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Phase composition and interface of starch–gelatin blends studied by synchrotron FTIR micro-spectroscopy

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ABSTRACT

The well recognized complex issue of compatibility between starch and gelatin was investigated based on their interface and phase composition using synchrotron FTIR micro-spectroscope. A high amylose (80%) corn starch grafted with flexible and hydrophilic hydroxpropyl groups and plasticized by poly(ethylene glycol) (PEG) was used in this work. The FTIR beam focused on a 5 μm × 5 μm detection region and the micro-spectroscope was scanned across the gelatin–starch interface. It was found that there was about a 20 μm thickness layer where gelatin and starch were in co-existence, indicating that gelatin and starch are compatible to a certain degree in this system. The ratio of the areas of the saccharide C—O bands (1180–953 cm^{-1}) and the amide I and II bands (1750–1483 cm^{-1}) was used to monitor the relative distributions of the two components of the blends. FTIR 2 and 3-dimensional maps indicated that gelatin constituted the continuous phase up to 80% of starch content. The PEG was homogeneously distributed in both gelatin and starch phases, and blurred the interface between gelatin and starch in the chemical maps, indicating that PEG acted not only as a plasticizer but as a compatibilizer for the gelatin–starch blends.

1. Introduction

Phase composition and interface of a polymer blend are important and attract much scientific attentions since they influence processing behavior and performance of the blend, in particular for immiscible blended systems. For various reasons developing edible films by blending starch with gelatin has attracted much attention. For example, a blended film of polysaccharides and proteins shows better gas barrier (O_2 and CO_2) than any pure film (Arvanitoyannis, Kalichevsky, Blanshard, & Psomiadou, 1994; Arvanitoyannis, Nakayama, & Aiba, 1998; Arvanitoyannis, Psomiadou, Nakayama, Aiba, & Yamamoto, 1997; Baldwin, Nisperos-Carriedo, & Baker, 1995). Previous researches have shown that gelatin and starch are immiscible (Abdulmola, Hember, Richardson, & Morris, 1996; Mousia, Farhat, Blachot, & Mitchell, 2000; Mousia, Farhat, Pearson, Chesters, & Mitchell, 2001), however, their morphologies and compatibility are affected by various factors, such as processing time (Firoozmand, Murray, & Dickinson, 2009), temperature (Arvanitoyannis et al., 1998, 1997), pH value (Ong, Whitehouse, Abeysekera, Al-Ruqaie, & Kasapis, 1998) and solid concentration (Al-Hassan & Norziah, 2012).

Like other immiscible blends, rheological and mechanical properties of starch–gelatin blends depends on their morphology, particularly in terms of their degree of homogeneity and the composition of their continuous and dispersed phases. Various techniques have been used to study phase separation of this complex blending system, including differential scanning calorimetry (DSC) (Jagannath, Nanjappa, Das Gupta, & Bawa, 2003), dynamic mechanical thermal analysis (DMTA) (Arvanitoyannis et al., 1997; Mousia et al., 2000), rheometry (Abdulmola et al., 1996; Ong et al., 1998), polarized optical microscopy (Mousia et al., 2001) and cofocus leases microscope CLMS (Firoozmand et al., 2009). Fourier transform infrared (FTIR) spectroscopy with microscope provides capability to combine optical microscopy image analysis with chemical analysis via FTIR spectroscopy for evaluating polymer blends (Bhargava, Wall, & Koenig, 2000; Chiono et al., 2009; Wetzel & LeVine, 1999). Investigation of composition and interface of a blend using FTIR micro-spectroscopy enables unique insight into the interface and morphologies of the system since it is based on chemical contrast between constituents. For example, Mousia and Farhat et al. (Mousia et al., 2000, 2001) have used a FTIR

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micro-spectrometer to study the composition of gelatin–amylopectin blends prepared by extrusion. FTIR two-dimensional maps were obtained based on the ratio of the peak areas of saccharide and amide bands. The role of the integrity of the starch granule in defining composition fluctuations that could control the performance of these blends was investigated. However, there is no report about the phase composition of film from solution that expected to have more homogeneous structure. Furthermore, investigation on the interface of starch–gelatin blends, in particular the polymer chain diffusion, could further explore the mechanisms of compatibility of the blend system.

