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Effect of Microwave Modification on Mechanical Properties and Structural Characteristics of Soy Protein Isolate and Zein Blended Film

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Abstract

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Soy protein isolate (SPI) and zein were blended at different ratios, and native and microwave-modified films were prepared at pH 12 in an ethanol/water (20 : 80) mixture. The effect of the microwave modification on the mechanical properties and structural characteristics of the SPI and zein blended films was investigated. The results show that the microwave-modified blended film of 3 : 1 SPI-zein demonstrated the best mechanical properties: the highest breaking strength (2900 g) and the highest fracturing distance (16.08 mm). The morphology of the microwave-modified film was more homogeneous and had fewer pinholes than the zein-only film. In addition, the glass transition temperature of the microwave-modified film rose by nearly half compared to the zein-only film, while the melting temperature increased by almost 2.5%. The secondary structure analysis indicates that a moderate amount of microwave treatment will promote a decrease in α-helix, β-turn, and random coil content and an increase in β-sheet content. This study provides an edible material with better flexibility for food packaging.

Keywords: protein film; protein modification; breaking strength; fracturing distance; structural characteristics

Zein is composed of polypeptides of α-, β-, γ-, and δ-zeins (Shukla & Cheryan 2001). It has a high proportion of nonpolar amino acid residues, such as leucine, proline, and alanine (Chen *et al.* 2013). However, zein is deficient in lysine and tryptophan, which results in poor nutritional properties, so further application of zein has been hindered in food products. However, it has been widely studied for application in biodegradable packaging materials (Kim *et al.* 2004). Although zein has a long history of being used as a coating for candies, fruits, nuts, and other encapsulated drugs and foods (Biswas *et al.* 2009), the shortcomings of zein-based materials still limit other applications of zein films (Chen *et al.* 2006; Gao *et al.* 2007; Zhong *et al.* 2008). Compared with other protein films, the zein film is brittle, stiff and has poor flexibility that cannot withstand industrial processing (Shi *et al.* 2011). A number of methods have been applied to improve the performance of zein films, such as the addition of lipids and polysaccharides. Some reports used carbohydrates (galactose, glucose, and fructose) and glycerol to reduce the brittleness of zein films (Ghanbarzadeh *et al.* 2006; Guo *et al.* 2008), but few reports have used plant protein to improve the mechanical properties of zein film or have researched the mechanism of this process.

Soy protein is an abundant, inexpensive, nutritional and biodegradable biopolymer (Kokoszka *et al.* 2010). Soy protein-based edible films have

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received considerable attention due to their excellent film-forming abilities and low cost (Rhim *et al.* 2006), and they have been used as resources for bioplastics in packaging applications (Cunningham *et al.* 2000; Swain *et al.* 2004). Recently, some papers reported that the addition of soy protein isolate (SPI) can improve the properties of polysaccharides and nanocomposite films (Kumar *et al.* 2010; Wang *et al.* 2013). However, reports of using SPI and zein to make a blended protein film are not very common. This is because SPI is easily dissolved in water but not in ethanol solution, while zein exhibits the opposite behaviour. Hence, if a blended protein film of SPI and zein was made under normal conditions, some insoluble substances would appear in the film-forming solution, which would have a negative effect on the properties of the blended films. The previous paper reported that the solubility of SPI substantially improved when it was heated at alkaline pH (Voutsinas *et al.* 1983). This may be due to the molecular interactions and soluble aggregates of protein molecules with water molecules, such as disulphide bonds, hydrogen bonds, hydrophobic forces, and van der Waals forces (Hua *et al.* 1996). However, to our knowledge, there are no reports on the preparation of films at alkaline pH.

