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Research Paper

Gelation of chicken batters during heating under high pressure

Hai-bo Zheng [a,b,c,d], Bao-cai Xu [a,*], Xing-lian Xu [c,*], Chao Li [d], Tomas Bolumar [e], Zong-yuan Zhen [b]

[a] School of Food and Biological Engineering, Hefei University of Technology, Hefei 230601, PR China
[b] College of Food Engineering, Anhui Science and Technology University, Fengyang 233100, PR China
[c] College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, PR China
[d] State Key Laboratory of Meat Processing and Quality Control, Jiangsu Yurun Meat Food Co., Ltd., 211899, PR China
[e] Max Rubner Institute (MRI), Department of Safety and Quality of Meat, Kulmbach 95326, Germany

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ABSTRACT

The gelling process of chicken meat batters, which were heated (75 °C) under atmospheric pressure or high pressure (200/400 MPa), was investigated by determining the hardness of batter, residual denaturation enthalpy, microstructure, and protein secondary structure. The results showed that meat batters heated at 200 MPa showed a similar increase in hardness to heat-only samples, but meat batters heated at 400 MPa showed a texture decreasing tendency after a limited increase. High pressure disrupted the myofibrils, promoted protein denaturation and aggregation in the first stage of heating under pressure treatment. In the second stage of treatment, heating was the main driving force for protein gelation, which was disturbed by hindering the structural transformation of proteins in the presence of high pressure during heating. The effect of 200 MPa on muscle proteins was relatively gentle and had a less negative effect. Excessive high pressure should be avoided when applying heating under pressure for gel-type meat products processing.

Industry relevance: High-pressure processing is increasingly applied in the meat industry. By combining high pressure with heating, their effects on texture improvement and microbial inactivation can be maximized. In this study, the influence of high pressure on the texture of meat products was analyzed, which showed that excessive pressure would significantly interfere with the thermal denaturation of the protein, thus adversely affecting the formation of the gel structure. High pressure at 400 MPa and above should be avoided when applying heating under pressure for gel-type meat products processing.

1. Introduction

High pressure processing (HPP) has many merits in food processing, such as its use to inactivate microorganisms (Heinz & Buckow, 2010) and to modify meat texture (Buckow, Sikes, & Tume, 2013), attracting more and more food companies to adopt HPP for food processing (Bajovic, Bolumar, & Heinz, 2012; Elamin, Endan, Yosuf, Shamsudin, & Ahmedov, 2015). The gelation property of meat proteins, which plays a vital role in gel-type meat products, can be improved by HPP treatment (Cheftel & Culioli, 1997; Simonin, Duranton, & de Lamballerie, 2012). Recent research has showed that HPP treatment at high temperature had a higher ability than HPP treatment at low temperature in improving gel qualities (Zheng et al., 2015; Zheng, Han, Bai, Xu, & Zhou, 2019), but also will have a higher impact on microbial inactivation (López-Caballero, Carballo, & Jiménez-Colmenero, 2002). In this regard, providing a means towards the industrial utilization of a single HPP process in the manufacturing of gel-like further-processed meat products (Bolumar et al., 2021).

Many researches have been done to illustrate the high pressure induced structural changes and properties of meat proteins (Orlien, 2021), however, most of them focused on the effect of high pressure under non-denaturing temperatures. The effect of high pressure can be very different at denaturing temperatures, as pressure-induced gelation depends on many factors such as meat systems and the processing conditions (Orlien, 2021). HPP treatment at a high temperature that is above the denaturing temperature of meat proteins can be considered a unique heating process under high pressure (instead of at atmospheric pressure). By heating under pressure (HUP), the heat-induced protein gelling was affected by pressure levels (Fernández Martín, Fernández, Carballo, & Colmenero, 1997; Zheng et al., 2017). It was reported that

* Corresponding authors.
E-mail addresses: baocaixu@163.com (B.-c. Xu), xlxus@njau.edu.cn (X.-l. Xu).

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HUP treatment at 200 MPa improved the texture while 400 MPa impaired the texture (Zheng et al., 2017). Many research ascribed the improvement in gelation to the high pressure induced disruption of myofibrils (Iwasaki, Noshiroya, Saitoh, Okano, & Yamamoto, 2006; Zheng et al., 2015; Zheng et al., 2017), and the impairment in gelation to the high pressure preserved non-denaturation (Fernández Martín et al., 1997; Fernández-Martín, 2007; Jiménez-Colmenero, Cofrades, Carballo, Fernández, & Fernández-Martin, 1998). However, these phenomena were found at both 200 MPa and 400 MPa (Fernández Martín et al., 1997; Zheng et al., 2015; Zheng et al., 2019), which can not be explained by these analyses. What is more, only a rather small amount of protein was preserved from being heat-denatured in 400 MPa-treated batters (Fernández-Martín, 2007). It is probably not suitable to ascribe the impairment found to the pressure preserved non-denaturation.

