Food Science and Human Wellness 8 (2019) 283–291

Contents lists available at ScienceDirect

Food Science and Human Wellness

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

Impact of hot alkali modification conditions on secondary structure of peanut protein and embedding rate of curcumin

Wei Li[a,b], Shugang Li[a], Yong Hu[a,b], Mengzhou Zhou[a,b], Chao Wang[a,b], Dongsheng Li[a,b,*], Deyuan Li[c,**]

[a] Hubei University of Technology, Wuhan, 430068, China
[b] Hubei Cooperative Innovation Center for Industrial Fermentation, Wuhan, 430068, China
[c] Function Food Engineering Center of Hubei province, Hubei University of Chinese Medicine, Wuhan, 430068, China

ARTICLE INFO

Article history:
Received 11 October 2018
Received in revised form 10 March 2019
Accepted 27 May 2019
Available online 28 May 2019

Keywords:
Curcumin
Embedding rate
Hot alkali modification
Peanut protein
Secondary structure

ABSTRACT

This study aimed to modify isolated and extracted peanut protein with hot alkali to study the impact of pH, heating temperature, processing time and other alkali liquor conditions on the molecular structure of the peanut.Curcumin was loaded in modified peanut protein.The results of the study are as follows:Within the alkaline range of 8 < pH < 12, the percentage of amino acid residue (AAR) and β-turns first increased and then decreased with the increasing pH, and the percentage of AAR reached a maximum 5.21 ± 0.33% when the pH was 11 (p<0.01). The percentage of α-helices and β-sheets gradually decreased with increasing pH, while that of random coils gradually increased with increasing pH, reaching a maximum 11.24 ± 0.87% when the pH was 11(p<0.05). Within the range of the heating temperature 75 °C < T < 95 °C, the percentage of random coils and β-sheets gradually increased with increasing heating temperature, while that of α-helices and AAR gradually decreased with increasing heating temperature; they remained unchanged when the heating temperature was 90 °C, and then decreased to (10.41 ± 1.18%; p<0.01) and (4.02 ± 2.12%; p<0.01), respectively. Within the range of 5 min < t<20 min, the percentage of random coils and AAR gradually increased with increasing heating time, while the percentage of α-helices decreased from 11.83 ± 1.04% to 10.75 ± 2.34% with increased heating time (p<0.01). The optimum conditions for hot alkali modification of peanut protein as followed: heating temperature of 90 °C, heating time of 20 min and a pH of alkali liquor of 11. Under these optimum conditions, the embedding rate of curcumin by the modified protein can reach 88.32 ± 1.29%.

1. Introduction

Peanuts are rich in unsaturated fatty acids and linolenic acid, and they contain 8 essential amino acids and up to 25%–30% crude protein. Peanut protein isolate (PPI) produced by alkaline extraction and subsequent isoelectric precipitation technology using slightly denatured and defatted peanut protein powder is a proteinaceous material with relatively uniform components and good physicochemical properties [1]. PPI is composed mainly of arachin (14S) and conarachin (7.8S and 2S) [2]. General properties of arachin and conarachinare shown in Table 1 [3]. They occupy an important economic position in world agricultural production and trade. In recent years, peanut protein has interested researchers because it is the best source of wall material for preparing nano-particles and microcapsule particles due to its good biocompatibility and biodegradability, excellent nanometer granulation, and protective slow-release effects [2,3]. However, research has found that certain proteins in peanuts have compact four-stage structure, with rarely exposed active sites.It is necessary to improve the

functionsof concentrated peanut protein to extend its application Protein molecules must be modified to expose more active groups and increase contact surfaces with core material to improve embedding activity [4].

* Corresponding author at: Hubei University of Technology, Wuhan, 430068, China.
** Corresponding author at: Function Food Engineering Center of Hubei province, Hubei University of Chinese Medicine, Wuhan, 430065, China.
E-mail addresses: lidongsheng86@163.com (D. Li), lideyuan66@sina.com (D. Li).
Peer review under responsibility of KeAi Communications Co., Ltd

Production and hosting by Elsevier

https://doi.org/10.1016/j.fshw.2019.05.004

Table 1
General properties of arachin and conarachin.

Property	Arachin	Conarachin(2 forms)	
Content	α-arachin 66%	Conarachin-1 16%	Conarachin-1 13%
Molecular weight	600KD	142KD	290KD
Sedimentation	11.7S	2S	8.4S
No.of molecular subunits	6	3	8
Molecular weight of subunits(KD)	72.4,60.3,39.8, 33.1,26.9,21.9	12,13.2,18.2	72.4,39.8,33.1,26.9, 24,21.9,18.6,15.8

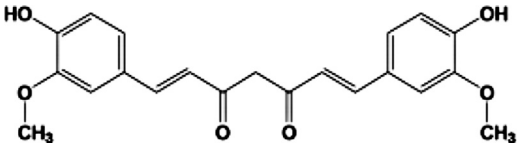

Fig. 1. Chemical structure of curcumin.

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)1,6-heptadiene-3,5-dione) is one of the most popular natural polyphenols derived from Curcuma longa L. Its compound demonstrates a wide spectrum of properties, especially appreciated in the pharmaceutical and food industries [5–7]. Chemical structure of curcumin shown in Fig. 1 [8].

However, bioavailability is low due to low solubility, poor stability and other factors; thus, the application of curcumin in food and medicine is limited [8,9]. In order to improve curcumin bioavailability and take advantage of it in functional foods and supplements, several carriers including emulsions [6], protein nanoparticles [7,16], protein nanotubes [8], complex coacervates [9], solid lipid nanoparticles [10], filled hydrogel beads [11], liposomes [12], and casein micelles [13–15] have been investigated. This experiment modified isolated and extracted peanut protein with hot alkali to study the impact of pH, heating temperature, processing time and other alkali liquor conditions on the molecular structure of the peanut protein and the embedding rate of curcumin. Finally, orthogonal tests were used to explore optimal technological conditions for the embedding of curcumin with modified peanut protein.The breakthrough and innovation in this study focus on the research method by embedding of curcumin. By modifying peanut protein, improving its properties and extending its application range, we can open up a new method adapted to produce, it is important in practice.