A major shortcoming of zein is its poor solubility in water and high hydrophobicity, which limits its application. It dissolves in a high concentration of an ethanol and water mixture, which means that the solution cannot be readily used for chemical modification, as the alcohol may react faster with electrophilic reagents than the zein does (Shi *et al.* 2011). Considerable efforts have been made to improve the protein solubility by physical methods such as heating and mechanical action (Hua *et al.* 1996). Microwave radiation is a non-ionising electromagnetic radiation, which can be used instead of convection to raise the temperature of polar substances by the interaction of the polar molecules with the electric constituent of the electromagnetic field (Zubov *et al.* 2009). The effects of microwaves such as oscillation, breaking of bonds, mutual friction, and collisions amongst particles can promote active groups within molecules to align, make contact, and react (Li *et al.* 2013). There is a study showing that microwaves offer a clean, rapid, convenient, and cost-effective method for heating that can cause an athermal effect by polarising macromolecules (Wang *et al.* 2013). Thus, it is increasingly used for investigating the properties of biomaterials (Copty *et al.* 2006).

In this paper, zein modified by microwaves was prepared and then blended with SPI to make edible protein films at pH 12. Subsequently, the mechanical properties and structural characteristics of the blended edible films were investigated. The goal was to obtain a blended edible film with better mechanical properties, while maintaining biodegradability and a high nutrition value. The microstructure, thermal properties, Fourier transform infrared spectroscopy (FTIR) characterisation and circular dichroism (CD) spectrum of the blended films were also analysed to confirm the change of the molecular structure by microwave modification.

MATERIAL AND METHODS

Material. Soy protein isolate (SPI) was obtained from Harbin High-tech Group Co., Ltd. (Harbin, China), containing (on dry basis) 3.7% moisture, 1.1% ash, 0.8% lipids, and 91% protein (determined by Kjeldahl method, N × 6.25). Zein was purchased from Wujiang Bache Medicinal Materials Factory (Jiangsu, China), containing (on dry basis) 7.1% moisture, 1.9% ash, 0.5% lipids, and 88% protein (determined by Kjeldahl method, N × 6.25). Distilled water was used throughout the experiments. Other chemicals including glycerol, glucose, polyethylene glycol 400, absolute ethanol, hydrochloric acid, and sodium hydroxide were of analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China).

Preparation of native blended edible films. SPI solution and zein solution with the same concentrations (70 g/l) were prepared respectively by stirring in distilled water (100 ml final volume) for 10 minutes. Then, the two solutions were blended at SPI/zein ratios of 3 : 1, 2 : 1, 1 : 1, 1 : 2, 1 : 3, and 0 : 1 (v/v). After that, absolute ethanol (20 ml/100 ml), glycerol (1 ml/100 ml), polyethylene glycol (MW 400) (1 ml/100 ml), and glucose (0.2 g/100 ml) were added to the blended protein solution. The pH of the mixture was adjusted to 12.0 by adding NaOH (1 mol/l), and then the solution was mixed using a magnetic stirrer for 30 min at room temperature. Afterwards, the solution was heated with stirring in a water bath at 80°C for 15 min, and after using a vacuum pump to remove air bubbles, 15 ml of the film-forming solution was poured onto smooth, circular, and rimmed sterile plastic plates with inner diameters of 10 cm that were placed on a level surface to obtain films of homogeneous, controlled thickness. The film-forming solutions were dried in a drying oven at 45°C for

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6 h, and the dried films were then carefully peeled from the plates. Before measurement, all the films were stored for at least 24 h in a desiccator of 50% relative humidity at ambient temperature.

Preparation of microwave-modified blended edible films. Zein solution with the concentration of 70 g/l was prepared by stirring in distilled water (100 ml final volume) for 10 minutes. Then, the pH was adjusted to 12.0 by adding NaOH (1 mol/l). A microwave reaction system (SL-SM50; Nanjing Shunliu Instruments Co., Ltd., Nanjing, China) was used to prepare the microwave-modified zein solution at 60°C and 350 W for 2 min, using an infrared probe inside the microwave reaction system to control the solution temperature. The SPI solution and microwave-modified zein solution were blended at ratios of 3 : 1, 2 : 1, 1 : 1, 1 : 2, 1 : 3, and 0 : 1 (v/v), and the same steps were followed as for the native films.

