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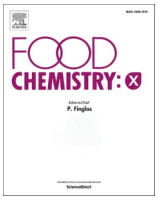

Effects of blackberry (*Rubus* spp.) polysaccharide on the structure and thermal behavior of the myofibrillar protein of chicken breast meat

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ABSTRACT

Blackberry crude polysaccharides (BCP) was added to chicken breast to inspect the intermolecular interaction with myofibrillar protein (MP). The influence of BCP on the thermal transformation behavior and protein microstructure during temperature rise period was studied. The results showed that the interaction between BCP and MP was mainly affected by the concentration of BCP and heating temperature. The results of infrared spectrophotometer and nano-particle/zeta potentiometer showed that a BCP-MP complex was generated through hydrogen bond and electrostatic interaction, which could promote the transformation of MP from β-folding to β-Angle transformation. The fluorescence spectra showed that the BCP was helped to the spread of protein structure of the MP. Moreover, synchronous thermal analyzer and rheometer results revealed that the BCP increased the enthalpy value and elastic modulus of MP. Scanning electron microscope verified pores inside the BCP-MP complex are more evenly distributed and smaller, which led to the high cross-linking of network and good stability of water distribution for the MP. The addition of BCP enhances the hydrogen bonds and disulfide bonds of MP molecules, which can strengthen the network structure and ultimately improve the performance of meat products.

Introduction

Chicken breast is a type of daily used food material which is cheap but contains a high protein content and low in fat and cholesterol contents, so it is favored by many consumers, especially for fitness enthusiasts (Cao et al., 2021). Nevertheless, the quality of chicken breast is less in juice and taste is tough, which greatly limits its market potential. Thus, it is very important to increase the tenderness for raw materials of chicken breast and improve its juiciness, increasing the product diversity of chicken breast products. Myofibrillar protein (MP) is an important component of chicken breast muscle, accounting for 60 % ~70 % of the total protein of chicken breast, mainly composed of actin, myosin, actomyosin, tropomyosin, and troponin (Dara et al., 2021). Functional properties of MP play a critical role in chicken breast products. For example, during heating processing, the MP molecule will chemically or physically interact with other non-protein components to form a gel network structure, which determines the texture structure, juiciness and stability of meat products during storage (Lundberg, 2005).

At present, multiple methods have been tried to interact with the MP to improve meat quality, such as enzyme treatment, complex phosphate treatment, ultrasonic treatment, or adding plant extracts, and etc. (Barekat & Soltanizadeh, 2018; Jayasooriya et al., 2004). Among them, recent studies found that the mixed system of protein/natural polysaccharide has been proved to effectively improve the characteristic of MP (Gao et al., 2022; Sharma et al., 2018). For examples, Ya-Kun Zhang reported that adding tremella polysaccharide could significantly improve the elasticity of MP gel (Zhang et al., 2017). Xinbo Zhuang reported that the changes of Raman spectra reflected that the addition of sugarcane insoluble dietary fiber had positive effects on the formation of solid and dense MP gel (Zhuang et al., 2018). Latest study by Keying Han found that the addition of inulin could enhance the interaction between

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MP molecules and the spatial network structure, thus improving the properties of MP gel (Han et al., 2022). In addition, compared with other methods, natural polysaccharides are numerous varieties with high yield, renewable, beneficial to health (Zhu et al., 2023). Numerous studies have shown that supplementation of natural polysaccharides can not only give specific flavor, taste, color and texture to meat products, but also reduce the number of microorganisms in meat products and extend their shelf life (Kallel et al., 2015; Eljoudi et al., 2022).

In the previous experiment, we extracted crude polysaccharide from blackberry (*Rubus* spp.) and analyzed it. The Blackberry crude polysaccharides (BCP) content was 822.13 mg/g, and it was composed of 95.44 % glucose, 2.01 % arabinose, 1.81 % galactose and 0.74 % glucuronic acid. Our previous study also discovered that BCP could significantly improve the quality of chicken breast and the production of volatile flavor substances. (He et al., 2023). BCP are natural functional compounds, and their application in meat products as antibacterial, antioxidant, flavoring agents and colorant has been reported, which can improve the nutritional value of meat products (Dou et al., 2021). But no relevant study has been covered on the usage as meat quality improvement agent.

Therefore, the present work studied the effect of different concentrations of the BCP on the micro-structure of MP from chicken breast, and investigated the thermal deformation of the MP during the heating process. Interaction between BCP and MP of chicken breast was analyzed by infrared spectrophotometer and nano-particle/zeta potentiometer. Then, the effects on the tertiary structure and thermal stability of the MP were analyzed by fluorescence spectroscopy, synchronous thermal analysis and rheometer. Finally, the changes in the microstructure of MP after the addition of BCP were observed by scanning electron microscopy (SEM). We hope our study could provide a theoretical basis from the perspective of protein confirmation and intermolecular forces to improve the quality of other gel-type meat products by using the BCP as a potential quality modifier.

2. Materials and methods

2.1. Materials

Chicken breast was purchased from Fujian Chia Tai Food Co. Ltd (Longyan city, China), and the powder of blackberry (*Rubus* spp.) crude polysaccharide was extracted in laboratory and stored at 4 °C until use. ColorMixed Protein Marker, Coomas bright blue quick dye solution, urea, Tris solution, and SDS solution were ordered from Solarbio Science & Technology Co. Ltd (Beijing, China). Other chemicals including EDTA and potassium bromide were purchased from Macklin biochemical Co. Ltd. (Shanghai, China).

