Food Hydrocolloids 97 (2019) 105174

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Gelatin and high methyl pectin coacervates crosslinked with tannic acid: The characterization, rheological properties, and application for peppermint oil microencapsulation

Bertrand Muhoza, Shuqin Xia*, Xiaoming Zhang

State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Collaborative Innovation Center of Food Safety and Quality Control in Jiangsu Province, Jiangnan University, Lihu Road 1800, Wuxi, Jiangsu, 214122, People's Republic of China

ARTICLE INFO

Keywords:
Gelatin
Pectin
Tannic acid
Coacervates
Crosslinking
Peppermint oil

ABSTRACT

In this study, gelatin and high methyl pectin coacervates were crosslinked with tannic acid (TA), and the protein conformation, microstructure, rheology, powder crystallinity, and thermal properties were investigated. Crosslinking of coacervates using TA altered the secondary structure of gelatin. Circular dichroism revealed significant changes in the alpha helix and random coil, while Fourier transform infrared spectra (FTIR) showed a spectra change of protein amine groups due to coacervate crosslinking. The hardening of gelatin and high methyl pectin coacervates with TA significantly increased the size from 18 μm to 36 ± 2.3 μm. Crosslinked coacervates had a higher storage modulus (*G'*), with significantly improved melting and gelling points; an indication of enhanced intermolecular bonding after crosslinking. X-ray diffraction peaks of the powder showed a higher intensity in coacervates treated with TA, which was attributed to the increase in gelatin helix. Thermal gravimetric analysis data revealed that the crosslinking of coacervates using TA improved thermal properties. TA-crosslinked coacervates were used to produce peppermint oil multinuclear microcapsules with an average particle size of 47 μm, encapsulation efficiency of 75.4% and improved thermal stability.

1. Introduction

The electrostatic attraction between gelatin and pectin leads to complexation and coacervation. Generally, coacervation favors phase separation into either a solvent or biopolymer rich phase (also known as coacervates). Coacervates have gel network properties arising from strong electrostatic attraction and charge neutralization (Espinosa-Andrews, Sandoval-Castilla, Vázquez-Torres, Vernon-Carter, & Lobato-Calleros, 2010; Qiaomei Ru, 2012). However, ionic strength at high concentration and pH variation may weaken or completely prevent the electrostatic interaction existing between proteins and polysaccharides which affect the rheological properties of coacervates.

Coacervate crosslinking is an irreversible process intended to improve mechanical properties, pH stability, ionic strength, and high temperature stability (Dong et al., 2008). Crosslinking requires the application of compounds endowed with properties to harden the structure of coacervates. Different chemical cross linkers such as formaldehyde and glutaraldehyde were previously used; however, these compounds have been reported to be toxic and therefore not suitable for food application. Enzymes such as transglutaminase have been used to harden different coacervates like soy protein isolate and chitosan encapsulating capsanthin (Huang, Xiao, Qiu, & Yang, 2014) and pig gelatin A and gum Arabic encapsulating peppermint oil (Dong et al., 2008). In a study by (Prata, Zanin, Re, & Grosso, 2008), crosslinking using transglutaminase resulted in minor changes in the wall membrane structure. Specificity of enzymes used for coacervate crosslinking to certain amino acid residues and substrates requirement may be a limitation. Additionally, optimum pH and temperature for optimal enzymatic activity may vary from coacervation pH and temperature which can affect coacervate properties. Contrary to enzymes, polyphenols are plant secondary metabolites with aromatic rings and OH groups that are highly reactive to proteins and amino acids. Moreover, previous studies reported that the interaction between polyphenol and protein can occur in a wide pH range (Thongkaew, Gibis, Hinrichs, & Weiss, 2014). In addition, polyphenols have functional properties such as antioxidant, antimicrobial properties and prevent various chronic diseases (Aewsiri, Benjakul, Visessanguan, Wierenga, & Gruppen, 2010; Shavandi et al., 2018; Wang et al., 2018). Findings revealed that polyphenols significantly improved the gel network property of fish gelatin/gum Arabic coacervates and gelatin gels (Anvari & Chung,

* Corresponding author.
E-mail addresses: sqxia2006@hotmail.com, sqxia@jiangnan.edu.cn (S. Xia).

https://doi.org/10.1016/j.foodhyd.2019.105174
Received 18 March 2019; Received in revised form 30 May 2019; Accepted 18 June 2019
Available online 19 June 2019
0268-005X/

2016; Strauss & Gibson, 2004).

The utilization of polyphenols in natural biopolymer crosslinking has attracted more attention partly because they are natural, renewable materials, their use may also enhance the trigger release and bioactive properties of the product (Gao et al., 2019; Zou et al., 2018). Protein crosslinking using polyphenols at different pH was reported to alter the protein secondary structure, improve heat stability and rheological properties due to the formed protein – polyphenol complex (Aewsiri et al., 2010; Koupantsis, Pavlidou, & Paraskevopoulou, 2016; Zhan, Yang, Li, Wang, & Li, 2018). Several studies have reported that the structure and molecular weight of tannic acid increases its ability to strongly bond or precipitate protein (Reitzer, Allais, Ball, & Meyer, 2018). Hydrogen bonds, electrostatic attraction, van der Waals force, hydrophobic interaction and covalent bonds may occur between gelatin amino acids side chain and the phenol group of the polyphenols (Picchio et al., 2018). Properties of gelatin and pectin cross-linked with transglutaminase and glycerol were previously reported (Gupta, Tummalapalli, Deopura, & Alam, 2014; Huang, Tu, Wang, Liu, et al., 2017a, b). Finding of (Zhang et al., 2015) showed that lysozyme and pectin deposited on cellulose nanofibrous mats prepared by electrostatic deposition possessed excellent thermal property. However, there has been no report on the preparation of gelatin and high methyl pectin coacervates that were crosslinked with tannic acid (TA), and the resulting effect on coacervate properties and the application for peppermint oil microencapsulation. Tannic acid binds to both hydrophobic and hydrophilic amino acids and these multiple interactions may offer opportunity to produce essential oils microcapsules prepared by complex coacervates with excellent physicochemical properties.

The main objective of this study was to investigate the crosslinking of gelatin and high methyl pectin coacervates with tannic acid and the application of coacervates for peppermint oil microencapsulation. The effect of crosslinking with tannic acid on the secondary protein structure of coacervates was investigated by circular dichroism and attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. The flow behavior, gelling and melting properties of tannic acid-crosslinked coacervates were determined using a rheometer. A light microscope was used to observe the microstructure of crosslinked and non-crosslinked samples. X-ray diffraction (XRD) and thermal gravimetric analysis (TGA) were used to study the crystallinity and thermal degradation of coacervates, respectively. Moreover, encapsulation efficiency (EE), morphology, particle and thermal properties of tannic acid crosslinked gelatin and high methyl pectin coacervates encapsulating peppermint oil were reported.

2. Material and methods

2.1. Materials

Gelatin (G, type B, bloom 225, Moisture Content 10 ± 0.8%), sodium hydroxide and acetic acid were purchased from Shanghai Chemical Reagent Corporation (Shanghai, China). High methyl pectin (HMP, Moisture Content 7.2 ± 0.5%) with esterification degree (76.6%) was supplied by CP Kelco (Shanghai, China). Tannic acid was purchased from Shanghai Qiangsheng Biochemical Ltd. (Shanghai, China). Peppermint oil (94%) was purchased from Ji'an Ju Peng Natural Flavor oil Co LTD (Jilin, China). All materials were used without any further purification. All aqueous solutions were prepared with deionized water (Milli-Q water).