The mapping resolution depends on the size of the detecting region. Theoretically you can concentrate an FTIR beam in a smaller region but that will result in lower sensitivity for a normal FTIR instrument. Synchrotron FTIR has a much higher signal-to-noise (S/N) ratio and higher spatial resolution, which allows mapping of the microstructure, as well as providing insight into the chemical distribution and interactions (Sin, Rahman, Rahmat, & Samad, 2010). It can provide a good tool to complementary other techniques for investigating starch and/or gelatin phases in the blends, in particular chemical composition.

The aim of this work was to blend gelatin with hydroxypropylated high amylose (80%) corn starch with poly(ethylene glycol) (PEG) as plasticizer through a solution method. An objective was to develop gelatin–starch blends for use in making drug capsules by well-established technology of dipping stainless mold into solution and then drying (Zhang et al., 2013). Equilibrated solutions will be studied in this work. A synchrotron FTIR with micro-spectroscopy facility will be used to study the interface and phase composition of gelatin–starch blends of cast films. The contribution of PEG on the morphology of the blends was investigated based on the mapped composition.

2. Materials and methods

2.1. 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 (A939) with MS (molar substitution) 0.11 was supplied by Penford (Australia). Poly(ethylene glycol) (PEG 400) was purchased from Sigma.

20% water solutions 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, 0:100) based on a total weight basis (100 g) in 400 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 rpm), then for a further 30 min at high speed (700 rpm) until a clear solution was obtained. Previous studies (Lan et al., 2010; Liu et al., 2010) 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. The solutions containing 5% PEG were also prepared by the same method.

2.2. Film and specimen preparations

Low concentration solutions were used to prepare very thin cost films. The solutions described above were diluted with water to 2% concentration and stirred at a speed of 500 rpm for 10 min. After degassing, 5 mL of solution was poured onto a PET dish (diameter 5 cm), which was kept same 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, and stored at 56% RH and 23 °C until required for analysis. All films were about 5–7 μm in thickness.

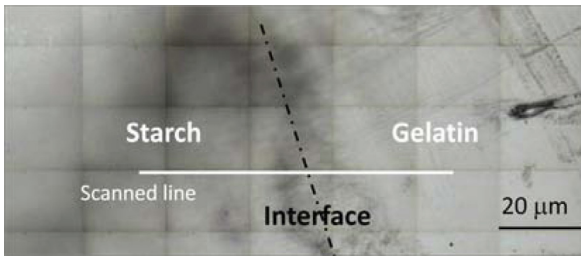

Fig. 1. Cross-section of the film with two parts of materials and scanning line on the interface.

The blend used for studying interfaces was prepared using a highly concentration solution (20%) described above. The pure starch solution was firstly added into a small plastic container, and then the pure gelatin solution was added on top of the starch solution at room temperature. Since the viscosities of both solutions were very high they did not mix together without stirring. After drying slowly at room temperature, a specimen with two layers of material was removed from the container. The specimen was embedded into epoxy resin so that vertical cross-section was exposed. A thin cross-section film (about 7–9 μm) with two parts of materials (see Fig. 1) was cut from the blend using a microtome. All the film were used FTIR-ATR (Bruker Tensor 37) first to search characteristic peaks of starch or gelatin.

2.3. Synchrotron Fourier transform infrared mico-spectroscopy (Synchro-FTIRM)

The specimens were analyzed using a Bruker Vertex V80v Fourier transform infrared 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 radiation–IR allowed an aperture of 5 μm × 5 μm to achieve a high S/N ratio and high spatial resolution. This allowed high quality mid-IR spectra to be achieved with a relatively low number of scans. Spectral collection was made in transmission mode at 4 cm^{-1} resolution, 32 scans were co-added and converted to absorbance using OPUS 6.5 software.