2.2. Preparation of MP

The MP was prepared according to the method of Ke Li (Li et al., 2020). First, Cutting the chicken breast into squares (5×5×5 mm) with uniform size and shape, and dividing them into three equal parts.: experimental group 1 (adding 1 g/kg BCP), experimental group 2 (adding 3 g/kg BCP), and blank group (adding no substance). BCP was dissolved in water and added to chicken breast at a solid-liquid ratio of 1: 3, and cured in a refrigerator at 4 °C for 24 h. All the meat pieces were then ground in a meat grinder for 30 s and divided into two groups: one group was placed at normal temperature (25 °C), and the other group was heated at 80 °C for 1 h. For the consideration of reducing experimental error from size difference among the meat samples, the shredded meat was enemaed into casing in this experiment. The intestine segments were divided into 10 cm sections with straw ropes, tied tightly, vented air bubbles, heated in 80 °C water bath for 1 h, and cooled at room temperature. Then, the MP of meat was extracted by mixing with the buffer solution (10 mM Na_2HPO_4/NaH_2PO_4, 1 mM EGTA, 2 mM $MgCl_2$, 0.1 M NaCl, pH 7.0) in a ratio of 1:4 and then homogenized in a high speed viscolizer (Ultra Turrax T-25 Basic, IKS, Germany) at 6000 r/min for 60 s. The connective tissue was filtered through 4 layers of gauze and centrifuged at 3500× g for 10 min. The sediment was washed twice with 0.1 M NaCl buffer solution (volume ratio of 4:1), and the final paste was MP. All the processes were preformed on ice. The obtained MP was stored in a 4 °C refrigerator until use, and all analysis was required to be completed within 24 h.

2.3. Solubility, turbidity, total sulfhydryl content and active sulfhydryl content

The solubility of MP was evaluated according to the research method of Li et al (Li, Wang, Kong, Shi & Xia, 2019), in which the extracted MP was dissolved in 5 % NaCl (10 mM PBS) and determined in triplicate. After centrifugation at 4 °C for 15 min at 5000 rmp, the content of MP was determined by Coomassie bright blue method. Protein solubility was expressed as the percentage of protein content in the supernatant and the total protein content of the sample.

Turbidity was assessed by the absorbance at 320 nm of the MP with adjusted concentration of 2 mg/ml (Li et al., 2019). The contents of total sulfhydryl and active sulfhydryl were analyzed as referred to a previous study with a minor modification (Du et al., 2021). The mixture of 0.5 mL was evenly mixed with 4.5 mL buffer solution (consisting of 0.2 mol/L Tris, 8.0 mol/L urea, 2 % SDS, and 10 mmol/L EDTA, pH 6.8). Then, 0.5 mL of Ellman reagent containing 0.1 % DTN was added to the test tube and kept at 40 °C for 25 min. The same method was used to determine the active sulfhydryl content without adding urea. Total sulfhydryl and active sulfhydryl were measured at 412 nm using the thermos scientific micro-plate reader (Varioskan Flash, Finland). Results were calculated according to the following equation:

$$-SH(nmol/mg) = 10^6 * A * D/(\varepsilon * C)$$

A: absorbance at 412 nm;
D: dilution ratio;
C: concentration of MP (mg/ml):
ε: coefficient of molar absorption, 13,600 L/mol·cm.

2.4. SDS-PAGE

The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out with reference to Xia's method with a slight modification (Xia et al., 2018). The extracted MP of 5 mg/ml was mixed with the sample buffer containing 250 mM Tris-HCl, 10 % (w/v) SDS, 50 % (v/v) glycerol, 5 %(v/v) β-mercaptoethanol (β-ME) and 0.5 % (w/v) bromophenol blue pH 6.8 at a volume ratio of 4:1. The mixture was heated in a boiling water bath for 5 min, 5 μL of mixture was then loaded onto a polyacrylamide gel consisting of 10 % separation gel and 5 % concentrated gel. The gel was stained with a Coomassie bright blue rapid staining solution and decolorized with ultra-pure water until the background became clear. After electrophoresis using GelDoc XR^+ gel imaging system (Bio-Rad Laboratories, Inc., USA).

2.5. Particle size distribution and zeta potential measurement

Particle size distribution and zeta potential of 0.1 mg/mL MP sample were measured using a Zetasizer Nano ZSE instrument (Malvern Instruments Corp., Malvern, England) according to a relative method (Wang et al., 2021).

2.6. Fourier transformation infrared (FTIR) spectroscopy

Fourier infrared spectroscopy was analyzed by using FTIR instrument (Bruker TENSOR 27, Germany) (Yang, Wang, Li-Sha & Chen, 2021). Freeze-dried MP samples were mixed well with KBr at a ratio of 1:150, and ground into powder for tablet pressing. The samples were

scanned 32 times under the wavelength range of the spectrum between 400 and 4000 cm^{-1} and the resolution of 4 cm^{-1} at room temperature.

2.7. Fluorescence spectrum

The fluorescence intensity of MP was measured according to the method of Feng R et al (Feng et al., 2022). The concentration of MP was diluted to 0.2 mg/mL with 10 mM Na_2HPO_4/NaH_2PO_4 (pH 7.0) and its fluorescence intensity was measured by RF-5301PC series spectrofluorometer (Shimadzu, Japan) under excitation wavelength of 295 nm, emission wavelength ranges 250 nm – 400 nm, and the width of excitation and emission slit of 5 nm.