2.2. Preparation of gelatin and pectin coacervates and crosslinking using tannic acid

Gelatin and high methyl pectin at mixing ratio (3:1) with total biopolymer concentration of 1% w/v were prepared at 60 °C, pH 7 for 2 h. Few droplets of sodium azide 0.2% were added to prevent microbial growth. The temperature of gelatin and pectin mixtures were kept at 45 °C under stirring at 300 rpm while adjusting the pH 4.23 for coacervation using acetic acid and NaOH (1%). An ice-water bath was used to instantly lower the temperature below 15 °C. Samples were kept at this temperature for 30 min at 300 rpm to induce gel network formation and coacervation (Muhoza et al., 2019). Tannic acid was used for coacervate crosslinking according to the method previously reported by (Anvari et al., 2016) with slight modifications. Briefly, tannic acid was dissolved in distilled water with the concentration of 10% (w/v) and then a solution of tannic acid was added to gelatin and pectin coacervates at a final concentration of (0.25% v/v) for crosslinking at 25 °C. Tannic acid crosslinked coacervates were kept for 6 h at 25 °C with a stirring rate of 300 rpm to induce crosslinking, followed by decanting at 6 °C overnight and centrifuging at 2000 rpm for 4 min. The crosslinked and non-crosslinked gelatin and high methyl pectin coacervate gels were used for rheology. The samples used for powder properties were centrifuged, frozen and then freeze-dried at 55 ± 7 bar and condenser temperature −78 °C for 24 h using Scientz-18N freeze dryer (Ningbo Scientz Biotechnology co. Ltd, China).

2.3. Circular dichroism analysis

Far-UV circular dichroism spectra of tannic acid crosslinked and non-crosslinked coacervates were acquired using Dichrograph Instrument, MOS-450 CD Spec-tropolarimeter (Biologic, Claix, France). The spectra obtained were presented in terms of ellipticity [θ] in the range of 185–260 nm.

2.4. Attenuated total reflectance fourier transform infrared (ATR-FTIR) spectroscopy

Tannic acid (TA), gelatin and high methyl pectin coacervates, TA crosslinked coacervates and crosslinked essential oil microcapsules were respectively mixed with potassium bromide (KBr) (1:50), ground with a pestle and mortar and then pressed onto the ZnSe plate using a high-pressure clamp. Infrared spectra was obtained using FTIR spectrophotometer (Nicolet iS10, Thermo Electron Corp., Madison, Wisconsin). The spectra data was collected in the range of 400–4000 cm^{-1} at a 4 cm^{-1} resolution and a zero-filling factor of 1 using a Happ-Genzel apodization and Mertz phase correction.

2.5. Thermal gravimetric analysis (TGA)

Thermal gravimetric analysis was performed using a thermal gravimetric analyzer (TGA/SDTA851e, Mettler-Toledo Corporation, Switzerland). Tannic acid crosslinked coacervates, uncross linked coacervates and crosslinked essential oil microcapsule powder (3–5 mg), were weighed in the TGA microbalance and heated at 20 °C/min from 25 to 500 °C. Nitrogen gas was used as the heating medium with a flow rate of 20 mL/min. Sample powders were analyzed for thermal behavior.

2.6. X-ray diffraction studies (XRD)

Gelatin and high methyl pectin coacervate powder were filled into the sample holder and exposed to Cu Kα radiation in an X-ray powder diffractometer (D8, Bruker AXS, Germany). Each sample was scanned in a continuous mode at a scanning rate of 5°/min with the diffraction angle 2θ from $5° < 2\theta < 50°$.

2.7. Rheological properties

Rheological measurements were conducted by using a controlled-stress rheometer (AR 2000; TA Instruments, Newcastle, DE, USA) equipped with a 40 mm parallel plate geometry with a gap of 1000 μm. All samples were covered to prevent moisture loss and drying during measurements. For frequency sweep experiments, the linear

viscoelastic region was determined, and a strain of 2%, frequency range of 0.1–10 Hz was used at 25 °C. The viscoelastic ratio (tang $\delta = G''/G'$) equation (2) was used to analyze the frequency of sweep curves. The crossover of storage modulus (G') and loss modulus (G'') during heating and cooling are defined as melting (T_m) and gelling (T_g) points of coacervates. Coacervates were heated from 5 to 40 °C for T_m while cooling from 40 to 5 °C was carried out for T_m determination. Apparent viscosity of coacervates were determined at 25 °C in a shear rate range from 0.1 to 100 s^{-1}. The power law model equation (1) was used to analyze the flow graphs and determine K and n values.

$$\eta = K\,\gamma^{*\,n-1} \tag{1}$$

$$\text{tang}\ \delta = G''/G' \tag{2}$$

Where η is the apparent viscosity, γ^* is the shear rate, n is flow behavior index, and K is the flow consistency index.

2.8. Size distribution and optical microscope image

Particle size distribution analysis was performed using a laser particle size analyzer (Mastersizer 2000; Malvern Corporation, England). The refraction index applied was 1.59 for material and 1.33 for water dispersant. The liquid coacervates and essential oil microcapsules were correctly mixed, then dropwise added to the dispersant into the wet dispersion accessory of the Mastersizer. The morphology of tannic acid crosslinked tannic coacervate, microcapsules and uncross linked were observed using optical microscopy (BX51, Olympus Corporation, Japan) at a magnification of 200 × .

2.9. Essential oil microencapsulation by complex coacervation

Gelatin and high methyl pectin coacervates encapsulating peppermint oil were prepared by the emulsification of essential oils (core to wall ratio 2:1 on dry basis) in a gelatin and high methyl pectin solution (ratio 3:1) at a total biopolymer concentration of 1% w/v for 3 min at 9000 rpm using an ultraturax homogenizer (T25-D model; IKA Werke GmbH & Co., Staufen, Germany). To promote coacervation, the pH was changed to 4.23 with (1% v/v) acetic acid at 45 °C under a stirring rate of 300 rpm; after which, the temperature was lowered below 15 °C and kept under a 300 rpm stirring rate for 30 min to complete complex coacervation (Muhoza et al., 2019). Subsequently, coacervates containing peppermint oil were crosslinked with tannic acid for 6 h at 25 °C according to the method described by (Anvari et al., 2016) as described in section 2.2. Afterwards, the peppermint oil microcapsules obtained were freeze dried into powder for further application.

