

High-moisture extrusion of peanut protein-/carrageenan/sodium alginate/wheat starch mixtures: Effect of different exogenous polysaccharides on the process forming a fibrous structure

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ABSTRACT

Of particular interest in this present work is to gain knowledge about the process forming the fibrous structure of peanut protein affected by carrageenan (CA), sodium alginate (SA), and wheat starch (WS), respectively. The multi-scale structure and physicochemical properties of exogenous polysaccharide modified-peanut protein powder (PPP) in the six extrusion zones were widely investigated to explain the process forming a fibrous structure. Results showed that 0.1% CA could significantly improve the tensile resistant force (about 1.92 kg), but it was not conducive to the fiber orientation. 0.1% SA could significantly improve the fibrous degree (up to about 1.24), springiness, and the tensile resistant force, but the hardness and chewiness increased significantly. The larger the amount of WS (0–8%), the lower the fibrous degree was, and the hardness and chewiness were both significantly reduced. Furthermore, 0.1% CA, 0.1% SA or 2% WS can protect the molecular chains of conarachin and arachin from the thermal transition. The selected exogenous polysaccharides, especially the 2% WS, can promote the aggregation of protein molecules mainly by breaking the intramolecular disulfide bonds, enhancing the hydrophobic interactions and increasing the apparent viscosity to stabilize the newly formed conformation. The protein subunit with a molecular weight of 22 kDa can be largely impacted by the exogenous polysaccharides due to the degradation of the subunits at 42 kDa and 39 kDa. Additionally, 2% WS can further promote the synthesis of protein subunits with larger molecular weight (66 kDa). The three exogenous polysaccharides can promote the opening of the α -helix and gradually converting into the β -turn and random coil structure. When mixing the PPP with 0.1% CA, the four protein secondary structures were present with β -sheet > α -helix > β -turn > random coil in the extrudate. While mixing the PPP with 0.1% SA or 2% WS, the order was β -sheet > β -turn > α -helix > random coil. This study has provided useful information for producing vegetable protein-based meat substitutes with rich fibrous structure by adding the exogenous polysaccharides.

1. Introduction

High-moisture extrusion technology for moisture contents higher than 40% has the advantages of lower energy input, no waste discharge, high efficiency, and higher quality of the texturized products, thus it is now considered as one of the best choices for developing vegetable protein-based meat substitutes (Akdogan, 1999; J.; Zhang, Liu, Liu, et al., 2018). According to our previous study, by texturizing the peanut protein powder (PPP, obtained as a by-product of the edible oil industry), it is possible to produce the peanut protein-based meat

substitutes through high-moisture extrusion technology (J. Zhang, Liu, Zhu, & Wang, 2018). However, there were no meat substitutes based on the high-moisture extruded texturized-peanut protein on the market so far. The market for vegetable protein-based meat substitutes is still quite small, probably because the meat substitutes currently available do not meet consumer preferences regarding sensory quality (Wild et al., 2014). To obtain a larger market share, peanut protein-based meat substitutes need to be comparable with the rich fibrous structure offered by real animal meat products (Asgar, Fazilah, Huda, Bhat, & Karim, 2010).

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The fibrous structure of peanut protein may be modified or improved by adjusting the process conditions or by incorporating food additives. Exogenous polysaccharides are one of the main additives generally used in food industries for texture optimization. Interactions with polysaccharides will influence the functional properties of food proteins, such as solubility (Ye, 2010), surface activity (Wong, Day, & Augustin, 2011), conformational stability (Pedro Guerrero, Kerry, & de la Caba, 2014), gel-forming ability (Velde, Hoog, Oosterveld, & Tromp, 2015), emulsifying properties (Evans, Ratcliffe, & Williams, 2013) and foaming properties (Schmitt, Sanchez, Desobrybanon, & Hardy, 1998). During the denaturation of protein in the extrusion process, the buried reactive sites in the protein molecular chains become exposed and the structure of the protein became flexible, which allows configurational adjustments and favors the protein-polysaccharide interactions (Chen, Wei, & Zhang, 2011; Ozbolat & Hospodiuk, 2016). Polysaccharides can be used as a crosslinker to alter the conformation of proteins, interact with them by binding to protein side groups through Maillard reaction, and produce a protein network structure (Caillard, Remondetto, & Subirade, 2010; Xin, Campbell, & Euston, 2009).

Carrageenan (CA) is a typical anionic polysaccharide, which more strongly interacts with protein aggregates to form a thermo-reversible gel (Chang, Li, Wang, Bi, & Adhikari, 2014). It is widely used in the food industry as a thickener, stabilizer and gelling agent. CA can increase the viscosity of the molten soybean protein mass inside the extruder by trapping more water in the interstitial spaces of the gel, which led to a compact protein network with thick uniform layers (Palanisamy, Töpfl, Aganovic, & Berger, 2017).

Sodium alginate (SA) is a sodium salt carbohydrate extracted from natural brown algae. It is capable of adsorbing water 200 to 300 times to its own weight, forming a thermo-irreversible viscoelastic colloid and giving the product a more delicate structure (Yao et al., 2017). Pan et al. (Pan et al., 2015) studied the thermo-gel properties of soy protein isolate/SA blends, demonstrating that SA can affect the speed of phase separation and improve the thermomechanical properties of blended gel membranes.

Starch is the most commonly selected exogenous polysaccharide for extrusion formulations and plays a major role in the formation of fibrous structure, but the adding amount should not exceed 10% of the feed mixture (Taranto, Kuo, & Rhee, 2006; H.; Wang, Ma, Zhou, & Lin, 2002). Zhang et al. (W. Zhang, Li, Zhang, Drago, & Zhang, 2016) showed that the starch gelatinization endotherm was significantly related with the fibrous structure, tensile properties, and the springiness of the products. Furthermore, wheat starch (WS) is more conducive in improving its texture characteristics.

However, data on peanut protein/exogenous polysaccharide mixtures during the high-moisture extrusion is limited and insufficient. Therefore, the objectives of this research are to compare the effect of exogenous polysaccharide types and contents on the color and textural properties of the high-moisture texturized peanut protein; to investigate the microstructure for the high-moisture extrusion process of peanut protein/exogenous polysaccharide mixtures using scanning electron microscope (SEM); to differentiate the flowing behavior combining with the thermal transition and decomposition properties of the peanut protein/exogenous polysaccharide mixtures in each extrusion zone; and to examine the effect of exogenous polysaccharides on the changes in protein conformation such as protein-protein interactions, in the molecular weight distribution, and in the secondary structure during the high-moisture extrusion process.

2. Materials and methods

2.1. Materials

Peanut protein powder (PPP) was supplied by Qingdao longevity Food Co., Ltd (Qingdao, China). Protein, lipid, and water contents of the PPP employed in this study were 60.75 ± 0.748 g/100 g (dry

basis), 6.95 ± 0.044 g/100 g (dry basis), and 5.82 g/100 g, respectively. The exogenous polysaccharides including carrageenan (CA) (thermo-reversible polysaccharide, extracted from marine red algae, purity of 99%, 10 mg/mL in water, molecular weight of 789), sodium alginate (SA) (thermo-irreversible polysaccharide, extracted from brown seaweed, purity of 94%, 10 mg/mL in water, molecular weight of 216) and native wheat starch (WS) (extracted from wheat, total content of 90%, amylose/amylopectin ratio = 1:3) were all obtained from Solarbio Technology Co., Ltd. (Beijing, China).

All the materials were ground (MM400, Retsch, China) and sieved (80 mesh). A mixer (HWJ25, Shandong Yinying Cooking Machinery Co., Ltd, China) was used for mixing the blends of PPP, CA, SA, and WS at different ratios (dry basis ratios) (Table S1) according to previous reports (Palanisamy et al., 2017; Yao et al., 2017; W.; Zhang et al., 2016). The conditioned mixtures were packed in plastic bags and kept at 4 °C overnight to equilibrate. The sample without exogenous polysaccharides was designated as the control.

2.2. Extrusion experiments and sample preparation

All of the extrusion experiments were conducted using a pilot-scale, co-rotating and intermeshing twin-screw food extruder (FMHE36-24, FUMACH, China). The PPP-CA/SA/WS mixtures were fed into the extruder at a constant speed of 6 kg/h (dry basis). Based on preliminary experiments (J. Zhang, Liu, Zhu, et al., 2018) and the working stability of the extruder, the feed moisture was selected as 55% (dry basis), the screw speed was 210 rpm, and the extruder barrel temperatures were kept at 60, 90, 155, 155, and 110 °C from the first zone to the fifth zone, respectively. A long cooling die was connected to the end of the extruder barrel and kept at 70 °C by running water.

According to the results of quality analysis in the section of 3.1 and 3.2, for the mixtures of PPP with 0.1% CA, PPP with 0.1% SA, and PPP with 2%WS, after reaching steady state (the extruder motor torque and die pressure remained constant values for 10 min), the extrusion operation was intentionally shut down (dead-stop) according to Chen et al. (Chen et al., 2011). Samples in the feeding zone (raw material), mixing zone, melting zone, die, cooling zone, and extrudate were collected and immediately sealed by a vacuum packaging machine (Lynx32, HENKELMAN, Netherlands) and frozen at -18 °C for future analysis.

2.3. Textural properties

The textural properties of the fresh extrudates including the cross-wise shear force, lengthwise shear force, fibrous degree, tensile resistant force, hardness, and springiness were measured with a TA.XT2 Texture Analyzer (Stable Micro Systems, UK) according to Fang et al. (Fang, Zhang, & Wei, 2014).

2.4. Microstructure

The microstructure of the sample in each extrusion zone was conducted by a scanning electron microscopy (SU8010, Hitachi Ltd., Tokyo, Japan). Frozen samples were kept at 30 min at room temperature prior to sample preparation. Structures were cut into small rectangles (the longest edge 8 mm) and mounted in a glutaraldehyde solution (2.5 v%) for 48 h. The next day, the sample was rinsed with cold phosphate buffer (0.1 M) and followed by a secondary fixation in osmium tetroxide (1%) for 2 h. Then samples were critical point dried in CO₂. Dehydrated samples were coated with gold particles with a sputter coater (IB-5, Hitachi Ltd., Tokyo, Japan). The images of the samples were taken with the scanning electron microscopy at magnifications of $\times 300$ and $\times 1500$, respectively, according to Grabowska et al. (Grabowska, Tekidou, Boom, & Goot, 2014).

2.5. DSC, TG and DTG measurements

The freeze-dried samples were ground then passed through an 80-mesh sieve. One gram of the powder sample was weighed into a 4-mL centrifuge tube, then deionized water was added until the water content of the mixture reached to 55% (w/w), the preferred moisture level for extrusion (J. Zhang, Liu, Zhu, et al., 2018). The differential scanning calorimetry (DSC) curves were obtained using a Thermal Analysis Systems Model Q-200 (TA Instruments, New Castle, DE, USA). The samples were first conditioned in a hermetic high-pressure capsule kit (TA Instruments), weighed (8–10 mg) using a precision balance (± 0.01 mg, Analytical Plus, Mettler-Toledo International Inc., Columbus, OH, USA), then heated at a rate of 5 °C/min from 25 to 130 °C in an inert atmosphere (50 mL/min of dry N₂).

