Food Hydrocolloids 60 (2016) 7–10

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Imaging the phase of starch–gelatin blends by confocal Raman microscopy

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ARTICLE INFO

Article history:
Received 13 July 2015
Received in revised form
28 January 2016
Accepted 1 March 2016
Available online 9 March 2016

Keywords:
Gelatin
Starch
Raman spectroscopy
Interface
Polymer compatibility

ABSTRACT

The well recognized complex issue of compatibility between starch and gelatin was studied based on their interfacial and phase composition using Raman microscopy. Blending films with different ratios of gelatin and starch was used in this work. Raman beam focused on a 1.7 μm × 1.5 μm detection region and the micro-spectroscope scanned across the gelatin–starch interface. The ratio of areas of saccharide bonds (1173-953 cm^{-1}) and amide I bands (1750-1550 cm^{-1}) was used to monitor the relative distributions of the two components of the blends. The Raman spectral maps confirmed that for all the blends investigated, gelatin formed a continuous matrix in which starch inclusions were dispersed. Intermediate phases consisted of amylose interacted with gelatin, which demonstrated this was an unique and effective method to investigate starch based blend and composite materials.

1. Introduction

Blends of gelatin with starch have attracted much attention since they are both renewable resources and they have film-forming ability. A blended film of polysaccharide and protein exhibited improved gas barrier (O_2 and CO_2) properties than either of the pure films (Arvanitoyannis, Nakayama, & Aiba, 1998; Baldwin, Nisperos-Carriedo, & Baker, 1995). Blends have been developed for use as medical capsule materials (L. Zhang et al., 2013; N. Zhang, H. Liu et al., 2013; N. Zhang, X. Liu et al., 2013). Previous research has shown that gelatin and starch are immiscible, their morphologies and compatibility are determined by several characteristics, such as processing time (Firoozmand, Murray, & Dickinson, 2009), temperature (Ioannis Arvanitoyannis et al., 1998), pH (Ong, Whitehouse, Abeysekera, Al-Ruqaie, & Kasapis, 1998) and volume fraction (Al-Hassan & Norziah, 2012).

Mechanical properties of starch–gelatin blends depend on their morphology, particularly the extent of homogeneity and the composition of their continuous and dispersed phases. Various techniques have been used to characterise phase composition and interface of this complex blended system by indirect methods such as differential scanning calorimetry (DSC) (Jagannath, Nanjappa, DasGupta, & Bawa, 2003), dynamic mechanical analysis (DMA) (Arvanitoyannis, Psomiadou, Nakayama, Aiba, & Yamamoto, 1997) and rheometry, as well as direct methods such as polarized optical microscopy and confocal laser scanning microscope (CLSM) (Firoozmand et al., 2009). In our previous paper (Liu et al., 2013), we have compared different microscopies such as polarized optical microscopy, scanning electron microscopy (SEM) and synchrotron-Fourier transform infrared spectroscopy microscopy (Synchro-FTIRM) to study gelatin–starch blends. Of these methods, the Synchro-FTIRM provided capability to detect and measure composition and interface. Spatial resolution of FTIRM was limited to 10 × 10 μm, even application of synchrotron light technology only enabled achievement of a spatial of 5 × 5 μm (Wetzel, Shi, & Reffner, 2010).

Corn-starch is an economic crop that is often used for the raw materials for edible or biodegradable materials. The average diameter of corn starch granules is from 8 to 12 μm (Chen, Yu, Chen, & Li, 2006), so high spatial resolution is needed to examine morphing of the granules into film by gelatinization. Raman microscopy is an effective method to study heterogeneous materials since it provides sub-micron spatial resolution with high sensitivity (Chrimes, Khoshmanesh, Stoddart, Mitchell, & Kalantar-zadeh,

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http://dx.doi.org/10.1016/j.foodhyd.2016.03.005
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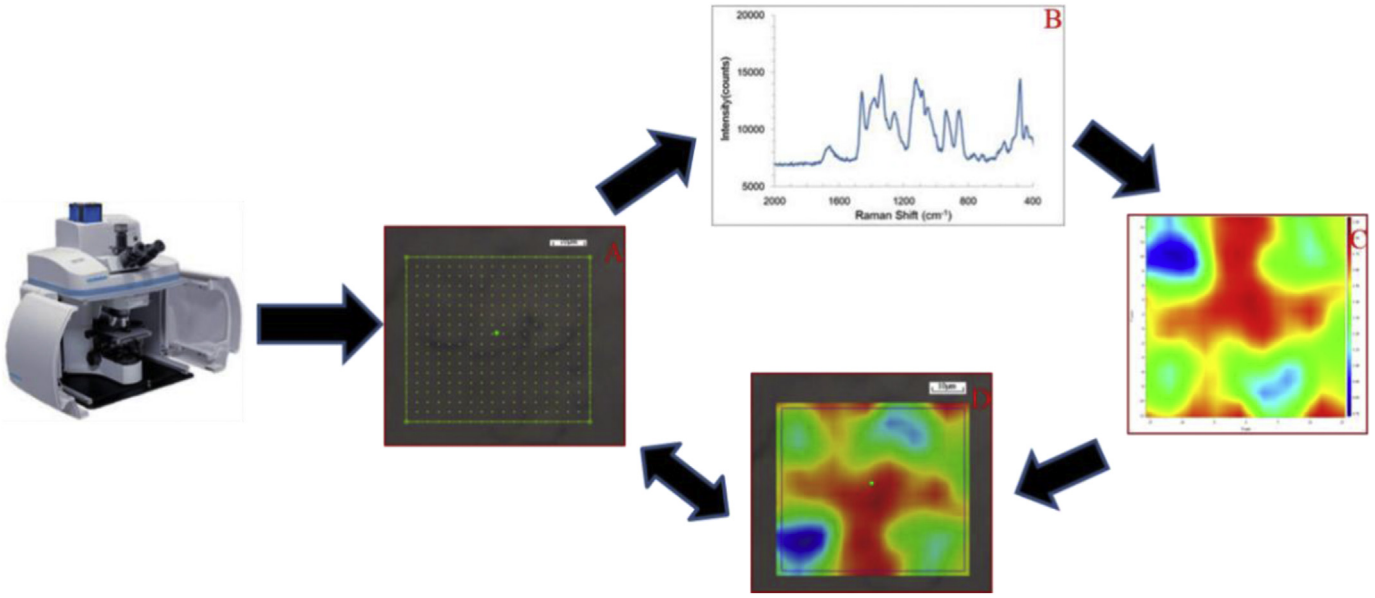