2.10. Determination of peppermint oil encapsulation efficiency

The ratio between the mass of peppermint oil to be encapsulated and total mass in the final freeze-dried powder was defined as encapsulation efficiency. The surface peppermint oil content was analyzed by spectrophotometer according to the method described by (Muhoza et al., 2019). The freeze-dried powder samples (2 g) were mixed with 20 mL of ethanol at 40 °C for 5 min without the destruction of the microcapsules. Surface essential oil was extracted by gentle shaking of the beaker. After extraction, the retained sample was collected to measure the quantity of essential content on the surface of the freeze-dried microcapsules. The total essential oil was extracted from freeze dried microcapsules by ultrasound sonication for 20 min at 40 °C for the total destruction of microcapsules. Sonication was followed by three successive washings with ethanol and centrifugation. After centrifugation, the retained sample was washed with ethanol. The obtained essential oil ethanol samples were evaporated and concentrated at 40 °C by rotary evaporator till 20 mL. The samples were collected to measure the total amount of essential oil and peppermint oil content on the surface of freeze-dried microcapsules. The absorbance of the above solution was determined by a spectrophotometer at 227 nm for peppermint. The essential oil was quantified using a standard curve. Encapsulation efficiency measurements were performed in triplicate for peppermint microcapsules. The encapsulation efficiency (EE) and encapsulation yield (EY) were calculated using the following equation:

$$EE(\%) = \frac{W1 - Ws}{W2} \times 100 \tag{3}$$

$$EY(\%) = \frac{W1}{W2} \times 100 \tag{4}$$

Where, W_1 is the total mass (g) of essential oils into microcapsules, W_2 is the mass (g) of peppermint oil loaded in the system *and* W_S is the mass (g) of peppermint oil on the surface of microcapsules.

2.11. Statistical analysis

Each experiment was done in triplicate under the same conditions. Statistical analysis was performed using SPSS 19.0 (IBM, Armonk, NY USA). Analysis of variance was used to compare means with Turkey multiple range tests for post hoc analysis. $P < 0.05$, was considered as significant.

3. Results and discussion

3.1. Circular dichroism and FTIR spectra of G-HMP coacervates crosslinked using tannic acid

The interaction between gelatin and tannic acid is realized by the side chains of protein amino acid and the hydroxyl and aromatic ring of tannic acid. Oxidation of tannic acid by oxygen molecules may lead to the formation of quinones during the crosslinking process. Quinones are electrophiles that are highly reactive to amino acids like tyrosine, proline, lysine, alanine, and methionine. The interaction mainly occurs through the hydroxyl of tannic acid and H^+ receptor of protein amino acids. Hydrophobic interaction occurs through the aromatic ring of tannic acid and hydrophobic amino acids such as leucine, isoleucine and proline (Poklar Ulrih, 2017). The covalent, hydrogen, hydrophobic and non-covalent interaction between side chains of protein amino acid and quinones could lead to complex formation and alter the secondary structure of gelatin in coacervates (Nie, Zhao, Wang, & Meng, 2017). From this background, the protein secondary structure of crosslinked and uncross linked coacervates were analyzed.

Circular dichroism (CD) was used to investigate the effect of coacervate crosslinking using tannic acid (TA) on gelatin secondary structure. As shown in Fig. 1A and Table 1, crosslinking of coacervates significantly increased the alpha helix ($p = 0.05$) from 26% in gelatin and high methyl coacervates to 49% in tannic acid crosslinked coacervates. The increase ($p = 0.05$) in alpha helix may be due to oxygen and hydrogen bonding together during the interaction of tannic acid and protein peptide to give rise to an alpha helix topology (Guo, Colby, Lusignan, & Whitesides, 2003). Moreover, random coil decreased ($p = 0.05$) from 36% in coacervates to 24% in crosslinked coacervates. Our CD spectra revealed a significant decrease in ellipticity band nearly 198–207 nm which was attributed to a decrease in random coil. Due to the intramolecular bonding of tannic acid and gelatin promoting protein aggregation, the CD spectra showed the lower ellipticity degree in 210–260 nm in crosslinked coacervates. Therefore, it was suggested that the crosslinking of gelatin and high methyl pectin using tannic acid increased intramolecular bonding and structure rearrangement (Rub, Asiri, Khan, Khan, & Kabir-ud, 2013).

FTIR spectra revealed significant changes due to coacervate crosslinking using tannic acid. As shown in Fig. 1B, FTIR spectra of tannic acid (TA), revealed a wider band in the region of 3600–3000 cm^{-1} due to – OH stretching. In TA spectrum, the bands at 1726 cm^{-1} indicate the presence of carboxyl carbonyl group. The bands displayed at 1612

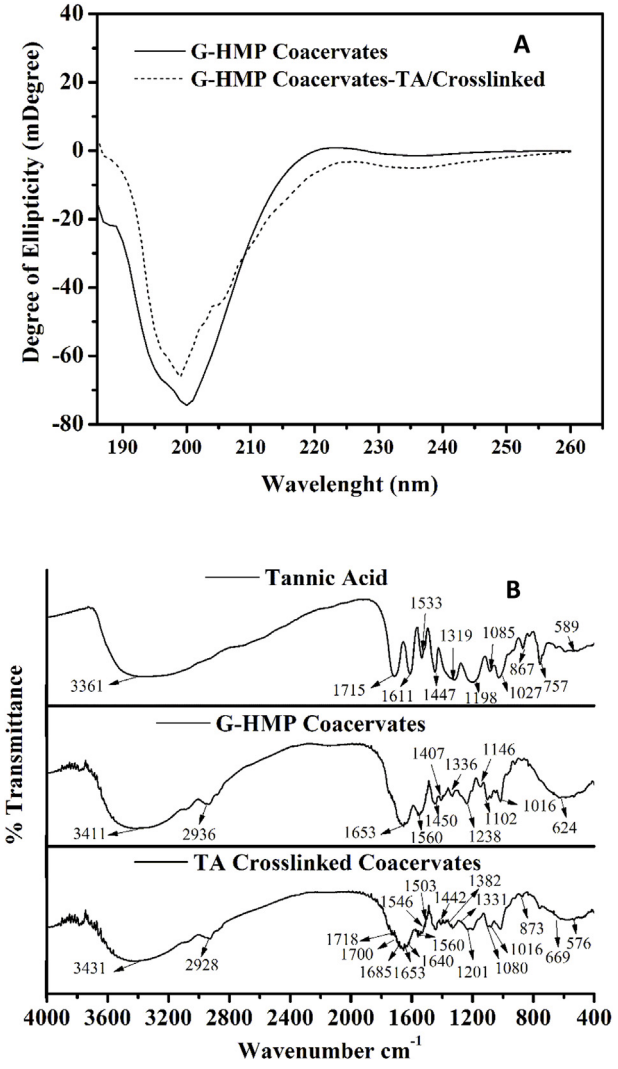

Fig. 1. (A) Circular dichroism (A) and Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) (B) of coacervates crosslinked using tannic acid.

and 1533 cm^{-1} revealed the presence –C= C- of the aromatic ring. The bands at 1447 cm^{-1} implied the deformation of -C-C- in the phenolic group, while the band 1319 cm^{-1} in TA spectrum was due to the phenol group. The band spectrum at 1198 cm^{-1} was due to C–H; additionally, vibration bands at 1100-1000 cm^{-1} were attributed to C–O and C–H deformation. The bands between 900 and 550 cm^{-1} were attributed to C–H bonds in the benzene ring. Our results are in agreement with previous findings (Erdem, Bursali, & Yurdakoc, 2013) on tannic acid characterization.