Mechanical properties. Referring to the method of Trinetta *et al.* (2011) and Kara *et al.* (2015), and making some improvements, these studies used the breaking strength and fracturing distance to measure the mechanical properties of the sample. Samples were cut into circles of 80 mm in diameter from the central region of the films and stored in 50% relative humidity desiccators under ambient temperature (25°C) for 48 h before conducting tensile tests. Mechanical properties were determined on a texture analyser (CT3; Brookfield, New Castle, USA) using TA41 probes, TA-DE fixture. The specimen was placed in a circular holder, and a pre-load of 5 g was applied to the film at a rate of 0.1 mm/s prior to the test. A hemispherical probe was used with a penetration rate of 0.5 mm/s and an object distance of 20 mm. A microcomputer was used to record the strength and distance data. For each film, at least three replicate measurements were performed.

Microstructure of the film. Microstructures of the surface and cross-section morphology of the films were examined using a scanning electron microscope (SEM) (S4800; Hitachi, Tokyo, Japan) with an accelerating voltage of 5 kV. Films were dehydrated at 60°C using a drying oven for 12 h (5.7% moisture content) and prepared for SEM by crushing following freezing in liquid nitrogen. Samples were sputtered with a thin layer of gold and photographed perpendicularly to the surface and cross-section.

Differential scanning calorimetry (DSC). DSC (TA-Q20; TA Instruments, New Castle, USA) was applied to determine the glass transition temperature (T_g – the midpoint of the glass transition), the melting temperature (T_m – the lowest point of melting peak) (Shi *et al.* 2011), and the enthalpy of fusion (ΔH_f) of the samples. They were automatically analysed from the thermogram by the TA Universal Analysis software that accompanied the instrument. Each film sample was cut into small pieces, and hermetically sealed aluminium pans containing approximately 5 mg of the samples were prepared. The samples were scanned at a heating rate of 10°C/min from 22°C to 220°C under nitrogen. An empty hermetically sealed aluminium pan was used as a reference. Triplicate readings were taken.

Fourier transform infrared spectroscopy (FTIR). Attenuated total reflection FTIR spectra were collected under ambient conditions using a FTIR spectrometer (Nicolet 6700; Thermo Fisher Scientific, Boston, USA) in the range of 4000~400 cm^{-1} to investigate the functional groups. Analyses of the obtained spectra were conducted by the OMNIC software that accompanied the instrument. The film was mounted directly in the sample holder, and data were collected after scanning the background.

Circular dichroism (CD) spectrum. A CD spectrometer (J-810; JASCO Corporation, Tokyo, Japan) was applied to evaluate the influence of the microwave modification and the heating process on the protein secondary structure. The film forming a SPI-zein solution at a 3 : 1 ratio was used as a sample. Referring to the Kurouski method (Kurouski *et al.* 2012) with improvements, the film-forming solution was diluted to 0.02% protein content with tris-HCl buffer solution (pH 7.5, 0.005 mol/l). After blending, the solution was poured into a cuvette and scanned in the CD spectrometer in the scanning range of 190 nm to 250 nm at a 100 nm/min scanning rate. Referring to Yang's algorythm to estimate the content of the secondary structure, the spectra were automatically analysed by the Jasco Secondary Structure Estimation program supplied with the instrument.

Statistical analysis. SPSS 20.0 software was used for the statistical analysis of means and standard error. Results were subjected to a one-way analysis of variance according to the general linear model procedure for the least squares mean effects. If required, Duncan's multiple range test ($P < 0.05$) was used to detect significant differences between mean values.