Fig. 1. Raman optical images, spectra and maps of G3S7 for excitation at 532 nm, (A) the selection zone chosen from the optical images; (B) one spectra of gelatin–starch blends of collecting data; (C) map obtained from the ratios between integrated intensities in the 1173-953 $cm^{-1}$and 1750-1550 cm^{-1} range; (D) overlaid images shows that the same area was mapped.

2013). For example, lateral and depth resolutions of approximately 0.25 and 1.7 μm, respectively, can be achieved when using a 633 nm laser source and an aperture of 50 mm in radius to give a 60 × /1.2 numerical aperture (NA) objective. Therefore, the aim in this study was to study morphology and phase composition of gelatin–starch blends using Raman microscopy.

2. Experimental

2.1. Materials and film preparation

Starch–gelatin blend film was prepared according to previous methods (N. Zhang, H. Liu et al., 2013; N. Zhang, X. Liu et al., 2013). Briefly, solutions were prepared with different ratios of starch (A1081, Penford, Australia):gelatin (GELITA UG719-N, Type A, 250 bloom, Sweden) equal 90:10, 70:30, 50:50, 30:70 and 10:90 including 1%·w/w sorbitol based on a total weight basis (2 g) in 100 mL distilled water. The mixed materials were dissolved in distilled water at 80 °C for an initial 30 min at a slow stirring speed (100 min^{-1}), then stirred for a further 30 min at high speed (700 min^{-1}) until a clear solution was obtained. The solution (5 mL) was poured onto a poly(ethylene terephthalate) (PET) dish (diameter 5 cm) that was kept level to control film thickness. The cast film was dried overnight at 37 °C. The dry films were peeled from the plate, placed in a desiccator containing saturated sodium bromide (NaBr) solution to control humidity, and stored at 56%·RH and 23 °C until required for analysis.

2.2. Raman microscopy

An XploRA plus Raman confocal microscope system (Horiba scientific) was used to analyze specimen surfaces. A532 nm diode laser (15 mW laser power) with an X100/0.90NA air objective was employed. Spatial resolution was obtained using 100 μm confocal pinholes. The Raman signal was acquired using a 1200 lines/mm grating centered between 200 and 2760 cm^{-1}. For each specimen, a 1.7 × 1.5 μm area of the surface was mapped at X and Y-axes. Data was analyzed using LabSpec 6. The integration time was 10 s for all measurements.

3. Results and discussion

Different ratios of starch:gelatin (90:10, 70:30, 50:50, 30:70 and 10:90, named G9S1, G7S3, G5S5, G3S7, G1S9) including 1%·w/w sorbitol plasticizer, were used as model materials, a Raman confocal microscope system with a 532 nm laser was used to study the gelatin–starch films. Fig. 1 shows the approach to acquisition of spectra, data analysis (image generation) and processing of the Raman images, Fig. 1(A) shows the heterogeneous nature of such blends and their phase distribution. Gelatin was a continuous phase while starch formed a separated phase distributed as spherulites of about 5 μm diameter. It should be noted that the diameters of starch particles depend on volume fraction of starch and drying temperature. The distribution of starch was more diffuse and the interfacial contrast became less distinct depending on volume fraction and water content.

Fig. 1(B) and Fig. 2 show the Raman spectra of gelatin–starch

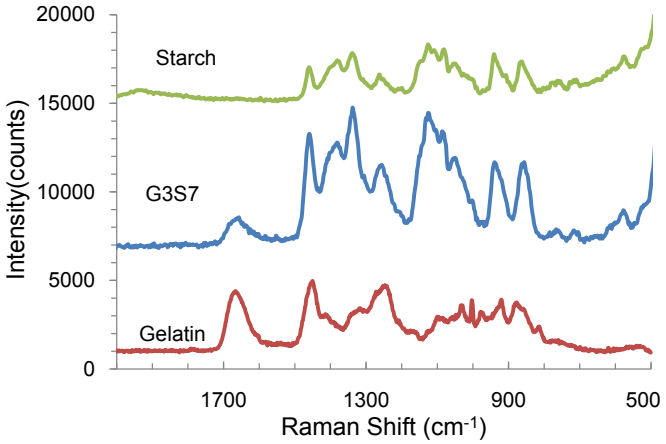

Fig. 2. Raman spectra of gelatin and gelatin–starch blend (G3S7).