Effect of bamboo shoot dietary fiber on gel quality, thermal stability and secondary structure changes of pork salt-soluble proteins

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ABSTRACT
The effect of bamboo shoot dietary fibre (BSDF) (0%-4%) on the gel quality, protein thermal stability and secondary structure of pork salt-soluble proteins (SSP) was investigated. The water holding capacity (WHC), breaking force and particle size were increased with the addition of 2% BSDF. Differential scanning calorimetry (DSC) analysis indicated that the addition BSDF changed the thermal denaturation temperature of myosin tail and actin, and led to the disappearance of myosin head as the increase of BSDF up to 4%. SDS-PAGE showed that the band intensity of actin was obviously enhanced by the addition of BSDF. Fourier transform infrared (FTIR) spectroscopy showed that BSDF could affect the protein secondary structure. The α-helix content was significantly decreased and the β-sheet content was significantly increased (P<0.05). In conclusion, BSDF could improve gel quality of salt soluble proteins and has a potential to be applied in meat products.

1. Introduction
The demand and consumption of healthier meat products have steadily increased worldwide, driven by the growing number of consumers who are convinced of the importance of nutrition and the necessity of reducing the risk of cardiovascular disease, obesity, and cancer (Zhang et al., 2017). Meat scientists and manufacturers concerned the development of high-quality processed meat products with lower calories and salt, reduced fat and cholesterol and higher nutritious and bioactive components, such as natural antioxidants and dietary fibres (Zhuang et al., 2016). Traditional emulsion-type meat products may contain up to 30% animal fat, especially meat patties, frankfurters and bologna sausage, which have higher saturated fat and cholesterol content contributing to a high intake of fat for consumers (Zhang et al., 2017). To lower the fat content and retain the texture and sensory quality of these meat products, lower caloric ingredients were added into the formulations using carrageenan (Gao et al., 2015), flaxseed gum (Liu et al., 2018) and dietary fibres (Choi et al., 2014; Zhuang et al., 2016). Dietary fibres are advantageous when designing the healthier emulsion-type meat products (Han & Bertram, 2017), because they not only have hydrocolloidal properties to achieve a desirable texture but also are conducive to prevent chronic diseases (Gibis, Schuh, & Weiss, 2015; Han & Bertram, 2017).

En general, muchos estudios han reportado la adición de varios tipos de fibra en los productos meat para mejorar la textura y la capacidad de retención de agua (WHC) de los productos finales (Ham et al., 2016; Kehlet, Pagter, Aaslyng, & Raben, 2017; Sánchez-Alonso, Haji-Maleki, & Borderías, 2007). Bengtsson, Montelius, y Tornberg (2011) reportaron que la adición de fibra de soja podría afectar la estructura secundaria de la proteína. El contenido de la hélice-α disminuyó significativamente y el contenido de la lámina β aumentó de manera significativa (P<0.05). En conclusión, la BSDF podría mejorar la calidad del gel de las proteínas solubles en sal y tiene potencial para ser aplicada a productos cárnicos.

RESUMEN
El presente estudio se propuso investigar el efecto de la fibra dietética de brotes de bambú (BSDF) (0%-4%) sobre la calidad del gel, la estabilidad térmica y los cambios de estructura secundaria de las proteínas de cerdo solubles en sal (SSP). Al adicionar 2% de BSDF, se detectaron aumentos en la capacidad de retención de agua (WHC), la fuerza de rotura y el tamaño de partícula. El análisis de calorimetría de barrido diferencial (DSC) indicó que la adición de BSDF cambió la temperatura de desnaturalización térmica de la cola de miosina y la actina, dando lugar a la desaparición de la cabeza de miosina al aumentar la adición de BSDF hasta 4%. La técnica SDS-PAGE mostró que la adición de BSDF mejoró notablemente la intensidad de la banda de actina. La espectroscopía infrarroja por Transformadas de Fourier (FTIR) mostró que la BSDF podría afectar la estructura secundaria de la proteína. El contenido de la hélice-α disminuyó significativamente y el contenido de la lámina β aumentó de manera significativa (P<0.05). En conclusión, BSDF podría mejorar la calidad del gel de las proteínas solubles en sal y tiene potencial para ser aplicada a productos cárnicos.

