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Characterization of agar/soy protein biocomposite films: Effect of agar on the extruded pellets and compression moulded films

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ABSTRACT

Agar/soy protein biocomposite films were successfully processed by extrusion and compression moulding, obtaining transparent and homogeneous films. The conformational changes occurred during the extrusion process and the effect of agar on the final properties were analyzed. As shown by differential scanning calorimetry (DSC) and specific mechanical energy (SME) values, during the extrusion process protein denatured and unfolded protein chains could interact with agar. These interactions were analyzed by Fourier transform infrared spectroscopy (FTIR) and the secondary structure was determined from the amide I band. Those interactions were supported by the decrease of film solubility. Furthermore, the good compatibility between agar and soy protein was confirmed by the images from scanning electron microscopy (SEM).

1. Introduction

The continuous increase in the use of non-biodegradable plastics has generated huge disposal problems and a serious impact on the environmental pollution and climate change, since these materials tend to accumulate in ecosystems due to their resistance to microbial degradation. In order to overcome these drawbacks, market stimuli and policies for biomass-sourced plastic production should be addressed (Song & Zheng, 2014). In an attempt to reduce the amount of plastic solid waste sent to landfills and also to reduce the dependency on fossil fuels, a great interest in the manufacture of biodegradable, renewable and cost-competitive materials has grown in recent years (Shankar & Rhim, 2015; Moreno, Atarés, & Chiralt, 2015; Guerrero, Kerry, & de la Caba, 2014). Renewable and biodegradable films can be prepared from mixtures of polysaccharides and proteins. Blending change physical and chemical properties of the final product as a function of the compatibility between the blend components. This compatibility is subjected to some characteristics of the macromolecules, such as the chemical structure and conformation, among others (Lee et al., 2016; Xu et al., 2015). In this context, agar is a cell-wall polysaccharide extracted from certain red seaweeds (*Gracilaria, Gelidium* and *Pterocladia*) and consists of a mixture of agarose and agaropectin, a sulphated polymer that varies according to the species of seaweed, but the main components of the chains are β-1,3-linked-D-galactose and α-1,4-linked 3,6-anhydro-L-galactose (Usov, 2011). This polysaccharide can interact with soy protein, a mixture of 18 amino acids, to form an interconnected network with enhanced physicochemical properties (Zhong & Sun, 2001). The synergistic effects resulting from blending these biopolymers are of great importance to create new functional structures and promote new applications (Rodríguez Patino & Pilosof, 2011).

Concerning the processing methods employed to prepare polysaccharide/protein blends, Tian, Xu, Yang, & Guo, (2011) compared the results obtained for the films prepared by solution casting and compression. These authors found that the processing method greatly affected the microstructure and mechanical properties of the blend films, obtaining higher tensile strength for the films prepared by solution casting in comparison to the hot-pressed films. In relation to extrusion, although it has become a common technique to produce pellets from some polysaccharides such as thermoplastic starch (Yan, Hou, Guo, & Dong, 2012), there are few reports related to blends of proteins and polysaccharides processed by extrusion (Guerrero, Kerry et al., 2014), so the application of this technology to the production of biocomposites is a challenge for researchers. It is worth noting that during extrusion the viscous dissipation of mechanical energy predominates, especially at low moisture contents, thus making this process highly energy efficient and cost effective. The effect of extrusion is to disassemble proteins and then reassemble them by disulfide bonds, hydrogen

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bonds and non covalent interactions, forming fibrous structures in the extrudates. It is generally accepted that proteins are denatured during the extrusion process, so these reactive unfolded protein chains can interact with other polymers (Akdogan, 1996). With this regard, mixtures of soy protein and red algae-derived waste, mainly composed of cellulose, were extruded in a previous work (Garrido, Peñalba, de la Caba, & Guerrero, 2016), and the mechanical forces undergone by the material were quantified by the measurement of the specific mechanical energy (SME). In that previous work, mixtures were extruded to obtain pellets, later injected to obtain rigid materials. Considering the knowledge of the process acquired in that previous work, soy protein and agar, extracted from red algae, have been extruded in this work with the aim to obtain pellets, subsequently hot-pressed to obtain flexible films.

Since few data are available about the molecular events occurred during blending, the aim of this work is to describe the conformational changes during the extrusion process and the effect of agar on the final structure. In this study, Fourier transform infrared (FTIR) spectroscopy was employed to obtain detailed information on the molecular and conformational modifications occurring during the blending process, while the structure of the films was analyzed by scanning electron microscopy (SEM). In addition, the functional properties of the films, such as physicochemical (moisture content, total soluble matter, water vapour permeability and water uptake), optical (colour, gloss and transparency) and mechanical (tensile strength and elongation at break) properties were measured.