2.8. DSC

The thermal denaturation of MP samples was analyzed by STA449F3 simultaneous thermal analyzer (NETZSCH, Germen) according to the method of Kang L (Liu et al., 2019). In brief, 5 mg of freeze-dried powder of MP was precisely weighted, transferred to an aluminum cell, and put the pot into the DSC sample clip. After balancing at 25 °C for 10 min, the sample was measured on the DSC calorimeter by scanning at 30–100 °C with a heating rate of 5 °C/min. Temperature at each endothermic peak was recorded.

2.9. Rheological analysis

Rheological properties of the sample were performed according to Nannan Yu with a slight modification (Yu, Xu, Jiang & Xia, 2017). Dynamic viscoelastic measurements were employed using a HAAKE MARS III rotary rheometer (thermerfeld, USA) with advance modules to measure the memory (G') and loss (G") moduli. Parallel plate geometry with a diameter of 20 mm and a clearance of 1 mm was used. Measurements were completed with 1 % strain and 1 Hz frequency and the dynamic temperature scanning ranged from 25 °C to 95 °C at a warming rate of 2 °C/min. The extracted MP (2 g) was poured directly onto the bottom plate of the rheometer, paved, and covered with silicone oil before measurement to avoid sample evaporation during the test. All dynamic rheologic data were obtained by using data analysis software of Rheo-Win Data Manager.

2.10. Scanning electron microscopy

The freeze-dried MP was fixed on the copper root, then coated with gold, and observed by a scanning electron microscope (SEM, ZEISS Sigma 300, Germany) under a magnification of 6,000 times.

2.11. Data analysis

Each trial in the present study was repeated at least three times and all experimental data were expressed as mean ± standard deviation (SD). Significant differences were assessed by one-way analysis of variance (ANOVA, 95 % significance level) and Duncan's test using SPSS software (version 16.0, SPSS Inc., Chicago, USA). Figures were drawn by Origin 2019 software (OriginLab, Co., Ltd., Northampton, MA, USA).

Results and discussions

3.1. The effect of BCP on the turbidity and solubility of MP

Fig. 1A shows the turbidity changes after the MP was added with different concentrations of BCP under room temperature and 80 °C heating. The turbidity is an index for the formation of a complex that was influenced by the interaction between MP and BCP. At room temperature, a significant increase in turbidity was confirmed when the BCP concentration increased to 3 g/kg ($p < 0.05$). And heating treatment at 80 °C for 1 h was able to get much higher increase in the turbidity. The increase of turbidity might be caused by competitive hydration of confirmed the interaction between MP and BCP, and this competitive

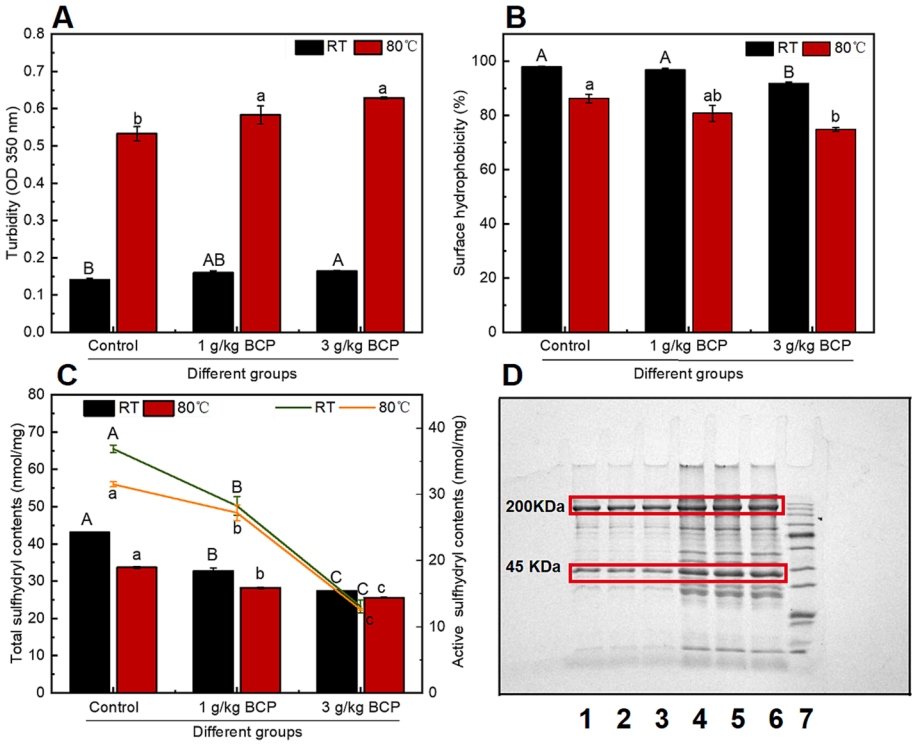

Fig. 1. The effect of different concentrations of BCP on (A) turbidity, (B) total sulfhydryl contents, (C) surface hydrophobicity, and (D) SDS-PAGE profile for the MP of chicken breast at room temperature and 80 °C heating treatment. Note: lane 1–3: lane 4–6: SDS-PAGE patterns of MP after the addition of 0, 1, 3 g/kg BCP heating at 80 °C for 1 h; lane 7: molecular weight standards. Different letters in the same treatment indicated significant differences ($p < 0.05$).

hydration resulted in the destruction of hydration layer on the surface of MP, which could be echoed with the change of solubility.