KEYWORDS
Salt-soluble proteins; bamboo shoot dietary fibre (BSDF); gel; thermal denaturation; protein conformation

PALABRAS CLAVE
proteínas solubles en sal; fibra dietética de brotes de bambú (BSDF); gel; desnaturalización térmica; conformación de proteínas
not improved by the addition of inner pea fibre. These different results indicated that different kinds of dietary fibres have different effects on the gel properties of meat proteins (Han & Bertram, 2017). It is important to further understand the potential mechanisms of how specific dietary fibres interact with meat proteins to affect the gel-like quality.

Bamboo shoot dietary fibre (BSDF) is a good source of functional ingredients and has been widely applied in milk pudding, fish balls and frozen dough (Zeng et al., 2016; Zhang et al., 2017; Zheng, Wu, Dai, Kan, & Zhang, 2017). Our previous research has revealed that compared with soybean dietary fibre and rice bran dietary fibre, BSDF has a higher water-binding capacity, swelling ability and oil-holding capacity (Zhang et al., 2017), which may be applied in meat products to improve both the nutritional value and certain functional properties. However, little information is available concerning the application of BSDF on the gel quality of meat proteins in model system. Recent researches have paid much attention to the interaction between exogenous additives such as non-meat proteins, flaxseed gum, and fibres, with SSP or myofibrillar proteins for improving the quality of protein gels (Feng et al., 2018; Liu et al., 2018; Niu, Xia, Wang, Kong, & Liu, 2018; Zhao, Zhou, & Zhang, 2019). However, few studies described the thermal stability and structural changes of dietary fibres-meat proteins composite gels, especially the addition of BSDF. A detailed understanding of the structural changes in SSP containing BSDF contributed to the effective application of dietary fibres and the development of healthier meat products while maintaining the gel properties of final products.

Therefore, the aims of this study were to evaluate the effects of various levels of BSDF on the WHC, breaking force, particle size, protein thermal denaturation and conformation of SSP composite gels to explore the mechanisms of interaction between BSDF and SSP.

2. Materials and methods

2.1. Materials

Fresh pork leg meat (72.98% moisture, 23.02% protein, 3.20% fat, 0.93% ash, pH 5.98 at 24 h post-mortem) (AOAC 2000) was obtained from a meat supermarket (Zhengzhou, China). All skin visible fat and connective tissue were removed. The meat was cut into cubes, mixed and ground by a meat grinder (MM-12, Guangdong, China) equipped with a slotted plate (6 mm diameter hole). The minced meat was divided and packaged (400 g each) in plastic bags and kept at −20°C until required for the extraction of SSP within 2 weeks. The BSDF was obtained from Gengshengtang Ecological Agriculture Co., Ltd. (Zhejiang, China). The proximate compositions of BSDF were as follows: 15.15% protein, 1.83% fat, 1.35% ash, 76.85% total dietary fiber (65.78% insoluble dietary fiber). The BSDF was ground to a superfine consistency after vacuum freeze-drying for 12 h and then collected by filtration fitted with a 100-mesh screen.

2.2. Extraction of SSP

SSP were extracted from pork leg meat according to the methods of Wang, Smith, and Steffe (1990) and Sun, Wu, Xu, and Li (2012) with slight modifications. The minced meat was thawed at 0–4°C overnight prior to extraction. The thawed meat samples were ground three times in a Waring Blender (GM200, Restch, Germany) at 3,000 r/min for 10 s. Then, the ground samples (approximately 250 g) were added to 1,000 mL of ice-cold extraction solution (0.6 mol/L NaCl, 0.75 g/L sodium hexametaphosphate, 1 g/L sodium pyrophosphate, 1.75 g/L sodium tripolyphosphate, pH 6.8) and homogenized (Ultraturrax T25, IKA, Staufen, Germany) at 10,000 r/min for 90 s. The dispersion was centrifuged (Beckman Avanti J-E, Beckman Coulter, USA) at 10,000 g for 20 min at 4°C. The supernatant was collected and placed at 4°C for 24 h. Next, the obtained supernatant was filtered with a 20-mesh screen. Then, the was blended in 2500 mL of sodium phosphate solution (20 mM Na₂HPO₄/NaH₂PO₄, pH 7.0) for 3 h. Then, the supernatant was centrifuged at 10,000 g for 15 min at 4°C. The precipitate obtained from the final centrifugation step was collected as SSP. All procedures were performed at 4°C. The SSP concentration was determined by the Biuret method (Gornall, Bardawill, & David, 1949), then diluted to a concentration of 40 mg/mL using the ice-cold extraction solution.

