

Morphology and Phase Composition of Gelatin-Starch Blends^{*}

Xing-xun Liu^{a,d}, Yan-fei Wang^a, Nuo-zi Zhang^{b,c}, Robert A. Shanks^b, Hong-sheng Liu^a,
Zhen Tong^d, Ling Chen^a and Long Yu^{a,c**}

^a Centre for Polymers from Renewable Resources, ERCPSP, SCUT, Guangzhou 510640, China

^b School of Applied Science, RMIT University, Melbourne 3000, Australia

^c CSIRO, Materials Science and Engineering, Clayton, Vic 3169, Australia

^d School of Materials Science and Engineering, SCUT, Guangzhou 510640, China

Abstract Morphology and phase compositions of different starch-gelatin blends were investigated by various microscopes: optical, SEM and synchrotron FTIR microscopy. A high amylose (80%) corn starch, grafted with hydroxypropyl to enhance flexibility and hydrophilicity, and plasticized by poly(ethylene glycol) (PEG), was used in this work. SEM revealed that the surface became smoother after adding PEG. Optical microscopy observation revealed that compatibility between gelatin and starch was improved by adding PEG. An FTIR beam focused on a $5\ \mu\text{m} \times 5\ \mu\text{m}$ detection area by the micro-spectroscope was used to map chemical composition. The ratio of areas of the saccharide bands ($1180\text{--}953\ \text{cm}^{-1}$) and the amide I and II bands ($1750\text{--}1483\ \text{cm}^{-1}$) was used to monitor the relative distributions of the two components in the blends. The FTIR maps indicated that gelatin constituted the continuous phase up to 80% of starch content. All of the FTIR spectra showed contributions from both starch and gelatin absorptions, therefore indicating that complete demixing with pure starch and gelatin domains did not occur. The PEG improved the compatibility of the gelatin-starch blends.

Keywords: Biopolymer; Drug capsule; Starch; Gelatin; Morphology; Phase; FTIR; Synchrotron.

INTRODUCTION

Blends of gelatin with starch have attracted much attention. For example, a blended film of polysaccharide and protein showed better gas barriers (O_2 and CO_2) than any of the pure films^[1–4]. Recently, blends were developed for use as medical capsule materials^[5, 6]. Starch is a relatively low cost material with good film-forming behavior^[7–11], and has showed great potential to be a component in gelatin products. The rheological and mechanical properties of starch-gelatin blends depend on their morphology, particularly in terms of their degree of homogeneity and the composition of their continuous and dispersed phases. Previous researchers have shown that gelatin and starch are immiscible^[12–14], however, their morphology and compatibility are affected by various factors, such as processing time^[15], temperature^[1, 3], pH^[16] and solids concentration^[17].

The aim of this work is to develop gelatin-starch blends for application in making drug capsules by the well-established technology of dipping a stainless mould into a gelatin-starch solution and then drying the blends in this work were prepared using equilibrated solutions. The interface of starch and gelatin improved by interfacial agent facilitates the formation of uniform blends. Poly(ethylene glycol) (PEG) was used as plasticizer as well as compatibilizer since it has widely used to improve compatibility between polymers and polymers with

^{*} This work was financially supported by the National Natural Science Foundation of China (Nos. 21174043 and 31301554), GNSF(S2012040006450), KLGPNS(201205) and FRFCU (2012ZZ0085).

^{**} Corresponding author: Long Yu (余龙), E-mail: long.yu@csiro.au

Received May 22, 2013; Revised June 20, 2013; Accepted July 2, 2013

doi: 10.1007/s10118-014-1377-1

fillers^[18–21]. An objective is to image and quantify the morphology and phase composition of different starch-gelatin blends using different microscopic techniques, and study the contribution of (PEG) on the blending system.

In this work, gelatin was blended with hydroxypropylated high amylose (80%) corn starch and plasticized by PEG through a solution mixing method. Morphology and phase composition of different starch-gelatin blends were investigated by various microscopes: scanning electron microscope (SEM), optical microscope and synchrotron FTIR-microscope. The comparison of the phase composition based on the synchrotron FTIR with images observed under SEM and polarized light microscope was discussed. The influence of PEG on the morphology and phase composition of the blends was investigated.