2. Materials and methods

2.1. Preparation of peanut protein isolate

We referred to the methods of Zhang et al. [2]. The peanut (purchased from the vegetable market at the South Gate of Hubei University of Technology, moisture content ≤ 5%) was removed after drying the peanut kernel; then the pent kernel was crushed and screened with 60 mesh screen. Five times mass volume of petroleum ether was added for degreasing. The mixture underwent extraction for 2 h at 4 °C (DF-101S heat collecting thermostatic heating magnetic stirrer, Gongyi Yuhua Instrument Co., Ltd.Henan) and centrifugation for 5 min at 6000 r/min (TGL-20 M desktop high speed refrigerated centrifuge from Hunan Xiangyi Laboratory Instrument Development Co., Ltd). Then, 10% NaOH was added to the solid residue from three times of repetitive extraction, degreasing, and centrifugation, according to a mass ratio of 1:5. The pH was adjusted to 9. The mixture was stirred and centrifuged at 6000 r/min for 5 min to collect the filtrate. Then, 8% HCl was used to adjust the pH of the filtrate to 4, after which the mixture was allowed to stand for 1 h to collect the sediment. The sediment was washed with deionized water until the pH was neutral, and then it was frozen. The sample was obtained by drying with the vacuum freeze drier (LGJ-12 vacuum freeze drier, Zhengzhou Honglang Instrument Equipment Co., Ltd.).

2.2. Modification of peanut protein

A 10 g sample of peanut protein was weighed and dissolved in 100 ml 0.5 M NaOH solution. The test was conducted as follows: (1) 10% NaOH and 8% HCl were used to adjust the pH of the protein solution to 8, 9, 10, 11 or 12, after which the tubes containing protein sample were immersed in a water bath at 85 °C for 15 min; (2) 10% NaOH and 8% HCl were used to adjust the pH of the protein solution to 10, after which the tubes containing aprotein sample were immersed in a water bath at 75, 80, 85, 90 or 95 °C for 15 min; and (3) 10% NaOH and 8% HCl were used to adjust the pH of the protein solution to 9, after which the tubes containing aprotein sample were immersed in a water bath at 85 °C for 5, 10, 15, 20 or 25 min.

Upon the completion of the above operations, the solution was allowed to cool to the ambient temperature, after which it was centrifuged at 6000 r/min for 5 min to collect the filtrate. The pH of the filtrate was adjusted to 4 with 8% HCl, after which the solution was allowed to stand for 1 h to collect the sediment. The sediment was washed with deionized water until the pH was neutral, and then it was frozen. The sample was obtained by drying with the vacuum freeze drier for 72 h. Modified peanut proteins prepared under different conditions was obtained. Then, 6 g modified peanut protein was weighed and dissolved in 100 ml double distilled water to prepare modified peanut protein solution.

2.3. Determination of secondary structure

Using the methods of Ye et al. [17] Fourier infrared transform spectrum (FTIR)(Nicolet5700, Thermo Fisher Scientific Inc., USA) was adopted to analyze the structural information of peanut protein before and after modification. Then, the 2 mg sample was accurately weighed and added to 100 mg KBr. The mixture was evenly ground in a mortar, after which it was tableted and analyzed 128 times with full-band scanning (400 –4000cm^{-1}) with an infrared spectrometer.The data (indoleamine I,1600 –1700 cm^{-1}) from the infrared spectrum were processed with the software PeakFit 4.12 (SPSS Inc., Chicago, IL, USA).

2.4. Preparation of curcumin granule embedded with modified protein

A curcumin sample was weighed to be 0.5 g and dissolved in 1 L absolute ethyl alcohol to prepare curcumin ethanol solution with a mass concentration of 0.05%. Then, 30 ml curcumin ethanol solution was collected to mix with the peanut protein solution prepared under different modification conditions in 1.3.2. The mixture was stirred and maintained in a dark place for reaction for 2 h. Then, 80 ml 5 mmol/L $CaCl_2$ was added to the mixed solution, and the mixture was allowed to stand at ambient temperature for 16 h, after which it underwent desalting treatment with 14,000 KD dialysis

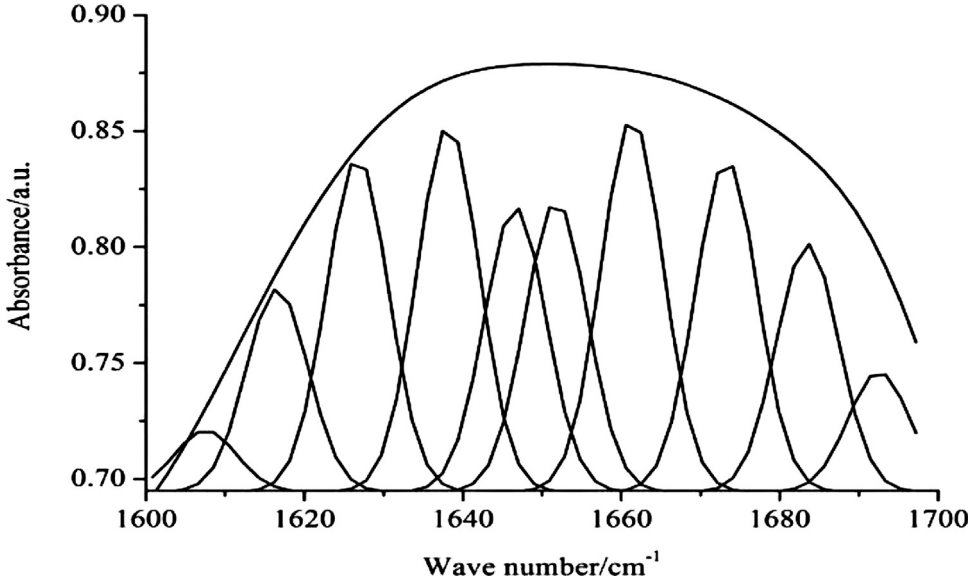

Fig. 2. Deconvolved and curve fit bands of amide I of peanut protein isolate.

bag for 72 h. Solid peanut protein granules with curcumin embedded therein were obtained by freeze drying.

2.5. Determination of embedding rate

Using the methods of Xun et al. [18] a certain amount of protein grains with curcumin embedded therein was collected and placed in 5 ml centrifuge tube. Then, ethanol was added, and the mixture was violently vibrated for 5 min and centrifuged at a speed of 10,000 r/min for 5 min; the supernatant was collected to determine its absorbency with ultraviolet-visible spectrophotometer (UV2450/2550 ultraviolet spectrophotometer from Shimazu International Trade (Shanghai) Co., Ltd.). For the ultraviolet analysis of the retained curcumin, the absorbance at 424 nm was measured.The sample in the centrifuge tube was further repeatedly washed by the same method until minimal changes in the curcumin content were observed. The sum of the curcumin measured in the supernatant, each time, was the amount of curcumin absorbed on the surface of the sample. The amount of residual curcumin in the sample was obtained by dissolving the sample in ethyl acetate. The embedding rate of curcumin was calculated with Formula (1): where m_1 is the total amount of curcumin in the sample, and m_2 is the amount of curcumin absorbed on the surface of the sample.

$$\text{Embeddingrate}/\% = (m_1\text{-}m_2)/m_1 \times 100 \quad (1)$$

2.6. Data processing

The data were processed with OriginPro 2007 (OriginLab Corporation, USA) and SAS 8.1(North Carolina State University, US). The measurement results were expressed with average value ± deviation(x ± std). The significance level was 0.05, and the highly significant level was 0.01.