Non-isothermal degradation measurements were made using TG/DTA (thermogravimetric/differential thermal analysis; Pyris Diamond, PerkinElmer Inc., Waltham, MA, USA). Tests were ran from room temperature up to 500 °C at a heating rate of 10 °C/min in a nitrogen atmosphere (50 mL/min) to avoid thermo-oxidative reactions. The mass loss curves were divided into five parts and the residue mass and peak temperature were recorded according to our previous study (J. Zhang et al., 2017).

2.6. Rheological measurements

The samples mentioned in section 2.6 were reconstituted at 20% (w/w) concentration in deionized water and gently stirred for 2 h or until dissolution was completed, and then stored overnight at 4 °C prior to testing. The steady shear test was performed at 25 °C, over the shear rate range of 1–100 s⁻¹ using a rheometer (Physica MCR 301, Anton Paar, Austria) with aluminum parallel plate geometry (50 mm diameter, 1 mm gap). A thin layer of silicone oil was added on the edge of the sample to avoid evaporation. All the tests were carried out allowing the sample to equilibrate for 2 min to eliminate the effect of loading of the sample on the plate. Shear stress (τ), shear rate ($\dot{\gamma}$), and apparent viscosity (η) were measured in these tests (Chang et al., 2014). The steady shear flow curves were fitted by Power law according to equation (2) below. The flow behavior index (n) and the consistency coefficient (K) were reported.

$$\tau = K \cdot \dot{\gamma}^n$$

2.7. Design of extracting systems and determination of protein solubility

The protein solubility of the samples described in section 2.6 was analyzed using the methods of Liu et al. and Chen et al. (Chen et al., 2011; Liu & Hsieh, 2008). The buffer systems used for protein extraction were as follows: (1) 0.035 mol/L pH 7.6 phosphate buffer solution (P); (2) 8 mol/L urea in the phosphate buffer solution (P + U); (3) 0.1 mol/L 2-mercaptoethanol (2-ME) in the phosphate buffer solution (P + M); (4) 1.5 g/100 mL sodium dodecyl sulphate (SDS) in the phosphate buffer solution (P + S); (5) 8 mol/L urea and 0.1 mol/L 2-ME in the phosphate buffer solution (P + U + M); (6) 1.5 g/100 mL SDS and 8 mol/L urea in the phosphate buffer solution (P + U + S); (7) 1.5 g/100 mL SDS and 0.1 mol/L 2-ME in the phosphate buffer solution (P + S + M); and (8) 8 mol/L urea, 1.5 g/100 mL SDS and 0.1 mol/L 2-ME in the phosphate buffer solution (P + U + S + M). The total protein content of the samples was determined using the Kjeldahl method, and the soluble protein in the supernatant by the Lowery method using a microplate reader assay at 650 nm (SpectraMax, Molecular Devices, San Jose, CA, USA). The protein solubility was calculated as the ratio of soluble protein in the supernatant to the total protein in the samples. Each measurement was performed in triplicate.

2.8. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed following the method of He et al. (He et al., 2014). Electrophoresis was conducted using the Mini-PROTEAN® System (Bio-Rad, Hercules, CA, USA). Images for the protein electrophoresis spectrum were taken using an AlphaEase FC gel imaging system (Genetic Technologies Inc, Miami, FL, USA). Densitometry analysis was performed using Image Studio Lite (version 5.2, LI-COR Biosciences, Lincoln, NE, USA) for the relative quantification of the different protein bands (66, 42, 39, 38, 27, 26, 22, and 18 kDa) (Pal et al., 2018).

2.9. Fourier transform infrared (FTIR) spectroscopy determination

FTIR analysis of the samples sieved through an 80-mesh sieve were recorded using a spectrometer (TENSOR 27, Bruker, Germany). The classic KBr-disk method was used to prepare analyzed samples, by using pre-dried 1 mg of the samples and 100 mg of KBr to obtain the compressed pellets. Spectra were collected in the 500–4000 cm⁻¹ infrared spectral range at room temperature. Each spectrum was an average of 64 scans at a 4 cm⁻¹ resolution. The amide I band (1600–1700 cm⁻¹) in each spectrum was analyzed using Omnic software (version 8.0, Thermo Nicolet Inc., MA, USA), and Peakfit software (version 4.12, SPSS Inc., Chicago, USA) was used to analyze the secondary structure changes.

2.10. Statistical analysis

All data were analyzed using analysis of variance (ANOVA) and Pearson correlation coefficients (r) between the extrusion response parameters and the textural properties of extrudates were performed using the Statistical Product and Service Solutions software (version 19.0, SPSS Inc., Chicago, USA). The comparisons between treatments were evaluated using Duncan's test. Statistical significance was set at a 0.05 probability level.

3. Results and discussion

3.1. Effect of exogenous polysaccharides on the textural properties of the extrudates

As shown in Table 1, when the contents of CA increased from 0 to 0.5%, the crosswise shear force and lengthwise shear force both increased significantly, but when the content of CA increased to 1%, their values both decreased significantly. It was found that CA could lead to a significant decrease in the fibrous degree (~ 0.1), but it did not have a significant change with the contents of CA increasing from 0.05% to 1% (Palanisamy et al., 2017). On the other hand, when increasing the content of SA from 0 to 1%, the crosswise shear force and lengthwise shear force both increased significantly. When the content of SA was 0.1%, the fibrous degree can reach up to 1.24, which was significantly higher than that without exogenous polysaccharide addition. WS had no significant effect on the crosswise shear force, but the lengthwise shear force increased significantly as the WS content increased from 0 to 8%. The fibrous degree decreased gradually and significantly with the increase in WS content from 0 to 8%. Therefore, excessive content of WS has disadvantage for the orientation of the fibrous structure in the extrusion direction.

It can be seen in Table 1, the coefficient of variation of the tensile resistant force was 42.53%, which proved that the exogenous polysaccharides can affect the tensile properties significantly. When the content of CA was 0.1%, the tensile resistant force reached the maximum value of about 1.92 kg. As the contents of CA increased from 0.1% to 1%, the tensile resistant force decreased gradually. When the content of SA was 0.05%, the tensile resistant force was significantly

Table 1

Effect of the types and contents of exogenous polysaccharides on the textural properties of the high-moisture texturized peanut protein.

Mixtures	Exogenous polysaccharide contents (%)	Crosswise shear force (kg)	Lengthwise shear force (kg)	Fibrous degree	Tensile resistant force (kg)	Hardness (kg)	Springiness
PPP	0	0.48 ± 0.04ef	0.41 ± 0.03 g	1.17 ± 0.06b	0.90 ± 0.11c	28.18 ± 0.37 g	0.83 ± 0.01cd
PPP + CA	0.05	0.53 ± 0.03cd	0.49 ± 0.01cde	1.08 ± 0.10c	0.59 ± 0.11ef	32.28 ± 0.13d	0.95 ± 0.04a
	0.1	0.56 ± 0.00bc	0.54 ± 0.01 ab	1.04 ± 0.01cdef	1.92 ± 0.21a	29.93 ± 1.17ef	0.79 ± 0.00e
	0.5	0.55 ± 0.01bc	0.51 ± 0.00bcd	1.08 ± 0.03cd	0.82 ± 0.04cd	29.36 ± 0.46f	0.88 ± 0.01bc
	1	0.45 ± 0.03f	0.43 ± 0.03 fg	1.06 ± 0.01cde	0.47 ± 0.02f	32.03 ± 2.88f	0.91 ± 0.03b
PPP + SA	0.05	0.46 ± 0.03f	0.46 ± 0.02ef	0.99 ± 0.05ef	0.62 ± 0.11def	33.42 ± 2.46c	0.92 ± 0.03a
	0.1	0.64 ± 0.01a	0.51 ± 0.02bcd	1.24 ± 0.03a	1.09 ± 0.02b	35.91 ± 0.5b	0.86 ± 0.00bc
	0.5	0.53 ± 0.00cde	0.54 ± 0.02 ab	0.97 ± 0.05f	1.18 ± 0.06b	40.21 ± 0.18a	0.89 ± 0.01b
	1	0.59 ± 0.04b	0.56 ± 0.04a	1.04 ± 0.01cdef	1.16 ± 0.05b	30.28 ± 5.85bc	0.84 ± 0.05b
PPP + WS	2	0.49 ± 0.02def	0.42 ± 0.02 fg	1.16 ± 0.02b	0.60 ± 0.01ef	25.43 ± 0.85i	0.83 ± 0.05e
	4	0.50 ± 0.01def	0.47 ± 0.02de	1.05 ± 0.03cdef	0.77 ± 0.06cde	25.76 ± 0.76i	0.86 ± 0.03bc
	6	0.50 ± 0.02def	0.50 ± 0.03bcde	1.00 ± 0.02def	0.78 ± 0.16cde	30.58 ± 0.61e	0.94 ± 0.04a
	8	0.52 ± 0.02cde	0.53 ± 0.03abc	0.98 ± 0.05ef	0.78 ± 0.21cde	27.13 ± 0.34 h	0.82 ± 0.01de
	Variable amplitude	0.39–0.60	0.42–0.64	0.94–1.26	0.45–2.15	0.78–0.99	24.23–40.35
	Coefficient of variation (%)	10.28	10.25	7.98	42.53	6.11	14.01

Note: Different letters in the same column mean significant differences ($P < 0.05$).

lower than that with no exogenous polysaccharide. While increasing the SA content to 1%, it showed a significant increase in the tensile resistant force (higher than 1 kg). The tensile resistant force can be affected significantly by WS, but it didn't have a significant change with the increase of WS contents.

The hardness, springiness, and the chewiness has been improved significantly by the addition of CA or SA. At a CA content of 0.05%, the high-moisture texturized peanut protein showed a hardness of 32.28 kg, a springiness of 0.95, and a chewiness of 18.94 kg, showing that the addition of CA led to a harder and higher springiness structure than that without CA (Palanisamy et al., 2017). Increasing the CA content to 1%, the hardness, springiness, and the chewiness were significantly decreased. When the content of SA was 0.05%, the springiness was 0.92, however, as the SA content increased to 1%, the springiness decreased significantly. The results of CA and SA indicate that other types of covalent bonds besides disulphide bonds were formed (Table 5) leading to the decrease in the springiness (Xin et al., 2009). Adding a content of WS of 2%, 4%, and 8%, respectively, the hardness decreased significantly, but the springiness and chewiness did not have a significant change. Surprisingly, at a WS content of 6%, the hardness, springiness, and chewiness all increased significantly.

Thus, for the PPP with 0.1% CA had the largest tensile resistant force, while the highest fibrous degree was with the addition of 0.1% SA to PPP. Additionally, increasing the WS contents from 2% to 8%, the fibrous structure became poor. Based on the quality analysis, the mechanisms for improving the fibrous structure of high-moisture texturized peanut protein by 0.1% CA, 0.1% SA, and 2% WS have been further investigated as below.

3.2. Microstructure of peanut protein with different exogenous polysaccharides during the high-moisture extrusion process

Fig. 1 shows the SEM micrographs taken at the break surface of the samples in each extrusion zones. In the mixing zone, for the PPP with 0.1% CA, the structure became loose and some blocky gels were formed, while the gel structure became more compact for the PPP with 0.1% SA. After mixing the PPP with 2% WS, the structure was loosened and no gels formation were noticed.