EXPERIMENTAL

Materials and Solution Preparation

A commercially available gelatin (GELITA UG719-N, Sweden) was used in this work. A food-grade hydroxypropylated high amylose (80%) corn starch (A1081) with MS (molar substitution) 0.11 was supplied by Penford (Australia). PEG 400 (400 g/mol) was purchased from Sigma.

30% solid content were initially prepared for various characterizations. Solutions were prepared with different ratios of starch:gelatin (100:0, 80:20, 60:40, 40:60, 80:20 and 0:100) based on a total weight basis (150 g) in 350 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 r/min), then for a further 30 min at high speed (700 r/min) until a clear solution was obtained. Previous studies^[22, 23] showed that the gelatinization of this hydroxypropylated starch occurred at about 57 °C, which is lower than the temperature of solution preparation used in this study. Thus the starch was considered to be gelatinized, though this was confirmed later by the morphology of films. The solutions containing 5% PEG (*W/W*) about 25 g were prepared in the same way.

Scanning Electron Microscopy

A Phillips XL-30 FEGSEM SEM was used to investigate the surfaces of the different films. Specimens were coated with iridium in a vacuum evaporator and viewed in the SEM at a low accelerating voltage of 2 kV.

Optical Microscope

An optical microscope (OM) (Axioskop 40 Pol/40 A Pol, Zeiss) equipped with a 35 mm SLR camera was used in the experimentals. The magnification 500X (50 × 10) was used to observe morphology of the starch-gelatin blends.

Film and Specimen Preparation

Low concentration solutions were used to prepare very thin cast films. More water was added to the solutions described above to give solutions of 1% concentration with stirring at a speed 500 r/min for 10 min. After degassing, 5 mL of solution was poured onto a PET dish (diameter 5 cm), which 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. All of the films were of about 7–9 μm thickness.

Fourier Transform Infrared Attenuated Total Reflection

A Tensor 37 (Bruker) spectrophotometer was used to detect the Fourier transform infrared (FTIR) spectra of specimens (films). In order to remove the effect of environment water, specimens were scanned using ATR mode with a ZnSe ATR Accessory. FTIR spectra were collected with 64 scans, and the spectral range and spectral resolution were 4000–800 cm⁻¹ and 4 cm⁻¹ respectively using OPUS 7.0 software.

Synchrotron Fourier Transform Infrared Micro-spectroscopy (Synchro-FTIRM)

The specimens were analyzed using a Bruker Vertex V80v Fourier transform infrared (FTIR) spectrometer coupled with a Hyperion 2000 microscope equipped with a liquid nitrogen cooled narrow-band MCT detector at

the Australian Synchrotron Infrared Beamline. The high brilliance of synchrotron IR-radiation allowed an aperture of $5\ \mu\text{m} \times 5\ \mu\text{m}$ while still achieving a high S/N ratio and high spatial resolution. This allowed high quality mid-IR spectra to be obtained with a relatively low number of scans. Spectral collection was made in transmission mode at $4\ \text{cm}^{-1}$ resolution, 64 scans were co-added and converted to absorbance using OPUS 7.0 software. The integration area of the saccharide bands ($1180\text{--}953\ \text{cm}^{-1}$), labeled as Band-1, was used to represent starch. The integration area of the amide I and II bands ($1750\text{--}1483\ \text{cm}^{-1}$), labeled as Band-2, was used to represent gelatin. Band-1 divided by Band-2 was used to evaluate the relative starch content while analyzing phase separation in mapped images.

RESULTS AND DISCUSSTION

Figure 1 shows the microstructures of the surfaces of the films of gelatin-starch blends with and without PEG observed under SEM. Some protrusions were observed on the surfaces of the blend films without PEG, and the density of these protrusions increased with increasing starch content. This phenomenon indicates that gelatin and starch were present as two phases, and that the shrinking ratios of gelatin and starch were different during drying. The individual surfaces are generally present as a continuous phase without indication of phase separation, meaning the gelatin and starch are compatible although they are immiscible. The surfaces of the blend films with PEG became much smoother. The protrusions had almost disappeared, indicating compatibility at the interface of gelatin and the starch was improved.