FTIR spectra of gelatin and pectin crosslinked by tannic acid exhibited shifts at the wavenumber of amine groups in coacervates (Amide- A, representative for NH and CH stretching of gelatin molecule) from 3411 cm^{-1} to 3431 cm^{-1}. The band shift was attributed to N–H and O–H partaking in hydrogen bonding in agreement with the results of (Anvari et al., 2016) on crosslinking of gelatin and gum Arabic using tannic acid. The major changes occurred from 1653 cm^{-1} to 1640 cm^{-1} and 1685 cm^{-1} (Amide I, attributed to C= O stretching/ hydrogen bonding) in coacervates due to the crosslinking of coacervates using tannic acid. A spectra shift was observed in the region (Amide- A, of gelatin molecule attributed to C–H stretching) at 2936 cm^{-1} in coacervates to 2928 cm^{-1} after coacervate treatment with tannic acid. FTIR spectra showed a change at a wavenumber of 1560 cm^{-1} (Amide II, representative for NH_2 bond stretching) in coacervates to 1546 and 1503 cm^{-1}due to crosslinking. Additionally, the wavenumber around 1238 cm^{-1} (Amide III, representative for C–N stretch bonding) present in G-HMP shifted to 1201 cm^{-1} after crosslinking of coacervates using tannic acid. The bands at 1450 cm^{-1} shifted to1442 cm^{-1} and the wavenumber around 1336 cm^{-1} moved to 1331 cm^{-1} due to crosslinking. New vibration bands appeared at wavenumber of 1718 cm^{-1} -1700 cm^{-1} indicating the presence of carboxyl carbonyl group of TA in crosslinked coacervates. Moreover, FTIR spectra of crosslinked coacervates showed wavenumber at 873 cm^{-1} -576 cm^{-1} responsible for the TA benzene ring. The shift of spectra wavenumber was due to the incorporation of tannic acid in gelatin and high methyl pectin. These results are in accordance with secondary structure alteration of gelatin in coacervates suggesting the interaction of gelatin and tannic acid.

3.2. Microstructure and size distribution of gelatin and high methyl pectin coacervates crosslinked with tannic acid

Fig. 2 shows the morphology and size distribution of gelatin and pectin coacervates crosslinked with tannic acid. Gelatin and high methyl pectin (G-HMP) coacervates had a smooth spherical shape, while tannic acid crosslinked coacervates were spherically shaped with some irregular, aggregated and rough surfaces due to the crosslinking effect. The particle size of G-HMP coacervates was 18 ± 0.5 μm (Fig. 2A), however crosslinking of G-HMP coacervates with tannic acid increased (p = 0.05) the size from 18 μm to 36 ± 2.3 μm (Fig. 2B). Our results are in accordance with FTIR and CD findings which revealed a strong interaction between tannic acid and gelatin in coacervates system. Interaction between gelatin side chain amino acids and the phenol group of tannic acid led to larger particle formation and a significant increase in size and shape variation. These findings are in accordance with our CD results and have been previously reported by (Thongkaew et al., 2014) on the interaction of whey protein isolate and pectin coacervates using different polyphenols.

3.3. Apparent viscosity

The results of viscosity at shear rates ranging from 0.1 to 100 s^{-1} of all coacervates are shown in Fig. 3. All the coacervates exhibited a shear thinning behavior due to structural loosening caused by increasing shear rate. Our findings are in accordance with previous findings on ovalbumin and gum Arabic coacervates (Niu et al., 2018). The crosslinking of coacervates using TA increased the viscosity of coacervates throughout the shear rate of 0.1–100 s^{-1}. FTIR and CD data revealed a significant change of gelatin secondary structure. The alpha helix control the strength of gelatin gels; thus, the significant increase of alpha helix resulted in strong coacervate gels. Tannic acid can bind to gelatin and form a complex with higher molecular weight which increases viscosity (Huang, Tu, Wang, Liu, et al., 2017a, b). These findings are in accordance with our results on gelatin conformation change

Table 1
Percentage of secondary structure of gelatin and pectin coacervates crosslinked with TA.

Samples	Alpha Helix	Antiparallel	Beta Sheet	B-Turn	Random Coil
Coacervates	26.2 ± 1.11[a]	9.8 ± 0.99[a]	10.0 ± 0.76[a]	18.0 ± 0.82[a]	36.0 ± 1.55[a]
TA-Coacervates	49.5 ± 2.38[b]	6.0 ± 0.45[b]	6.0 ± 0.34[b]	14.6 ± 0.66[b]	23.9 ± 1.04[b]

Different subscript letters in the same column indicate significantly different (P < 0.05).

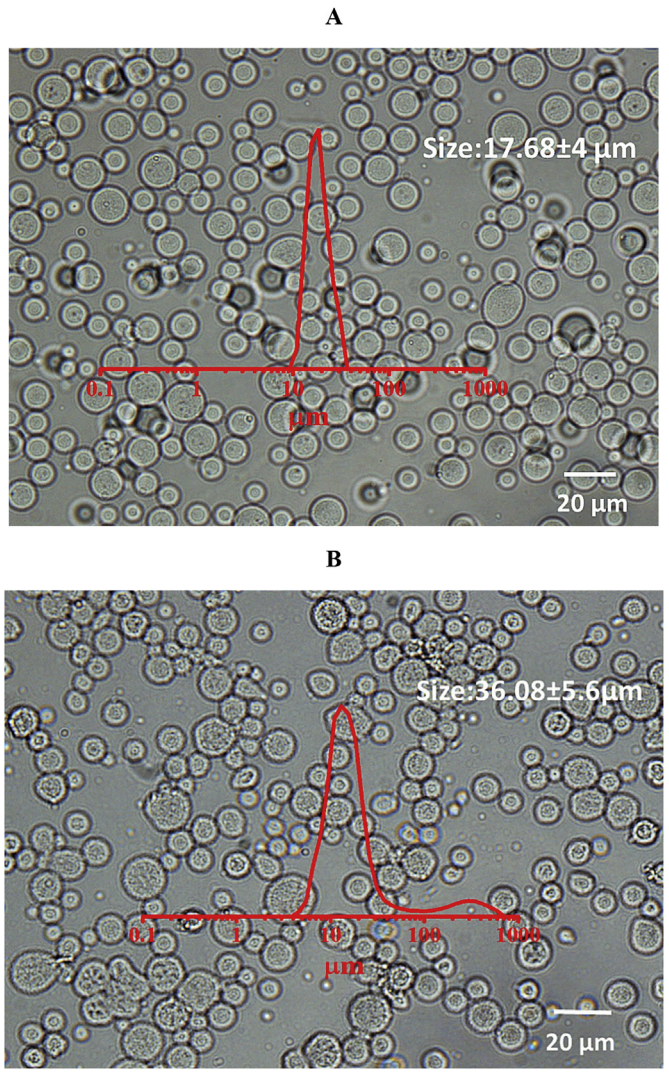

Fig. 2. Morphology and size distribution of (A) gelatin and high methyl pectin (B) gelatin and high methyl pectin crosslinked with tannic acid.

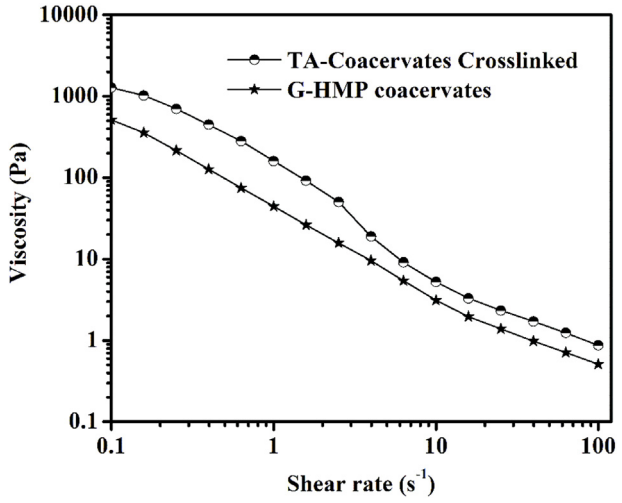

Fig. 3. Viscosity of Gelatin and High methyl pectin coacervates as a function of shear rate.