3. Results

3.1. Identification of structure of infrared Spectrum of unmodified peanut protein

The infrared spectrum of Amide Band I was an atlas formed by the superposition of the peak spectrum component *via* different secondary structures of protein caused by the stretching vibration of the C=O and C—N in protein structure. The undistinguashed peaks in Amide Band I in the original protein infrared spectrum were further decomposed into subpeaks by second order derivation and the deconvolution technique. Qualitative analyses were conducted on protein secondary structure in combination with the curvefitting method [19].

Table 2
Fitted results of amide I band of peanut protein.

Peakposition (cm^{-1})	Corresponding structure	Secondary structure (%)
1605–1610	AAR	4.73 ± 0.21
1617–1618	β-sheet	9.38 ± 0.33
1627–1628	β-sheet	11.49 ± 1.02
1637–1639	β-sheet	12.32 ± 1.74
1645–1648	random coil	11.03 ± 1.59
1651–1653	α-helix	11.35 ± 1.23
1660–1662	β-turn	12.48 ± 1.06
1672–1673	β-turn	12.14 ± 0.72
1682–1684	β-sheet	9.35 ± 0.51
1691–1692	β-sheet	5.73 ± 1.06

Fig. 2 shows the atlas of Amide Band I in the infrared spectrum of unmodified peanut protein after curvefitting. From Fig. 2, there were 10 structure-relevant peaks after deconvolution, indicating that the secondary structure of the peanut protein was very complicated. Table 2.1 shows the results of the fitting analysis of the figure. According to the structural features of peanut protein and the pertinent literature [17], the structural information and percentage corresponding to the above 10 peaks are shown in Table 2.

3.2. Impact of modification conditions on secondary structure of peanut protein

3.2.1. Impact of pH of alkali liquor on secondary structure of peanut protein

Table 3 demonstrates the fitting results of Acylamide Band I of the sample from the alkali liquor with different pH values, heated by the water bath at 85 °C for 15 min. When the pH was less than 11, as the pH of the alkali liquor increased, the percentage of AAR (1605–1610cm-1) gradually increased from 4.32 ± 0.43% to 5.21 ± 0.33% ($p < 0.01$). The percentage of random coil(1645–1648 cm-1) gradually increased from 10.21 ± 1.96% to 11.24 ± 0.87% ($p < 0.05$), while the percentage of β-sheets at 1637–1639 cm-1 gradually decreased from 12.27 ± 0.87% to

Table 3
Fitted results of amide I band of peanut protein treated with alkaline liquor.

Peak Position (cm^{-1})	Corresponding structure	Secondary structure (%)				
		8	9	10	11	12
1605–1610	AAR	4.32 ± 0.43	4.65 ± 0.36	4.97 ± 0.64[b]	5.21 ± 0.33[a]	5.04 ± 0.38[b]
1617–1618	β-sheet	9.46 ± 0.24	9.31 ± 0.86	9.45 ± 0.01	9.62 ± 0.1[b]	9.08 ± 0.18[b]
1627–1628	β-sheet	11.23 ± 0.18	11.05 ± 1.93	11.68 ± 0.11[b]	11.25 ± 0.74	11.55 ± 0.74
1637–1639	β-sheet	12.27 ± 0.87	12.13 ± 0.25	12.12 ± 0.63	11.85 ± 0.97[a]	12.05 ± 0.97[b]
1645–1648	random coil	10.21 ± 1.96	10.52 ± 2.18	11.04 ± 1.49	11.24 ± 0.87[b]	11.42 ± 1.27[b]
1651–1653	α-helix	11.76 ± 2.04	11.27 ± 0.92[b]	11.01 ± 0.45	10.85 ± 2.67[b]	10.52 ± 1.76[b]
1660–1662	β-turn	12.57 ± 1.66	12.06 ± 0.13	12.18 ± 1.3	12.42 ± 0.63	12.24 ± 0.36
1672–1673	β-turn	12.56 ± 0.49	12.23 ± 0.26	12.82 ± 0.32	12.55 ± 0.14	12.42 ± 0.14
1682–1684	β-sheet	8.99 ± 0.35	8.84 ± 0.02	9.01 ± 0.52	9.13 ± 0.17	9.22 ± 0.26
1691–1692	β-sheet	6.63 ± 0.89	6.94 ± 0.67	5.80 ± 0.24[a]	5.88 ± 0.46	6.01 ± 0.55[b]

Note: a:$p<0.01$; b: $p<0.05$; compared with the percentage of pH = 8 heating group.

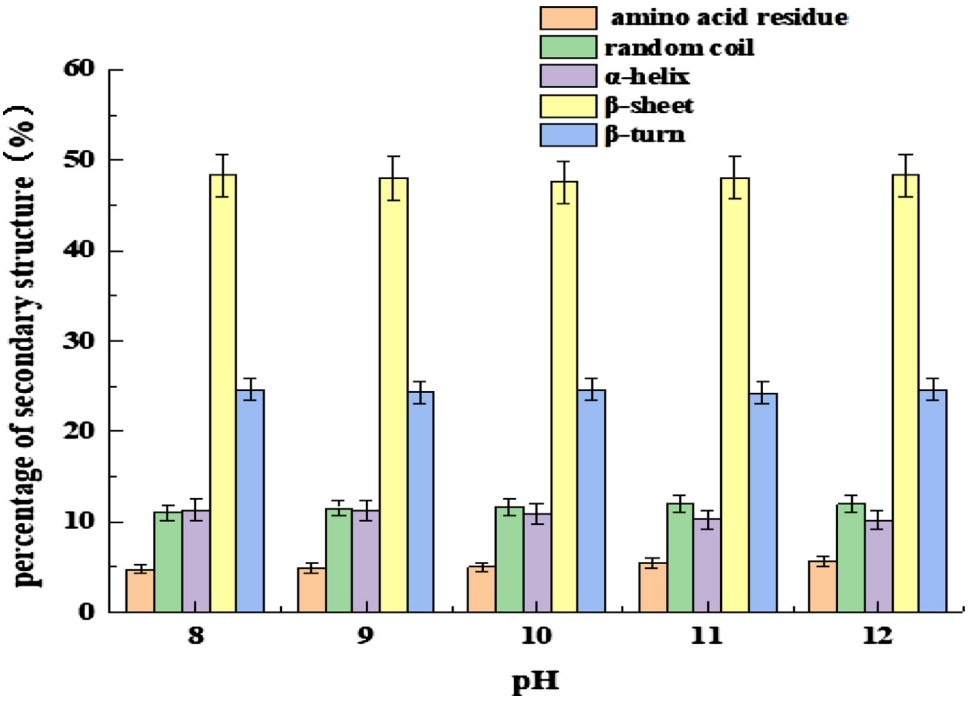

Fig. 3. Changes in secondary structure of peanut protein with increasingly alkaline pH.