In the melting zone, for the PPP with 0.1% CA, the gel structure became denser. For the PPP with 0.1% SA, some larger gels with a dense structure were formed. After mixing the PPP with 2% WS, some large gels with loose structure were formed.

In the die, for the PPP with 0.1% CA, some larger gels were found. For the PPP with 0.1% SA, the gel structure became denser and relatively flat. After mixing the PPP with 2% WS, the gel structure was loose.

In the cooling zone, for the PPP with 0.1% CA, the gel structure became denser but with no fiber orientation. For the PPP with 0.1% SA, the large gels were observed with a layered structure and the fiber orientation occurred. After mixing the PPP with 2% WS, the gels were further formed and cross-linked to each other representing the fiber orientation.

For the extrudate, for the PPP with 0.1% CA, it was difficult to find the fibrous structure other than a compact structure, which was consistent with the result of the fibrous degree. For the PPP with 0.1% SA, the oriented fiber structures were closely crosslinked together. After mixing the PPP with 2% WS, the fibrous structure became fine but with a poor cross-linking.

3.3. Differential scanning calorimetry (DSC) analysis of peanut protein with different exogenous polysaccharides during the high-moisture extrusion process

The DSC data on peanut protein/exogenous polysaccharide mixtures during the extrusion process are summarized in Fig. 2 and Table 2. Two endothermic peaks appeared in the DSC curves of each sample, corresponding to the denaturation of conarachin (low-temperature endotherm) and arachin (high-temperature endotherm) (Andres Colombo, Ribotta, & Leon, 2010). It can be seen in Fig. 2 that when the mixtures passing through extruder from the mixing zone to the melting zone, the endothermic peaks of conarachin and arachin were both greatly reduced due to the denaturation of protein molecules (D. Li & Swanson, 2013).

In the mixing zone, 2% WS could cause a significant decrease in the T_p value (the peak thermal transition temperature) and a significant increase in the ΔH value of conarachin (refer to Table 2). This result indicated that 2% WS accelerated the thermal transition of conarachin, but the energy required to open its molecular structure increased (S. Li, Wei, Fang, Zhang, & Zhang, 2013). 0.1% CA or 2% WS could lead to a significant decrease in the T_p value of arachin. However, all the three exogenous polysaccharides had no significant effect on the ΔH value of arachin, which was related to the relatively tight structure of arachin (Guo et al., 2012).

In the melting zone, all the three exogenous polysaccharides could significantly decrease the T_p value of conarachin. When mixing the PPP with 0.1% CA, the ΔH value of conarachin decreased significantly from 0.20 J g^{-1} to 0.10 J g^{-1} , indicating that 0.1% CA reduced the thermal stability of conarachin and increased the flexibility of its molecular structure (Yue, Cui, Shuttleworth, & Clark, 2012). While, 0.1% SA or 2% WS could lead to a significant increase of the ΔH of conarachin, making its molecular structure more stable and difficult to open (Gabrielaa et al., 2009). For the arachin, all the three exogenous

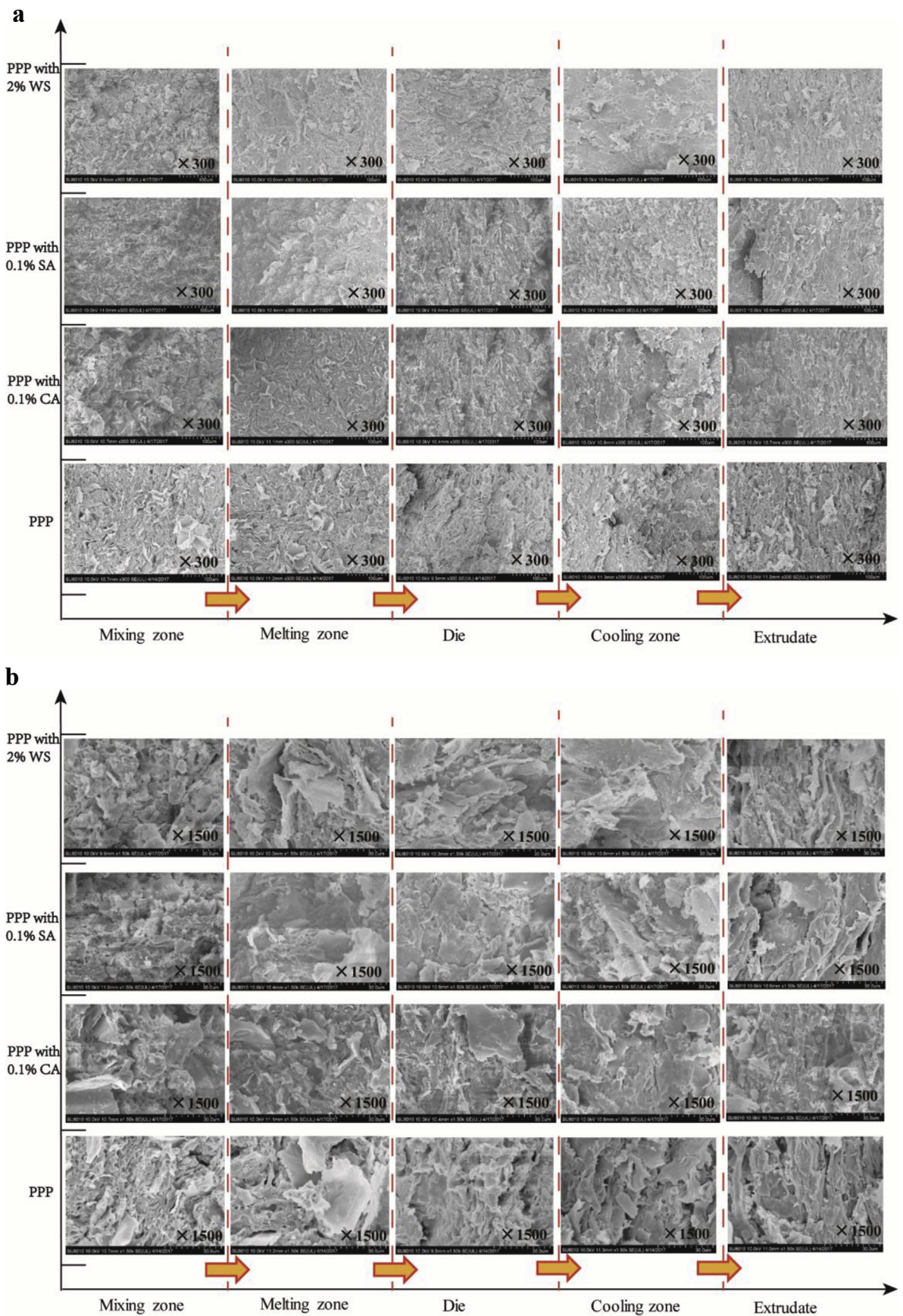


Fig. 1. Microstructure of PPP-CA/SA/WS mixtures during the high-moisture extrusion process obtained by SEM, a) $\times 300$, b) $\times 1500$.

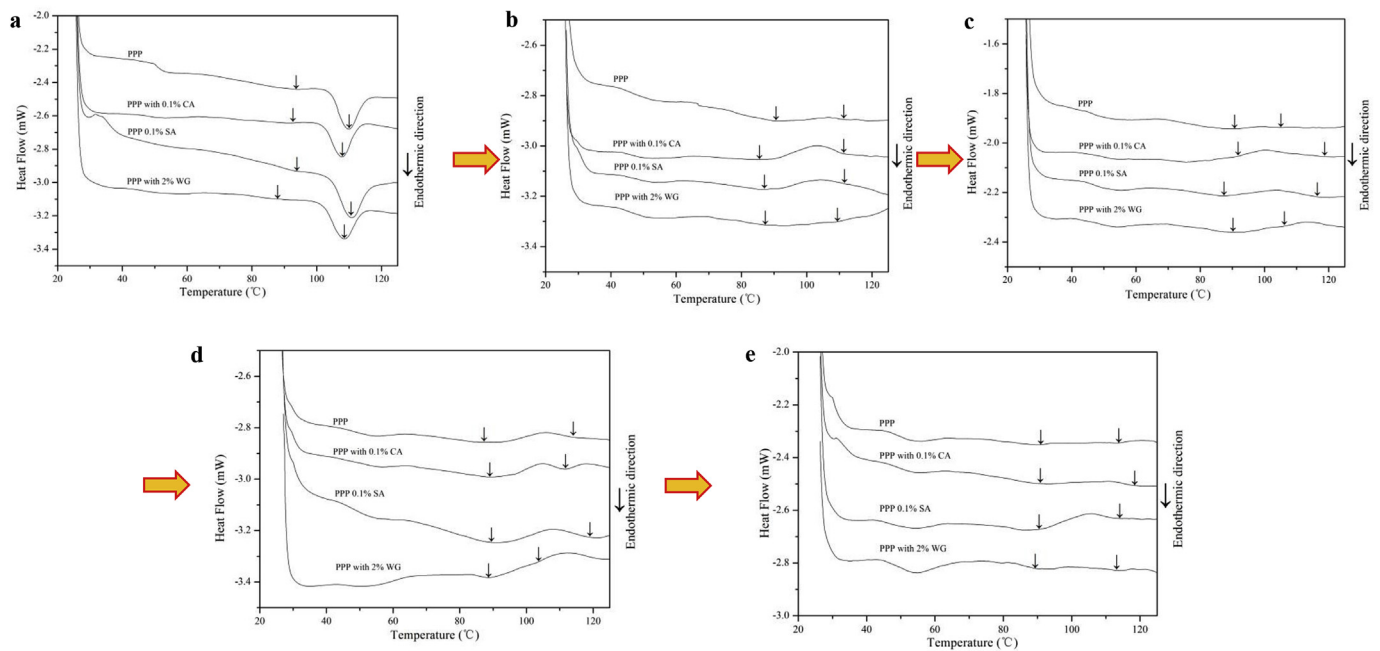


Fig. 2. DSC endotherms of PPP-/CA/SA/WS mixtures in a) mixing zone, b) melting zone, c) die, d) cooling zone, and e) extrudate during the high-moisture extrusion process.

polysaccharides had no significant effect on the T_p . When mixing the PPP with 0.1% CA, the ΔH value of arachin increased significantly from 0.07 J g^{-1} to 0.35 J g^{-1} , indicating that 0.1% CA was not conducive to the opening of the arachin molecular structure in the melting zone.