Table 2
Power law model for gelatin and pectin coacervates crosslinked with TA.

Sample	*K* (Pa·s)	*n*	R^2
Coacervates	44.83 ± 0.11[a]	0.13 ± 0.01[a]	0.99 ± 0.02[a]
TA-Coacervates	122.30 ± 0.15[b]	0.17 ± 0.02[b]	0.99 ± 0.01[a]

Different subscript letters in the same column indicate significantly different ($P < 0.05$).

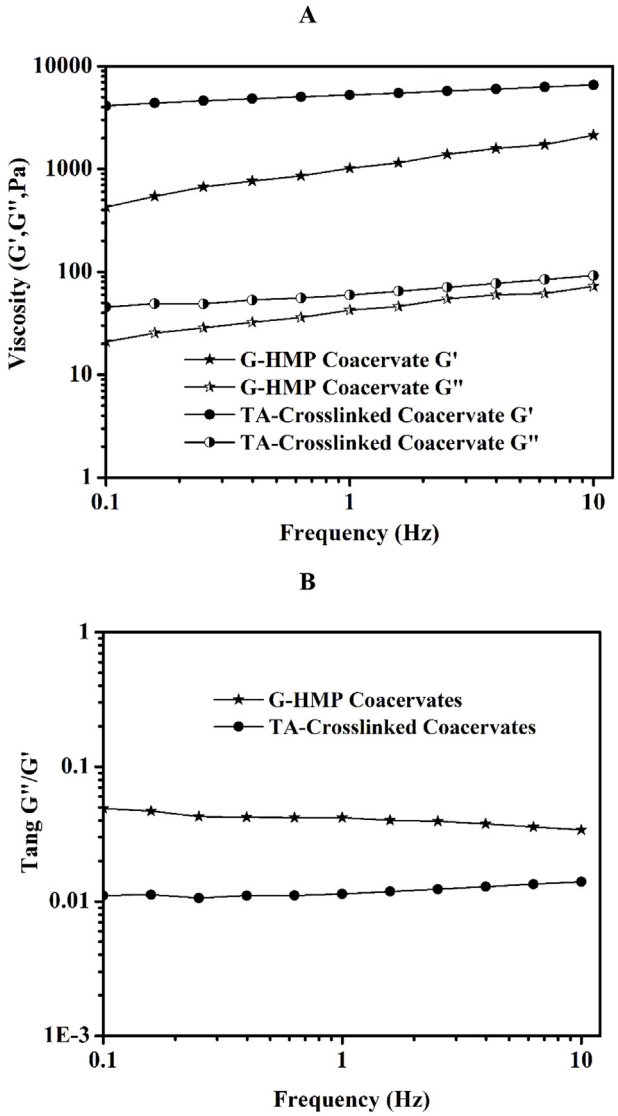

Fig. 4. Viscoelastic behavior of coacervates using frequency sweep (A) Gelatin and high methyl pectin changes in storage modulus (G′) and loss of modulus (G″) (B) Gelatin and high methyl pectin changes in the viscoelastic ratio (tang δ).

and coacervate morphology.

The evaluation of flow indices with the power law model are shown in Table 2. The flow behavior index revealed shear thinning behavior indicating that viscosity was shear dependent. All the coacervates had flow indices of less than 1 ($n < 1$), typical of non-Newtonian fluids. The flow consistency index (K) was high for samples crosslinked with tannic acid indicating that crosslinking significantly improved the flow behavior of coacervates at $p < 0.05$.

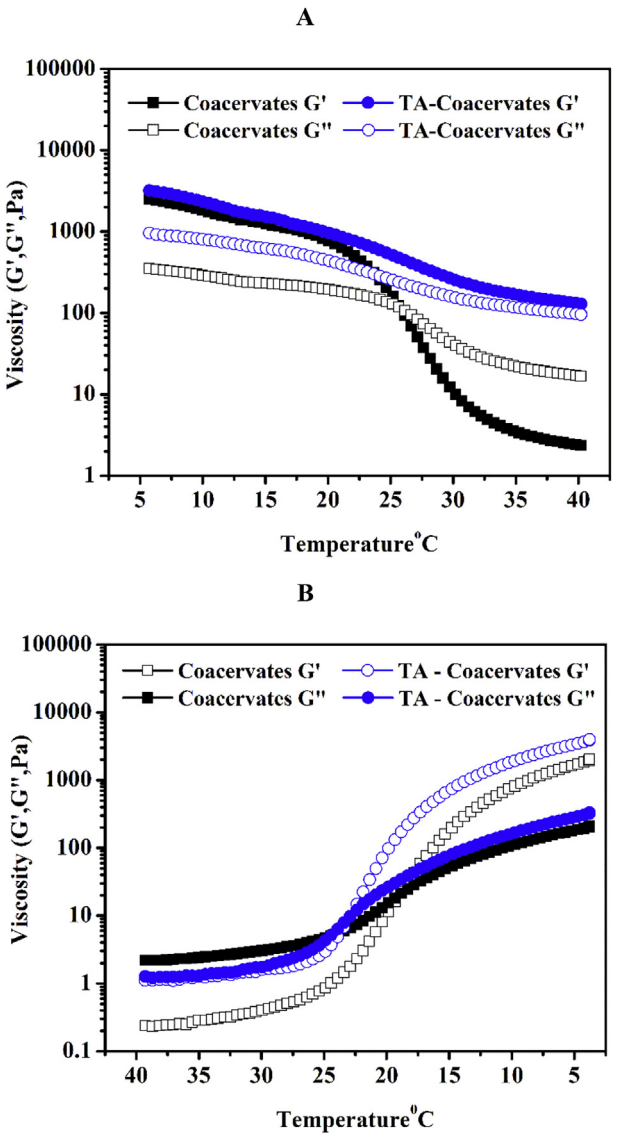

Fig. 5. Change in storage modulus (G′) and loss of modulus (G″) of gelatin and pectin coacervates (A) Heating of gelatin and high methyl pectin coacervates (B) Cooling of gelatin and high methyl pectin coacervates.