RESULTS AND DISCUSSION

Mechanical properties. The breaking strength and fracturing distance of the edible films prepared from

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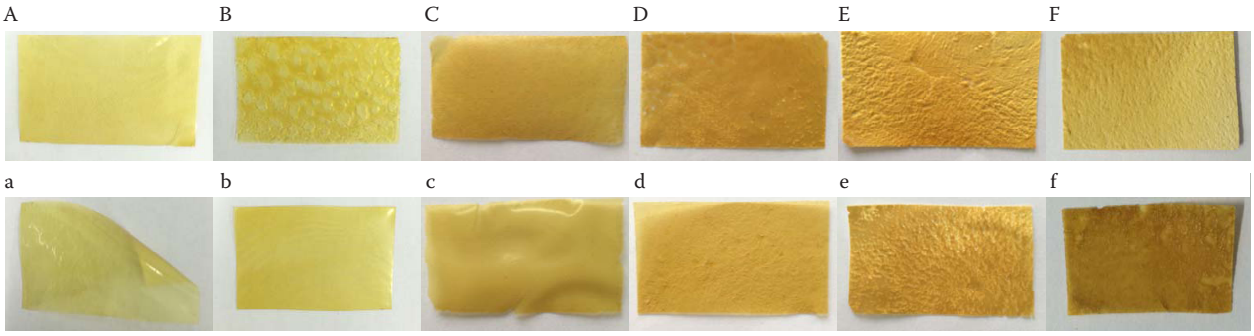

Figure 1. Macroscopic appearance of the films: the images marked with A–F represent the native and a–f the microwave-modified blended edible films of 3 : 1, 2 : 1, 1 : 1, 1 : 2, 1 : 3, and 0 : 1 SPI-zein, respectively

native and microwave-modified SPI and zein blends are presented in Table 1. The breaking strength of the native zein-only edible film was 192.25 g, and the fracturing distance was 2.65 mm; it presented as generally brittle (Figure 1F) and ruptured at low strain (Shi *et al.* 2011). The breaking strength and fracturing distance of the blended edible films were all much higher than the zein-only film, and the values increased with the increasing content of SPI. When the ratio of SPI to zein was 3 : 1, the maximum breaking strength was 2624.88 g, which was 12 times higher than that of the native zein-only film and nearly 10 times more than that of the 1 : 3 SPI-zein blended film. Additionally, the value of the fracturing distance decreased from 13.30 mm for the 3 : 1 SPI-zein blended film to 2.65 mm for the native zein-only film. The microwave-modified films revealed higher hardness and toughness (Figure 1A) than the native blended films. Compared to the native blended films, the breaking strength of the microwave-modified blended edible films was increased by 5～25%, and the fracturing distance was also slightly increased after modification. The maximum breaking strength of the modified edible film was 2900 g, and the fracturing distance was 16.08 mm, when the ratio of SPI/zein was 3 : 1. The increases in the breaking strength and fracturing distance values might be attributed to the interactions of protein molecules (Wang *et al.* 2013). The microwave modification might change the structure of the blended protein, which might be the reason for the increased mechanical properties of the microwave-modified blended protein films.

Microstructure. The surface and cross-section morphology of the SPI-only film, zein-only film, and native and microwave-modified blended edible films of 3 : 1 SPI-zein is presented in Figure 2. It is evident that the films differ in their microtopography, with pores observed. The surface of the zein-only film had many large, deep holes, and similar results have been reported in the literature (Gu *et al.* 2013). The microwave-modified film had a smooth appearance, with the fewest pores. The cross-section micrographs showed more pronounced differences. The microwave-modified film was smoother, more homogeneous and had fewer pinholes than the native blended film or the SPI-only film, and the zein-only film was clearly non-homogeneous and had numerous holes. The microwave-modified blended film of

Table 1. Breaking strength and fracturing distance of the native and microwave-modified blended edible films prepared from different ratios of SPI and zein

SPI/zein ratio	Breaking strength (g)		Fracturing distance (mm)	
	native	microwave	native	microwave
0 : 1	192 ± 21^{a}	317 ± 14^{b}	2.65 ± 0.25^{a}	3.69 ± 0.13^{ab}
3 : 1	2624 ± 21^{h}	2900 ± 30^{i}	13.30 ± 0.30^{e}	16.08 ± 0.43^{f}
2 : 1	2139 ± 31^{f}	2471 ± 46^{g}	12.66 ± 0.19^{e}	12.98 ± 0.17^{e}
1 : 1	1432 ± 23^{e}	1505 ± 54^{e}	8.63 ± 0.17^{d}	9.31 ± 0.39^{d}
1 : 2	726 ± 37^{c}	891 ± 22^{d}	5.70 ± 0.16^{c}	5.38 ± 0.27^{c}
1 : 3	278 ± 44^{ab}	347 ± 53^{b}	3.99 ± 0.08^{b}	4.12 ± 0.31^{b}