2.4. Analysis of the spectra

For all spectra, the intergration area of the saccharide bands (1180–953 cm^{-1}), labeled as Band-1, was used to represent starch. The intergration area of the amide I and II bands (1750–1483 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 during analyzing mapped images. OPUS 6.5 was used to reconstruct the 3D image using the ratio of integrated areas of starch and gelatin.

For a more detailed analysis, the corresponding spectral data were reconstructed with CytoSpec 1.4 (CytoSpec Inc., New York, USA) software to obtain chemical maps of the ratios of integrated areas under starch and gelatin peaks. Images and maps were contrasted using the "Jet" color scheme available in CytoSpec, with red indicating the highest relative concentration of starch, while blue indicated the highest relative concentration of gelatin.

3. Results and discussion

Fig. 2 shows typical transmission FTIR spectra of a starch and gelatin film acquired using the FTIR microscope with an aperture size of 5 μm × 5 μm, respectively. The distinctive spectral features for starch were C—O and C—C vibrational modes that are highly coupled from 1300 to 800 cm^{-1}, which is sensitive to conformational and crystalline order of starch (Mousia et al., 2000, 2001;

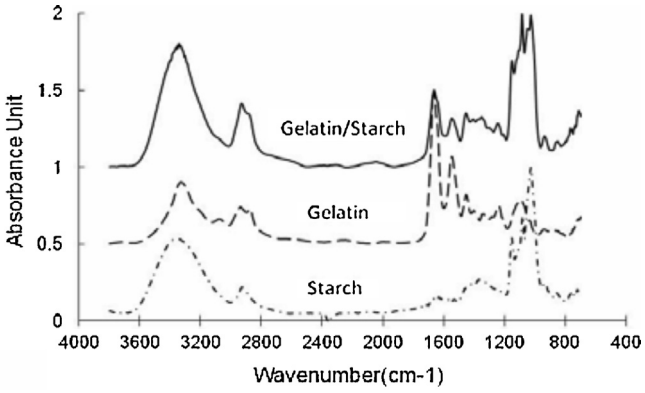

Fig. 2. The transmission FTIR of thin films of pre-gelatinized starch, gelatin, and a 2:1 starch–gelatin blend.

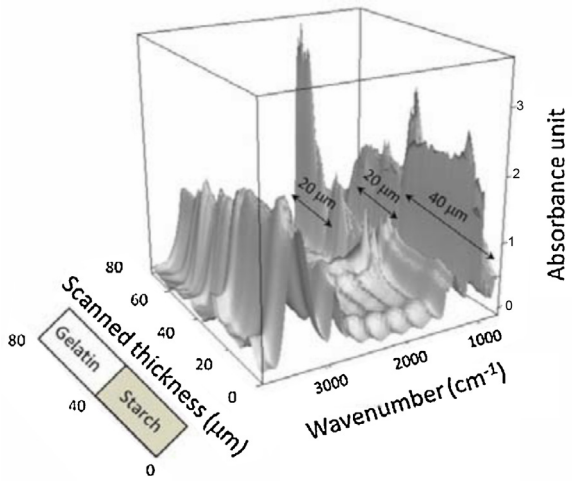

Fig. 3. The transmission FTIR scanned from the interface of the films with two parts of materials (corresponded with Fig. 1).

Mutungi et al., 2011). 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 CH_2 related modes. For the starch, a series of overlapping peaks located in the region of 1180–953 cm^{-1}, were the most intense bands in the mid-IR spectrum. Furthermore, 3400–3100, 3000–2700 cm^{-1} belong to OH and CH stretching vibrations, respectively. The typical spectral features for the protein were strong amide I and II bands located at approximately at 1650 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.

Fig. 2 also shows a typical FTIR-ATR spectra of starch–gelatin (2:1) blend. The bands associated with individual components in addition to the contributions of the water absorptions at 3300 (OH stretching), 1630 (COH bending), with a broad combination band centered around 2200 cm^{-1}. The bands for starch and gelatin were identified in the spectra and no new bands were detected, which means it is a phase separated system. In order to characterize the starch or gelatin content quantitatively, the integrated area of the saccharide bands (1180–953 cm^{-1}), labeled as Bands-1, was used to represent starch; while the integrated area of the amide I and II bands (1740–1486 cm^{-1}), labeled as Bands-2, was used to represent gelatin. The Bands-1 divided by Bands-2 was used to evaluate the starch content during analysis of mapped images, which is effective in eliminating the absorbed water and effects due to the blend itself.