2. Materials and methods

2.1. Materials

Agar (AG) was extracted from the red algae *Gelidium sesquipedale (Rodophyta)*, collected on the northern coast of Spain (Hondarribia beach: 43°23′50N, 01°47′50W) in autumn (September). The extraction process was described in a previous work (Guerrero, Etxabide, Leceta, Peñalba, & de la Caba, 2014). Briefly, the dried red algae (30 g) were immersed in boiling water (900 mL) for 4 h and the solution was separated from the residue by filtration on 50 μm blutex nylon. Then, the solution was gelled at room temperature and freeze-dried to obtain the powder. Soy protein isolate (SPI), PROFAM 974, was supplied by ADM Protein Specialites Division (Netherlands). According to the information provided by the protein supplier, SPI has 90% protein (min.), 6% moisture (max.), 4% fat (max.), and 5% ash (max.), and its isoelectric point is 4.6. Glycerol (Gly) with a purity of 99.01%, obtained from Panreac (Spain), was used as plasticizer.

2.2. Agar characterization

^{1}H and ^{13}C NMR spectra were recorded with a Bruker Avance (Bruker, Spain), equipped with BBO z-gradient probe. Agar solutions, 5% (w/v) in D_2O, were prepared and data were recorded at 90 °C. Experimental conditions for ^{1}H NMR were as follows: 500 MHz, 64 scans, spectral window of 5000 Hz, and recovery delay of 1 s. For ^{13}C NMR, the conditions used were 125.75 MHz, 14,000 scans, spectral window of 25,000 Hz, and recovery delay of 2 s.

2.3. Pellets preparation

AG, SPI, and Gly were blended in a Stephan UMC 5 mixer (Stephan, UK) for 5 min at 1500 rpm in order to obtain a good blend. Control films were prepared with SPI and 30 wt% Gly on SPI dry basis and were designed as SPI/Gly/AG0. SPI was replaced by 3, 6 and 9 wt% AG to prepare the films designated as SPI/Gly/AG3, SPI/Gly/AG6 and SPI/Gly/AG9, respectively.

Blends were added into the feed hopper and mixed with water in the barrel of a twin-screw extruder. The MPF 19/25 APV Baker extruder (Baker Perkins group, UK) used in this study had 19 mm diameter-barrel and a length/barrel diameter ratio of 25:1. Barrel temperatures were set at 70, 80, 95, and 100 °C for the four zones from input to output, and die temperature was set at 100 °C, based on a previous work (Guerrero, Beatty, Kerry, & de la Caba, 2012). Water was pumped directly into the extruder barrel at a constant speed of 250 rpm using a peristaltic 504U MK pump (Watson Marlow Ltd., UK). All trials were carried out using a water speed of 2.68 g/min (0.16 kg/h). The feed rate of extruder was adjusted to 1 kg/h and a single die of 3 mm diameter was used, giving a throughput per unit area of 0.141 kg/h mm^2. The specific mechanical energy (SME) was determined using the following expression (Hu, Hsieh, & Huff, 1993):

$$\mathrm{SME(kJ/kg)} = \frac{\text{Screw speed} \times \text{Power(kW)} \times \text{Torque(\%)}}{\text{Max. screw speed} \times \text{Throughput(kg/h)} \times 100}$$

2.4. Pellets characterization

2.4.1. Piece density (PD)

PD of each extrudate was obtained by dividing the mass of the extrudate piece (W_{piece}) by its volume (V_{piece}), which was calculated from its dimensions (diameter, d; and length, l). The dimensions of ten pieces (2 cm long) were measured by using a hand-held digimatic QuantuMike Mitutoyo micrometer (Neurtek, Spain) and PD value was calculated as:

$$\mathrm{PD} = \frac{W_{piece}}{V_{piece}} = \frac{4 \times W_{piece}}{\pi \times d^2 \times l}$$

Measurements were taken ten times for each composition.

2.4.2. Expansion ratio (ER)

ER is the ratio of the extrudate cross-sectional area to the die orifice cross-sectional area, and was calculated as:

$$\mathrm{ER} = \frac{d^2}{d_{die}^2}$$

where d_{die} is the die diameter and d is the extrudate diameter (average of 10 samples).

2.5. Films preparation

The pellets obtained by extrusion were placed between two aluminium sheets and introduced into a caver laboratory press (Neurtek, Spain), previously heated at 150 °C. The pellets were pressed at 130 bar for 2 min to obtain compression-moulded films. All samples were conditioned in an ACS Sunrise 700 V bio-chamber (Alava Ingenieros, Spain) at 25 °C and 50% relative humidity for 48 h before testing.

2.6. Films characterization

2.6.1. Film thickness

Films thickness was measured to the nearest 0.001 mm with a QuantuMike Mitutoyo hand-held digimatic micrometer (Neurtek, Spain). The values obtained for each sample at five different locations were averaged. All the films had values between 80 and 100 μm.