11.85 ± 0.97% ($p < 0.01$) and that of the α-helices gradually decreased from 11.76 ± 2.04% to 10.85 ± 2.67% ($p < 0.05$)

Fig. 3 demonstrates the superposition results of the same secondary structure classification identified at different peak positions in Table 3. When pH was within the range of 8–12, the percentage of random coils and β-turns gradually increased with increasing pH, while the percentages of α-helices and β-sheets gradually decreased with increasing pH. When pH was below 11, the percentage of AAR gradually increased with increasing pH.

3.2.2. Impact of heating time on secondary structure of peanut protein

Table 4 demonstrates the fitting results of Acylamide Band I of the sample from the treated alkali liquor with pH = 10, heated by a water bath at 85 °C for different times. Within 20 min, with increased heating time, the percentage of AAR (1605–1610 cm^{-1}) gradually increased from 4.56 ± 1.01% to 5.22 ± 1.54% ($p < 0.05$). The percentage of random coils (1645–1648 cm^{-1}) gradually increased from 10.49 ± 3.12% to 11.43 ± 2.16% ($p < 0.05$), and that of β-sheets at 1617–1618 cm^{-1} gradually increased from 9.31 ± 0.09% to 10.7 ± 0.97% ($p<0.01$). The percentage of β-sheets at 1691–1692 cm^{-1} gradually decreased from 5.57 ± 0.61% to 4.82 ± 0.57% ($p<0.05$). Within 25 min, the percentage of α-helices decreases from 11.83 ± 1.04% to 10.75 ± 2.34% ($p < 0.05$).

Fig. 4 demonstrates the superposition results of the same secondary structure classification identified at different peak positions in Table 4. Within the range of 5–25 min, the percentage of random coils and AAR increased with increased heating time, and the percentage of α-helices gradually decreased from 11.77 ± 0.37% to 10.41 ± 1.18% ($p<0.01$).

3.2.3. Impacts of heating temperature on secondary structure of peanut protein

Table 5 demonstrates the fitting results of Acylamide Band I of the sample from the treated alkali liquor with a pH = 10, heated by a water bath at different temperatures for 15 min. When the heating temperature was within the range of 75–95 °C, with increasing the heating temperature, the percentage of AAR (1605–1610 cm^{-1}) gradually decreased from 4.94 ± 1.11% to 3.92 ± 1.54% ($p<0.01$), and the percentage of random coils(1645–1648 cm-1) gradually increased from 10.01 ± 2.37% to 11.93 ± 2.16% ($p<0.05$). While the percentage of β-sheets at 1627–1628 cm-1 gradually increased

Table 4
Fitted results of amide I band of peanut protein treated with heating time.

Peak Position (cm^{-1})	Corresponding structure	Secondary structure (%)				
		5 min	10 min	15 min	20 min	25 min
1605–1610	AAR	4.56 ± 1.01	4.77 ± 0.47	4.93 ± 0.22	5.22 ± 1.54[b]	4.87 ± 0.54
1617–1618	β-sheet	9.31 ± 0.09	9.97 ± 1.23[b]	10.35 ± 2.12[b]	10.7 ± 1.02[a]	10.57 ± 0.32[b]
1627–1628	β-sheet	11.02 ± 0.11	10.94 ± 0.46[b]	11.41 ± 3.09	10.96 ± 1.46	11.63 ± 0.46[b]
1637–1639	β-sheet	12.42 ± 0.75	12.36 ± 1.05	12.54 ± 1.54	12.71 ± 1.07	12.66 ± 0.07
1645–1648	random coil	10.49 ± 3.12	10.79 ± 2.79[b]	11.12 ± 2.07[b]	11.43 ± 2.16[b]	11.26 ± 2.86[b]
1651–1653	α-helix	11.83 ± 1.04	11.72 ± 2.05	11.36 ± 1.38	11.16 ± 1.34	10.75 ± 2.34[b]
1660–1662	β-turn	12.34 ± 1.21	12.98 ± 2.04[b]	12.15 ± 1.31	12.44 ± 1.12	12.04 ± 1.09
1672–1673	β-turn	12.22 ± 1.74	12.19 ± 0.41	11.83 ± 0.38	12.36 ± 1.18[b]	11.21 ± 1.74[a]
1682–1684	β-sheet	9.11 ± 0.07	8.9 ± 0.74	9.53 ± 0.22	9.02 ± 0.23	9.02 ± 0.53
1691–1692	β-sheet	5.57 ± 0.61	5.38 ± 0.49	4.96 ± 0.83[b]	4.82 ± 0.57[b]	5.08 ± 0.23[b]

a:p<0.01; b: p<0.05; compared with the percentage of 5-min heating group.