Solubility is a key functional property reflecting aggregation degree of protein (Du et al., 2021). Fig. 1B shows the changes of solubility of MP added with different BCP concentrations under room temperature or after heating at 80 °C for 1 h. The protein solubility decreased significantly with the addition of 3 g/kg BCP ($p < 0.05$) at room temperature, and heating treatment (80 °C for 1 h) resulted in a comparatively severe decline in the protein solubility. The possible reason mainly due to the addition of BCP promotes the electrostatic interaction of MP to form a complex, which were also co-demonstrated by the zeta potential data in Fig. 2C and the increased turbidity in Fig. 1A. Moreover, the heating treatment contribute to spread the structure of protein, and the internal hydrophobic groups and sulfhydryl groups leak out. It also causes a generation of disulfide bonds, as well as the cross-linking and aggregation of protein, leading to decreases in solubility (Na et al., 2023).

3.2. Total sulfhydryl content and active sulfhydryl content

Fig. 1C shows the total sulfhydryl content and active sulfhydryl content of MP after reacted with different concentrations of BCP at room temperature or heating at 80 °C for 1 h. As compared to the control, the total sulfhydryl and active sulfhydryl contents of MP decreased significantly when BCP ($p < 0.05$) was supplemented and reacted at room temperature with a dose-dependent manner. And heating treatment at 80 °C for 1 h led to declines in total sulfhydryl content of MP. It was worth noticing that the heating treatment (80 °C, 1 h) caused a significant decrease in total sulfhydryl and active sulfhydryl content of the control, but the BCP supplementation could effectively reduce the content variation induced by different temperatures. This may be due to the fact that the sulfhydryl group located in the head of MP are exposed due to the expansion of the head-to-head structure. MP molecules aggregate with the exposed sulfhydryl group by the thermal energy to form disulfide or sulfur-containing group, resulting in low total sulfhydryl and active sulfhydryl contents (Yu et al., 2017). Therefore, the addition of BCP expanded the protein structure and promoted the transformation of sulfhydryl groups to disulfide bonds. And a recent study by Zhang et al. showed that the content of disulfide bonds could significantly affect the gel properties of porcine myosin, which were consistent with our results (Zhang et al., 2020).

3.3. SDS-PAGE

The change of electrophoresis band can reflect the degradation of myofibrils after heating. The main body of MPs consists of two main heavy chains with myosin (MHC, 200 kDa) and actin (45 kDa) as well as several low strength molecular weight (Grossi et al., 2016). Myosin, actin, and actomyosin complexes are the most abundant proteins and are thought to be responsible for functional properties, while gels and other functional properties are affected by the overall effects of all proteins in the food system. It can be seen from Fig. 1D that the number of bands at 80 °C is more than that at room temperature, indicating that high temperature degrades MP. Notably, the strength of the MHC bands without the addition of BCP was weaker than the bands with the addition of BCP, which may be attributed to cross-linking between myofibril and BCP residues. As noting above, the MP-BCP complex could effectively alleviate the decrease of myofibrillar heavy chain concentration and reduce protein degradation.

3.4. Particle size and zeta potential

Protein particle size can directly reflect the degradation and aggregation of proteins (Cai et al., 2019). Fig. 2(A and B) shows that the addition of BCP significantly increased the particle size of MP ($p < 0.05$) at room temperature/heating at 80 °C for 1 h, indicating that BCP may aggregate with MP molecules. On the one hand, this may be due to the fact that the addition of BCP causes MP molecules to unfold and lead to protein aggregation. On the other hand, the polymer formed by non-covalent cross-linking of BCP and MP greatly increased the particle size.

Zeta potential reflects the electrostatic interaction between molecules (Ravindran et al., 2018). With or without the addition of BCP, the zeta potential results for MP were negative (Fig. 2C). BCP contains uronic acid which has a negative charge, and the zata potential of BCP we measured was −30 mV, pH (7.0) > pI (5.8) protein shows negative charge, but the local region of the protein still has $–NH_3^+$ with positive charge (Xue et al., 2021). The absolute value of MP potential did not increase with the addition of BCP. This suggests that the more BCP added, the more positive charge ($–NH_3^+$) was exposed, promoting the development of myofibrillar structure. As a result, they interact with each other by electrostatic bond. At the same time, the turbidity of MP was enhanced after the addition of BCP, which confirmed that the electrostatic interaction occurred between MP and BCP molecules, forming a complex.

3.5. Secondary structure analysis of MP

In the absorption band, 3000–3500 cm^{-1} is hydroxyl hydration stretch, indicating the existence of hydrogen bond interaction. Amide I belt emerged in 1600–1700 cm^{-1} suggests C—O stretching, which can be used for the analysis of protein secondary structure. The absorption range between 800 and 1200 cm^{-1} is associated with the stretching of C—O and C—C, and the bending of C—H, which is considered to be the absorption region of polysaccharide (Yang et al., 2021).