All values shown are means ± standard error; $^{a–i}$data marked by different letters in a column indicate significant difference ($P < 0.05$)

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Table 2. Differential scanning calorimeter (DSC) measurement results of SPI-only film, zein-only film, native and microwave-modified blended edible films of 3 : 1 SPI-zein

Film	T_g (°C)	T_m (°C)	ΔH_f (J/g)
SPI-only	87.19 ± 0.27[a]	167.91 ± 0.26[b]	46.14 ± 0.80[b]
Zein-only	55.05 ± 0.07[d]	167.54 ± 0.67[b]	61.77 ± 0.68[a]
Native 3 : 1 SPI-zein	77.95 ± 0.56[c]	168.80 ± 0.84[b]	33.62 ± 0.30[d]
Microwave 3 : 1 SPI-zein	81.11 ± 0.48[b]	172.69 ± 0.61[a]	40.10 ± 0.46[c]

T_g – glass transition temperature; T_m – melting temperature; ΔH_f – enthalpy of fusion; all values shown are means ± standard error; [a–d]data marked by different letters in a column indicate significant difference ($P < 0.05$)

3 : 1 SPI-zein had the highest breaking strength and fracturing distance and also the most homogeneous surface and cross-section micrographs, while the zein-only film exhibited the opposite results. It could therefore be considered that the smoothness and the pinhole number were responsible for the mechanical properties of the film, as a homogeneous and smooth surface is usually preferred (Shi *et al.* 2011). This might be because the intermolecular forces increased with the decreasing number of holes and the change in the protein secondary structure (Gu *et al.* 2013; Wang *et al.* 2013).

Thermal properties. The DSC thermogram is conventionally used to analyse the thermal properties of films by comparing T_g, T_m, and ΔH_f in different samples. The DSC thermograms of the SPI-only film, the zein-only film, and the native and microwave-modified blended films of 3 : 1 SPI-zein are shown in Figure 3, and their T_g, T_m, ΔH_f are shown in Table 2. These results show that the T_g of the microwave-modified film was 81.11°C, an increase of 4% compared to the native blended film and an increase of nearly 50% compared to the zein-only film. The melting peak of the microwave-modified film appeared at approximately 172°C, higher than for the other films. Moreover, the T_m of the blended films (native and microwave) was higher than that of the SPI-only and zein-only films by approximately 2.5%. The report of Kokoszka *et al.* (2010) confirmed the above results. They proposed that the increasing protein–protein interaction could promote aggregation in the presence of heating treatment (Kokoszka *et al.* 2010). The ΔH_f of the microwave-modified film was 19% higher than that of the native blended film, and the T_g and T_m had the same results. This might be due to changes in the molecular structure of the protein caused by the microwave treatment, driving the hydrogen bonds and hydroxyls to form a more compact structure, and the thermal properties of the film were accordingly improved (Wang *et al.* 2013). The results of DSC were similar to those of the breaking strength, indicating a positive correlation between the thermal properties and the mechanical properties of the film. The reports showed that DSC is related to the mechanical, WVP and OP properties of the film,