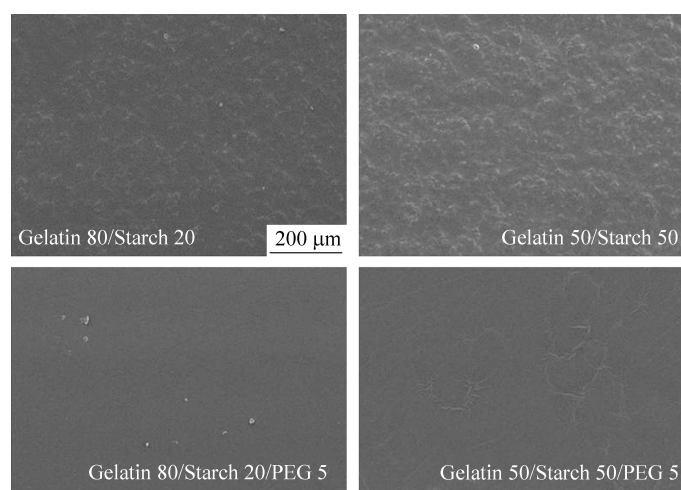


Fig. 1 SEM of various starch/gelatin blends with and without PEG

Figure 2 shows the morphologies of the starch 50-gelatin 50 blends with and without PEG, observed under optical microscopy. In order to enhance the phase contrast, the starch was stained with iodine, which increased darkness of starch phase. The results demonstrated the highly heterogeneous nature of such blends and their phase separation. It is seen that generally gelatin is a continuous phase while starch is a separated phase distributed as spherulites of about $80\ \mu\text{m}$ diameters in gelatin. It has been noted that the distribution of starch is more homogeneous and the interfacial details became dimmer even at the edge of particles that were more identifiable after adding PEG, indicating that compatibility between gelatin and starch was improved.

Figure 3 shows the FTIR-ATR spectra of various blends of gelatin-starch. For the pure starch film, the region between $3600\ \text{cm}^{-1}$ and $3000\ \text{cm}^{-1}$ is due to O—H stretching while $3000\text{--}2800\ \text{cm}^{-1}$ belongs to the C—H stretching vibrations region. The FTIR band at $1642\ \text{cm}^{-1}$ was attributed to water adsorbed in the starch. The region between $1500\ \text{cm}^{-1}$ to $800\ \text{cm}^{-1}$ is the fingerprint region that provided highly overlapping and complex spectra making exact band assignments impossible. However, typical FTIR spectra features were C—O and

C—C vibrational modes that are highly coupled from 1300–800 cm^{-1} , which are sensitive to the conformational and crystalline order of starch^[13, 14, 24–27]. The absorption bands at approximately 1155, 1125, and 1105 cm^{-1} due to CO, CC stretching with some COH contributions, while 1080, 1047, 1022, 995, and 928 cm^{-1} belong to COH bending and CH₂ related modes. For, the pure gelatin film, typical spectral features for the protein were strong amide I and II bands located approximately at 1650 cm^{-1} and 1540 cm^{-1} , respectively. The amide I absorption was primarily due to the stretching vibration of the C=O bond and the amide II band was due to the coupling of the bending of the N—H bond and the stretching of the C—N bond^[14, 28, 29].

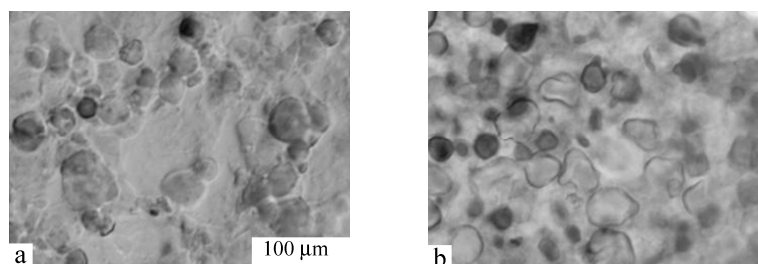


Fig. 2 The blends of gelatin 50/starch 50 without (a) and with 5% PEG (b) observed under optical microscope (the starch was died by iodine.)