Fig. 3A and B are Fourier infrared spectral curves of MP after the

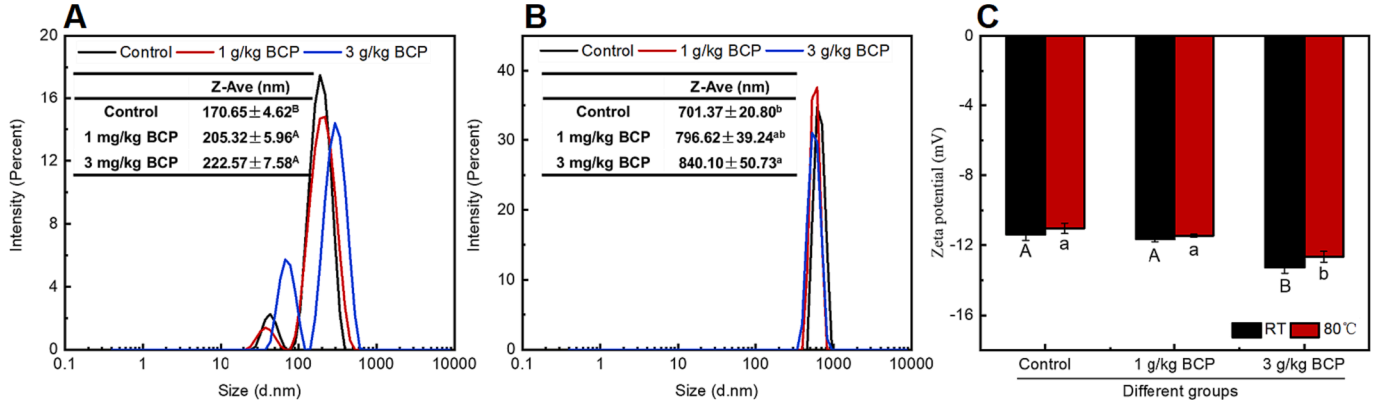

Fig. 2. The effect of different concentrations of BCP on the size distribution at room temperature(A), 80 °C heating treatment (B), and zeta potential (C) of MP from chicken breast. Different letters in the same treatment indicated significant differences ($p < 0.05$).

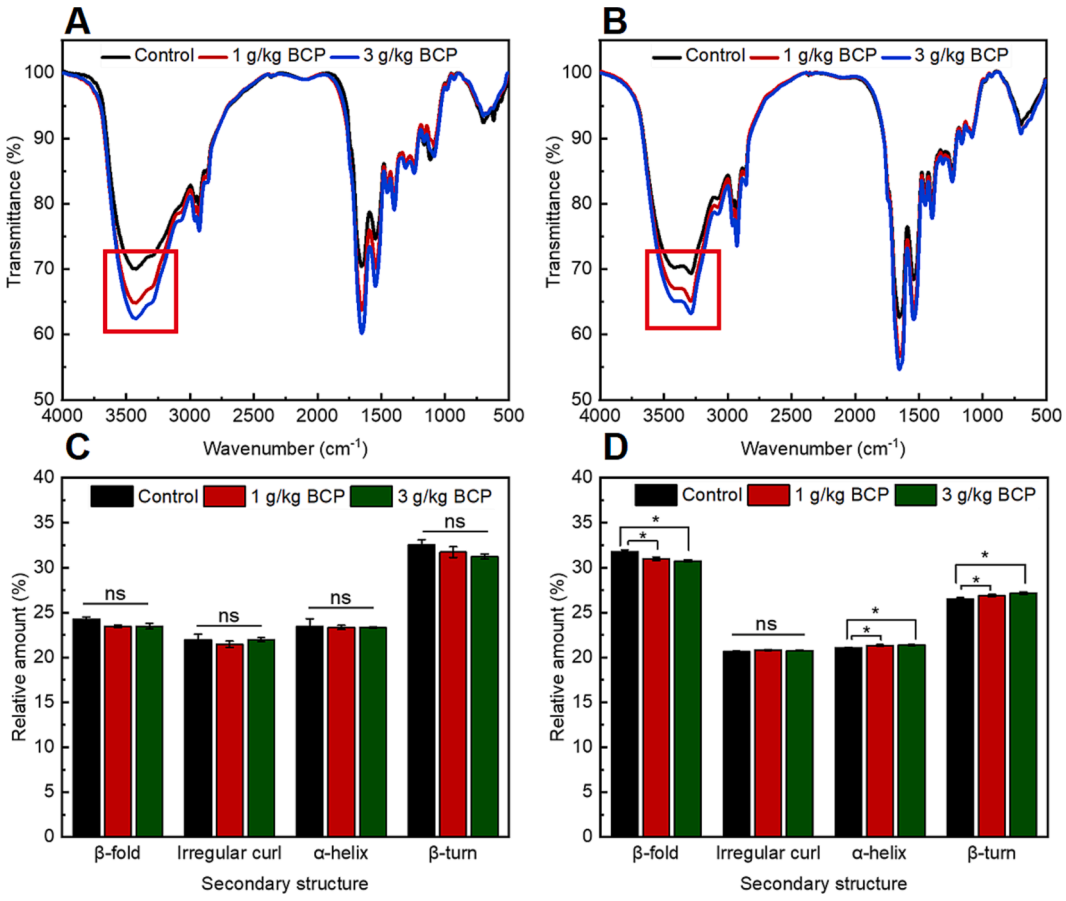

Fig. 3. Fourier infrared spectrogram variation of MP of chicken breast after supplementing with different concentration of BCP at room temperature (A) or at 80 °C (B). And the relative amount of secondary structure of the protein for the corresponding sample obtained at room temperature (C) or heating at 80 °C(D). "*" indicated the significant difference of compared groups. "ns"indicated no significant difference of compared groups ($p > 0.05$).

addition of different concentrations of BCP (0, 1, and 3 g/kg) at room temperature/heating at 80 °C for 1 h, respectively. The results showed that the transmittance of MP at the range of 3200–3400 cm^{-1} decreased when mixed with BCP at room temperature or after heating, indicating that the hydrogen bond interaction between them was strengthened. A similar result was reported by Ya-Kun Zhang working on the interaction between tremella polysaccharide and MP (Zhang et al., 2017).