2.3. Preparation of BSDF-SSP sols

The BSDF was added at four concentrations as follows: 1, 2, 3 and 4 g/100 g of SSP. The mixed SSP solutions were stirred and homogenized with a high-speed disperser (XHF-D, Xinzheng Biological Science and Technology Co., Ltd, Ningbo, China) three times for 30 s. Then the BSDF-SSP samples were stored at 4°C and used in the succeeding tests.

2.4. WHC

The WHC was determined by a centrifugal method as reported by Chen et al. (2014) and Li, Liu, Fu, Zhao, and Bai (2019) with minor modifications. Each BSDF-SSP sol sample (approximately 8 g) was weighted (W1) and transferred into a 10 mL capped centrifuge tube and heated in a water bath at 80°C for 30 min. Then, the tubes of composite gels were cooled to reach room temperature. After cooling, the tubes were directly centrifuged (Beckman Avanti J-E, Beckman Coulter, USA) at 10,000 g for 10 min at 4°C. The amount (g) of released water after centrifugation were determined (W2). So, the weight of heat-induced compositied gel in the tubes is calculated as the weight difference between the BSDF-SSP sols sample (W1) and the released water (W2). The WHC was calculated as a percentage of the gel’s weight (W1-W2) after centrifugation divided by the original weight of the samples (W1). Each sample was analysed six times.

2.5. Breaking force

The breaking force was determined by a Texture Analyzer (TA-XT2i Plus, Stable Micro System Co., Godalming, England) according to the methods of Zhang, Xue, Xu, Li, and Xue (2013) and Zhou and Yang (2019) with a slight modification. Each BSDF-SSP sol (approximately 15 g) was added to a 10-mL glass breaker (25 mm diameter and 35 mm height). Then, the beakers were sealed with parafilm, heated and kept in a water bath at 80°C for 30 min. Subsequently, the gels were placed at 4°C overnight for analysis. The obtained gels were equilibrated to ambient temperature (20°C). An aluminium cylindrical probe (P/0.5 inch) was used to press the gels under a compression test. The compression mode was as follows: a trigger-type button with a 2 mm/s pre-test speed, a 1.0 mm/s test speed, and a 1.0 mm/s post-test speed with a 10 mm displacement and a 3 g trigger force. Peak load after compression was
recorded. The peak force to rupture to the gels was recorded as the breaking force (g). Each sample was analysed six times.

2.6. Particle size determination
The particle size of the BSDF-SSP sols was measured according to the method of Wu et al. (2019) using a Zetasizer Nano-ZS 90 (Malvern Instruments, Malvern, England). The concentration of BSDF-SSP sols was adjusted to 5.0 mg/mL (0.6 mol/L NaCl, 1 g/L sodium pyrophosphate, 1.75 g/L sodium tripolyphosphate, 0.75 g/L sodium hexametaphosphate, pH 6.8). Then, approximately 1 mL of each sample was loaded in a quartz cuvette and measured using dynamic light scattering. Based on the scattering intensity, the size of the particles and particle size distribution were estimated and reported. Each sample was analysed four times.

2.7. DSC
Thermal transition temperatures and denaturation enthalpy (ΔDH) of BSDF-SSP sols were performed using a calorimeter (Q20 DSC, TA Instruments, USA) according to the method of Debussa, Tahergorabi, Beamer, Partington, and Jaczynski (2013) with a slight modification. Each sample (approximately 30 mg) was weighed in an aluminium pan and then hermetically sealed. The sample was tempered at 20°C for 5 min and then heated from 20 to 80°C at a scanning rate of 5 °C/min. These thermal parameters were estimated by the software (Universal Analysis 2000, TA Instruments, USA). Each sample was analysed three times.

2.8. SDS-PAGE
SDS-PAGE was performed according to the procedure described by Laemmli (1970) with slight modifications. The SSP and BSDF-SSP composite sols were diluted to the concentration of 10 mg/mL using the buffer (125 mM Tris–HCl pH 6.8, 20% glycerol, 4% SDS, 3% (w/v) β-mercaptoethanol, and 0.005% bromophenol blue). Each sample was mixed well, tempered at 95°C for 5 min, and then stored at –80°C for succeeding SDS-PAGE. The gel was loaded with 15 μg of sample solution per well. SDS-PAGE was performed using a 10% acrylamide resolving gel and a 5% acrylamide stacking gel. The gel was run for approximately 40 min. Then, the gel was stained with 0.25% (w/v) Coomassie brilliant blue R-250, 45.5% ethanol, and 9% glacial acetic acid for 5 min, and then stored at 20°C for succeeding SDS-PAGE. The WHC and breaking force of heat-induced gel of pork salt-soluble proteins with various BSDF concentrations. WHC: water-holding capacity; BSDF: bamboo shoot dietary fibre; T0: SSP; T1: SSP+1% BSDF; T2: SSP+2% BSDF; T3: SSP+3% BSDF; T4: SSP+4% BSDF. Different letters (a–d) indicate significant differences (p < 0.05) among the samples with various BSDF concentrations.