Fig. 3 shows the transmission FTIR scanned cross the interface of the film containing two materials (corresponding with Fig. 1). It is seen that the bands on both starch and gelatin sides are clearly identified and showed as continuous signal phases smoothly. It is interesting to note that there is a layer of about 7–9 μm thickness, in which both the Bands-1 and Bands-2 were detected, indicating that gelatin and the starch were co-existence. The result means starch and gelatin are partly compatible; the polymer chains defused into each other's phase on the interface. The starch used in this work has more flexible linear amylose chains, with more hydrophilic hydroxpropylene chain improving the compatibility with gelatin.

Fig. 4 shows a typical count contour map for starch–gelatin 40:60 blend. The image was generated from the data of a single measurement set by allocating a color to each pixel based on the ratio of Bands-1 and Bands-2, which was used to characterize the starch–gelatin content. The red peak in the scale denotes a high value of starch while the blue botom line denotes a high value of gelatin. It was observed that the starch phase (Bands-1) distribution was a dispersed phase, while gelatin (Bands-2) was a continuous phase. However, the sharp peak, not the column, of Bands-1 indicates there are certain chain diffusions on 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 interface (Fig. 3).

In order to analyze quantitatively the chemical maps of the ratios of integrated areas under starch and gelatin bands, the 2D count contour maps were established for the blends with different starch:gelatin contents. Fig. 5 shows the chemical map of starch distribution acquired from the MCT detector for the various starch–gelatin blends. The color code represents the concentration of a component. The red in the scale denotes a high value of starch while blue denotes a high value of gelatin. The FTIR microscopy images confirmed that the starch phase distribution was a dispersed phase. The size of starch domains increased with increasing starch content.

The FTIR concentration maps suggested that for all mixtures investigated gelatin formed a continuous matrix in which starch inclusions were dispersed. The results demonstrated the highly heterogeneous nature of such blends with starch domains dispersed into a gelatin continuous phase even with higher starch content blends. All FTIR spectra showed contributions from both starch and gelatin absorptions and therefore indicated that complete demixing, with pure starch and gelatin domains, did not occur. The FTIR results support the observation that starch and gelatin are partly compatible.

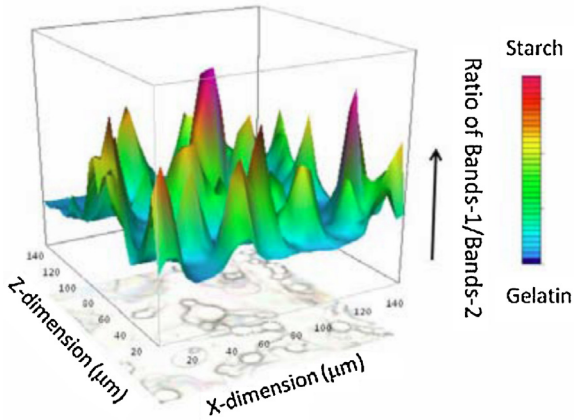

Fig. 4. Variation of the ratio of the area of the Bands-1/Bands-2 plotted as 2D and 3D contour maps for starch–gelatin (60:40) blend.