In the die, 0.1% CA or 2% WS could lead to a significant increase in the T_p value of conarachin. Moreover, when mixing the PPP with 0.1% CA, the ΔH value of conarachin decreased significantly from 0.53 J g^{-1} to 0.07 J g^{-1} , however, 2% WS could increase the ΔH value significantly to about 0.90 J g^{-1} . When mixing the PPP with 0.1% SA, the ΔH value was about 1.10 J g^{-1} , which was the largest. Therefore, all the three exogenous polysaccharides could improve the thermal stability of conarachin (Guo et al., 2012). Further, 0.1% CA promoted

the opening of its molecular structure, while 0.1% SA or 2% WS hindered the opening of the molecular structure by controlling the change of ΔH values (S. Li et al., 2013). As for the arachin, 0.1% CA or 0.1% SA could increase the T_p value of arachin significantly from $105.67 \text{ }^\circ\text{C}$ to $120.07 \text{ }^\circ\text{C}$ and $117.91 \text{ }^\circ\text{C}$, respectively. While the addition of 2% WS would decrease the T_p value significantly to about $104.93 \text{ }^\circ\text{C}$. Moreover, all the three exogenous polysaccharides could lead to a significant increase in the ΔH value of arachin. Therefore, 0.1% CA or 0.1% SA improved the thermal stability of arachin and its molecular structure became more stable, which was not conducive to the opening or re-arrangement of protein molecular chains in the die (Xin et al., 2009). 2% WS reduced the thermal stability of arachin, making its molecular structure susceptible to the thermal transition, but the energy required

Table 2

Thermal transition properties of PPP-CA/SA/WS mixtures during the high-moisture extrusion process.

Extrusion zones	Mixtures	Conarachin		Arachin	
		$T_p/^\circ\text{C}$	$\Delta H/\text{J.g}^{-1}$	$T_p/^\circ\text{C}$	$\Delta H/\text{J.g}^{-1}$
Mixing zone	PPP	$91.70 \pm 2.84a$	$0.16 \pm 0.00b$	$109.88 \pm 0.01a$	$7.45 \pm 0.78a$
	PPP with 0.1 %CA	$90.89 \pm 0.40 ab$	$0.16 \pm 0.00b$	$108.29 \pm 0.77b$	$6.78 \pm 0.95a$
	PPP with 0.1% SA	$93.05 \pm 0.58a$	$0.23 \pm 0.03b$	$110.54 \pm 0.30a$	$8.37 \pm 0.14a$
	PPP with 2% WS	$86.89 \pm 0.69b$	$0.50 \pm 0.16a$	$108.19 \pm 0.35b$	$7.31 \pm 0.26a$
Melting zone	PPP	$90.39 \pm 0.56a$	$0.20 \pm 0.01b$	$111.28 \pm 0.99a$	$0.07 \pm 0.00bc$
	PPP with 0.1 %CA	$84.55 \pm 0.20c$	$0.10 \pm 0.01c$	$111.34 \pm 0.02a$	$0.35 \pm 0.04a$
	PPP with 0.1% SA	$87.35 \pm 0.30b$	$0.54 \pm 0.04a$	$110.28 \pm 3.05a$	$0.01 \pm 0.00c$
	PPP with 2% WS	$86.67 \pm 1.08b$	$0.56 \pm 0.03a$	$109.48 \pm 0.30a$	$0.08 \pm 0.02b$
Die	PPP	$87.41 \pm 0.56b$	$0.53 \pm 0.04c$	$105.67 \pm 0.06c$	$0.01 \pm 0.00c$
	PPP with 0.1 %CA	$90.74 \pm 1.38a$	$0.07 \pm 0.01d$	$120.07 \pm 0.57a$	$0.05 \pm 0.00b$
	PPP with 0.1% SA	$86.74 \pm 0.21b$	$1.10 \pm 0.13a$	$117.91 \pm 1.18b$	$0.13 \pm 0.01a$
	PPP with 2% WS	$90.76 \pm 0.13a$	$0.90 \pm 0.03b$	$104.93 \pm 1.25d$	$0.05 \pm 0.00b$
Cooling zone	PPP	$87.72 \pm 0.60a$	$0.20 \pm 0.03c$	$114.14 \pm 0.09b$	$0.23 \pm 0.01c$
	PPP with 0.1 %CA	$87.92 \pm 0.98a$	$0.23 \pm 0.00c$	$112.27 \pm 0.84b$	$0.56 \pm 0.06b$
	PPP with 0.1% SA	$90.15 \pm 1.12a$	$1.36 \pm 0.07a$	$121.75 \pm 0.25a$	$1.96 \pm 0.03a$
	PPP with 2% WS	$88.14 \pm 1.31a$	$0.54 \pm 0.11b$	$104.02 \pm 1.72c$	$0.04 \pm 0.00d$
Extrudate	PPP	$89.77 \pm 0.34 ab$	$0.22 \pm 0.00c$	$113.48 \pm 0.11b$	$0.09 \pm 0.00b$
	PPP with 0.1 %CA	$89.56 \pm 0.54 ab$	$0.43 \pm 0.03b$	$118.23 \pm 0.04a$	$0.18 \pm 0.02a$
	PPP with 0.1% SA	$90.93 \pm 1.27a$	$0.82 \pm 0.03a$	$113.25 \pm 1.00b$	$0.04 \pm 0.00c$
	PPP with 2% WS	$88.14 \pm 0.31b$	$0.23 \pm 0.01c$	$109.27 \pm 1.27c$	$0.04 \pm 0.01c$

Note: The temperatures denoted in the dashed rectangle indicate the thermal transition peak temperature (T_p) and the calorific value represents enthalpy changes (ΔH); different letters in the same column mean significant differences ($P < 0.05$).

was increased.

In the cooling zone, all the three exogenous polysaccharides had no significant effect on the T_p value of conarachin. When mixing the PPP with 0.1% SA or 2% WS, the ΔH value of conarachin increased significantly from 0.20 J g^{-1} to 1.36 J g^{-1} and 0.54 J g^{-1} , respectively. This phenomenon would hinder the opening or rearrangement of the molecular structure of conarachin, which was consistent with the results in the die (S. Li et al., 2013). As for the arachin, the addition of 0.1% CA or 0.1% SA could increase the ΔH value of arachin significantly from 0.23 J g^{-1} to 0.56 J g^{-1} and 1.96 J g^{-1} . Moreover, 0.1% SA could also increase the T_p value of arachin significantly from 114.14°C to 121.75°C . This was consistent with the results in the die, indicating that 0.1% CA or 0.1% SA impacted the molecular structure of arachin to more stable, which was not easy to undergo thermal transition during molecular rearranging (Kaiqiang et al., 2016; Verbeek & Berg, 2010). While, the addition of 2% WS could decrease the T_p value of arachin significantly from 114.14°C to 104.02°C , and the ΔH value from 0.23 J g^{-1} to 0.04 J g^{-1} . It can be seen that 2% WS made the structure of the arachin molecules more susceptible to the thermal transition during the rearranging of the molecular chains.

For the extrudate, all the three exogenous polysaccharides had no significant effect on the T_p value of conarachin. 0.1% CA or 0.1% SA increased the ΔH value of conarachin significantly from 0.22 J g^{-1} to 0.43 J g^{-1} and 0.82 J g^{-1} , respectively. This result was consistent with the die and the cooling zone, that more energy was required to open the molecular structure of the conarachin when mixing the PPP with 0.1% CA or 0.1% SA (S. Li et al., 2013). As for the arachin, 0.1% CA would increase the T_p value of arachin significantly from 113.48°C to 118.23°C , and the ΔH value from 0.09 J g^{-1} to 0.18 J g^{-1} . This was consistent with the die and the cooling zone, indicating that 0.1% CA made the molecular structure of arachin not flexible to the thermal transition (Y. Wang et al., 2017). It was found that the addition of 0.1% SA or 2% WS resulted in a significant decrease in both T_p and ΔH values of arachin, showing that 0.1% SA or 2% WS made it easier to occur the thermal transition.

3.4. Thermal gravimetric analysis (TGA) of peanut protein with different exogenous polysaccharides during the high-moisture extrusion process

The TGA curves of PPP/exogenous polysaccharide mixtures during the extrusion process are shown in Fig. 3 and analyzed in Table 3. It was found from Fig. 3a that in the mixing zone, after mixing the PPP with 0.1% SA, there was still a peak near 110°C due to the water evaporation (P Guerrero, Beatty, Kerry, & De, 2012). However, the peak disappeared when mixing the PPP with 0.1% CA or 2% WS. As shown in Table 3, in the mixing zone, the addition of exogenous polysaccharides increased the Ft_2 , especially when 0.1% CA or 2% WS was added. This result showed that the exogenous polysaccharides had enhanced the protein-lipid interaction (Alzagtat & Alli, 2009). The exogenous polysaccharides could decrease the Ft_3 , especially when 2% WS was added and the value decreased significantly from 310.11°C to about 302.46°C . This indicated that exogenous polysaccharides could accelerate the breaking of the intramolecular disulfide bonds in the mixing zone (Verbeek et al., 2010; J.; Zhang et al., 2017). When mixing the PPP with 0.1% SA or 2% WS, the Ft_4 values were about 374.25°C and 375.62°C , respectively, which was significantly higher than that without exogenous polysaccharides, indicating that 0.1% SA or 2% WS hindered the thoroughly degradation of protein in the mixing zone (Verbeek et al., 2010).

Fig. 3b showed that in the melting zone, the peaks near 110°C disappeared due to the fact that the protein-water interaction was weakened (J. Zhang et al., 2017). In the melting zone, all the three exogenous polysaccharides had no significant effect on the Ft_2 value, which might be due to the drastic reaction conditions (high temperature and strong shear force) (Alzagtat et al., 2009). When 0.1% CA or 2% WS was added, the Ft_3 value decreased significantly from 309.46°C to 307.76°C and 304.88°C , respectively, indicating the breaking of the

intramolecular disulfide bonds. When mixing the PPP with 0.1% CA, the Ft_4 value decreased significantly from 358.00°C to about 356.32°C , while it increased significantly ($\sim 20^\circ\text{C}$) when 2% WS was added. Therefore, 0.1% CA could accelerate the degradation of protein in the melting zone, while 2% WS played the opposite role.

In the die, all the three exogenous polysaccharides promoted a significant increase in the Ft_2 value. When 0.1% SA was added, the Ft_2 value could reach up to about 231.07°C , indicating that the protein-lipid interaction has been enhanced (Alzagtat et al., 2009). The Ft_3 and Ft_4 values can't be significantly influenced by the three exogenous polysaccharides in the die.

In the cooling zone, when mixing the PPP with 2% WS, the Ft_2 value increased significantly, which may be related to the formation of polysaccharide-lipid complexes (J. Zhang, Liu, Liu, et al., 2018). There were no significant changes in the Ft_3 and Ft_4 values, which was consistent with the results in the die. It indicated that after the drastic reaction in the melting zone, the intramolecular disulfide bonds can't be influenced by the exogenous polysaccharides furthermore.

For the extrudate, when 0.1% SA was added, the Ft_2 value decreased significantly from 229.29°C to 227.87°C , indicating that the protein-lipid interaction has been destroyed to some extent. The Ft_3 value increased significantly upon the addition of 0.1% CA or 0.1% SA, further stabilizing the newly formed conformation. All the three exogenous polysaccharides caused a significant increase in the Ft_4 value, which showed that exogenous polysaccharides can effectively control the protein degradation in the extrudate.

3.5. Rheological properties of peanut protein with different exogenous polysaccharides during the high-moisture extrusion process

The rheological properties including apparent viscosity, K value and n value can be seen as the external manifestation of protein association, aggregation, and reaggregation (Verbeek et al., 2010). Fig. 4 and Table 4 present the steady shear flow curves and the power law equation fitting results of the PPP/exogenous polysaccharide mixture dispersions (20% w/w) at different shear rates. In the mixing zone, the apparent viscosity and K value increased significantly after mixing the PPP with the exogenous polysaccharides. However, the n values decreased significantly, leading to a poor flowability of the mixtures.