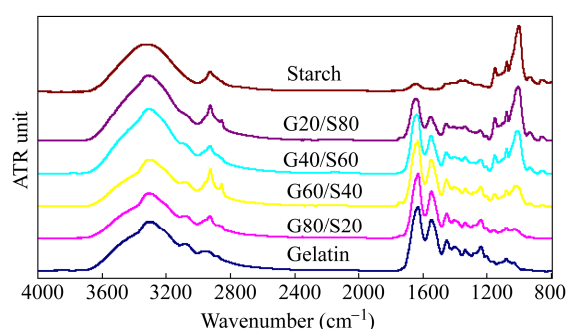


Fig. 3 The FTIR spectra of various starch/gelatin blends

For the various blend films, a series of overlapping peaks located in the region of 1180–953 cm^{-1} (labeled as Band-1), were the most intense bands in the mid-IR spectrum and they were chosen as the characteristic peaks of starch; while the integrated area of the amide I and II bands (1750–1483 cm^{-1}), labeled as Band-2, was used to represent gelatin. From Fig. 3 it is observed that the intensity of Band-1 decreased with increasing gelatin content while the intensity of Band-2 increased with increasing gelatin content.

In order to quantitatively study the relationship between the ratio of band areas and concentration functions, the ratio of the area of the starch bands (Band-1) to that of gelatin bands (Band-2) for each blend composition is shown in Fig. 4. It can be seen that the ratio of Band-1 to Band-2 increased with increasing starch content, but it is not a linear correlation. Based on the differences between starch and gelatin in the FTIR spectral region, the distribution of starch or gelatin in the blends could be obtained from a 3-D contour map (see Fig. 5) that relates the ratio of the peak area of the starch bands to that of gelatin bands. The fact that the ratio of band areas rather than absolute values was used enabled normalization of the results, correcting for possible variations in thickness both within the same film and between different films.

Figure 5 shows variation of the ratio of the area of the Band-1/Band-2 plotted as 3D contour maps for various starch-gelatin blends respectively. This image was generated from the data of a single measurement set by allocating a color to each pixel based on the ratio of Band-1 and Band-2, which was used to characterize the starch-gelatin content. In the 3D contour maps for various starch-gelatin blends, the Y axis show the ratios of band-1/band-2, which means that the relative content of starch increases with increasing Y axis height. Similarly

the monocolour optical images represent the ratio of various starch-gelatin blends, with darkness reflecting the content of starch distribution. From the 3D images, the red peak in the scale denotes a high value of starch while the blue bottom line denotes a high value of gelatin. G40/S60 is a typical count contour map for starch-gelatin 40:60 blend. It was observed that the starch phase (Band-1) distribution was a dispersed phase, while gelatin phase (Band-2) was a continuous phase. However the sharp peak, not the column, of Band-1 indicates there are certain chain diffusions across the interface of starch domains. That means the complete demixing of starch and gelatin domains did not occur, which corresponded with the results of scanned interfaces (Fig. 1). From Fig. 5, it can be seen that the starch phase distribution was a dispersed phase in various starch-gelatin blends, and the size of starch domains increased with increasing starch content, which is similar to the results from optical images.