As the gelation process is the unfolding and refolding course of protein denaturation and conformational changes, during the gelation setting at certain P/T conditions, the intermolecular connections inevitably will occur and ultimately form a three-dimensional network. High pressure may affect the unfolding and re-folding process, so thus, influence the gel quality. This work was aimed to investigate the role of pressure in heating under pressure processing on the gelation properties of chicken meat batter during gelling by examining the gel texture, protein denaturation, protein secondary structure, and microstructure of meat batter.

2. Materials and methods

2.1. Materials and chemicals

Chicken breast meat (*M. pectoralis major*) was purchased from a supermarket (Nanjing Suguo Supermarket Co., Ltd., China). All chemicals used were of analytical grade, except for sodium chloride and sodium tripolyphosphate that were food grade.

2.2. Preparation of batters

The chicken breast meat was trimmed to remove connective tissue and visible fat, then minced through a 5 mm plate (MOD TC/12E, Sirman, Marsango, Italy). The minced meat was mixed and equally divided into four parts and then chopped separately. The meat mince (80%) was chopped in a bowl chopper (K15E, Talsabell S. A., Spain) at 1800 rpm with ice (17.7%), sodium chloride (2%), and sodium tripolyphosphate (0.3%) for 5 min. The temperature of chicken batters was no more than 13 °C during the cutting. The meat batters were vacuumed to remove air before being stuffed into plastic casings (diameter: 25 mm, Kangyuan Food Raw Materials Co., Ltd., China), and the stuffed casings were clipped every 10–12 cm with 60–70 g each. Last, the sausages were vacuum-packaged in plastic bags (Shanghai Yihe Packaging Machinery Co., Ltd., China) individually and stored in a cold room (0–4 °C) overnight for subsequent treatments the next day.

2.3. Pressure/thermal treatment

Samples were heated in a chamber at 75 °C under high pressure (200/400 MPa) or heated under atmospheric pressure (0.1 MPa) for 0, 3, 6, 9, 12, 18, 24, and 30 min, respectively. After heating, all samples were cooled by running tap water for 30 min and then kept in a cold room (0–4 °C), unless required for analysis. Analytical determinations were performed immediately and completed within a few days.

The heating under pressure treatment was performed in a 0.3 L high pressure unit (S-FL-850-9-W/FPG5620YHL, Stansted Fluid Power Ltd., Stansted, UK) by using a mixture of water and propylene glycol (7:3, *v*/v) as the compression fluid. The high-pressure chamber was heated to 75 °C and remained constant by a water bath (ILB-WCS, STIK Shanghai Co., Ltd.) connected to the thermos jacket outside the high-pressure chamber. Pressurization and depressurization rates were about 5 MPa/s and 20 MPa/s, respectively. The heat-only treatment (0.1 MPa) was performed in a water bath (TW20, JULABO Technology Co. Ltd., Seelbach, Germany) with heated water kept at 75 °C.

Center temperature of the chicken sausage heated under atmospheric pressure was determined by a digital thermometer (TA8112, Suzhou TASI Electronics Co. Ltd., Suzhou, China). Due to the requirement of sealing, the internal temperature of high pressure treated samples cannot be measured directly. A plastic tube (Thermo Fisher Scientific Inc., USA) filled with chicken batter was used to imitate the chicken sausage. The center temperature of samples was determined by a thermocouple inserted into the center of the plastic tube and connected to the built-in temperature detection device. The center temperature of chicken batter during processing is shown in Fig. 1.

2.4. Appearance measurement

The chicken sausages were placed horizontally on a table and were perpendicularly cut into 20 mm long cylindrical sub-samples with a knife. Then, the sub-samples were placed in a tray with black background and photographed individually with a camera (Redmi4, Xiaomi Inc. Beijing, China), which was fixed on a bracket.

2.5. Hardness measurement

The hardness of chicken sausage was assessed on a TA-XT plus texture analyzer (Stable Micro Systems Ltd., UK) at room temperature according to the methods described by Trespalacios and Pla (2007). Sausages were cut into 20 mm long cylindrical sub-samples, which were axially compressed to 40% of their original height at a speed of 1 mm/s by a 50-mm flat-bottom cylindrical probe. The trigger force used for the assessment was 5 g. The data were collected by Exponent Stable Microsystem (version 5.1.2.0, Stable Microsystems Ltd., UK).

2.6. Differential scanning calorimetry (DSC)

The thermal behavior of the meat batter was determined by differential scanning calorimeter DSC1 (Mettler-Toledo International Inc., Switzerland). A sample of 15–20 mg was picked out from the central part of the sausage, accurately weighed, capsulated in aluminium pans, and hermetically sealed. The samples were equilibrated for 2 min at the initial scanning temperature and then heated from 25 to 90 °C at 5 °C/min. An empty pan was used as a reference. The thermal data was normalized to dry matter content determined by desiccating the sample at 105 °C for 16 h.