In the melting zone, the K value was lower than that in the mixing zone due to the dissociation of aggregates after heat treatment (P Guerrero et al., 2012; Xin et al., 2009). When mixing the PPP with 2% WS, the K value increased significantly from $14.75 \times 10^{-3} \text{ Pa.s}^n$ to about $31.75 \times 10^{-3} \text{ Pa.s}^n$, however, it decreased to $5.90 \times 10^{-3} \text{ Pa.s}^n$ when 0.1% SA was added. All the three exogenous polysaccharides could lead to an increase in the n value, which can reach up to about 1.44 when 0.1% SA was added.

In the die, it was found in Fig. 4c that the apparent viscosity for the dispersions of PPP with 2% WS was the largest. In Table 4, the K value for the dispersions of PPP with 2% WS was as higher as $677.10 \times 10^{-3} \text{ Pa.s}^n$ with an absolutely lower n value (0.60) due to the significant increase of the amount of hydrogen bonds (Table 5) (Andrés Colombo, Ribotta, & León, 2014). When mixing the PPP with 0.1% CA or 0.1% SA, the K value decreased and the n value increased significantly, which might be related with the phase separation (Schmitt & Turgeon, 2011).

In the cooling zone, the largest apparent viscosity was about $462.10 \times 10^{-3} \text{ Pa.s}^n$, which was obtained in the case of adding 2% WS in PPP. This result was consistent with that in the die. Interestingly, when mixing the PPP with 0.1% CA or 0.1% SA, the K value increased and the n value decreased. This result indicated that the three exogenous polysaccharides could stabilize the newly formed conformation of peanut protein by increasing the apparent viscosity (Bueno, Pereira, Menegassi, Arêas, & Castro, 2009).

For the extrudate, the flowing behavior of the dispersions was the same as that in the cooling zone. The K values were all increased

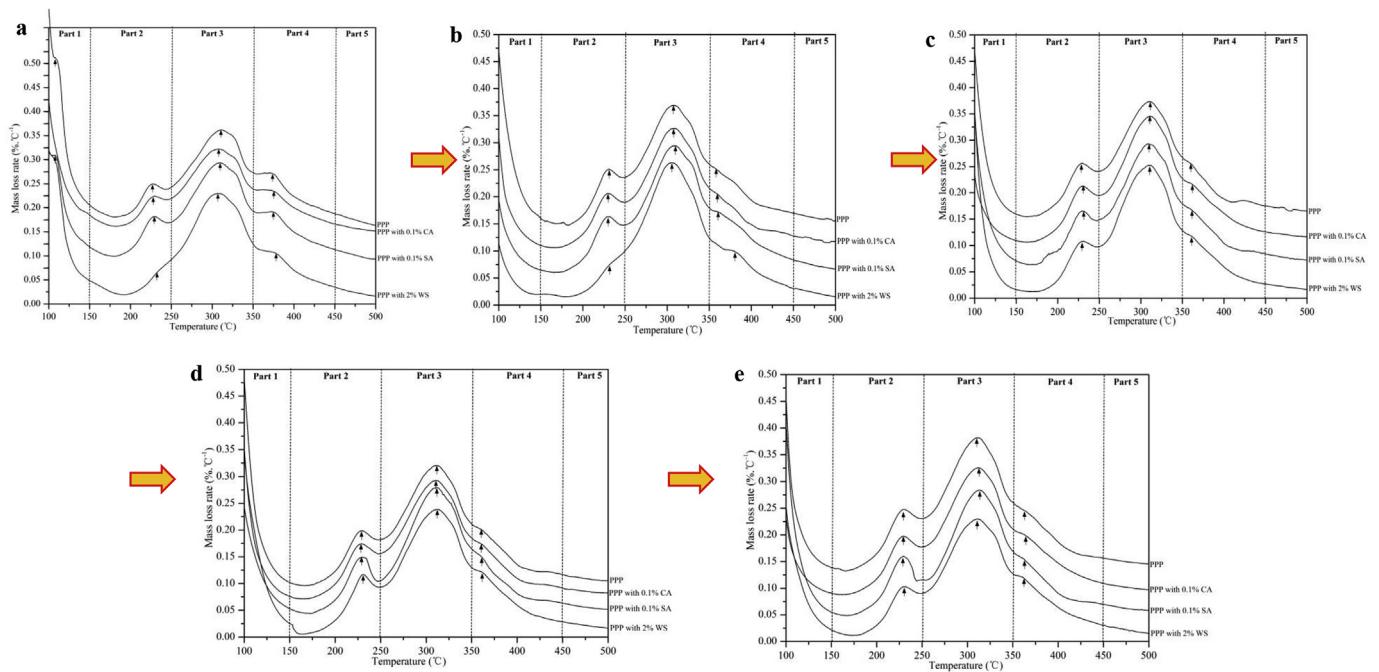


Fig. 3. DTG curves of PPP-CA/SA/WS mixtures in a) mixing zone, b) melting zone, c) die, d) cooling zone, and e) extrudate during the high-moisture extrusion process.

significantly after mixing the PPP with exogenous polysaccharides, especially for the PPP with 2% WS with a K value about $431.75 \times 10^{-3} \text{ Pa}\cdot\text{s}^n$.

3.6. Chemical cross-linking of peanut protein with different exogenous polysaccharides during the high-moisture extrusion process

According to the protein solubility in the eight extraction solutions, the relative importance of each specific chemical bond and their interactions can be calculated, as shown in Table 5 (Chen et al., 2011; Liu et al., 2008). Protein in natural state (1); hydrogen bonds, (2)–(1); disulfide bonds, (3)–(1); hydrophobic interactions, (4)–(1); interactions between hydrogen bonds and disulfide bonds, (5) + (1)–(2)–(3); interactions between hydrophobic interactions and hydrogen bonds, (6)

+ (1)–(2)–(4); interactions between hydrophobic interactions and disulfide bonds, (7) + (1)–(3)–(4); interactions among hydrophobic interactions, hydrogen bonds and disulfide bonds, (8) + (2) + (3) + (4)–(1)–(5)–(6)–(7).

In the mixing zone, when 0.1% CA was added, the hydrophobic interactions were significantly weakened, while the interactions between hydrophobic interactions and disulfide bonds were significantly enhanced. When 0.1% SA was added, the amount of disulfide bonds was significantly reduced and the hydrophobic interactions were significantly weakened. For the PPP with 2% WS, the hydrophobic interactions, the interactions between hydrogen bonds and disulfide bonds, and the interactions between hydrophobic interactions and disulfide bonds were all significantly enhanced, but the amount of disulfide bonds was significantly reduced, the interactions among the hydrogen

Table 3 Thermal decomposition properties of PPP-CA/SA/WS mixtures during the high-moisture extrusion process.

Extrusion zones	Mixtures	F _{t1} /°C	F _{t2} /°C	F _{t3} /°C	F _{t4} /°C	residual mass/%
Mixing zone	PPP	111.47 ± 0.32a	226.61 ± 0.60b	310.11 ± 0.99a	367.55 ± 0.10b	13.90 ± 0.02a
	PPP with 0.1 %CA	–	230.89 ± 0.48a	309.02 ± 1.03a	364.33 ± 1.25b	14.94 ± 0.32a
	PPP with 0.1% SA	112.89 ± 0.03a	228.12 ± 0.88b	309.28 ± 0.02a	374.25 ± 0.44a	15.38 ± 0.27a
	PPP with 2% WS	–	232.44 ± 0.27a	302.46 ± 0.70b	375.62 ± 0.40a	15.15 ± 1.00a
Melting zone	PPP	–	229.84 ± 0.01a	309.46 ± 0.68a	358.00 ± 0.14b	15.48 ± 0.32a
	PPP with 0.1 %CA	–	229.71 ± 0.06a	307.76 ± 0.11b	356.32 ± 0.82c	15.25 ± 1.05a
	PPP with 0.1% SA	–	230.32 ± 0.18a	309.04 ± 0.05a	358.83 ± 0.14b	15.12 ± 1.18a
	PPP with 2% WS	–	229.88 ± 0.72a	304.88 ± 0.34c	375.43 ± 0.03a	14.74 ± 0.89a
Die	PPP	–	227.15 ± 0.14c	311.01 ± 0.06a	358.38 ± 0.50a	15.05 ± 0.15b
	PPP with 0.1 %CA	–	229.41 ± 0.37b	310.88 ± 0.51a	359.90 ± 1.29a	14.17 ± 0.18c
	PPP with 0.1% SA	–	231.07 ± 0.76a	310.47 ± 1.80a	358.50 ± 0.36a	15.57 ± 0.16a
	PPP with 2% WS	–	229.60 ± 0.39b	311.69 ± 0.33a	359.60 ± 0.84a	15.22 ± 0.21 ab
Cooling zone	PPP	–	228.11 ± 0.35b	311.10 ± 0.26a	358.72 ± 0.58a	15.24 ± 0.15a
	PPP with 0.1 %CA	–	228.48 ± 0.26b	310.40 ± 0.13a	357.95 ± 1.04a	15.44 ± 0.56a
	PPP with 0.1% SA	–	227.99 ± 0.05b	310.54 ± 0.61a	360.40 ± 1.18a	15.36 ± 0.13a
	PPP with 2% WS	–	230.16 ± 0.01a	310.54 ± 0.43a	359.42 ± 0.59a	14.97 ± 0.29a
Extrudate	PPP	–	229.29 ± 0.09a	310.89 ± 0.17b	356.27 ± 0.24b	15.52 ± 0.27a
	PPP with 0.1 %CA	–	229.55 ± 0.77a	312.17 ± 0.29a	358.07 ± 0.05a	14.73 ± 1.15a
	PPP with 0.1% SA	–	227.87 ± 0.01b	312.85 ± 0.21a	358.13 ± 0.72a	15.16 ± 0.13a
	PPP with 2% WS	–	229.46 ± 0.36a	310.42 ± 0.48b	358.83 ± 0.65a	13.91 ± 0.13a

Note: The temperatures denoted in the dashed rectangle indicate the thermal decomposition peak temperature (F_{t1}, F_{t2}, F_{t3}, F_{t4}); different letters in the same column mean significant differences (P < 0.05).