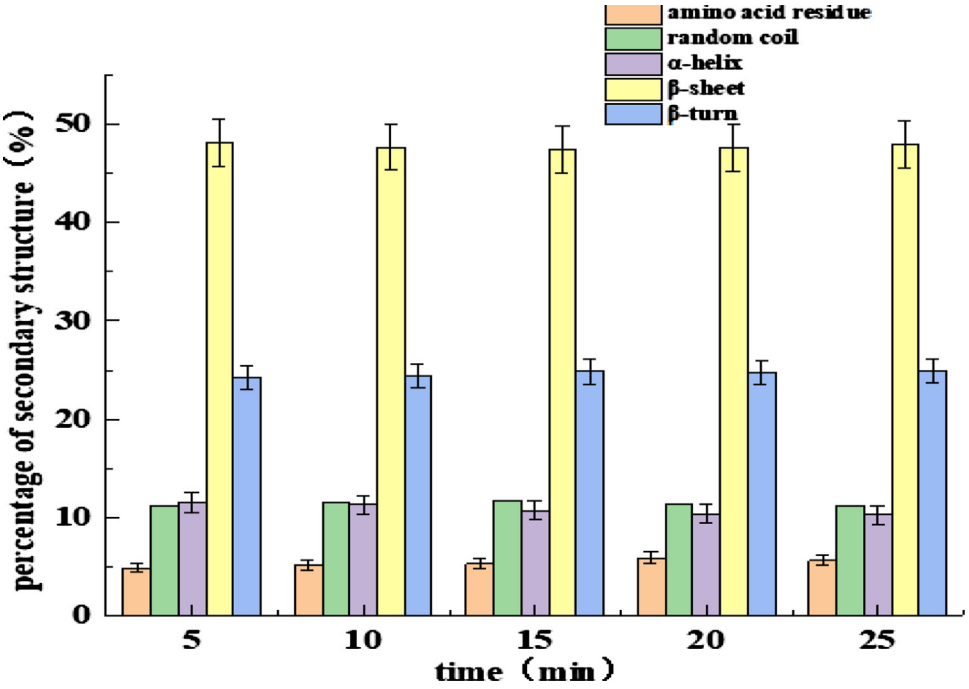

Fig. 4. Changes in secondary structure of peanut protein with increased heating time.

Table 5
Fitted results of amide I band of peanut protein treated with heating temperature.

Peak Position (cm^{-1})	Corresponding structure	Secondary structure (%)				
		75 °C	80 °C	85 °C	90 °C	95 °C
1605–1610	AAR	4.94 ± 1.11	4.65 ± 0.97	4.41 ± 2.07	4.02 ± 2.12[a]	3.92 ± 1.54[a]
1617–1618	β-sheet	9.51 ± 1.02	9.4 ± 1.23	9.52 ± 0.47	9.73 ± 1.34	9.7 ± 1.02
1627–1628	β-sheet	11.29 ± 0.06	11.66 ± 0.74	11.84 ± 1.51[b]	11.96 ± 3.1[b]	12.14 ± 1.46[a]
1637–1639	β-sheet	11.89 ± 2.12	12.02 ± 3.19	12.53 ± 1.29[b]	12.27 ± 1.62[b]	11.71 ± 1.07[b]
1645–1648	random coil	11.01 ± 2.37	10.96 ± 2.11	11.22 ± 3.02	11.53 ± 2.02	11.93 ± 2.16[b]
1651–1653	α-helix	11.77 ± 0.37	11.01 ± 1.54	10.73 ± 0.67[b]	10.41 ± 1.18[a]	10.16 ± 0.34[a]
1660–1662	β-turn	12.45 ± 1.1	12.12 ± 0.57	12.38 ± 1.74	12.52 ± 1.07	12.44 ± 1.12
1672–1673	β-turn	12.51 ± 0.82	12.19 ± 0.68	12.02 ± 1.05	12.29 ± 1.28	12.36 ± 1.18
1682–1684	β-sheet	9.43 ± 0.61	9.79 ± 0.62	9.52 ± 0.37	10.03 ± 0.38[b]	10.02 ± 0.23[b]
1691–1692	β-sheet	5.19 ± 0.82	5.20 ± 0.77[a]	5.83 ± 0.63	5.24 ± 0.27	5.62 ± 0.39

a:p<0.01; b: p<0.05; compared with the percentage of 75 °C heating group.

from 11.29 ± 0.06% to 12.14 ± 1.46% (p<0.01), the percentage of β-sheets at 1682–1684 cm-1 gradually increased from 9.43 ± 0.61% to 10.03 ± 0.38% (p<0.05). The percentage of α-helices gradually decreased from 11.76 ± 2.04% to 10.85 ± 2.67% (p<0.05).

Fig. 5 demonstrates the superposition results of the same secondary structure classification identified at different peak positions in Table 4. When the heating temperature was within the range of 75–95 °C, the percentage of random coils and β-sheets gradually increased with increasing of heating temperatures, while the percentage of α-helices and AAR gradually decreased with increasing heating temperature (Table 6).

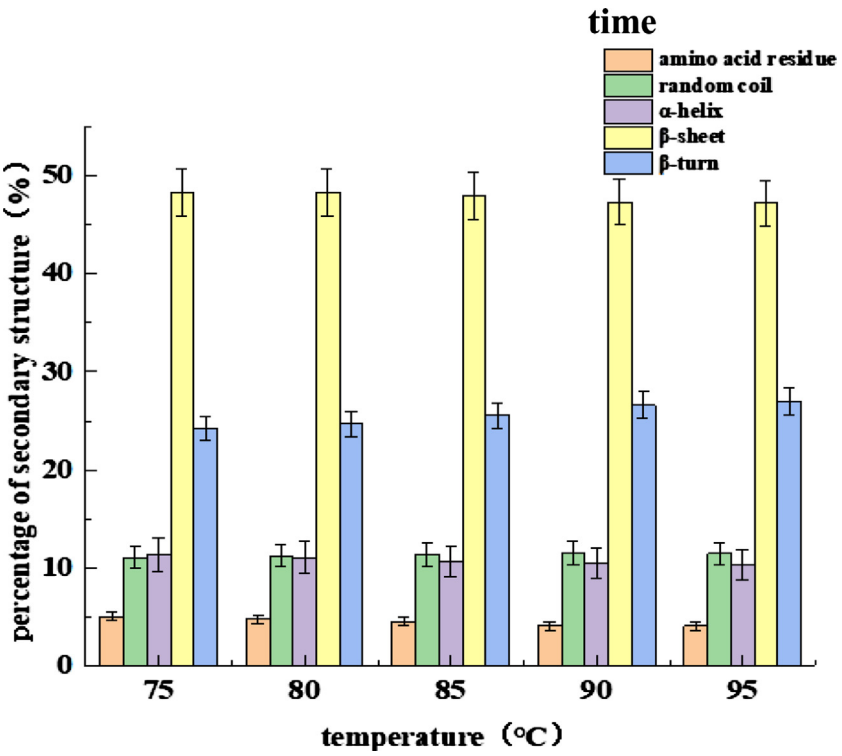

Fig. 5. Changes in secondary structure of peanut protein with increasing of heating temperature.

Table 6
Factors and levels.