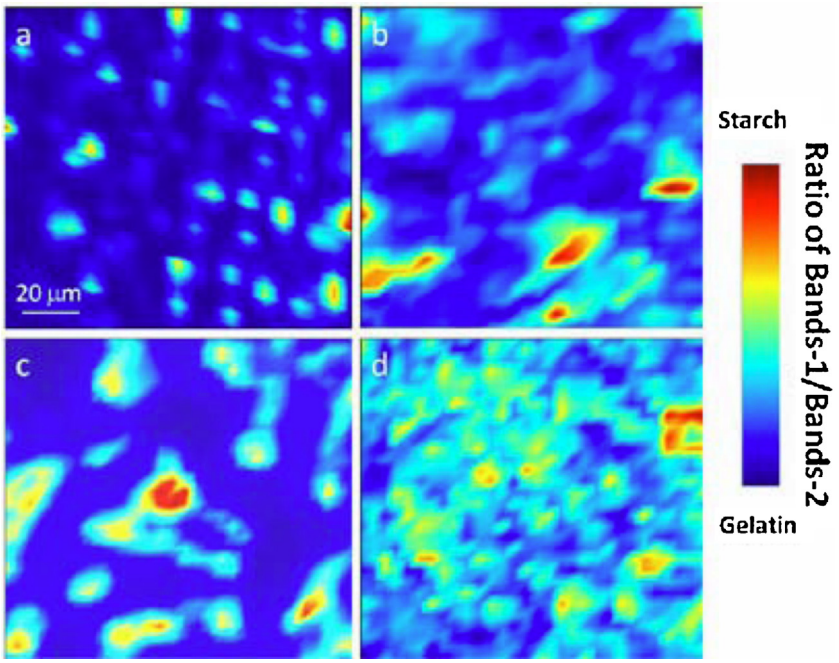

Fig. 5. Contour plots of the variation of the Bands-1/Bands-2 ratio for different starch–gelatin blends: (a) 80:20; (b) 60:40; (c) 40:60 and (d) 20:80.

Fig. 6 shows contour plots for variation of the Bands-1/Bands-2 ratio for the starch–gelatin blends with and without PEG. It is seen that the ratio of Bands-1 was increased significantly after addition of PEG, even only 5%. The increase of the Bands-1 was contributed by PEG resulting in an improvement of the interface between starch and gelatin. Fig. 7 shows the FTIR-ATR spectra of PEG and starch containing PEG. It is noted that there are some bands for PEG (Kim, Kim, & Cho, 2009; Pasquali, Andanson, Kazarian, & Bettini, 2008) just in the range of ca. 1180–953 that overlap with the Bands-1 used for representing starch. The overlap of the bands of PEG and starch enhanced the intensity of the Bands-1 in the maps, resulting in enhanced color of Bands-1 and enlarging its area. It is noted that the blue colored area (Bands-2) became weaker after adding PEG, indicating the PEG could dissolve homogeneously into gelatin. PEG is miscible with starch (Kim et al., 2009; Laohakunjit & Noomhorm, 2004). It is expected that PEG at the inter-phase between starch and gelatin improved their compatibility. The improvement of the compatibility or interface enlarged the area of the Bands-1.