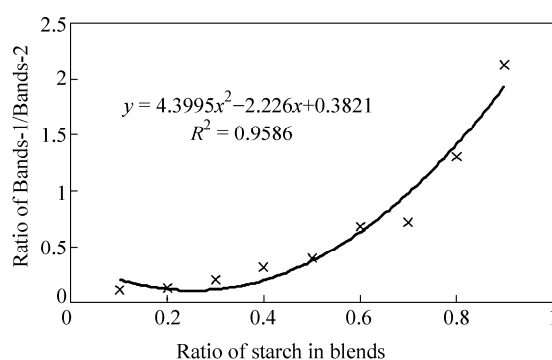


Fig. 4 Integrated plot of the Bands-1/Bands-2 ratios

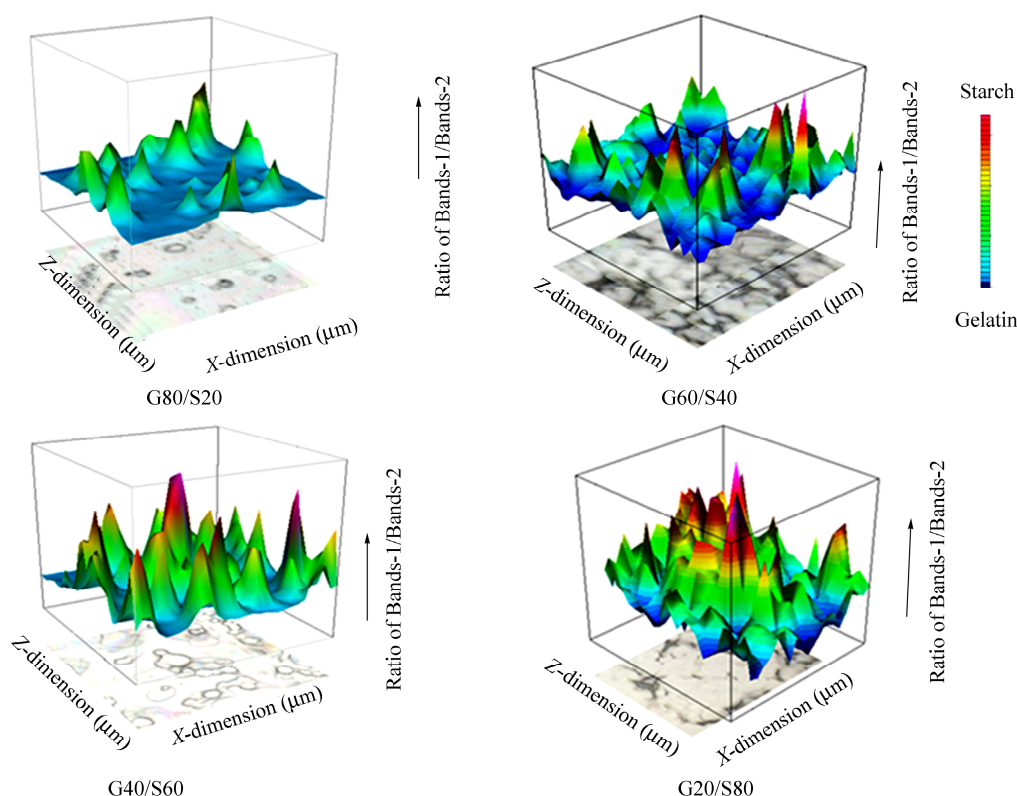


Fig. 5 Variation of the ratio of the area of the Bands-1/Bands-2 plotted 3D contour maps for different starch/gelatin blends

Figure 6 shows variation of the ratio of the area of the Band-1/Band-2 plotted as 3D contour maps for the starch-gelatin blends with and without PEG. It is seen that the ratio of Band-1 to Band-2 was decreased significantly after addition of PEG. The decrease of Band-1 was attributed to PEG creating an improvement of the interface between starch and gelatin. It is noted that there are some bands for PEG^[18, 30, 31] just in the range of about 1180–953 cm^{-1} that overlap with Band-1 used for representing starch. The overlap of the bands of PEG and starch enhanced the intensity of the Band-1 in the maps, resulting in enhanced color of Band-1 and enlarging its area. However, the ratio of Band-1 to Band-2 became weaker after adding PEG, indicating that PEG could dissolve homogeneously into gelatin. PEG is miscible with starch^[18, 19]. It is expected that PEG at the interphase between starch and gelatin improved their compatibility. The improvement of compatibility or interface enlarged the area of Band-1.