3. Results and discussion

3.1. WHC
The WHC reflects the capability of proteins to hold water and is used to evaluate the cooking yield of meat products. Generally, better water retention signifies a stronger binding ability between the protein and water. Figure 1 shows the effect of various amounts of BSDF on the WHC of SSP. The WHC of gels were significantly increased when the concentrations of BSDF increased from 1% to 4% (P<0.05). The increase in WHC indicated that more favourable physical entrapment of water in the gels, and the more addition of BSDF could enhance this effect of entrapment (Yao et al., 2017). Choi et al. (2011) reported that the incorporation of rice bran fibre (0.1%, 0.5%, 1%, and 2%) into pork salt-soluble proteins increased the cooking yield. The WHC of SSP was significantly increased (P<0.05) with the increase in rice bran fibre according to the proportion until the addition of rice bran fibre at 1%. The WHC results were also consistent with previous observations in carboxymethyl cellulose (Han et al., 2018), peanut protein isolate (Sun et al., 2012) and soy protein isolates (Wang et al., 2015). However, Cardoso, Mendes, Vaz-Pires, and Nunes (2011) found that pea fibre addition had no positive effect on the WHC of the gels. These findings suggested that the factors affecting WHC by the addition of specific dietary fibres need to be further understood.

2.10. Statistical analysis
The experiments were performed in three independent replications at different occasions. All data were expressed as the mean ± SD. The results were analysed with one-way ANOVA (P<0.05) using SPSS 21.0 software (IBM Corporation, NY, USA). The difference between least-square means was determined by Duncan’s multiple range test. The graphs were performed by Origin 8.5 (Origin Lab Corporation, MA, USA).

Figure 1. The WHC and breaking force of heat-induced gel of pork salt-soluble proteins with various BSDF concentrations. WHC: water-holding capacity; BSDF: bamboo shoot dietary fibre. Different letters (a–d) indicate significant differences (p < 0.05) among the samples with various BSDF concentrations.

Figure 1. WHC y fuerza de rotura del gel, inducidas por calor de proteínas de cerdo solubles en sal con diversas concentraciones de BSDF. WHC: capacidad de retención de agua; BSDF: fibra dietética de brotes de bambú. Las letras diferentes (a-d) indican diferencias significativas (p < 0.05) entre las muestras con varias concentraciones de BSDF.
3.2. Breaking force

As shown in Figure 1, BSDF had a significantly positive effect on the breaking force. The breaking force significantly increased when the BSDF concentration increased from 0% to 2% ($P<0.05$), while the breaking force had no significant increase ($P>0.05$) when the BSDF concentration increased from 2% to 4%. Similar results were found by Zhuang et al. (2018), who reported that the addition of various amounts of sugarcane dietary fibre could increase the breaking force of myofibrillar proteins. Cruz et al. (2010) and Han and Bertram (2017) reported that some dietary fibres acted as “active” fillers and binders, thus promoted to the formation of a stronger gel network through nonspecific interactions with the primary gelling component. The improved breaking force could be attributed to the hydrophilic polysaccharide components of BSDF. Hydrophilic groups can be effectively combined with water to increase the number of active groups, such as sulfhydryl groups or the polar amino acid residues, and change the interaction forces between proteins to promote the occurrence of cross-linking reactions for forming more stable network structure during thermal induced gelation of SSP (Hayakawa et al., 2012). However, the excess addition of dietary fibres might produce the excessive aggregation of protein-polysaccharides, leading to the disruption or dilution of the protein matrix during thermal induced gelation (Cardoso, Mendes, & Nunes, 2007; Debusca, Tahergorabi, Beamer, Matak, & Jaczynski, 2014).

3.3. Particle size

The particle size and the particle size distribution of SSP with different amounts of BSDF are shown in Figure 2(a and b). The average particle size and particle size distribution of pork salt-soluble proteins (SSP) with various BSDF concentrations. (a): the average particle size of pork salt-soluble proteins with various concentrations of BSDF; (b): the particle size distribution of pork salt-soluble proteins with various concentrations of BSDF. BSDF: bamboo shoot dietary fibre. Different letters (a–c) indicate significant differences ($P<0.05$) between samples with various BSDF concentrations.