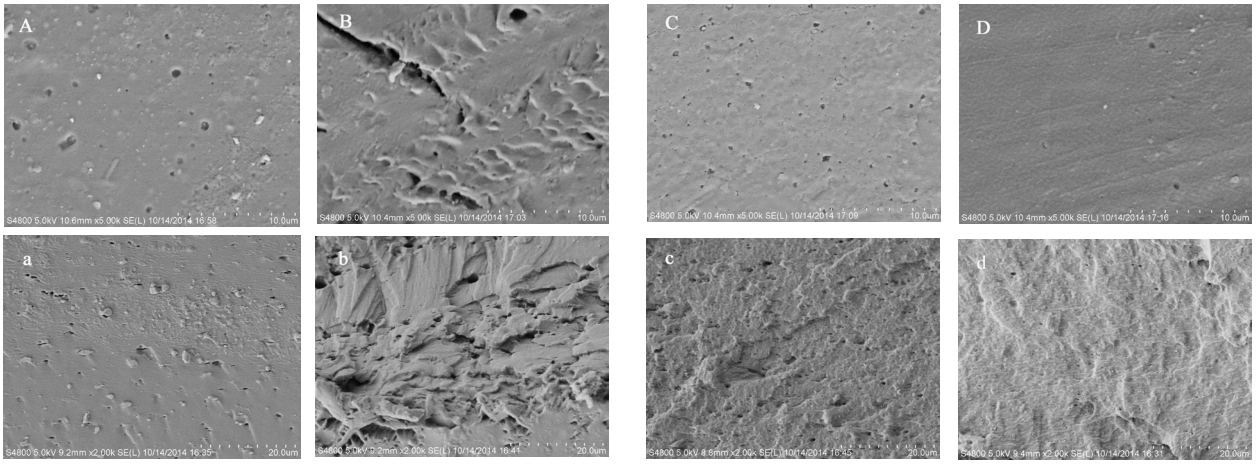

Figure 2. Scanning electron micrographs of (A/a) SPI-only film, (B/b) zein-only film, (C/c) native blended edible film of 3 : 1 SPI-zein, and (D/d) microwave-modified blended edible film of 3 : 1 SPI-zein (the images marked with A–D represent the surface of the film, and a–d the cross-section of the film)

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Table 3. Secondary structural content of native and microwave-modified film-forming solution before heating and after heating estimated from circular dichroism spectra by the software

Sample	α-Helix (%)	β-Sheet (%)	β-Turn (%)	Random coil (%)
Native 3 : 1 SPI/zein (before heating)	23.9 ± 0.4[a]	11.0 ± 0.2[d]	30.9 ± 0.6[a]	34.2 ± 0.2[a]
Microwave 3 : 1 SPI/zein (before heating)	21.7 ± 0.3[b]	16.6 ± 0.5[c]	28.6 ± 0.3[b]	33.1 ± 0.1[a]
Native 3 : 1 SPI/zein (after heating)	10.0 ± 0.3[d]	51.4 ± 0.4[a]	13.0 ± 0.1[d]	25.5 ± 0.5[b]
Microwave 3 : 1 SPI/zein (after heating)	11.9 ± 0.4[c]	48.0 ± 0.3[b]	14.8 ± 0.4[c]	25.3 ± 0.3[b]

All values shown are means ± standard error; [a–d]data marked by different letters in a column indicate significant difference ($P < 0.05$)

it is in agreement with the above results (Trinetta *et al.* 2011; Wang *et al.* 2013).

FTIR characterisation. Figure 4 shows the FTIR spectra of the SPI-only film, zein-only film, and native and microwave-modified blended edible films of 3 : 1 SPI-zein. The main absorbance peaks for these films have been identified as C=O stretching at 1630 cm^{-1} (amide I), N-H bending at 1530 cm^{-1} (amide II), C-H deformation at 1450 cm^{-1}, and C-N stretching and N-H bending vibrations at 1240 cm^{-1} (amide III). The absorbance band at nearly 1080 cm^{-1} is attributed to different groups such as out-of-plane C-H bending (from aromatic structures). The broad absorbance band observed at nearby 3280 cm^{-1} is attributable to the free and bounded O-H and N-H groups. The characteristic C-H stretching of the CH_2- and CH_3- groups of saturated structures generally appears in the range of 2980–2850 cm^{-1} (Schmidt *et al.* 2005). Therefore, the FTIR spectra of the SPI-only and zein-only films exhibit three adjacent peaks near 2920, 2870, and 2850 cm^{-1}, while the native and microwave-modified blended films had only two peaks near 2920 and 2870 cm^{-1}. This might be because the intermolecular interaction upon blending SPI and zein changed the structure of the protein. Another band was formed near 1740 cm^{-1} (C=O) only in the spectra of the zein-only film and native blended film. The absorbance peak in the region of 1700 cm^{-1} is used most widely in the study of the protein secondary structure, and the vibrational frequency reflects the second structure (α-helix, β-sheet, β-turn, and random) of polypeptides or proteins (Surewicz *et al.* 1993). As seen from the FTIR spectra of the microwave-modified blended film, the absence of the absorbance band at approximately 1740 cm^{-1} indicates that the microwave procedure effectively changes the secondary structure of the protein.