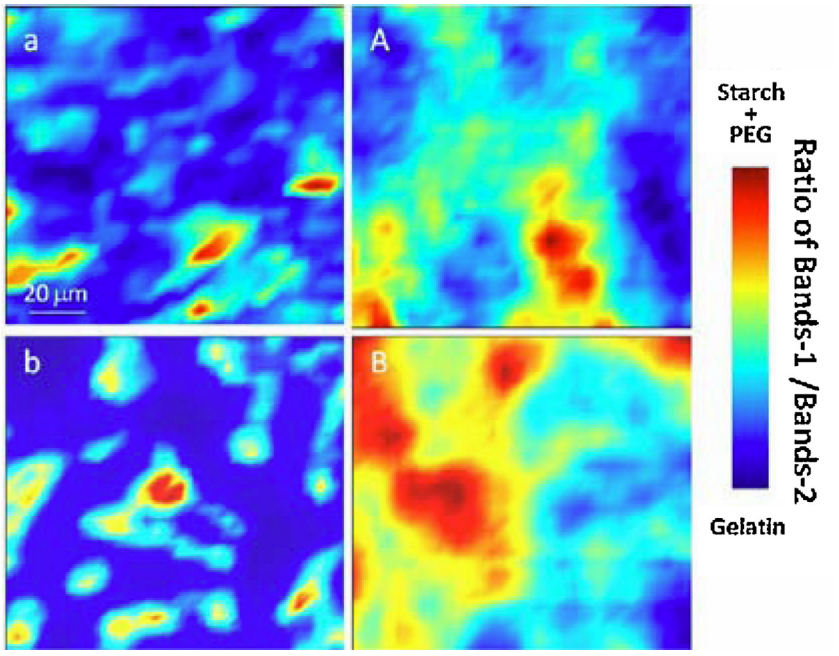

Fig. 6. Contour plots of the variation of the Bands-1/Bands-2 ratio for different starch–gelatin blends: (a) 60:40; (b) 40:60; and with 5% PEG: (A) 40:60; (B) 60:40.

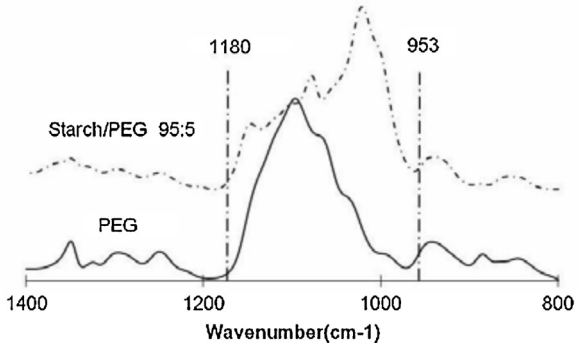

Fig. 7. FTIR spectra of PEG and starch containing PEG.

4. Conclusions

Interfacial and phase composition of various starch–gelatin blends were investigated by FTIR with various extended techniques, from scanning cross interface to 2D and 3D mapped by synchrotron FTIR micro-spectroscopy. The peaks of the saccharide bands (1180–953 cm^{-1}) and the amide I and II bands (1750–1483 cm^{-1}) were used to identify the starch and gelatin, respectively. The ratio of the areas of the starch and gelatin bands was used to determine the relative distributions of the two components in the blends. The FTIR concentration maps suggested that for all the mixtures investigated, gelatin formed a continuous matrix in which starch inclusions were dispersed. All FTIR spectra showed contributions from both starch and gelatin absorptions and therefore indicated that complete demixing with pure starch and pure gelatin domains did not occur. There was an about 20 μm thickness layer where gelatin and the starch were in co-existence, indicating gelatin and the starch were compatible to a certain degree in this system. The PEG homogeneously distributed in both gelatin and starch phases, and blurred the interface between gelatin and starch, indicating that PEG acted not only as a plasticizer but also as a compatibilizer for the gelatin–starch blends. The starch used in this work has more flexible linear amylose chains, and with the more hydrophilic hydroxpropyl substitutents improving the compatibility with gelatin.

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References

Abdulmola, N. A., Hember, M. W. N., Richardson, R. K., & Morris, E. R. (1996). Application of polymer blending laws to starch–gelatin composites. *Carbohydrate Polymers*, *31*, 53–63.

Al-Hassan, A. A., & Norziah, M. H. (2012). Starch–gelatin edible films: Water vapor permeability and mechanical properties as affected by plasticizers. *Food Hydrocolloids*, *26*, 108–117.

Arvanitoyannis, I., Kalichevsky, M., Blanshard, J. M. V., & Psomiadou, E. (1994). Study of diffusion and permeation of gases in undrawn and uniaxially drawn films made from potato and rice starch conditioned at different relative humidities. *Carbohydrate Polymers*, *24*, 1–15.

Arvanitoyannis, I., Nakayama, A., & Aiba, S.-i. (1998). Edible films made from hydroxypropyl starch and gelatin and plasticized by polyols and water. *Carbohydrate Polymers*, *36*, 105–119.

Arvanitoyannis, I., Psomiadou, E., Nakayama, A., Aiba, S., & Yamamoto, N. (1997). Edible films made from gelatin, soluble starch and polyols: Part 3. *Food Chemistry*, *60*, 593–604.

Baldwin, E. A., Nisperos-Carriedo, M. O., & Baker, R. A. (1995). Use of edible coatings to preserve quality of lightly (and slightly) processed products. *Critical Reviews in Food Science and Nutrition*, *35*, 509–524.