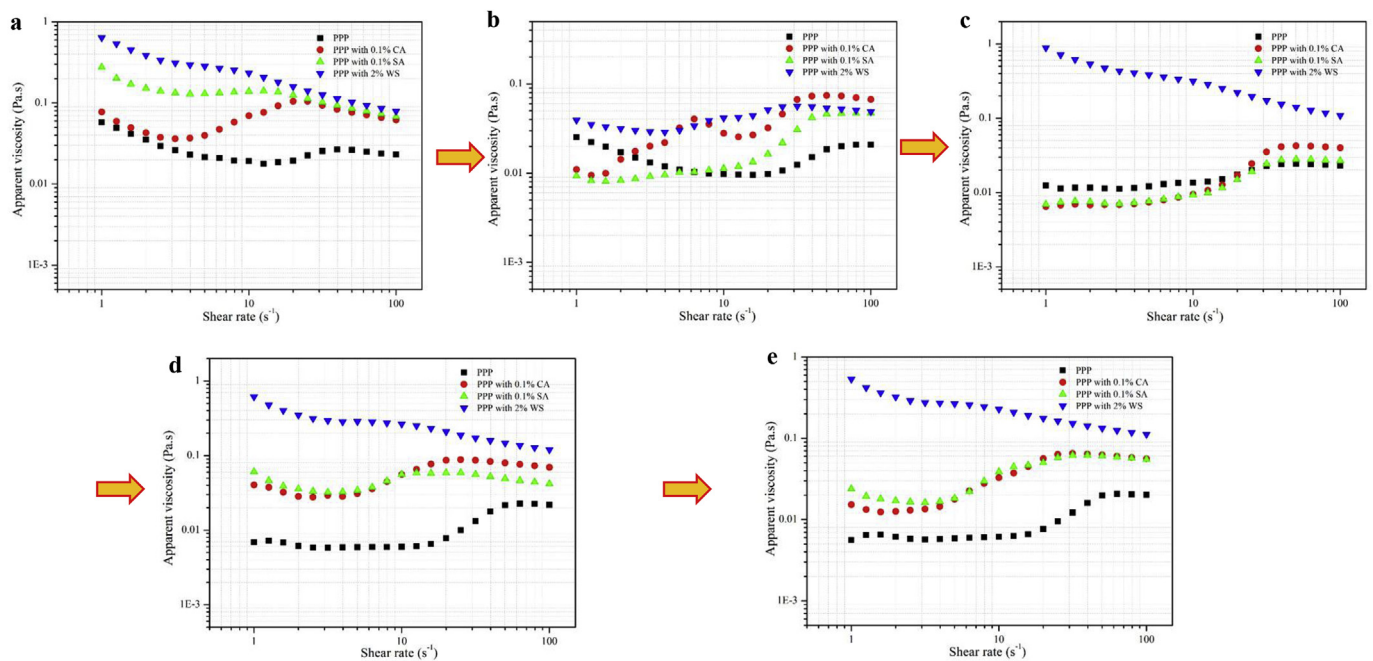


Fig. 4. Steady shear flow curves of dispersions containing 20% (w/w) PPP-/CA/SA/WS mixtures in a) mixing zone, b) melting zone, c) die, d) cooling zone, and e) extrudate during the high-moisture extrusion process.

bonds, hydrophobic interactions and disulfide bonds were significantly weakened. It can be seen that in the mixing zone, hydrogen bonds were the main force maintaining the protein structure, and all the three exogenous polysaccharides had no significant effect on the hydrogen bonds.

In the melting zone, the protein structure was mainly maintained by the hydrogen bonds, however, all the three exogenous polysaccharides could lead to a significant reduction of the hydrogen bonds, especially the 2% WS. This phenomenon can be explained by the fact that the polysaccharide competed for the water as the protein unfolding (Andrés Colombo et al., 2014). 0.1% CA could significantly enhance the hydrophobic interactions and the interactions between hydrophobic interactions and disulfide bonds. 0.1% SA destroyed the hydrogen bonds, hydrophobic interactions and disulfide bonds significantly, but enhanced the interactions among these forces. It can be clearly seen that

the hydrophobic interactions and the interactions between hydrogen bonds and disulfide bonds were enhanced due to the addition of 2% WS.

In the die, the protein structure was mainly maintained by the interactions between hydrophobic interactions and disulfide bonds, but the interactions can be weakened by all the three exogenous polysaccharides due to the Maillard reaction, especially the 0.1% SA (P Guerrero et al., 2012). 0.1% CA could reduce the disulfide bonds, and promote the hydrogen bonds, hydrophobic interactions, and their interactions. When 0.1% SA was added, the main forces maintaining the protein structure were hydrogen bonds and hydrophobic interactions. For the PPP with 2% WS, the amount of hydrogen bonds was increased significantly, and the hydrophobic interactions, the interactions between hydrogen bonds and hydrophobic interactions were both enhanced significantly to maintain the protein structure.

Table 4

Power law equation fitting results of steady shear flow curves of PPP-CA/SA/WS mixtures during the high-moisture extrusion process.

Extrusion zones	Mixtures	K ($\times 10^{-3}$ Pa.s ⁿ)	n	R ²
Mixing zone	PPP	19.40 ± 4.67c	1.02 ± 0.06a	0.969
	PPP with 0.1 %CA	50.30 ± 6.22c	1.12 ± 0.02a	0.976
	PPP with 0.1% SA	218.25 ± 21.99b	0.77 ± 0.02b	0.985
	PPP with 2% WS	508.45 ± 82.38a	0.60 ± 0.05c	0.991
Melting zone	PPP	14.75 ± 0.07b	0.97 ± 0.02b	0.942
	PPP with 0.1 %CA	14.70 ± 5.37b	1.36 ± 0.12a	0.985
	PPP with 0.1% SA	5.90 ± 0.28c	1.44 ± 0.00a	0.985
	PPP with 2% WS	31.75 ± 2.90a	1.13 ± 0.01b	0.994
Die	PPP	9.45 ± 0.49b	1.21 ± 0.02c	0.995
	PPP with 0.1 %CA	4.00 ± 0.42b	1.52 ± 0.02a	0.981
	PPP with 0.1% SA	5.10 ± 0.14b	1.36 ± 0.02b	0.986
	PPP with 2% WS	677.10 ± 140.43a	0.60 ± 0.04d	0.993
Cooling zone	PPP	4.20 ± 0.28b	1.31 ± 0.00a	0.972
	PPP with 0.1 %CA	20.90 ± 9.76b	1.35 ± 0.12a	0.982
	PPP with 0.1% SA	35.30 ± 6.65b	1.09 ± 0.05b	0.979
	PPP with 2% WS	462.10 ± 27.29a	0.71 ± 0.01c	0.990
Extrudate	PPP	4.20 ± 0.14d	1.32 ± 0.02b	0.981
	PPP with 0.1 %CA	11.35 ± 0.78c	1.44 ± 0.01a	0.987
	PPP with 0.1% SA	14.20 ± 1.13b	1.33 ± 0.03b	0.981
	PPP with 2% WS	431.75 ± 0.92a	0.71 ± 0.01c	0.996

Note: The flow behavior index (n) and consistency coefficient (K) were reported; different letters in the same column mean significant differences ($P < 0.05$).

Table 5
Effect of exogenous polysaccharides on the specific chemical bonds and their interactions of peanut protein during the high-moisture extrusion process.

Extrusion zones	Mixtures	Hydrogen bonds	Disulfide bonds	Hydrophobic interactions	Interactions between hydrogen bonds and disulfide bonds	Interactions between hydrophobic interactions and hydrogen bonds	Interactions between hydrophobic interactions and disulfide bonds	Interactions among hydrophobic interactions, hydrogen bonds and disulfide bonds
Mixing zone	PPP	26.84 ± 1.30a	13.24 ± 0.15a	17.97 ± 1.41b	2.81 ± 0.99bc	-20.55 ± 2.63a	-6.45 ± 1.01b	-6.73 ± 2.84a
	PPP with 0.1 %CA	25.80 ± 0.63a	6.87 ± 0.71 ab	2.53 ± 0.08d	5.29 ± 1.68b	-25.13 ± 1.16a	13.77 ± 0.82a	-7.92 ± 4.52a
	PPP with 0.1% SA	24.13 ± 1.39a	6.24 ± 1.51b	10.07 ± 3.66c	-3.54 ± 0.63c	-24.22 ± 5.30a	-0.33 ± 3.49b	7.37 ± 8.66a
	PPP with 2% WS	26.49 ± 1.70a	8.71 ± 4.24 ab	27.37 ± 3.36a	23.66 ± 4.71a	-25.22 ± 1.03a	12.82 ± 4.22a	-28.56 ± 6.01b
	PPP	30.19 ± 0.63a	13.61 ± 0.59a	19.30 ± 0.11b	6.46 ± 0.95b	-21.16 ± 0.17b	4.78 ± 0.84b	-12.14 ± 1.70b
Melting zone	PPP with 0.1 %CA	17.65 ± 2.86bc	10.46 ± 0.08b	22.04 ± 1.87 ab	8.20 ± 0.80b	-7.36 ± 4.60a	22.59 ± 0.63a	-41.03 ± 3.43d
	PPP with 0.1% SA	20.09 ± 0.13b	8.95 ± 0.50b	12.88 ± 0.57c	6.51 ± 1.64b	-22.81 ± 0.65b	-1.17 ± 1.32c	-2.75 ± 0.86a
	PPP with 2% WS	14.13 ± 2.33c	8.25 ± 1.95b	22.65 ± 0.97a	33.92 ± 6.07a	-12.75 ± 0.84a	6.63 ± 1.09b	-29.29 ± 0.74c
	PPP	18.53 ± 1.70b	16.76 ± 1.51a	12.01 ± 1.09b	10.39 ± 2.84b	1.52 ± 1.30a	22.11 ± 2.06a	-30.30 ± 1.53b
	PPP with 0.1 %CA	19.67 ± 0.21b	12.72 ± 0.08b	16.36 ± 0.06b	15.04 ± 0.63b	-3.61 ± 0.61a	14.90 ± 0.06b	-36.81 ± 2.16b
Die	PPP with 0.1% SA	21.47 ± 0.06 ab	11.55 ± 0.02b	20.09 ± 2.23b	15.08 ± 1.66b	-27.61 ± 2.61b	-7.50 ± 2.12d	-7.39 ± 3.97a
	PPP with 2% WS	24.21 ± 1.24a	17.19 ± 0.57a	29.23 ± 5.53a	29.79 ± 6.12a	-24.38 ± 3.13b	1.13 ± 2.98c	-14.01 ± 5.65a
	PPP	18.53 ± 1.49a	22.53 ± 0.71b	16.03 ± 2.21 ab	24.41 ± 0.06a	-1.31 ± 3.03a	17.24 ± 2.69 ab	-36.12 ± 4.94b
	PPP with 0.1 %CA	18.41 ± 1.95a	13.08 ± 0.21c	14.79 ± 3.17 ab	15.36 ± 0.34b	-11.43 ± 3.59b	7.79 ± 0.46c	-8.43 ± 0.65a
	PPP with 0.1% SA	20.63 ± 0.25a	13.89 ± 0.57c	13.23 ± 0.55b	3.27 ± 0.50c	-17.67 ± 1.83b	12.26 ± 4.52bc	1.10 ± 0.84a
Cooling zone	PPP with 2% WS	23.11 ± 3.85a	28.62 ± 1.68a	20.42 ± 1.56a	13.02 ± 4.75b	0.82 ± 3.89a	22.01 ± 1.62a	-41.62 ± 5.19b
	PPP	12.10 ± 0.13d	12.59 ± 0.53b	12.51 ± 0.21c	27.46 ± 2.82a	-3.49 ± 0.32a	8.51 ± 0.95c	-34.30 ± 2.98b
	PPP with 0.1 %CA	21.95 ± 0.06c	8.78 ± 0.19c	18.75 ± 2.56b	15.23 ± 0.61 ab	-16.70 ± 1.85b	12.01 ± 2.23bc	-17.97 ± 1.70a
	PPP with 0.1% SA	29.51 ± 1.26b	13.14 ± 0.13b	21.19 ± 2.19 ab	10.61 ± 0.71b	-17.64 ± 1.58bc	14.03 ± 1.30b	-20.09 ± 2.31a
	PPP with 2% WS	35.81 ± 2.77a	21.89 ± 1.07a	24.55 ± 1.18a	12.33 ± 10.42 ab	-21.5 ± 2.25c	20.25 ± 1.87a	-25.54 ± 8.51 ab

Note: Different letters in the same column mean significant differences ($P < 0.05$).