Secondary structure. The CD spectra of the film-forming solutions all showed negative peaks near 208 and 222 nm and a positive peak at 192~195 nm, indicating the presence of α-helix; negative peaks at 220~230 nm and a positive peak near 205 nm for

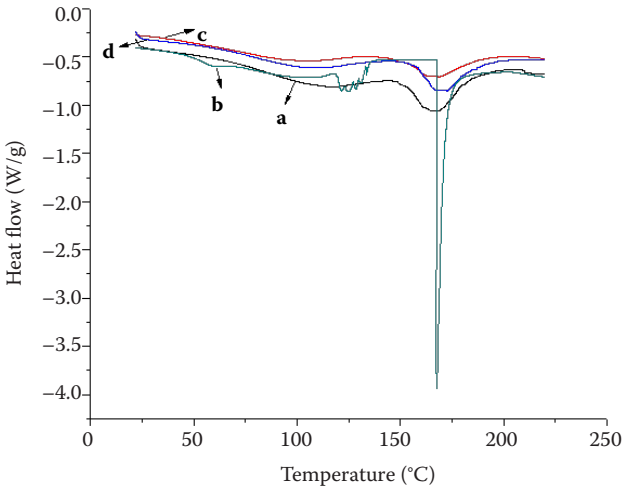

Figure 3. Differencial scanning calorimetry thermograms of (a) SPI-only film, (b) zein-only film, (c) native and (d) microwave-modified blended edible film of 3 : 1 SPI-zein

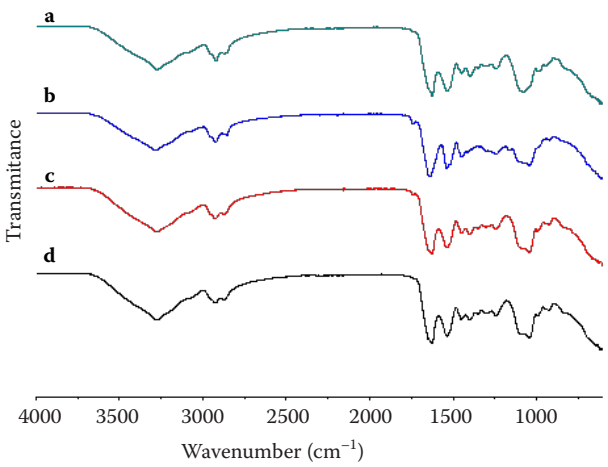

Figure 4. Fourier transform infrared spectra of (a) SPI-only film, (b) zein-only film, (c) native blended edible film of 3 : 1 SPI-zein and (d) microwave-modified blended edible film of 3 : 1 SPI-zein

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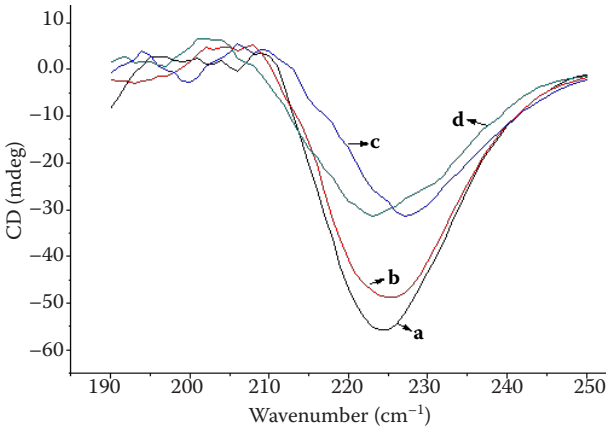