Bhargava, R., Wall, B. G., & Koenig, J. L. (2000). Comparison of the FT-IR mapping and imaging techniques applied to polymeric systems. *Applied Spectroscopy*, *54*, 470–479.

Chiono, V., Vozzi, G., D'Acunto, M., Brinzi, S., Domenici, C., Vozzi, F., et al. (2009). Characterisation of blends between poly([epsilon]-caprolactone) and polysaccharides for tissue engineering applications. *Materials Science and Engineering: C*, *29*(7), 2174–2187.

Firoozmand, H., Murray, B. S., & Dickinson, E. (2009). Microstructure and rheology of phase-separated gels of gelatin + oxidized starch. *Food Hydrocolloids*, *23*(4), 1081–1088.

Jagannath, J. H., Nanjappa, C., Das Gupta, D. K., & Bawa, A. S. (2003). Mechanical and barrier properties of edible starch–protein-based films. *Journal of Applied Polymer Science*, *88*(1), 64–71.

Kim, C.-H., Kim, D.-W., & Cho, K. (2009). The influence of PEG molecular weight on the structural changes of corn starch in a starch/PEG blend. *Polymer Bulletin*, *63*(1), 91–99.

Lan, C., Yu, L., Chen, P., Chen, L., Zou, W., Simon, G., et al. (2010). Design, preparation and characterization of self-reinforced starch films through chemical modification. *Macromolecular Materials and Engineering*, *295*(11), 1025–1030.

Laohakunjit, N., & Noomhorm, A. (2004). Effect of plasticizers on mechanical and barrier properties of rice starch film. *Starch/Stärke*, *56*(8), 348–356.

Liu, H., Li, M., Chen, P., Yu, L., Chen, L., & Tong, Z. (2010). Morphologies and thermal properties of hydroxypropylated high-amylose corn starch. *Cereal Chemistry*, *87*(2), 144–149.

Mousia, Z., Farhat, I. A., Blachot, J. F., & Mitchell, J. R. (2000). Effect of water partitioning on the glass-transition behaviour of phase separated amylopectin–gelatin mixtures. *Polymer*, *41*(5), 1841–1848.

Mousia, Z., Farhat, I. A., Pearson, M., Chesters, M. A., & Mitchell, J. R. (2001). FTIR microspectroscopy study of composition fluctuations in extruded amylopectin–gelatin blends. *Biopolymers*, *62*(4), 208–218.

Mutungi, C., Onyango, C., Doert, T., Paasch, S., Thiele, S., Machill, S., et al. (2011). Long- and short-range structural changes of recrystallised cassava starch subjected to in vitro digestion. *Food Hydrocolloids*, *25*(3), 477–485.

Ong, M. H., Whitehouse, A. S., Abeysekera, R., Al-Ruqaie, I. M., & Kasapis, S. (1998). Glass transition-related or crystalline forms in the structural properties of gelatin/oxidised starch/glucose syrup mixtures. *Food Hydrocolloids*, *12*(3), 273–281.

Pasquali, I., Andanson, J.-M., Kazarian, S. G., & Bettini, R. (2008). Measurement of CO_2 sorption and PEG 1500 swelling by ATR-IR spectroscopy. *The Journal of Supercritical Fluids*, *45*(3), 384–390.

Sin, L. T., Rahman, W. A. W. A., Rahmat, A. R., & Samad, A. A. (2010). Computational modeling and experimental infrared spectroscopy of hydrogen bonding interactions in polyvinyl alcohol–starch blends. *Polymer*, *51*(5), 1206–1211.

Wetzel, D. L., & LeVine, S. M. (1999). Imaging Molecular Chemistry with Infrared Microscopy. *Science*, *285*(5431), 1224–1225.

Zhang, N., Liu, H., Yu, L., Liu, X., Zhang, L., Chen, L., & Shanks, R. (2013). Developing gelatin -starch blends for use as capsule materials. *Carbohydrate Polymer*, *92*, 455–461.