Level	temperature (A)/°C	Time (B) /min	pH (C)
1	85	15	10
2	90	20	11
3	95	25	12

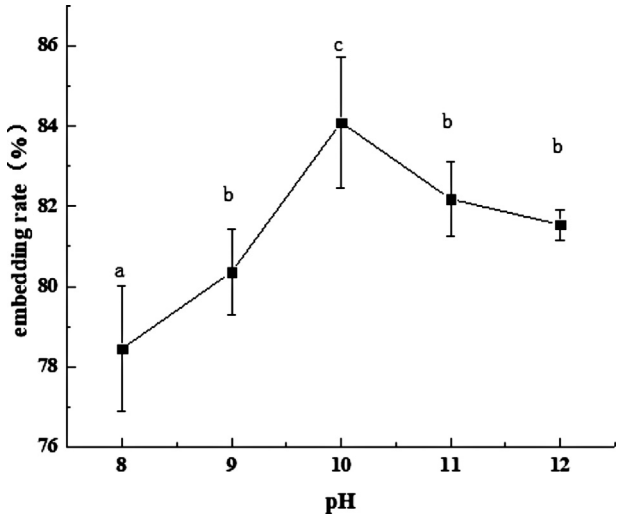

Fig. 6. Changes in embedding rate of curcumin with increased alkaline pH.

3.3. Embedding rate of curcumin

3.3.1. Single factor experiment

Fig. 6 demonstrates the impact of the peanut protein sample treatment(from alkali liquor at different pH values, heated by a water bath at 85 °C for 15 min) on the embedding rate of curcumin. When the pH of alkali liquor was within the range of 8–12, increased pH first increased the embedding rate of curcumin and then decreased it; when the pH was 10, the embedding rate was the highest, 82.09 ± 1.64%. Therefore, pH values of 9, 10 and 11 were selected for the orthogonal test.

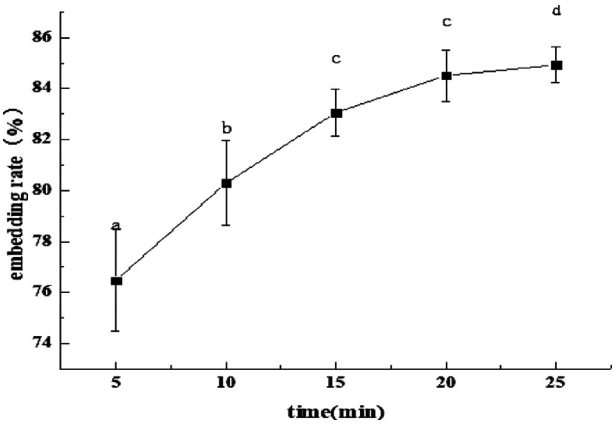

Fig. 7. Changes in embedding rate of curcumin with increased heating time.

Fig. 7 demonstrates the impacts of the peanut protein sample(obtained from alkali liquor at pH = 10 heated with a water bath at 85 °C for different times) on the embedding rate of curcumin. When the heating time was within the range of 5–25 min, with increasing time, the embedding rate of curcumin increased first and then remained the same. When the heating time was 20 min, the embedding rate was highest, 84.73 ± 1.65%. Therefore, the heating times of 15, 20 and 25 min were selected for the orthogonal test.

Fig. 8 demonstrates the impacts of the peanut protein sample(obtained from alkali liquor at pH = 10 and heated by a water bath at different temperatures for 20 min) on the embedding rate of curcumin. When the heating temperature was within the range of 85–95 °C, increased heating temperature first increased and then decreased the embedding rate of curcumin. When the heating tem-

Table 7
Arrangement and results of orthogonal test of L_9 (3^4).

Test number	Temperature (A) (°C) A	Time (B) (min) B	pH C	Error column	Embedding rate (%)
1	1	1	1	1	80.24
2	1	2	2	2	85.32
3	1	3	3	3	83.28
4	2	3	2	1	84.86
5	2	1	3	2	85.31
6	2	2	1	3	83.36
7	3	2	3	1	86.62
8	3	3	1	2	82.19
9	3	1	2	3	84.55
k1	82.95	83.37	81.93	83.91	
k2	84.51	85.10	84.91	85.06	
k3	84.45	83.44	85.07	83.73	
Range	1.56	1.73	3.14	1.33	
Factors order	C→B→A				
Optimal solution	$A_2B_2C_3$				

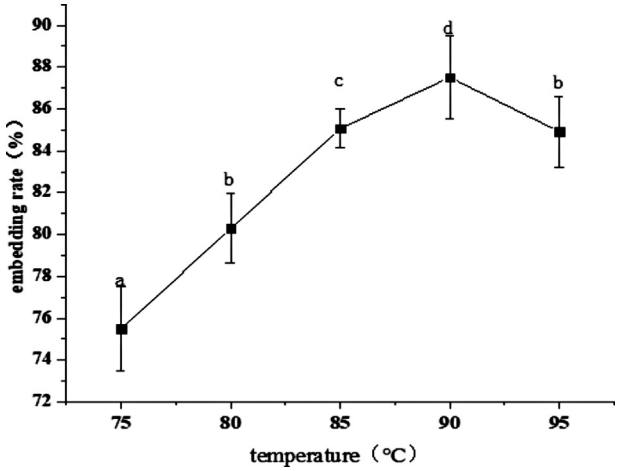

Fig. 8. Changes in embedding rate of curcumin with increased heating temperature.

Table 8
Result of orthogonal test variance analysis.

Source of difference	SS	Df	MS	F value	Significance level
Temperature	4.72	2.00	2.36	10.24	*
Time	5.75	2.00	2.88	12.49	*
pH	18.77	2.00	9.38	40.72	**
Error	0.46	2.00	0.23		
Total Error	29.70	8.00			

perature was 90 °C, the embedding rate was highest, 85.23 ± 1.08%. Therefore, the heating temperatures 85 °C, 90 °C and 95 °C were selected for the orthogonal test.

3.3.2. Design of orthogonal test

Using the embedding rate of curcumin as the optimizing index for the single factor experiments, the three levels of water bath temperatures (A), heating times (B) and the pH of alkali liquor (C) were selected for the orthogonal design according to orthogonal table L_9 (3^4): see Table 5 for the factor level table; see Table 7 for experimental results; and see Table 8 for variance analysis results.

From the range analysis in Table 7, the order of influence level of three factors on the embedding rate of curcumin was as follows: the pH of alkali liquor (C) > heating time (B) > heating temperature (A). Within the range of the experimental design, the optimum conditions for hot alkali modification of peanut protein were $B_2A_2C_3$. That is to say, the conditions of heating temperature of 90 °C, heating time of 20 min and pH of alkali liquor of 11, enabled a maximum embedding rate of curcumin. From Table 8, the pH of alkali liquor had extremely significant impacts on the embedding rate of curcumin, and the heating time and temperature had significant impacts of the embedding rate of curcumin.