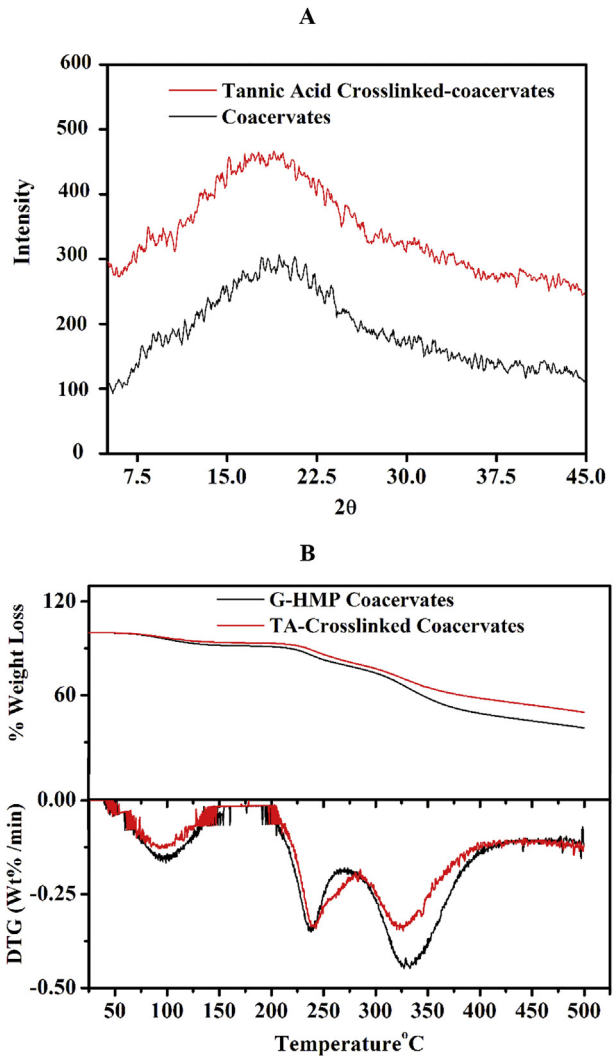

Fig. 6. Gelatin and high methyl pectin coacervates crosslinking with tannic acid (A) X-ray diffraction (XRD) (B) Thermal gravimetric analysis (TGA).

3.4. Investigation of viscoelastic behavior of gelatin and pectin coacervates crosslinked by tannic acid using frequency sweep test

The strength of the gel network formed during crosslinking of gelatin and pectin coacervates with tannic acid was evaluated by investigating the frequency dependence modulus. Fig. 4 shows the frequency dependence of tang δ (G"/G'), storage modulus (G') and loss of modulus (G") for G-HMP coacervates.

In the whole frequency range, the storage modulus (G') of all gelatin and pectin coacervates was higher ($P < 0.05$) than the loss modulus (G''), indicating that the elastic behavior was dominant in the samples. Tannic acid-crosslinked coacervates exhibited higher storage modulus (G′), which indicated that the intermolecular bonding was enhanced after crosslinking. The phenomenon is in agreement with CD and FTIR results. The viscoelastic ratio tang δ (G"/G') value ranged from 0.01 to 0.09 for all the coacervates; however, crosslinked samples had the lowest tang δ confirming that tannic acid improved the elastic behavior of coacervates. Our findings revealed that gelatin and pectin coacervates exhibited the characteristics of a strong gel system which is consistent with previous reports on the interaction of gelatin and carrageenan (Sow, Nicole Chong, Liao, & Yang, 2018). The interaction of tannic acid with gelatin contributed to the formation of coacervates with improved gel network properties.

3.5. Investigation of sol-gel transformation of gelatin and pectin crosslinked using tannic acid

To explore the effect of crosslinking coacervates with tannic acid on the thermo behavior of storage modulus (G') and loss modulus (G''), coacervates were subjected to heating (5–40 °C) and cooling (40-5 °C). Fig. 5 shows the effect of heating and cooling on storage modulus (G') and loss of modulus (G'') of gelatin and pectin treated with or without tannic acid. Heating and cooling of coacervates can result in a geometric point where storage modulus (G') and loss modulus (G") traverse. These intersection points are called melting points (T_m) during heating and gelling points (T_g) while cooling coacervates.

Gelation and melting point of coacervates arises due to the transition of single strand to triples helix of gelatin chains via hydrogen bonds, chains interactions, protein aggregation or self-assembly and hydrophobic interaction (Huang et al., 2018). All coacervates exhibited temperature dependence of storage modulus (G') and loss modulus (G'') during heating as shown in Fig. 5. The storage modulus (G') and loss modulus (G") decreased with the increase of temperature. Heating of gelatin and pectin coacervates from 5 to 40 °C revealed a melting point

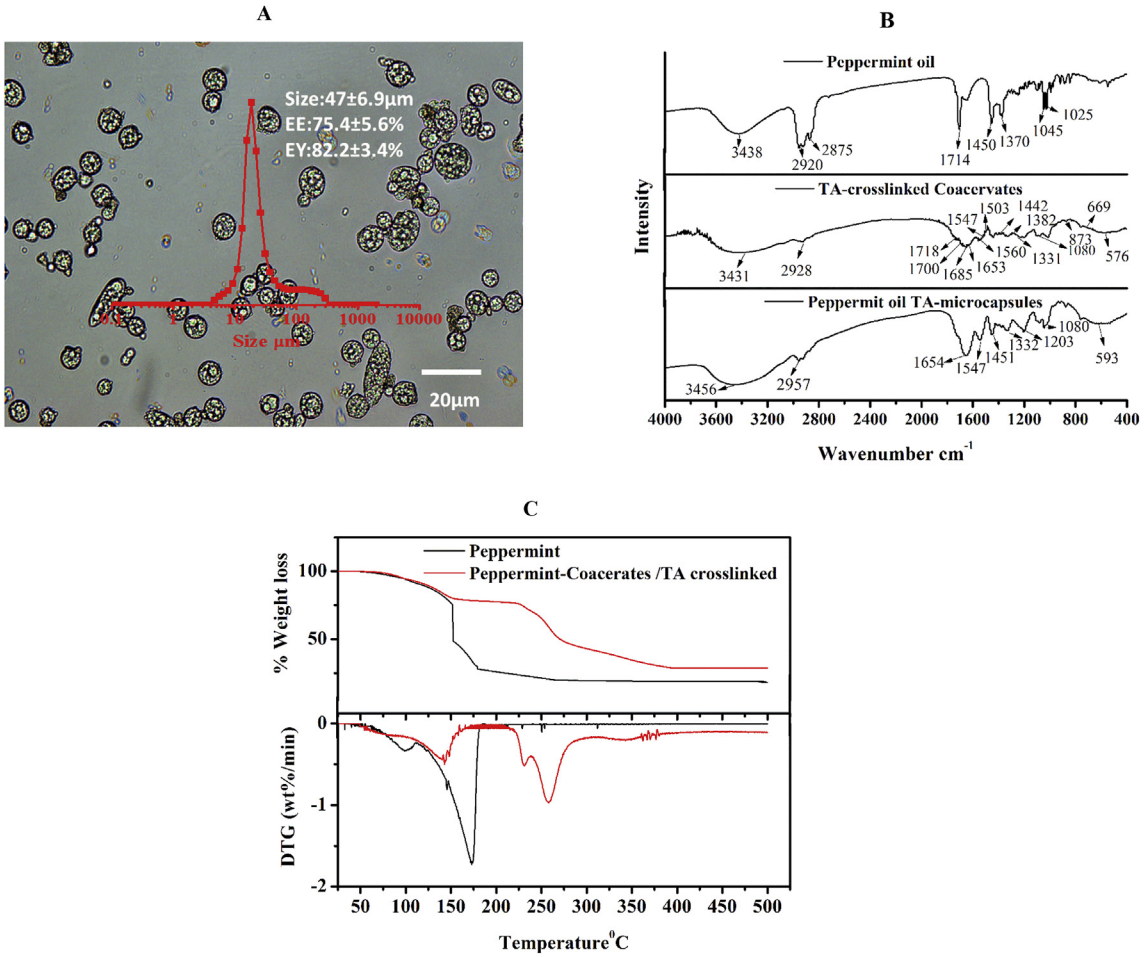

Fig. 7. Peppermint oil microcapsules prepared by complex coacervation using gelatin and high methyl pectin crosslinked using tannic acid (A) Morphology, size distribution and encapsulation efficiency (EE) (B) Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) (C) Thermal gravimetric analysis (TGA).

of 26 °C for G-HMP coacervates (Fig. 5A). Tannic acid-crosslinked coacervates did not exhibit a melting point within the range of 5–40 °C indicating that crosslinking improved the gel network properties of gelatin and pectin coacervates. The cooling of coacervates revealed gelling temperature of 19 °C and 23 °C for G-HMP and TA-crosslinked G-HMP (Fig. 5B), respectively. This was attributed to hydrogen bonding between tannic acid and gelatin that contributed to the formation of more rigid coacervates.