The relative contents of secondary structure (α-helix, β-folding, β-turn, random curling) were obtained by second derivative, peak separation and fitting of the amido I band. The results showed that when the temperature increased to 80 °C, the α-helix content of all samples decreased, while the β-folding content increased. The stability of the α-helical structure is primarily dependent on the hydrogen bond between the carbonyl group (C—O) and the amino group (–NH_2). In addition, the stability of β-folding structure is also related to the formation of hydrogen bonds between peptide chains. Elevated temperature will lead to the uncoiling of the α-helix structure of MP, and the α-helix structure will be transformed into β-folding. The increase of β-folding content after heat treatment indicates the increased degree of protein aggregation. Adding BCP without heating treatment (at room temperature) had no significant effect on the variation of the secondary structure of MP. But β-folding was significantly decreased and β-turn was significantly increased ($p < 0.05$) after the MP reacted with BCP at 80 °C for 1 h, indicating that the secondary structure tended to be disordered and stretched, leading to the spread of the protein structure.

3.6. Tertiary structural analysis of MP

The polarity of microenvironment around protein has great influence on the fluorescence intensity of endogenous tryptophan. Generally, when the protein is n a partially or completely unfolded state., tryptophan residues will be more vulnerable to the surface of the protein molecule in the polar environment of the solvent. At this time, the fluorescence intensity for excited tryptophan decreases, and the fluorescence spectrum provides us with information about the tertiary structure of the protein (Wang et al., 2022).

The fluorescence spectra for the MP after the addition of different concentrations of BCP (0, 1 and 3 g/kg) at room temperature or by heating at 80 °C for 1 h is shown in Fig. 4A and Fig. 4B, respectively. Compared with MP obtained at room temperature (Fig. 4A), fluorescence intensity decreased at 80 °C, which was because protein molecules were stretched by heat, the exposure degree of tryptophan residues increased, leading to the decrease in fluorescence intensity. When BCP was added, it reduced the fluorescence intensity of the MP in a concentration-dependent manner. As shown in Fig. 4B, the decrease in fluorescence intensity may involve the further stretching of MP structure and the interaction between BCP and MP tryptophan residues, getting to the decrease in surface hydrophobicity. In other words, BCP could unfold the structure of MP through the formation of complex during the heating process. To a certain extent, the BCP accelerated the expansion of myofibril structure under the action of tryptophan residues, therefore, quenching of internal fluorescence is promoted and the fluorescence intensity is reduced. This was in line with the results of the interaction between heat-induced myosin and polysaccharide of Cytophania Sinica studied by Gang You (You et al., 2023). Besides, Shuai Jiang found that the addition of TRC or TIRC significantly reduced the fluorescence intensity of tryptophan extracted from MP composite sol in a concentration-dependent manner, as compared with MP alone (Jiang

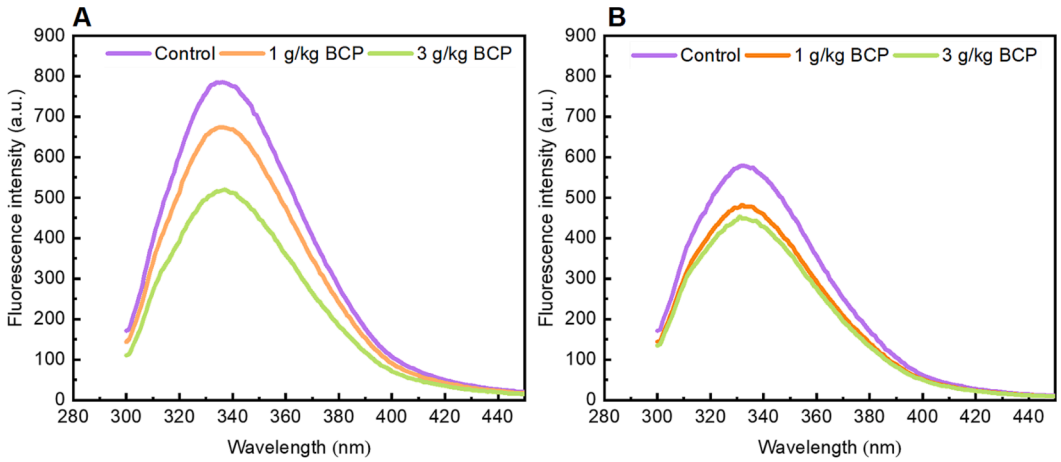

Fig. 4. Fluorescence spectrogram for the interaction effect of MP and BCP under room temperature (A) or after heating at 80 °C for 1 h (B).

et al., 2020).

3.7. Analysis of thermal properties of MP

Differential scanning calorimetry (DSC) was employed to analyze enthalpy changes required for the denaturation of MP that added with varied concentrations of BCP (0, 1 and 3 g/kg). Fig. 5A shows the DSC diagram during the heating process of MP. In the process of endothermic denaturation of proteins, the polypeptide chain opens and the intermolecular force is undermined. When a definite protein reaches its denaturation temperature, the corresponding absorption peak will appear, and enthalpy value which is the heat required for endothermic denaturation of proteins becomes higher (Cheng et al., 2019). After adding BCP, the enthalpy of MPs increased from 0.4 J/g to 0.55 J/g. Since the BCP-MP molecules bind together to form tight structures through hydrogen bonds and electrostatic interactions, it requires more energy to break energy bands.