Figure 5. Circular dichroism spectra of (a) native film-forming solution before heating, (b) microwave-modified film-forming solution before heating, (c) native and (d) microwave-modified film-forming solution after heating

the presence of β-turn, and a negative characteristic peak of the random coil near 200 nm (Johnson 1990; Sreerama *et al.* 2000; Whitmore & Wallace 2008; Shi *et al.* 2014) (Figure 5). The negative peaks at 215~220 nm are a characteristic feature of β-sheet, suggesting a prominent β-sheet content in the film-forming solutions. The estimated secondary structure contents of the native and microwave-modified film-forming solutions before and after heating are shown in Table 3. The secondary structure of the native and microwave-modified solutions underwent changes in their contents of α-helix, β-sheet, β-turn, and random coil during the heating process. The contents of α-helix, β-turn, and random coil decreased by 23.6~58.2%, and the content of β-sheet increased significantly after heating. The heating process could be considered as an important factor that affected the protein structure upon forming the film. The data are in agreement with the previously reported results of Santosa and Padua (2000), who considered that the α-helix content decreased as a result of the irreversible thermal denaturation of protein, so the spectrum was different before heating and after heating (Santosa & Padua 2000). The secondary structure contents of the film-forming solution also changed after microwave modification. Before heating treatment, compared to the native film-forming solution, the α-helix, β-turn, and random coil of the microwave-modified film-forming solution decreased by 9.2, 2, and 3%, respectively, while there was a significant increase in the β-sheet content by nearly 51%. This indicates that the protein intrinsic structure was extensively damaged by the microwave treatment (Chinnadayyala *et al.* 2012). The effect of the microwave modification was similar to that of the heating treatment. This suggests that a moderate amount of microwave modification could promote a decrease of α-helix, β-turn and the random coil and an increase of the β-sheet content before the heating treatment. The previous literature reported that the secondary structure content of protein contributed to the mechanical properties of the films (Gu *et al.* 2013). These results therefore imply that the change in the secondary structure of the film-forming solution by microwave modification (the decrease in α-helix, β-turn, and random coil and the increase in β-sheet content) is helpful to forming a film with better mechanical properties, more homogeneous morphology and higher T_g and T_m.

CONCLUSIONS

Blended edible protein films of SPI and zein at different ratios were prepared, and the mechanical properties and structural characteristics of the films were determined. The results showed that the breaking strength of the microwave-modified blended films increased 5~25% compared to native blended films, and the fracturing distance was also slightly heightened after modification. The maximum breaking strength (2900 g) of the modified blended film was 15 times that of the native zein-only film, and the fracturing distance (16.08 mm) was 6 times higher when the SPI to zein ratio was 3 : 1. The 3 : 1 SPI-zein films were chosen for studies on the structural characteristics due to their remarkable mechanical properties. The morphology of the films showed that the microwave-modified film was more homogeneous and had fewer pores, and the surface and cross-section morphologies of the blended films were both better than those of the zein-only film. In addition, according to the data of DSC, the T_g of the microwave-modified film rose by nearly half compared to the zein-only film, and the T_m increased by almost 2.5%. A secondary structure analysis was performed via FTIR characterisation and circular dichroism spectra, indicating that the effect of microwave modification was similar to that of the heating treatment, and a moderate amount of microwave treatment will promote an increase in the β-sheet content and a decrease in α-helix, β-turn, and random coil contents, which may be one of the reasons for the improved mechanical properties.

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Meanwhile, the change of secondary structure may be helpful to forming a film with more homogeneous morphology and higher T_g and T_m.

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