.8), dithiothreitol (DTT), glycerol and bromophenol blue. The stacking and the separating gels were 12% polyacrylamide. Electrophoresis voltage used was 200 V. After electrophoresis, the gel was washed with water and then, stained with a staining solution (Coomassie Brilliant Blue R-250, BIO-RAD). Subsequently, the sample was distained with a mixed solution (water, methanol, glacial acetic acid). The protein standard (Precision plus protein all blue standards, BIO-RAD) was used for calibration.

In order to determine sulfur content, elemental analysis was carried out using an Euro EA Elemental Analyzer (EuroVector, Italy). Furthermore, amino acid analysis of hydrolyzed keratin was performed with a Biochrom 30 + amino acid analyzer physiological system (Biochrom, UK). Instead, SPI amino acid content was supplied by ADM Protein Specialties Division, Netherlands.

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