In the cooling zone, hydrogen bonds, disulfide bonds, and their interactions were the main forces that maintained the protein structure, and all the three exogenous polysaccharides had no significant effect on the hydrogen bonds. Although 0.1% CA had a destructive effect on the main forces, it didn't change the type of the main forces. 0.1% SA could significantly weaken the interactions between hydrogen bonds and disulfide bonds. 2% WS could significantly increase the amount of disulfide bonds, and enhance the hydrophobic interactions.

For the extrudate, the protein structure was mainly maintained by the interactions between hydrogen bonds and disulfide bonds. When 0.1% CA was added, the amount of hydrogen bonds was increased, and the hydrophobic interactions were significantly enhanced to maintain the protein structure. The interactions between hydrogen bonds and disulfide bonds could be weakened by the 0.1% SA, and the main forces that maintained the protein structure were converted into hydrogen bonds and hydrophobic interactions, which was consistent with the action of 0.1% CA. 2% WS could increase the amount of hydrogen bonds and disulfide bonds significantly.

3.7. Molecular weight distribution of peanut protein with different exogenous polysaccharides during the high-moisture extrusion process

Fig. 5 shows the SDS-PAGE profiles of PPP/exogenous polysaccharide mixtures during the extrusion process in the presence of β -mercaptoethanol. Eight bands were exhibited ranging in molecular weight from 18 to 64 kDa for the peanut protein in each extrusion zone. In the mixing zone, the addition of exogenous polysaccharides had a significant effect on the protein subunits with lower molecular weight, especially for the subunits of 18 kDa. As shown in Table S2, the ratio of the protein subunit at 18 kDa decreased significantly from 3.87% to about 1.26%, 0.84%, and 1.06%, respectively, for the PPP with 0.1% CA, PPP with 0.1% SA, and PPP with 2% WS.

In the melting zone, when mixing the PPP with 0.1% CA, the ratios of the protein subunits at 38 kDa and 18 kDa decreased significantly. While the ratio of the protein subunit at 22 kDa increased significantly from 19.88% to 28.40%. When mixing the PPP with 0.1% SA, the ratios of the protein subunits at 66 kDa and 22 kDa increased significantly. While the ratio of the protein subunit at 18 kDa decreased significantly from 4.70% to 1.18%. For the PPP with 2% WS, the ratios of the protein subunits at 66 kDa and 22 kDa increased significantly and that at

18 kDa decreased significantly, which was similar to that of PPP with 0.1% SA.

In the die, when mixing the PPP with 0.1% CA, a significant increase in the ratios for the subunits at 66 kDa, 27 kDa, 26 kDa, and 22 kDa can be seen in Table S2. At the same time, the ratios for the subunits at 39 kDa and 38 kDa both decreased significantly. When mixing the PPP with 0.1% SA, the ratios for the subunits at 66 kDa and 22 kDa both increased significantly. While, the ratios for the subunits at 42 kDa, 39 kDa, and 18 kDa decreased significantly. For the PPP with 2% WS, the ratios for the subunits at 66 kDa, 26 kDa, and 22 kDa increased significantly and those at 42 kDa, 39 kDa, and 38 kDa decreased significantly. These results indicated that the three selected exogenous polysaccharides could promote the degradation of large protein subunits such as 42 kDa, 39 kDa into 22 kDa and also to synthesis more larger subunits like the 66 kDa (Fuente, Hemar, & Singh, 2004).

In the cooling zone, when mixing the PPP with 0.1% CA, the ratio for the subunit at 22 kDa increased significantly from 18.75% to 28.02%. While, the ratios for the subunits at 39 kDa and 18 kDa both decreased significantly. The 0.1% SA could only lead to a significant decrease in the ratio for the subunit at 18 kDa. For the PPP with 2% WS, the ratio for the subunit at 22 kDa increased significantly.

For the extrudate, when mixing the PPP with 0.1% CA, the ratios for the subunits at 39 kDa and 18 kDa both decreased significantly. The addition of 0.1% SA would lead to a significant decrease in the ratios for the subunits at 42 kDa and 18 kDa. For the PPP with 2% WS, the ratio for the subunit at 66 kDa increased significantly and the ratios for the subunits at 42 kDa and 39 kDa decreased significantly at the same time. Thus, these results suggest that during the extrusion process, the exogenous polysaccharides promoted the disintegration of the native protein from high molecular weight to low molecular weight forms, which subsequently reform into very large networks (Shah, 2003).

3.8. Secondary structure changes of peanut protein with different exogenous polysaccharides during the high-moisture extrusion process

The FTIR data from the PPP/exogenous polysaccharide mixtures are summarized in Table 6 and shown in Fig. 6. The amide I region ($1700\text{--}1600\text{ cm}^{-1}$) of the FTIR spectrum was selected for detailed investigation as it provides most information on the secondary structure of proteins (Georget & Belton, 2006). In the mixing zone, it can be seen in

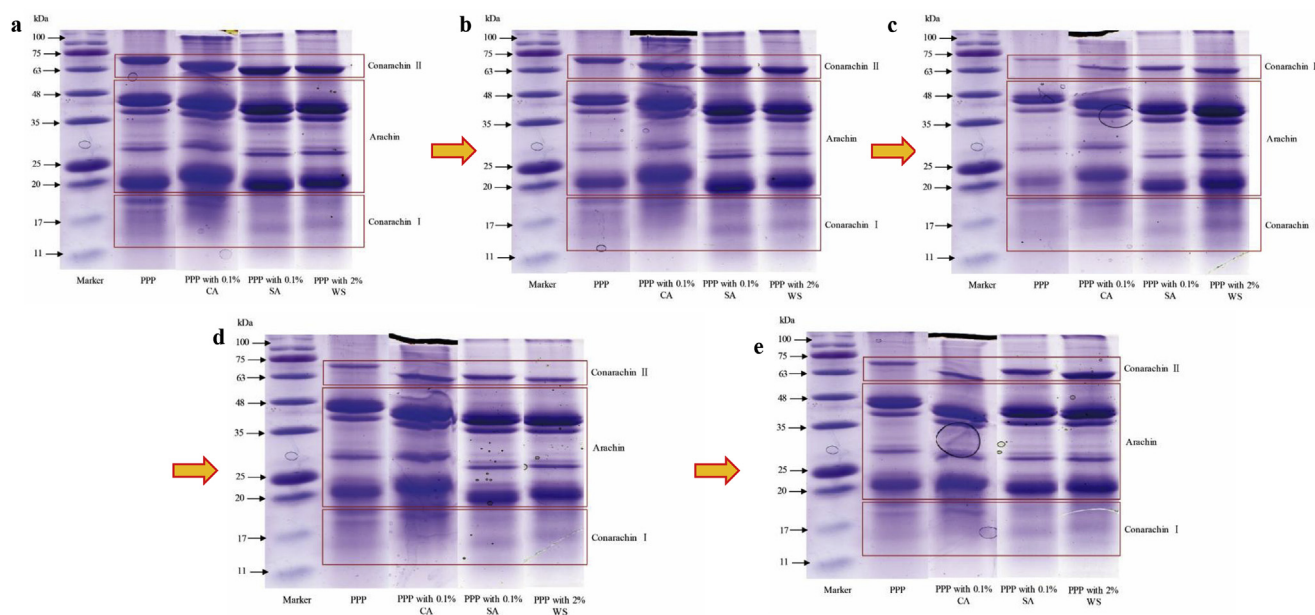


Fig. 5. Molecular weight distribution of peanut protein in the PPP-/CA/SA/WS mixtures in a) mixing zone, b) melting zone, c) die, d) cooling zone, and e) extrudate during the high-moisture extrusion process.

Table 6
Effect of exogenous polysaccharides on the changes in secondary structure ratios of peanut protein during the high-moisture extrusion process.

Extrusion zones	Mixtures	Secondary structure ratios/%				
		α -Helix	β -Sheet	β -Turn	Random coil	α -Helix/ β -Sheet
Mixing zone	PPP	37.93 \pm 0.24a	38.82 \pm 2.22b	8.47 \pm 0.70c	14.78 \pm 1.27a	0.98 \pm 0.06a
	PPP with 0.1 %CA	27.16 \pm 3.41b	39.86 \pm 3.05b	19.05 \pm 1.24b	13.93 \pm 0.87a	0.59 \pm 0.16b
	PPP with 0.1% SA	19.11 \pm 2.24c	34.92 \pm 0.89b	29.41 \pm 3.35a	16.56 \pm 2.00a	0.49 \pm 0.01bc
	PPP with 2% WS	19.87 \pm 1.34c	48.32 \pm 3.12a	18.68 \pm 0.77b	13.13 \pm 2.55a	0.41 \pm 0.05c
Melting zone	PPP	33.77 \pm 0.46a	47.82 \pm 2.99a	13.86 \pm 4.12b	4.55 \pm 1.59b	0.71 \pm 0.05b
	PPP with 0.1 %CA	32.85 \pm 0.70a	35.08 \pm 2.62b	24.42 \pm 0.29a	7.65 \pm 1.63b	0.94 \pm 0.09a
	PPP with 0.1% SA	11.37 \pm 1.69b	35.01 \pm 1.48b	29.22 \pm 0.29a	24.40 \pm 0.09a	0.33 \pm 0.06c
	PPP with 2% WS	11.45 \pm 0.58b	41.48 \pm 3.50 ab	27.18 \pm 0.39a	19.89 \pm 2.53a	0.28 \pm 0.04c
Die	PPP	35.84 \pm 1.82a	45.58 \pm 0.41a	12.98 \pm 2.13c	5.59 \pm 0.10c	0.79 \pm 0.03a
	PPP with 0.1 %CA	15.41 \pm 0.38c	39.33 \pm 0.03b	23.31 \pm 2.80 ab	21.96 \pm 3.21a	0.39 \pm 0.01b
	PPP with 0.1% SA	16.69 \pm 0.20c	39.20 \pm 0.25b	26.53 \pm 0.74a	17.59 \pm 0.79 ab	0.43 \pm 0.01b
	PPP with 2% WS	21.76 \pm 1.94b	44.11 \pm 1.44a	20.10 \pm 1.71b	14.04 \pm 2.22b	0.49 \pm 0.06b
Cooling zone	PPP	45.05 \pm 2.84a	36.26 \pm 3.19 ab	15.11 \pm 3.78b	3.59 \pm 2.24b	1.24 \pm 0.03a
	PPP with 0.1 %CA	16.77 \pm 1.14b	40.12 \pm 2.78a	26.42 \pm 2.54a	16.68 \pm 0.90a	0.42 \pm 0.06bc
	PPP with 0.1% SA	14.62 \pm 0.88b	39.52 \pm 0.45a	23.69 \pm 2.79a	22.17 \pm 3.22a	0.37 \pm 0.03c
	PPP with 2% WS	19.39 \pm 3.14b	33.12 \pm 1.39b	31.08 \pm 2.45a	16.40 \pm 0.70a	0.59 \pm 0.12b
Extrudate	PPP	42.84 \pm 4.44a	30.52 \pm 1.62b	21.49 \pm 2.31b	5.15 \pm 0.51b	1.41 \pm 0.22a
	PPP with 0.1 %CA	22.02 \pm 0.49b	44.91 \pm 4.13a	19.53 \pm 0.34b	13.54 \pm 4.27a	0.49 \pm 0.03b
	PPP with 0.1% SA	21.97 \pm 2.50b	34.03 \pm 1.67b	28.16 \pm 0.39a	15.84 \pm 1.22a	0.65 \pm 0.11b
	PPP with 2% WS	19.94 \pm 3.00b	35.66 \pm 1.63b	28.25 \pm 0.44a	16.14 \pm 0.94a	0.56 \pm 0.11b

Note: Different letters in the same column mean significant differences ($P < 0.05$).