3.3.3. Confirmation experiment

Three parallel experiments were conducted according to the composition conditions $A_2B_2C_3$ for the orthogonal test. The embedding rate of curcumin reached 88.32 ± 1.29%, which was higher than all test results in Table 7. Therefore, $A_2B_2C_3$ was the optimum modification condition for the embedding of curcumin with peanut protein.

4. Discussion

Peanuts are usually consumed by millions of people in the world mainly as toasted or salted snacks and as peanut butter or sweets. Peanuts are also an important source of cooking oil considered premium [2]. This material is used mainly in the Asian continent for the preparation of meals and the residue of the process is a flour with high protein content (47%–55%) commonly used as ingredient in animal feedstocks. Despite its mild taste, good color and digestibility, the defatted peanut flour is rarely used in human foods [3]. The feasibility of using peanut protein is often limited because of its poor solubility,gel property and this feature is associated with the high molecular weight of the protein structure and the low number of ionizable groups. Such functionalities can be modified to improve solubility by Mechanical or chemical action.

Secondary structure elements of protein include α-helices, β-sheets, β-turns, random coils and other conformations, α-helices and β-sheets are ordered arrangements in proteins that are maintained by hydrogen bonds [20], the secondary structure of a protein directly impacts its functional properties. The secondary structure and functional properties of protein may be affected by factors that affect the stability of the hydrogen bonds between protein molecules, facilitating the gel [21], emulsifying [17,22] and foaming properties [23] of a protein.

Some studies showed that the electrostatic field [24,25] and pH [26] affect the distribution of amino acid side chain charges and the stability of hydrogen bonds in salt-soluble myofibrin molecules. Ying et al. [27] found that increased pH promoted the conformation of tilapia myosin; Liu et al. [28,29] found that, under acidic conditions, changes in electrostatic interactions of myofibrillar protein molecules affect the stability of hydrogen bonds in pork and cause the loss of α-helices in these proteins. When studying the impacts of heating temperature and the pH of alkali liquor on the secondary structure of peanut protein, the α-helix was easily lost under acidic conditions and gradually decreased with increasing

pH, that is, alkaline conditions. Based on previous studies [28–30], it is likely that intermolecular electrostatic interactions and the stability of hydrogen bonds in peanut protein are weaker in alkaline or acidic condition than neutral condition. Therefore, when modifying peanut proteins, determining appropriate pH conditions will be beneficial for opening the compact four-stage structure of peanut protein. The increased content of AAR in Table 3 and Fig. 3 further verified that the compact and stable long-chain structure of peanut protein began to depolymerize, and the modification was in progress.

Ling et al. [31] found, by studying the impacts of heating on the structure of myofibrin in chicken breast, that changes in heating temperature significantly changed the secondary structure of protein; Jian et al. [32] studied the impact of heating conditions on the molecular structure of soybean protein isolate. With increased heating temperature, they found that hydrogen bonds in the α-helix structure gradually fractured, and the structure uncoiled, decreasing the proportion of α-helix structures; Stathopulos et al. [33] studied temperature increases during heating and observed the polymerization of group R, and that the stability of α-structure was damaged when group R was too large. The results in Table 4 and Fig. 4 demonstrated that, within the range of 75 °C–95 °C, the percentage of α-helices decreased with increased temperature, which may indicate that the degree of molecular expansion of the peanut protein increased. The increased percentage of β-sheets and decreased percentage of AAR may indicate increased aggregation between protein molecules.

Peanut protein isolates were successfully modifed using hot alkali. Within the levels evaluated, reaction parameters such as alkali pH, temperature and time affected the conditions of secondary structure. All these are the efforts to make innovations.The optimum conditions for hot alkali modification of peanut protein as followed: heating temperature of 90 °C, heating time of 20 min and a pH of alkali liquor of 11. Under these optimum conditions, the embedding rate of curcumin by the modified protein can reach 88.32 ± 1.29%. HPLC and peanut protein isolate.will be used separating and purifying in the next step, It is expected that results will be useful to assess the feasibility of these ingredients as novel food components for human consumption,

Conflict of interest statement

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled, “Impact of Hot Alkali Modification Conditions on Secondary Structure of Peanut Protein and Embedding Rate of Curcumin.

Acknowledgements

This work was financially supported by The foundation for young scientists of hubei province (grant number 610112246), the foundation for Doctoral startup project of Hubei University of Technology (grant number 337/338). We would like to thank LetPub (www.letpub.com) for providing linguistic assistance during the preparation of this manuscript.