**Figure 2.** The average particle size and particle size distribution of pork salt-soluble proteins (SSP) with various BSDF concentrations. (a): the average particle size of pork salt-soluble proteins with various concentrations of BSDF; (b): the particle size distribution of pork salt-soluble proteins with various concentrations of BSDF. BSDF: bamboo shoot dietary fibre. Different letters (a–c) indicate significant differences ($P<0.05$) between samples with various BSDF concentrations.

**Figura 2.** Tamaño promedio de partícula y distribución del tamaño de partícula de las proteínas de cerdo solubles en sal (SSP) con varias concentraciones de BSDF. A: tamaño medio de partícula de proteínas de cerdo solubles en sal con diversas concentraciones de BSDF; B: distribución del tamaño de partícula de proteínas de cerdo solubles en sal con diversas concentraciones de BSDF. BSDF: fibra dietética de brotes de bambú. Las letras diferentes (a–c) indican diferencias significativas ($P<0.05$) entre las muestras con varias concentraciones de BSDF.
b). Compared to the control, the average particle size of SSP increased significantly ($P<0.05$) as the BSDF addition increased from 0% to 2%. While no significant differences ($P>0.05$) were observed in the average particle size of SSP among the control, T3 and T4. Figure 2(b) shows that the particle size distribution of T1 and T2 moved to both sides and T3 and T4 moved to the centre compared to T0. These results suggested that BSDF has significant effects on the average particle size and distribution of SSP. Gao et al. (2016) reported that a smaller particle size was unfavourable for the water retention capacity of the gel. The WHC of gels was associated with the formation of the gel network structure. A higher WHC value demonstrated that the gels had a much more stable three-dimensional network structure. This means that the larger particle size was favourable to the three-dimensional network structure. The appropriate addition of fibres may promote the formation of a stronger gel network through nonspecific interactions with the primary gelling components (Cruz et al., 2010; Han & Bertram, 2017). The particle size of T1 and T2 gradually increased and the particle size distribution became more concentrated, which indicates that the addition of BSDF contributes to the gel-forming ability and promotes the formation of the hydrophobic interactions and hydrogen bonds. These interactions may give rise to the effects on particle size distribution. However, excessive addition of BSDF (T3 and T4) did not affect in the average particle size of SSP compared with the control, which may be due to the destruction of non-covalent associative forces, such as electrostatic and hydrophobic interactions and hydrogen bonds, resulting in more protein aggregates (Feng et al., 2018; Gao et al., 2016). These results were consistent with above result of breaking force, indicating that the appropriate amount of BSDF can promote gel formation while excessive amounts of BSDF had a negative on the formation of the gel.

3.4. DSC

Heat-induced irreversible protein thermal transitions were a prerequisite for gelation (Debusca et al., 2013). Generally, three typical thermal transition temperatures are observed for muscle proteins as follows: 43–67°C was assigned to myosin and its subunits, 67–69°C was assigned to sarcoplasmic proteins and 71–83°C was assigned to actin (Ma et al., 2012). Ma et al. (2013) and Yao et al. (2017) reported that SSP were mainly composed of myosin. As shown in Figure 3 and Table 1, three typical thermal transition temperatures of SSP without BSDF

**Figure 3.** Differential scanning calorimetry (DSC) thermograms of pork salt-soluble proteins (SSP) with various BSDF concentrations. BSDF: bamboo shoot dietary fibre.

**Figure 3.** Termogramas de calorimetría diferencial de barrido (DSC) de proteínas de cerdo solubles en sal (SSP) con diversas concentraciones de BSDF. BSDF: fibra dietética de brotes de bambú.

**Table 1.** Denaturation temperature ($T_{\text{peak}}$) and endothermic enthalpy ($\Delta H$) of pork salt-soluble proteins (SSP) with various BSDF concentrations.