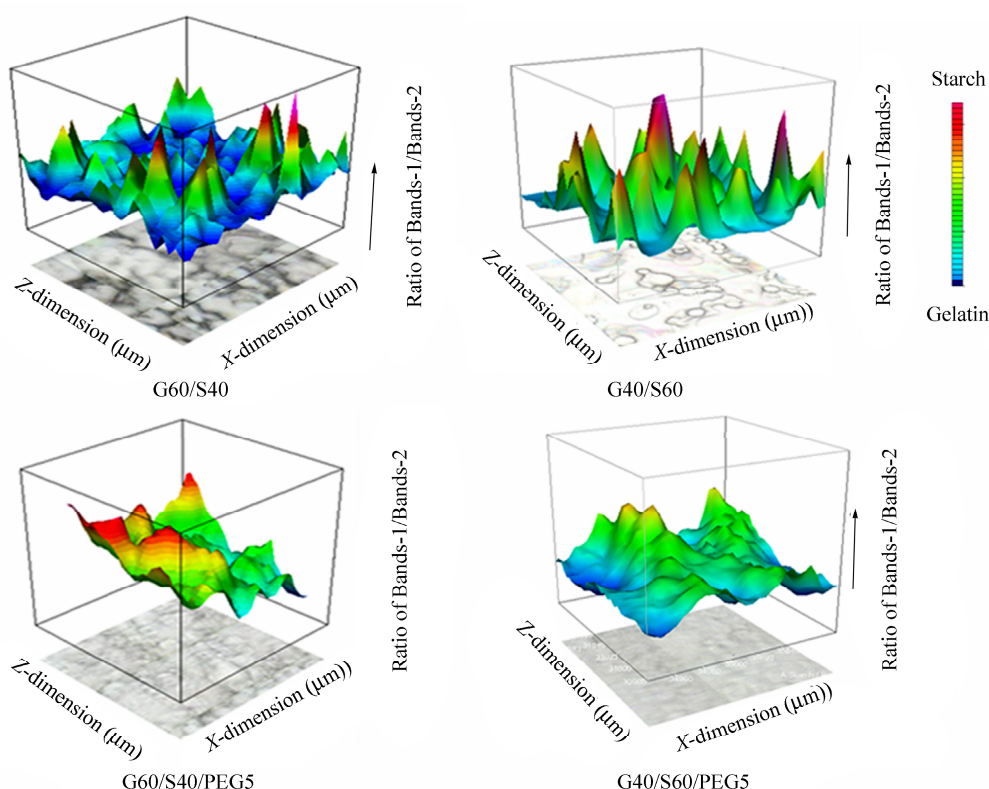


Fig. 6 Contour plots of the variation of the Bands-1/Bands-2 ratio plotted as 3D for different starch/gelatin blends with and without 5% PEG

CONCLUSIONS

SEM results showed that blends of gelatin and starch form two separated phases. The surfaces of the blending films with PEG became much smoother, indicating compatibility at the interface of gelatin and the starch was improved. The optical microscope demonstrated the highly heterogeneous nature of such blends and their phase separation. The results show that generally gelatin was a continuous phase while starch was a separated phase, distributed as spherulites of about 80 μm diameter in gelatin. Based on the differences between starch and gelatin in the FTIR spectral region, the distribution of starch or gelatin in the blends was obtained from the 3-D and 2-D contour maps, which are plots of the ratio of the peak area of the starch bands to that of gelatin bands. It was observed that the starch phase (Band-1) distribution was a dispersed phase, while gelatin (Band-2) was a continuous phase. PEG at the interphase between starch and gelatin improved their compatibility. The results from SEM, optical microscopy and FTIR micro-imaging corresponded. Compatibility between gelatin and starch was improved by PEG.