References

[1] W.B. Marty, G.A. Kruizinga, M.C. Rubington, W.R. Ren, F.H. Geert, Assessing food allergy risks from residual peanut protein in highly refined vegetable oil, Food Chem. Toxicol. 106 (2017) 306–313.
[2] X.C. Zhang, W.Z. Xing, T.Z. Cai, K. Huan, L.D. Yun, L. Yi, Composite modification of peanut protein by enzyme-microfluidization and its application in microcap, China Oils Fats 39 (2014) 32–35.
[3] E. Hoffen, H.P. Kleij, C.F. Den, Hartog Jager, W.A. Doorn, E.F. Knol, D.J. Opstelten, S.J. Koppelman, A.C. Knulst, Chemical modification of peanut conglutin reduces Ig E reactivity and T cell reactivity in peanut-allergic patients, Clin. Transl. Allergy 44 (2014) 1558–1566.
[4] L.L. Shen, Q. Zhu, Q. Feng, W. Huang, H. Xu, L. Wu, H.F. Xiao, K. Zhou, Z.G. Liu, Effect of heat treatment on structure and immunogenicity of recombinant peanut protein Ara h 2.01, LWT - Food Sci. Technol. 60 (2015) 964–969.
[5] M. Esmaili, S.M. Ghaffari, Z. Atri, M.S. Sharifizadeh, Beta casein-micelle as a nano vehicle for solubility enhancement of curcumin; food industry application, LWT - Food Sci. Technol. 44 (2011) 2166–2172.
[6] C. Wang, Z. Liu, G. Xu, B. Yin, P. Yao, BSA-dextran emulsion for protection and oral delivery of curcumin, Food Hydrocoll. 61 (2016) 11–19.
[7] G. Xu, C. Wang, P. Yao, Stable emulsion produced from casein and soy polysaccharide compacted complex for protection and oral delivery of curcumin, Food Hydrocoll. 71 (2017) 108–117.
[8] S. Hu, T. Wang, M.L. Fernandez, Y. Luo, Development of tannic acid crosslinked hollow zein nanoparticles as potential oral delivery vehicles for curcumin, Food Hydrocoll. 61 (2016) 821–831.
[9] R. Sadeghi, A. Moosavi-Movahedi, Z. Emam-Jomeh, A. Kalbasi, A. Razavi, The effect of different desolvating agents on BSA nanoparticle properties and encapsulation of curcumin, J. Nanoparticle Res. 16 (2014) 2565.
[10] S.M. Hosseini, Z. Emam-Djomeh, S. Razavi, A. Moosavi-Movahedi, A. Saboury, P. Van der Meeren, Complex coacervation of β-lactoglobulin–κ-K-carrageenan aqueous mixtures as affected by polysaccharide sonication, Food Chem. 141 (2013) 215–222.
[11] T. Wang, X. Ma, Y. Lei, Y. Luo, Solid lipid nanoparticles coated with crosslinked polymeric double layer for oral delivery of curcumin, Colloids Surf. B Biointerfaces 148 (2016) 1–11.
[12] B. Zheng, Z. Zhang, F. Chen, X. Luo, D.J. McClements, Impact of delivery system type on curcumin stability: comparison of curcumin degradation in aqueous solutions, emulsions, and hydrogel beads, Food Hydrocoll. 71 (2017) 187–197.
[13] Y. Liu, D. Liu, L. Zhu, Q. Gan, X. Le, Temperature-dependent structure stability and in vitro release of chitosan-coated curcumin liposome, Food Res. Int. 74 (2015) 97–105.
[14] M.R. Bower, H.S. Aiyer, C.G. Robert, Chemoprotective effects of curcumin in esophageal epithelial cells exposed to bile acids, World J. Gastroenterol. 16 (2010) 4152–4158.
[15] A.M. Chuah, B. Jacob, Z. Jie, S. Ramesh, S. Mandal, J. Puthan, P. Deshpande, V.V. Vaidyanathan, R.W. Gelling, G. Patel, T. Das, S. Shreeram, Enhanced bioavailability and bioefficacy of an amorphous solid dispersion of curcumin, Food Chem. 156 (2014) 227–233.
[16] W. Li, M.Z. Zhou, N. Xu, Y. Hu, C. Wang, D.S. Li, L.G. Liu, Comparative analysis of protective effects of curcumin,curcumin-p-cyclodextrin nanoparticle and nanoliposomal curcumin on unsymmetrical dimethyl hydrazine poisoning in mice, Bioengineered 7 (2016) 334–341.
[17] L. Ye, Y. Liao, M. Zhao, W. Sun, Effect of protein oxidation on the conformational properties of peanut protein isolate, J. Chem. 8 (2013) 1–6.
[18] N. Xun, F.J. Zabihi, J. Fu, J.L. Ping, Z.Y. Ping, Optimizing supercritical anti-solvent preparation of curcumin-loaded PLGA nanoparticles by response surface methodology, Food Sci. 35 (2014) 1–5.
[19] I. Carton, U. Bocker, R. Ofstad, Monitoring secondary structural changes in salted and smoked salmon muscle myofiber proteins by FT-IR microspectroscopy, J. Agric. Food Chem. 57 (2009) 3563–3570.
[20] W.H. Liu, C.D. Stevenson, T.C. Lanier, Rapid heating of alaska pollock and chicken breast myofibrillar proteins as affecting gel rheological properties, J. Food Sci. 78 (2013) 971–977.
[21] S.R. Zhong, F.Z. Ping, W.S. Ye, Effect of metal ions on gel-forming characteristics of peanut protein isolate, Food Sci. 7 (2010) 173–176.
[22] Q.H. Li, Q.B. Kun, W. Hai Qing, Hong Li, J. Lian Zhou, Yang Li, X.N. Sui, Effect of enzymatic hydrolysis time on emulsifying properties and interaction of soybean protein isolate-lysophospholipid emulsion, Food Sci 38 (2017) 7–13.
[23] Y. Li, S. Bo, H.D. Xuan, L. Jie, Y.B. Shuang, Z.Q. Zhi, J.L. Zhou, S.X. Nan, Effects of non-covalent/covalent interactions between anthocyanins and soybean protein isolate on its interfacial function, Food Sci. 39 (2018) 32–38.
[24] S.Y. Ke, L. Xia, Z. Chun hui, Q. Shuyi, S. Zhen, Effect of electrostatic field-assisted freezing-thawing on water-holding capacity and physicochemical characteristics of beef muscle proteins, Food Sci. 39 (2018) 157–162.
[25] G. Jia, S. Nirasawa, X. Ji, Y. Luo, H. Liu, Physicochemical changes in myofibrillar proteins extracted from pork tenderloin thawed by a high-voltage electrostatic field, Food Chem. 4 (2018) 240–248.
[26] W.D. Cheng, Z. Zhen, C. Rong, Z.C. Qiong, Changes of protein in sour meat during fermentation and its effects on digestive characteristics, Food Ferment. Ind. (2018) 1–11.
[27] F. Ying, H. Yi, M.Y. Han, Z.G. Hong, X.X. Lian, P.Z. Qi, Studies on the secondary structure and heat-induced gelation of pork myofibrillar proteins as affected by pH, Sci. Agric. Sin. 43 (2010) 164–170.
[28] R. Liu, S.M. Zhao, Y. Liu, H. Yang, S.B. Xiong, L. Xie, Effect of pH on the gel properties and secondary structure of fish myosin, Food Chem. 121 (2009) 196–202.
[29] R. Liu, S. Zhao, Y. Liu, H. Yang, S.B. Xiong, L. Xie, D.Y. Qin, Role of secondary structures in the gelation of porcine myosin at different pH values, Meat Sci. 80 (2008) 632–639.

[30] A. Dumetz, A.M. Chockla, E.W. Kaler, A.M. Lenhoff, Effects of pH on protein–protein interactions and implications for protein phase behavior, BBA - Proteins Proteomics 1784 (2007) 600–610.
[31] Y.Y. Ling, Y. Yuan, P.X. Pei, C.Y. Ji, Influence of heating on structure and gel properties of myofibrillar proteins from chicken breast muscle, Sci. Agric. Sin. 10 (2014) 2013–2020.
[32] D. Jian, Z.Q. Zhi, H.T. Xiang, S.X. Nan, D.J. Xuan, L. Yang, Effects of heat treatment on properties of soybean oil body emulsion, Food Sci. 37 (2016) 8–14.
[33] P.B. Stathopulos, G.A. Scholz, Y.M. Hwang, Sonication of proteins causes formation of aggregates that resemble amyloid, Protein Sci. 13 (2004) 3017–3027.