3.6. XRD and thermal behavior of gelatin and pectin coacervates crosslinked by tannic acid

The XRD pattern of coacervates with and without tannic acid is shown in Fig. 6A. The coacervates exhibited a wide peak at 20°. The presence of a wide peak at 2θ nearly 20° is consistent with a triple helical crystalline structure of collagen (Qiao, Ma, Zhang, & Yao, 2017). Our findings revealed that the crosslinking of gelatin and high methyl pectin with tannic acid did not change the crystallinity of coacervates. However, a significant increase in intensity was observed in gelatin and high methyl pectin coacervates crosslinked using tannic acid, which was due to an increase in gelatin helix resulting from the crosslinking effect of tannic acid (Liu, Antoniou, Li, Ma, & Zhong, 2015). X-ray diffraction findings were in agreements with the results on circular dichroism and FTIR.

Thermal degradation of tannic acid-crosslinked coacervates are shown in Fig. 6B. The first steps was due to water evaporation that occurred at nearly 100 °C. A slight difference was noticed in the mass loss between tannic acid-crosslinked coacervates and uncrosslinked coacervates. This was attributed to the interaction of tannic acid and gelatin that may have replaced the water molecules in the coacervate system through covalent, non-covalent and hydrogen bonding as a result of higher affinity between tannic acid and gelatin. Our results were similar to previously reported findings by (Anvari et al., 2016) on the preparation of gelatin and gum Arabic crosslinked using tannic acid.

The second stage was observed around 250 °C with a mass loss of 17% for G-HMP coacervates and 14% for G-HMP coacervates crosslinked with tannic acid. The third degradation steps occurred nearly at 300–350 °C with a mass loss of 35% for gelatin and high methyl pectin coacervates, while tannic acid crosslinked coacervates exhibited a mass loss of 30%. This was attributed to the breakdown of intramolecular bonding (Wu, Liao, Zhang, & Chen, 2018).

Tannic acid-crosslinked coacervate decomposition resulted in a total mass loss of 51%, while gelatin and high methyl pectin coacervates were at 61%. It was suggested that gelatin and tannic acid interaction led to the formation of complexes with improved thermal properties. These findings were in accordance with previous results on protein and phenolic interaction in silver carp myofibrilliar protein film (Nie et al., 2017).

3.7. Properties of peppermint oil microcapsules coacervates crosslinked using tannic acid

The properties of peppermint oil microcapsules prepared by complex coacervation method using G-HMP coacervates crosslinked with tannic acid were investigated. The average particle size ($D_{4,3}$) of peppermint oil microcapsules was 47 ± 6.9 µm, which was multinuclear

and unimodal with an encapsulation efficiency of 75 ± 5.6% (Fig. 7A) and encapsulation yield of 82 ± 3.4%. FTIR spectra of peppermint oil exhibited different peaks at 3438, 2920, 2875, 1714, 1450, 1370, 1045 and 1025 cm^{-1} (Fig. 7B). G-HMP coacervates encapsulating peppermint oil revealed spectra bands at 3456, 2957, 1654, 1547, 1451, 1332, 1203, 1080 and 593 cm^{-1}. Comparing the spectra of unloaded and peppermint oil-loaded coacervates, there was spectra variation indicating that peppermint oil was encapsulated in the coacervates. Fig. 7C showed the thermal degradation curve of free peppermint oil and peppermint oil-loaded coacervates. Peppermint oil revealed a total degradation at 175 °C, however peppermint oil microcapsules exhibited a three stage degradation pattern. The first step is nearly between 150 and 200 °C due surface peppermint oil. The second and third degradation were observed at nearly 225 and 275 °C, respectively. Our findings suggested that tannic acid-crosslinked coacervates could significantly improve the thermal properties of peppermint oil.

4. Conclusion

The findings of this study revealed that crosslinking of gelatin and pectin coacervates using tannic acid significantly altered the secondary structure of protein. CD data and FTIR spectra confirmed a change in protein secondary structure in tannic acid-crosslinked coacervates. Additionally, crosslinking reduced random coil, increased alpha helix and displayed spectra shifts at various gelatin amide groups. Tannic acid and coacervate interaction led to the formation of larger coacervates with irregular shape, rough surface and aggregates as shown by light microscope and particle size distribution. Hardening of gelatin and high methyl pectin using tannic acid improved the elastic behavior and gel network properties. X-ray diffraction findings showed that the crosslinking of coacervates using tannic acid did not change the crystallinity of coacervates though it was revealed that alpha helix increased. Thermal gravimetric analysis results revealed that tannic acid-crosslinked coacervates could be relatively more heat resistant. Moreover, gelatin and high methyl pectin coacervates crosslinked using tannic acid were used to produce peppermint microcapsules with high encapsulation efficiency and improved thermal stability. Our results showed that gelatin and high methyl pectin coacervate properties can be improved using tannic acid and applied to prepare microcapsules for the food and pharmaceutical industries.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

This research was financially supported by projects of the National key R & D program (2016YFD0400801), National Natural Science Foundation of China (31471624), National First-class Discipline of Food Science and Technology (JUFSTR20180204), and program of “Collaborative innovation center of food safety and quality control in Jiangsu Province”.

References

Aewsiri, T., Benjakul, S., Visessanguan, W., Wierenga, P. A., & Gruppen, H. (2010). Antioxidative activity and emulsifying properties of cuttlefish skin gelatin–tannic acid complex as influenced by types of interaction. *Innovative Food Science & Emerging Technologies, 11*(4), 712–720.

Anvari, M., & Chung, D. (2016). Dynamic rheological and structural characterization of fish gelatin – gum Arabic coacervate gels cross-linked by tannic acid. *Food Hydrocolloids, 60*, 516–524.

Dong, Z. J., Xia, S. Q., Hua, S., Hayat, K., Zhang, X. M., & Xu, S. Y. (2008). Optimization of cross-linking parameters during production of transglutaminase-hardened spherical multinuclear microcapsules by complex coacervation. *Colloids and Surfaces B: Biointerfaces, 63*(1), 41–47.

Erdem, P., Bursali, E. A., & Yurdakoc, M. (2013). Preparation and characterization of tannic acid resin: Study of boron adsorption. *Environmental Progress & Sustainable Energy, 32*(4), 1036–1044.