| Samples | $T_{\text{peak}1}$ (°C) | $T_{\text{peak}2}$ (°C) | $T_{\text{peak}3}$ (°C) | $\Delta H_1$ (J/g) | $\Delta H_2$ (J/g) | $\Delta H_3$ (J/g) |
|---------|--------------------------|--------------------------|--------------------------|-------------------|-------------------|-------------------|
| T0      | 45.30 ± 0.13$^d$         | 55.01 ± 0.44             | 63.23 ± 0.59$^d$         | 0.0535 ± 0.042    | 0.0922 ± 0.0026   | 0.0372 ± 0.0103   |
| T1      | 48.56 ± 0.23$^c$         | 55.07 ± 0.22             | 64.54 ± 0.93$^c$         | 0.0398 ± 0.0003   | 0.0676 ± 0.0062   | 0.0195 ± 0.0111   |
| T2      | 50.28 ± 0.34$^b$         | 54.85 ± 1.10             | 65.81 ± 0.13$^ab$        | 0.0265 ± 0.0023   | 0.0499 ± 0.0023   | 0.0371 ± 0.0113   |
| T3      | 51.03 ± 0.11$^a$         | 55.87 ± 0.26             | 66.62 ± 0.29$^a$         | 0.0217 ± 0.0006   | 0.0247 ± 0.0023   | 0.0342 ± 0.0030   |
| T4      | 52.65 ± 0.35$^bc$        | 55.06 ± 0.88$^bc$        | 65.06 ± 0.88$^bc$        | 0.1826 ± 0.0033   | 0.0587 ± 0.0124   | 0.0342 ± 0.0030   |

BSDF: bamboo shoot dietary fibre; T0: SSP; T1: SSP+1% BSDF; T2: SSP+2% BSDF; T3: SSP+3% BSDF; T4: SSP+4% BSDF. Different letters (a–d) in the same column indicate significant differences ($P < 0.05$) between samples with various BSDF concentrations.
were found at 45.30°C (Tpeak1), 55.01°C (Tpeak2), and 63.23°C (Tpeak3). Previous studies reported that the Tpeak1 transition at 45.30°C was ascribed to subfragment 1 (S-1) of the myosin head (heavy meromyosin), the Tpeak2 transition at 55.01°C was ascribed to subfragment 2 (S-2) of the myosin head (heavy meromyosin), and the Tpeak3 transition at 63.23°C is due to the myosin tail (light meromyosin) (Lőrinczy & Belágyi, 1995; Nagai et al., 1999). These transition temperatures indicated protein conformational changes during thermal denaturation, one of which was transformed from an α-helix into a random coil for promoting thermal induced gelation of SSP.

Thermal denaturation temperatures (Tpeak) reflect the thermal stability of muscle proteins. A lower Tpeak signifies greater susceptibility to thermal denaturation. Endothermic enthalpy (∆H) reflects the degree of denaturation of muscle proteins. Changes in the endothermic enthalpy (∆H) of the reaction were mainly due to the rupture of intramolecular hydrogen bonds (Chen, Xu, & Wang, 2007). As shown in Table 1, the addition of BSDF significantly affected the thermal stability of myosin. First, the Tpeak1 increased significantly (P<0.05) and the ∆H1 had no change with the addition of BSDF, which indicated that the addition of BSDF improved the thermal stability of S-1 of the myosin head, but the hydrogen bonds may be not disrupted. Debusca et al. (2014) reported that the long chains of fibre probably made water embedded in the gel matrix and then the water was further stabilized by chemical bonding with proteins. Second, the endothermic enthalpy (∆H2) was reduced significantly (P<0.05), but the denaturation temperature (Tpeak2) did not change with the addition of BSDF. This indicated that BSDF could enhance the interactions in relation to S-2 of the myosin head, produce a large number of hydrogen bonds, and provide enhanced thermal stability (Pighin, Sancho, & Gonzalez, 2008). When the addition of BSDF increased to 4%, the ∆H2 decreased sharply and the Tpeak1 increased continuously, which resulted in the merger of peak1 and peak2. The denaturation temperature occurred at 52.65°C and the endothermic enthalpy was 0.1826 J/g. Finally, the values of Tpeak3 and ∆H3 increased significantly (P<0.05), which suggested that the thermal stability of myosin tails improved with an increase in BSDF. Deng et al. (2002) found that the thermal stability of myosin tails was positively correlated to the WHC. These results suggested that the addition of BSDF may contribute to increase the WHC of SSP. The addition of BSDF could affect the thermal stability of SSP and probably interact with myosin during thermal denaturation, leading to the changes in the breaking force.