REFERENCES

- 1 Arvanitoyannis, I., Nakayama, A. and Aiba, S., *Carbohydr. Polym.*, 1998, 36: 105
- 2 Arvanitoyannis, I., Kalichevsky, M., Blanshard, J.M.V. and Psomiadou, E., *Carbohydr. Polym.*, 1994, 24: 1
- 3 Arvanitoyannis, I., Psomiadou, E., Nakayama, A., Aiba, S. and Yamamoto, N., *Food Chem.*, 1997, 60: 593
- 4 Baldwin, E.A., Nisperos-Carriedo, M.O. and Baker, R.A., *Crit. Rev. Food Sci. Nutr.*, 1995, 35: 509
- 5 Zhang, L., Wang, Y., Liu, H., Zhang, N., Liu, X., Chen, L. and Yu, L., *Acta Polymerica Sinica (in Chinese)*, 2013, (1): 1
- 6 Zhang, N., Liu, H., Yu, L., Liu, X., Zhang, L., Chen, L. and Shanks, R., *Carbohydr. Polym.*, 2013, 92: 455
- 7 Wang, X., Zhang, Y. and Wang, Y., *Acta Polymerica Sinica (in Chinese)*, 2011, (1): 24
- 8 Li, Y., Zhong, F., Ma, J. and Gu, X., *Acta Polymerica Sinica (in Chinese)*, 2008, (7): 720
- 9 Li, B. and Shao, Z., *Acta Polymerica Sinica (in Chinese)*, 2010, (4): 395
- 10 Zhang, L. and Johnson, A.F., *Chinese J. Polym. Sci.*, 1993, V11(2): 144
- 11 Liu, H.S., Xie, F.W., Yu, L., Chen, L. and Li, L., *Prog. Polym. Sci.*, 2009, 34: 1348
- 12 Abdulmola, N.A., Hember, M.W.N., Richardson, R.K. and Morris, E.R., *Carbohydr. Polym.*, 1996, 31: 53
- 13 Mousia, Z., Farhat, I.A., Blachot, J.F. and Mitchell, J.R., *Polymer*, 2000, 41: 1841
- 14 Mousia, Z., Farhat, I.A., Pearson, M., Chesters, M.A. and Mitchell, J.R., *Biopolymers*, 2001, 62: 208
- 15 Firoozmand, H., Murray, B.S. and Dickinson, E., *Food Hydrocolloid.*, 2009, 23: 1081
- 16 Ong, M.H., Whitehouse, A.S., Abeysekera, R., Al-Ruqaie, I.M. and Kasapis, S., *Food Hydrocolloid.*, 1998, 12: 273
- 17 Al-Hassan, A.A. and Norziah, M.H., *Food Hydrocolloid.*, 2012, 26: 108
- 18 Kim, C.H., Kim, D.W. and Cho, K., *Polym. Bull.*, 2009, 63: 91
- 19 Laohakunjit, N. and Noomhorm, A., *Starch-Stärke*, 2004, 56: 348
- 20 Fang, H., Feng, L. and Wu, L., *Chinese J. Polym. Sci.*, 2009, V27(3): 327
- 21 Zhu, Y., Wang, B., Yu, J. and Guo, Z., *Chinese J. Polym. Sci.*, 2007, V25(3): 291
- 22 Lan, C., Yu, L., Chen, P., Chen, L., Zou, W., Simon, G. and Zhang, X.Q., *Macromol. Mater. Eng.*, 2010, 295: 1025
- 23 Liu, H., Li, M., Chen, P., Yu, L., Chen, L. and Tong, Z., *Cereal Chem.*, 2010, 87: 144
- 24 Mutungi, C., Onyango, C., Doert, T., Paasch, S., Thiele, S., Machill, S., Jaros, D. and Rohm, H., *Food Hydrocolloid.*, 2011, 25: 477
- 25 Liu, X., Yu, L., Xie, F., Li, M., Chen, L. and Li, X., *Starch-Stärke*, 2010, 62: 139
- 26 Liu, X., Wang, Y., Yu, L., Tong, Z., Chen, L., Liu, H. and Li, X., *Starch-Stärke*, 2013, 65: 48
- 27 Dean, K.M., Do, M.D., Petinakis, E. and Yu, L., *Compos. Sci. Technol.*, 2008, 68: 1453
- 28 Muyonga, J.H., Cole, C.G.B. and Duodu, K.G., *Food Chem.*, 2004, 86: 325
- 29 Ahmad, M. and Benjakul, S., *Food Hydrocolloid.*, 2011, 25: 381
- 30 Pasquali, I., Andanson, J.M., Kazarian, S.G. and Bettini, R., *J. Supercrit. Fluids*, 2008, 45: 384
- 31 Zhang, N., Liu, X., Yu, L., Shank, R., Petinaks, E. and Liu, H., *Carbohydr. Polym.*, 2013, 95: 649