Espinosa-Andrews, H., Sandoval-Castilla, O., Vázquez-Torres, H., Vernon-Carter, E. J., & Lobato-Calleros, C. (2010). Determination of the gum Arabic–chitosan interactions by Fourier Transform Infrared Spectroscopy and characterization of the microstructure and rheological features of their coacervates. *Carbohydrate Polymers, 79*(3), 541–546.

Gao, S., Tang, G., Hua, D., Xiong, R., Han, J., Jiang, S., et al. (2019). Stimuli-responsive bio-based polymeric systems and their applications. *Journal of Materials Chemistry B, 7*(5), 709–729.

Guo, L., Colby, R. H., Lusignan, C. P., & Whitesides, T. H. (2003). Kinetics of triple helix formation in semidilute gelatin solutions. *Macromolecules, 36*(26), 9999–10008.

Gupta, B., Tummalapalli, M., Deopura, B. L., & Alam, M. S. (2014). Preparation and characterization of in-situ crosslinked pectin-gelatin hydrogels. *Carbohydrate Polymers, 106*, 312–318.

Huang, T., Tu, Z. C., Shangguan, X., Wang, H., Sha, X., & Bansal, N. (2018). Rheological behavior, emulsifying properties and structural characterization of phosphorylated fish gelatin. *Food Chemistry, 246*, 428–436.

Huang, T., Tu, Z. C., Wang, H., Liu, W., Zhang, L., Zhang, Y., et al. (2017a). Comparison of rheological behaviors and nanostructure of bighead carp scales gelatin modified by different modification methods. *Journal of Food Science & Technology, 54*(5), 1256–1265.

Huang, T., Tu, Z. C., Wang, H., Shangguan, X., Zhang, L., Zhang, N. H., et al. (2017b). Pectin and enzyme complex modified fish scales gelatin: Rheological behavior, gel properties and nanostructure. *Carbohydrate Polymers, 156*, 294–302.

Huang, G. Q., Xiao, J. X., Qiu, H. W., & Yang, J. (2014). Cross-linking of soybean protein isolate-chitosan coacervate with transglutaminase utilizing capsanthin as the model core. *Journal of Microencapsulation, 31*(7), 708–715.

Koupantsis, T., Pavlidou, E., & Paraskevopoulou, A. (2016). Glycerol and tannic acid as applied in the preparation of milk proteins – CMC complex coacervates for flavour encapsulation. *Food Hydrocolloids, 57*, 62–71.

Liu, F., Antoniou, J., Li, Y., Ma, J., & Zhong, F. (2015). Effect of sodium acetate and drying temperature on physicochemical and thermomechanical properties of gelatin films. *Food Hydrocolloids, 45*, 140–149.

Muhoza, B., Xia, S., Cai, J., Zhang, X., Duhoranimana, E., & Su, J. (2019). Gelatin and pectin complex coacervates as carriers for cinnamaldehyde: Effect of pectin esterification degree on coacervate formation, and enhanced thermal stability. *Food Hydrocolloids, 87*, 712–722.

Nie, X., Zhao, L., Wang, N., & Meng, X. (2017). Phenolics-protein interaction involved in silver carp myofibrilliar protein films with hydrolysable and condensed tannins. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 81*, 258–264.

Niu, F., Kou, M., Fan, J., Pan, W., Feng, Z. J., Su, Y., et al. (2018). Structural characteristics and rheological properties of ovalbumin-gum Arabic complex coacervates. *Food Chemistry, 260*, 1–6.

Picchio, M. L., Linck, Y. G., Monti, G. A., Gugliotta, L. M., Minari, R. J., & Alvarez Igarzabal, C. I. (2018). Casein films crosslinked by tannic acid for food packaging applications. *Food Hydrocolloids, 84*, 424–434.

Poklar Ulrih, N. (2017). Analytical techniques for the study of polyphenol-protein interactions. *Critical Reviews in Food Science and Nutrition, 57*(10), 2144–2161.

Prata, A. S., Zanin, M. H., Re, M. I., & Grosso, C. R. (2008). Release properties of chemical and enzymatic crosslinked gelatin-gum Arabic microparticles containing a fluorescent probe plus vetiver essential oil. *Colloids and Surfaces B: Biointerfaces, 67*(2), 171–178.

Qiao, C., Ma, X., Zhang, J., & Yao, J. (2017). Molecular interactions in gelatin/chitosan composite films. *Food Chemistry, 235*, 45–50.

Qiaomei Ru, Y. W., Lee, J., Ding, Y., & Huang, Q. (2012). Turbidity and rheological properties of bovine serum albumin pectin coacervates Effect of salt concentration and initial protein polysaccharide ratio. *Carbohydrate Polymers,* (88), 838–846.

Reitzer, F., Allais, M., Ball, V., & Meyer, F. (2018). Polyphenols at interfaces. *Advances in Colloid and Interface Science, 257*, 31–41.

Rub, M. A., Asiri, A. M., Khan, J. M., Khan, R. H., & Kabir-ud, D. (2013). Interaction of gelatin with promethazine hydrochloride: Conductimetry, tensiometry and circular dichroism studies. *Journal of Molecular Structure, 1050*, 35–42.

Shavandi, A., Bekhit, A. E. A., Saeedi, P., Izadifar, Z., Bekhit, A. A., & Khademhosseini, A. (2018). Polyphenol uses in biomaterials engineering. *Biomaterials, 167*, 91–106.

Sow, L. C., Nicole Chong, J. M., Liao, Q. X., & Yang, H. (2018). Effects of κ-carrageenan on the structure and rheological properties of fish gelatin. *Journal of Food Engineering, 239*, 92–103.

Strauss, G., & Gibson, S. M. (2004). Plant phenolics as cross-linkers of gelatin gels and gelatin-based coacervates for use as food ingredients. *Food Hydrocolloids, 18*(1), 81–89.

Thongkaew, C., Gibis, M., Hinrichs, J., & Weiss, J. (2014). Polyphenol interactions with whey protein isolate and whey protein isolate–pectin coacervates. *Food Hydrocolloids, 41*, 103–112.

Wang, C., Sang, H., Wang, Y., Zhu, F., Hu, X., Wang, X., et al. (2018). Foe to friend: Supramolecular nanomedicines consisting of natural polyphenols and bortezomib. *Nano Letters, 18*(11), 7045–7051.

Wu, J., Liao, W., Zhang, J., & Chen, W. (2018). Thermal behavior of collagen crosslinked with tannic acid under microwave heating. *Journal of Thermal Analysis and Calorimetry, 135*(4), 2329–2335.

Zhang, T., Zhou, P., Zhan, Y., Shi, X., Lin, J., Du, Y., et al. (2015). Pectin/lysozyme bilayers layer-by-layer deposited cellulose nanofibrous mats for antibacterial application. *Carbohydrate Polymers, 117*, 687–693.

Zhan, F., Yang, J., Li, J., Wang, Y., & Li, B. (2018). Characteristics of the interaction mechanism between tannic acid and sodium caseinate using multispectroscopic and thermodynamics methods. *Food Hydrocolloids, 75*, 81–87.

Zou, Y., Zhang, L., Yang, L., Zhu, F., Ding, M., Lin, F., et al. (2018). Click” chemistry in polymeric scaffolds: Bioactive materials for tissue engineering. *Journal of Controlled Release, 273*, 160–179.