Fig. 5B and C show the effect of different concentrations of the BCP (0, 1 and 3 g/kg) on the G' and G" of MP with variations in heating temperature. In the process of deformation, G' represents the stored energy of the sample caused by elastic deformation, while G" represents energy loss (Ji et al., 2017). As can be observed in Fig. 5B and C, during the entire heating process, G' is always higher than G", indicating that the gel formed is mainly elastic. Supplemental level of BCP had little

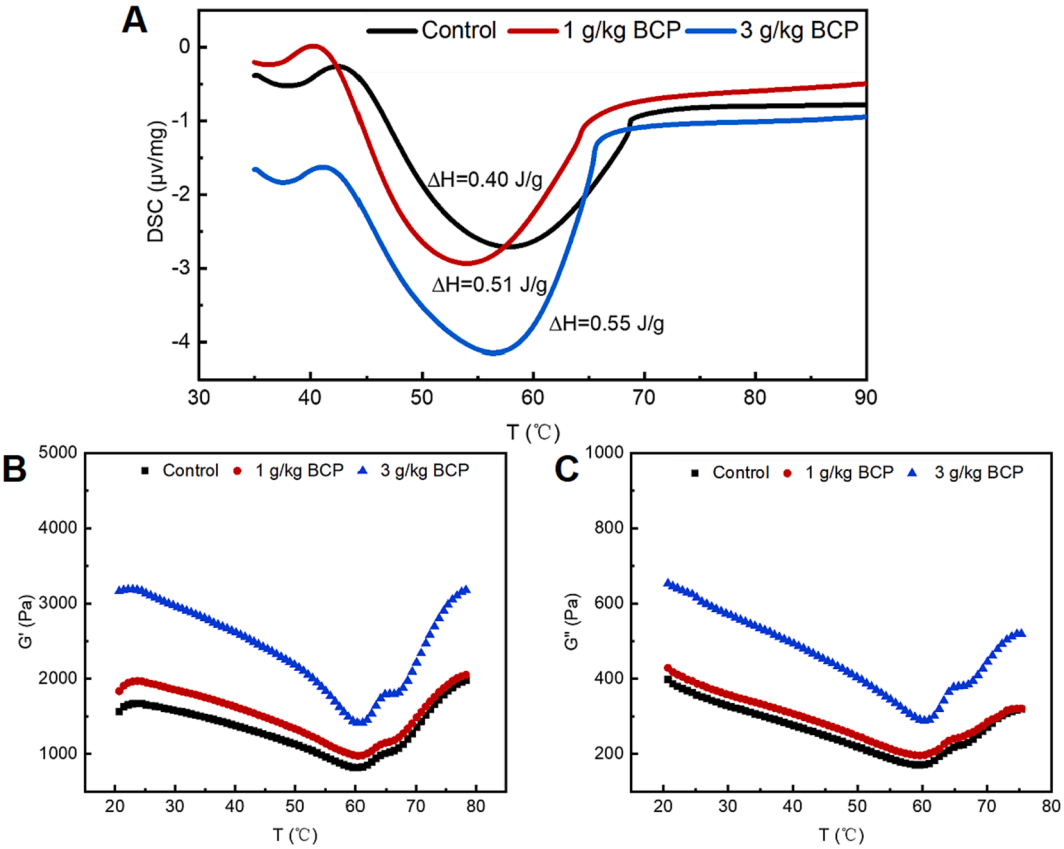

Fig. 5. Differential scanning calorimetry (DSC) diagram during the heating process of MP(A). and the effect of different concentrations of the BCP (0, 1 and 3 g/kg) on the G' (B) and G" (C) of MP with variations in heating temperature.

effect on the overall trend of G'. As can be seen from Fig. 5B, the initial G' value of MP was in descending order, i.e. 3 g/kg BCP group > 1 g/kg BCP group > control group. During the whole heating process, G" values of each group still follow the same order. At the initial stage of heating, the early stage of G' shows a trend of sharp decline, which may be because the chemical bonds in MP are destroyed during heating and the protein denaturation leads to the break of the protein chain, resulting in a loose structure of the protein. At 60 °C, G' of MP gradually increases. At this time, heads of myosin begin to cross-link through dimerization, forming an elastic protein network structure under the action of disulfide and non-covalent bonds (Wang et al., 2022). The G' of the samples added with BCP was always higher than that of the control group during the whole heating process, and the G' of MP tended to increase gradually with the addition of BCP. This may be because the BCP mainly increases the elastic modulus of MP and can induce cross-linking of protein molecules, thus making the gel network structure more dense and stable. These results indicated that appropriate addition of BCP could improve the elasticity and gel properties of MP.

3.8. Micromorphology analysis of MP

Fig. 6 shows the micro-morphology of MP after the addition of different BCP concentrations at room temperature (left column) or heating at 80 °C for 1 h (right column). Under the scanning electron microscope, the gray and white areas represent the network structure of MP, and the circular black areas are the pores in MP structure. In the SEM image (Fig. 6A), the raw MP shows a network that is rough, porous and uneven. Additionally, its cross-section is undulating and uneven either, showing a continuous "hill-shaped" protrusion. As a contrast, addition of 3 g/kg BCP (Fig. 6C) could get a more dense and well-polymerized network structure of MP. The pores inside are more evenly distributed and smaller in size. Compared with the MP obtained at room temperature, Fig. 6D exhibits that the heat treated MP is divided into several clusters by numerous "water channels". It might be due to that in the process of thermal induction, the molecular degeneration of MP causes the initial water absorbed by MP to seep out and eventually form a large number of water holes or interconnected water channels in the gel network structure. The spatial network structure of MP is divided into several cluster areas by interconnected waterways, which destroys the three-dimensional network structure of MP. (Fig. 6F). The hydroxyl group contained in the BCP can form hydrogen bonds with the hydroxyl group of proteins and water molecules during heat induction, stabilizing the water near MP molecules (Zheng et al., 2019). In addition, when the BCP absorbed water and expanded, it exerted local pressure on the protein matrix and promoted the compact and stable structure of MP. In conclusion, the addition of 3 g/kg BCP is beneficial for the high cross-linking of MP network and the stability of water distribution.