Table 6 that the random coil structure can't be significantly affected by the selected exogenous polysaccharides. When mixing the PPP with 0.1% CA, the α -helix ratio decreased significantly and it was converted into β -turn structure. In this case, the α -helix to β -sheet ratio decreased significantly from 0.98 to 0.59, demonstrating that 0.1% CA could promote the stretching of protein molecular chains (Kaiqiang et al., 2016). The addition of 0.1% SA could also further promote the conversion of α -helix into β -turn, resulting in the stretching of protein molecular chains. For the PPP with 2% WS, the α -helix ratio decreased significantly from 37.93% to 19.87%, while the β -sheet and β -turn ratios both increased significantly. The α -helix to β -sheet ratio was the lowest (about 0.41). Thus, all the three exogenous polysaccharides could promote the stretching of protein molecular chains in the mixing

zone, especially the 2% WS.

In the melting zone, when mixing the PPP with 0.1% CA, the β -sheet ratio decreased significantly, while the β -turn ratio increased significantly at the same time. Interestingly, the α -helix to β -sheet ratio increased significantly from 0.71 to 0.94, indicating that under drastic reaction conditions, 0.1% CA could destroy the β -sheet structure (Yao et al., 2017). When mixing the PPP with 0.1% SA, the α -helix and β -sheet ratios both decreased significantly, while the β -turn and random coil ratios both increased significantly. The α -helix to β -sheet ratio decreased significantly from 0.71 to 0.33, indicating that 0.1% SA promoted the stretching of protein molecular chains with a more disordered state, but the β -sheet structure would be destroyed. For the PPP with 2% WS, the α -helix ratio decreased significantly, leading to a

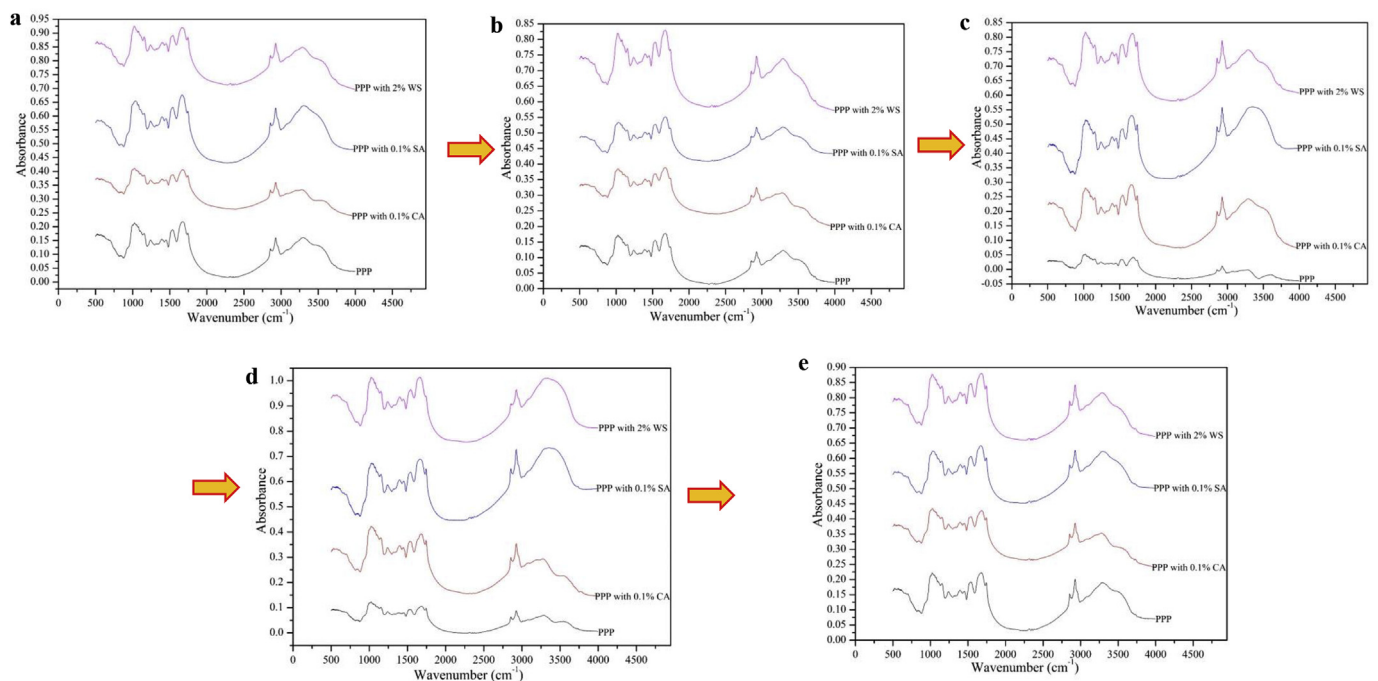


Fig. 6. FTIR curves of PPP-/CA/SA/WS mixtures in a) mixing zone, b) melting zone, c) die, d) cooling zone, and e) extrudate during the high-moisture extrusion process.

significant increase of both β -turn and random coil ratios. The significant decrease of the α -helix to β -sheet ratio suggested that 2% WS was beneficial to the stretching of protein molecular chains, which was similar to the effect of 0.1% SA.

In the die, when mixing the PPP with 0.1% CA, the α -helix and β -sheet ratios both decreased significantly, while the β -turn and random coil ratios both increased significantly. The α -helix to β -sheet ratio decreased significantly from 0.79 to 0.39, indicating that the 0.1% CA could promote the stretching of protein molecular chains with a more disordered state in the die. When mixing the PPP with 0.1% SA, the α -helix and β -sheet ratios both decreased significantly, while the β -turn and random coil ratios both increased significantly. Similar to the effect of 0.1% CA, 0.1% SA could lead to a significant decrease of the α -helix to β -sheet ratio. For the PPP with 2% WS, the α -helix ratio decreased significantly, leading to a significant increase of both β -turn and random coil ratios. The significant decrease of the α -helix to β -sheet ratio suggested that 2% WS was beneficial to the stretching of protein molecular chains, which was similar to the effect of other exogenous polysaccharides.

In the cooling zone, when mixing the PPP with 0.1% CA, the α -helix ratio decreased significantly, while the β -turn and random coil ratios both increased significantly. The α -helix to β -sheet ratio decreased significantly from 1.24 to 0.42, which was consistent with the result in the die that 0.1% CA could promote the stretching of protein molecular chains with a more disordered state. When mixing the PPP with 0.1% SA, the α -helix ratio decreased significantly, while the β -turn and random coil ratios both increased significantly. Similar to the effect of 0.1% CA, 0.1% SA could also lead to a significant decrease in the α -helix to β -sheet ratio in the cooling zone. For the PPP with 2% WS, the α -helix ratio decreased significantly, leading to a significant increase of both β -turn and random coil ratios. The significant decrease of the α -helix to β -sheet ratio suggested that 2% WS was beneficial to the stretching of protein molecular chains, which was similar to the effect of other exogenous polysaccharides. It can be concluded that the three exogenous polysaccharides would promote the conversion of α -helix into β -turn and random coil structure but not had a significant influence on the β -sheet structure in the cooling zone (P Guerrero, Kerry, & De, 2014).

For the extrudate, when mixing the PPP with 0.1% CA, the α -helix ratio decreased significantly, while the β -sheet and random coil ratios both increased significantly. The α -helix to β -sheet ratio decreased significantly from 1.41 to 0.49, indicating that 0.1% CA could promote the stretching of protein molecular chains by converting the α -helix into β -sheet and random coil structure. When mixing the PPP with 0.1% SA, the α -helix ratio decreased significantly, while the β -turn and random coil ratios both increased significantly. The α -helix to β -sheet ratio decreased significantly from 1.41 to 0.65, indicating that 0.1% SA could promote the stretching of protein molecular chains by converting the α -helix into β -turn and random coil structure. For the PPP with 2% WS, the α -helix ratio decreased significantly, leading to a significant increase of both β -turn and random coil ratios. Similar to the effect of 0.1% SA, the significant decrease of the α -helix to β -sheet ratio suggested that 2% WS was also beneficial to the stretching of protein molecular chains by converting the α -helix into β -turn and random coil structure.

4. Conclusions

For the PPP/exogenous polysaccharide mixtures, 0.1% CA could significantly improve the tensile resistant force (about 1.92 kg), but it was not conducive to the fiber orientation. 0.1% SA could significantly improve the fibrous degree (up to about 1.24) and springiness, which was conducive to the fiber orientation, and it could also increase its tensile resistant force, hardness and the chewiness significantly. The larger the amount of wheat starch (0–8%), the lower the fibrous degree was, and the hardness and chewiness were both significantly reduced.

0.1% CA, 0.1% SA or 2% WS can protect the molecular chains of conarachin and arachin by increasing the thermal transition temperature T_p or increasing the ΔH value, slowing down the thermal transition speed of peanut protein. The selected exogenous polysaccharides, especially the 2% WS, can promote the aggregation of protein molecules by breaking the intramolecular disulfide bonds, enhancing the hydrophobic interactions and increasing the apparent viscosity to stabilize the newly formed conformation. The protein subunit with a molecular weight of 22 kDa can be largely impacted by the exogenous polysaccharides due to the degradation of the subunits at 42 kDa and 39 kDa. Additionally, 2% WS can further promote the synthesis of protein subunits with large molecular weight (66 kDa). The three exogenous polysaccharides can promote the opening of the α -helix and gradually converting into a β -turn and random coil structure. When mixing the PPP with 0.1% CA, the four protein secondary structures were present with β -sheet > α -helix > β -turn > random coil in the extrudate. While mixing the PPP with 0.1% SA or 2% WS, the order was β -sheet > β -turn > α -helix > random coil. Thus, a certain amount of the exogenous polysaccharides can improve the fibrous structure of peanut protein through influencing the protein aggregation during the extrusion process, which should be better when two or more exogenous polysaccharides were combined together.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodhyd.2019.105311>.

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