3.5. SDS-PAGE

The electrophoretic patterns of SSP with various amounts of BSDF are shown in Figure 4. The protein profiles mainly consisted of myosin heavy chains (MHC) at 205 kDa, myosin light chains (MLC) at 16–25 kDa, actin at 45 kDa and other proteins, such as tropomyosin (34–36 kDa) and α-actinin (95 kDa), which were mainly connected with myosin and actin (Asghar, Samejima, Yasui, & Henrickson, 1985; Margossian & Lowey, 1982). MHC become aggregated and formed a gel by removal from the expelled water following heat treatment while MLC was dissociated and solubilized to remain in a dissolved state after heat treatment, which suggested that MHC was involved in formation of heated-induced gelation and was responsible for the functional properties of proteins (DeFreitas, Sebranek, Olson, & Carr, 1997; Samejima, Yamauchi, Asghar, & Yasui, 1984). The MHC band intensity was enhanced when BSDF was increased from 1% to 2% and then weakened in line with the control.
group when BSDF was increased from 3% to 4%. This suggested that moderate amounts of BSDF can enhance the interaction of MHC with BSDF, but excessive amounts of BSDF may hinder the connection between MHC and BSDF. The number of MLC bands increased from 1 to 3 and the actin band enhanced visibly with increasing BSDF. These results showed that the electrophoretic banding pattern for SSP sol were affected by the addition of BSDF. BSDF as an “active” filler and binder, may promote to the contact of α and β shows the percentages of the four secondary groups more likely interacted with other side chains (Sinthusamran, Benjakul, Swedlund, & Hemar, 2017), suggesting that the interaction occurred between proteins and BSDF. Zhuang et al. (2018) reported that sugarcane insoluble dietary fibre could also significantly affect the hydrophobic aliphatic amino acid residues to improve the hydrophobic interactions of aliphatic residues. Zhang et al. (2018) reported that soluble nanofibre cellulose enhanced the peak intensity at 2,960 cm⁻¹ because of the addition of cNFC and the formation of hydrogen bonds between the protein and cNFC.

The amide I band was one of the most important information parts of the FTIR spectrum when analysing the characterization of protein secondary structure (Yao et al., 2017). Between the wave numbers 1,600 cm⁻¹ and 1,700 cm⁻¹, the characteristic absorption peak of the amide I region was caused by the C = O stretching vibration of the amide group and the C – N stretching vibration (Carbonaro & Nucara, 2010; Hu, Zhao, Sun, Zhao, & Ren, 2011). The protein secondary structure contained α-helix, β-sheet, β-turn and random coil structure, respectively. Importantly, the changes of α-helix and β-sheet fractions were closely related to the gelation process (Bouraoui, Nakai, & Li-Chan, 1997). According to previous reports (Byler, Brouillet, & Susi, 1986), the 1,600–1,640 cm⁻¹ band was regarded as β-sheets, the 1,640–1,650 cm⁻¹ band was regarded as random coils, the 1,650–1,660 cm⁻¹ band was regarded as α-helices, and 1,660–1,695 cm⁻¹ band was regarded as β-turns.

Table 2 shows the percentages of the four secondary structures. Compared to the SSP gels (T0), the addition of BSDF could significantly (P < 0.05) increase the β-sheet and random coil structures and decrease the percentages of α-helix and β-turn structures. Moreover, the percentage of α-helices significantly decreased, and the percentage of β-sheets significantly (P < 0.05) increased with the increase in BSDF from 1% to 4%. Feng et al. (2018) explained that the loss of α-helix structures and the gain of β-sheet structure were closely related to the unfolding of the muscle proteins. Some exposed active groups such as such as sulfhydryl and hydrophobic groups, induced the decrease of α-helical content. Liu, Zhao, Xie, and Xiong (2011) reported that the unfolded α-helices and β-sheets formation favoured gel formation. These results were similar to those findings by Sánchez-González, Rodríguez-Casado, Careche, and Carmona (2009), who reported the changes in protein secondary structures of surimi gelation when different concentrations of wheat dietary fibre were added. They found that the β-sheet content of gelation had a great increase. Sánchez-González et al. (2009) considered that this change induced by addition of wheat dietary fibre resulted from the perspective of protein structure.