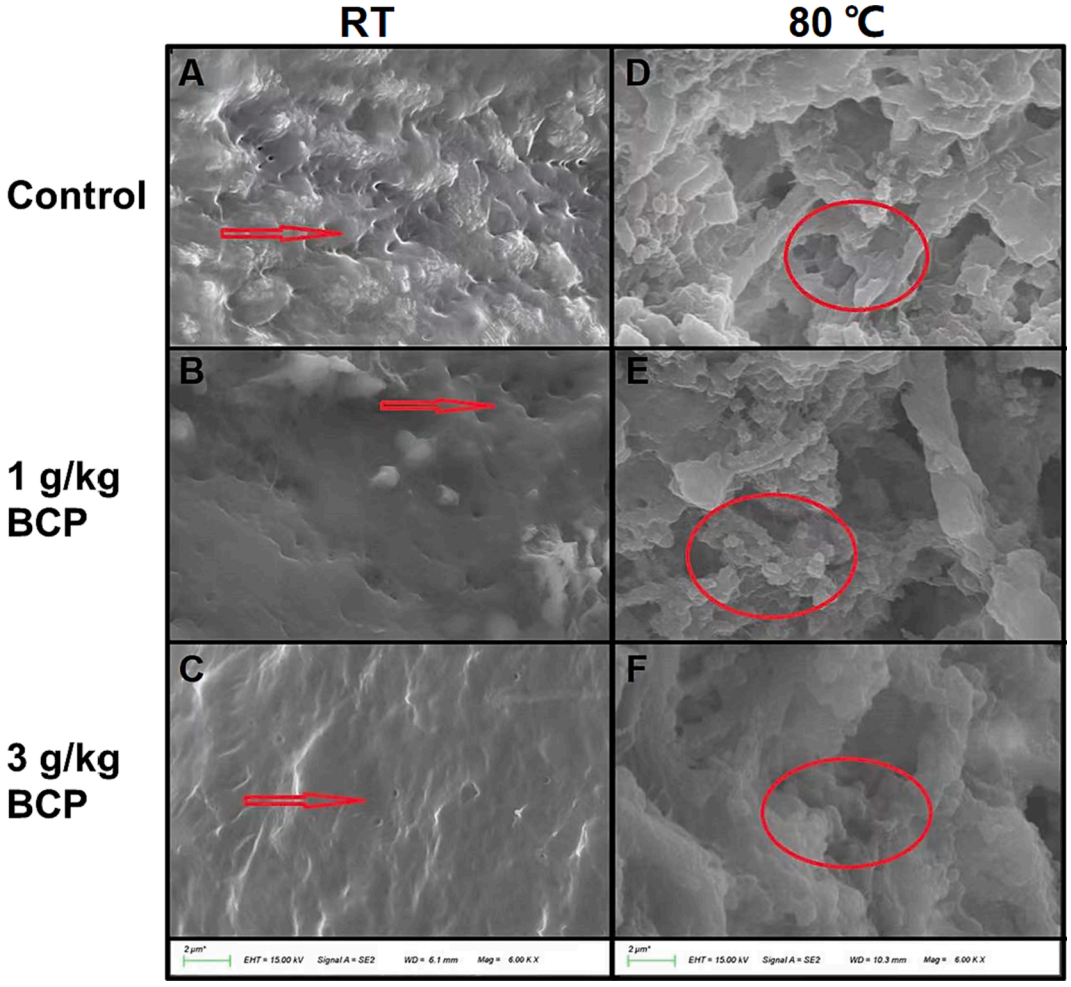

Fig. 6. Scanning electron microscopy graphs of MP of chicken breast meat when different concentrations of BCP (0, 1, 3 g/kg BCP) was added at room temperature (left column) or reacted at 80 °C for 1 h (right column). Note: A, B, and C are the microcosmic shape for myofibrillar protein after the addition of 0, 1, 3 g/kg BCP at room temperature and reacted for 1 h, respectively. D, E, and F are the microcosmic shape for myofibrillar protein after the addition of 0, 1, 3 g/kg BCP heating at 80 °C for 1 h, respectively.

Conclusion

The present study investigated the effect of BCP supplementation on physicochemical properties of the MP of chicken breast meat at normal temperature and 80 °C. We found that a BCP-MP complex was generated by the interaction effect of polysaccharide and MP, and its thermal change behavior during temperature rise was inspected. Interaction between BCP and MP was mainly affected by the concentration of BCP and the heating method. The BCP-MP complex was formed through hydrogen bond and electrostatic interaction, which facilitated the development of MP structure and promoted β-folding to β-turn transformation. In addition, the elastic modulus of MP was enhanced by the addition of BCP. DSC results showed that the enthalpy value of MP increased with the addition of crude polysaccharide, and higher BCP concentration (3 g/kg) was beneficial to the high cross-linking of MP network and the stability of water distribution, which could be effective in the quality improvement of meat products. Thus, we hope our study can provide a theoretical basis for using blackberry polysaccharide as a natural food additive in the food industry to enhance the structure of gel-type meat products.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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