### 3.6. FTIR

The FTIR spectra of SSP gels with various concentrations of BSDF are shown in Figure 5. Two characteristic absorption peaks (Peak 1 and Peak 2) appeared with the addition of BSDF. The intensity of the peaks revealed a sustained increasing trend with an increase in BSDF. The pork salt-soluble protein gel had no peak observed at the region of 3,700–3,600 cm⁻¹ and 1,100–1,000 cm⁻¹. When BSDF was added, Peak 1 arose due to the stretching vibration of the O-H bond in cellulose. Similarly, Peak 2 was due to the symmetrical and asymmetric stretching vibration of the C-O bond in cellulose and hemicelluloses (Yu, Liu, Yu, Wu, & He, 2012). Both of the above peaks were characteristic absorption peaks of BSDF. Peak 3 at 2,900–2,800 cm⁻¹ was assigned to CH₃ and CH₂ bending vibrations in cellulose, proteins, peptides and aliphatic amino acids (Yu et al., 2012; Zhuang et al., 2018). The peak intensity was obviously enhanced compared to the infrared spectrum of the SSP gel without BSDF (T0). These results indicated that the intensity and wavenumbers of Peak 3 were significantly affected by the addition of BSDF and the CH₂ groups more likely interacted with other side chains (Sinthusamran, Benjakul, Swedlund, & Hemar, 2017), suggesting that the interaction occurred between proteins and BSDF. Zhuang et al. (2018) reported that sugarcane insoluble dietary fibre could also significantly affect the hydrophobic aliphatic amino acid residues to improve the hydrophobic interactions of aliphatic residues. Zhang et al. (2018) reported that soluble nanofibre cellulose enhanced the peak intensity at 2,960 cm⁻¹ because of the addition of cNFC and the formation of hydrogen bonds between the protein and cNFC.

![Figure 5. FTIR of pork salt-soluble proteins (SSP) heat-induced gel with various concentrations of BSDF. BSDF: bamboo shoot dietary fibre.](image)

**Figure 5.** FTIR of pork salt-soluble proteins (SSP) heat-induced gel with various concentrations of BSDF. BSDF: bamboo shoot dietary fibre.

| Samples | α-helix (%) | β-sheet (%) | β-turn (%) | random coil (%) |
|---------|-------------|-------------|------------|-----------------|
| T0      | 47.87 ± 2.54₄ | 31.33 ± 1.48₄ | 20.24 ± 0.31₃ | 1.49 ± 0.12₂ |
| T1      | 45.27 ± 0.65₄ | 35.06 ± 0.21₁ | 17.43 ± 0.73₁ | 2.24 ± 0.09₁  |
| T2      | 41.69 ± 3.05₄ | 38.19 ± 3.81₄ | 17.40 ± 0.34₁ | 2.71 ± 0.25₁  |
| T3      | 35.16 ± 2.54₄ | 39.58 ± 0.81₁ | 22.93 ± 1.61₁ | 2.23 ± 0.11₁  |
| T4      | 33.11 ± 0.57₄ | 45.05 ± 1.41₁ | 20.48 ± 0.95₁ | 1.37 ± 0.10₁  |

BSDF: bamboo shoot dietary fibre; T0: SSP; T1: SSP+1% BSDF; T2: SSP+2% BSDF; T3: SSP+3%; T4: SSP+4% BSDF. Different letters (a–c) in the same column indicate significant differences (P < 0.05) between samples with various BSDF concentrations.

**Tabla 2.** Contenido de la estructura secundaria de proteínas del gel inducido por calor de las proteínas de cerdo solubles en sal (SSP) con varias concentraciones de fibra dietética de brotes de bambú (BSDF).

**Tabla 2.** Protein secondary structure content of pork salt-soluble proteins (SSP) heat-induced gel with various BSDF concentrations.
matrix dehydration, i.e. wheat dietary fibre either acted as a dehydrating agent of the gel protein matrix or an absorber of the water released from this matrix upon the formation of β-structure. These results regarding the secondary structure changes indicate that the BSDF addition was related to the improvement of gel quality and WHC. The above results make it clear that when BSDF is added to pork salt-soluble proteins, the gel properties are improved. The higher WHC value and breaking force were indicators for the formation of a more uniform and stable gel structure. Moreover, the stronger interaction between SSP-BSDF were obtained from the changes of thermal stability (Table 1) and protein bands (Figure 4). The larger proportion of β-structures formation contributed to the functional properties of the gel. Therefore, BSDF could significantly improve the WHC and gel strength by the cross-linking interaction with SSP.

4. Conclusion

The experiments performed in the current study indicated that BSDF has significant effects on the gel properties, thermal transition temperature and secondary structures of pork salt-soluble proteins. The addition of 2% BSDF could effectively improve the WHC and breaking force of heat-induced gel and increase the particle size of SSP composite sols. DSC showed that the appropriate addition of BSDF (2%) can produce cross-linking reactions with pork salt-soluble proteins to enhance the gel structure. SDS-PAGE showed that there were interactions between BSDF and myosin and/or actin. FTIR showed that the α-helix content was significantly decreased while the β-sheet content was significantly increased when BSDF was increased. These results suggested that BSDF can improve the gel properties and WHC of pork SSP and could be used in the meat products.

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Declaration of interest statement

The authors have no conflict of interest.

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