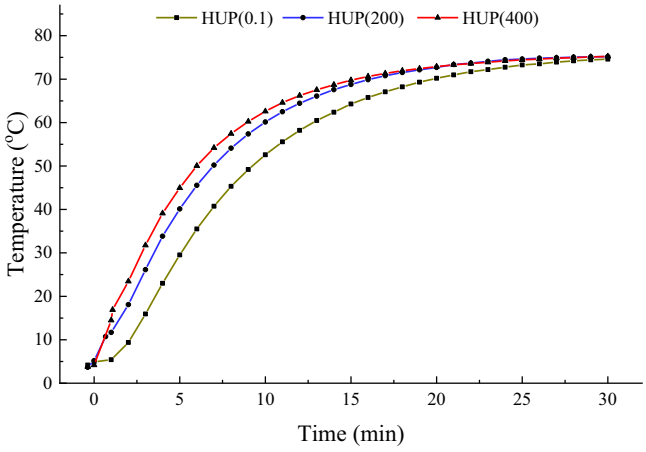

Fig. 1. Center temperature of chicken batters during heating at 75 °C and 0.1, 200, and 400 MPa. All values are the mean ± SD ($n = 3$).

2.7. Raman spectroscopy

Raman experiments were conducted with a Labram HR EV0 spectrometer (HoribaJobin, Longjumeau, France). A microscope equipped with a 50× lens was used to focus the excitation laser beam on the sample and to collect the Raman signal in the backscattered direction. The chicken sausage was taken and cut into 1 mm thick slices, and only the central part of the slices was selected and placed on the stainless-steel plate. The steel plate with the sample was then put on the microscope stage in the Raman spectrometer. The Raman spectra of 400–3000 cm^{-1} were collected at the excitation wavelength of 523 nm. Each spectrum was obtained under the following conditions: 3 scans, 30 s exposure time. Spectra were smoothed, baseline corrected, and normalized against the phenylalanine band at 1003 cm^{-1} using Labspec 6 (HoribaJobin, Longjumeau, France). The Phe ν-ring band located near 1003 cm^{-1} was used as internal to normalize the spectra, as it has been reported to be insensitive to the microenvironment (Herrero, 2008; LiChan, 1996; Li-Chan, Nakai, & Hirotsuka, 1994). Protein secondary structures were calculated from the amide I spectra around 1655 cm^{-1} using Alix's method (Alix, Pedanou, & Berjot, 1988).

2.8. Scanning electron microscopy

Samples were cut into 0.5 mm × 1 mm × 2 mm cubes and were fixed in a 0.1 mol/L phosphate buffer solution (pH 7.0) with 2.5 g/100 g glutaraldehyde. After fixing for 36 h, the cubes were cut into thin slices (0.2 mm × 0.5 mm × 1 mm). Ethanol dehydration, freeze-drying and sputter-coating were performed according to the procedure of Zheng et al. (2017). The microstructure of the sample was observed and photographed by a scanning electron microscope (S-3000 N, Hitachi Ltd., Japan).

2.9. Statistical analysis

The results were presented as mean ± standard deviation. One-way ANOVA was carried out to analyze the significance of the effect of pressure and time on the meat batters by Turkey test ($P < 0.05$) using SAS 9.1 (SAS Institute Inc., USA).

3. Results and discussion

3.1. Appearance

The cross-sectional changes of chicken batters with heating under pressure are shown in Fig. 2. In the first few minutes, the heat-induced gel-ring increased sharply, and then the whole sausage was gelled at 9 min and did not show a significant change in appearance afterward. The gelling process of the pressurized or unpressurized samples was basically the same, no matter whether there was high pressure or not, indicating that the main driving force for gelation was heating itself.

The effects of high pressure were distinguishable, especially on color, the sausage heated under 400 MPa (HUP(400)) showed that its color was almost totally faded at the very beginning as the center color turned white after being pressurized for 3 min. The color of the batter heated under 200 MPa (HUP(200)) did not change as much as that of the HUP (400) treated batter, which showed almost the same color as that of the batter heated at 0.1 MPa (HUP(0.1)). It was reported that high-pressure treatment caused significant color modification at pressures higher than 280 MPa for pork (Tintchev et al., 2010).

The different effects of high pressure can also be seen on texture. After heating for 3 min, the batter HUP(200)-treated sample had formed a soft gel, which was flat and smooth, while the HUP(400)-treated sample, however, was loose and watery. With continuous heating, the cross-section of the HUP(200)-treated sample was more smooth than that of both the HUP(0.1), and HUP(400) treated samples. These findings indicated that the effect of high pressure could be very distinct at different pressure levels and heating stages.

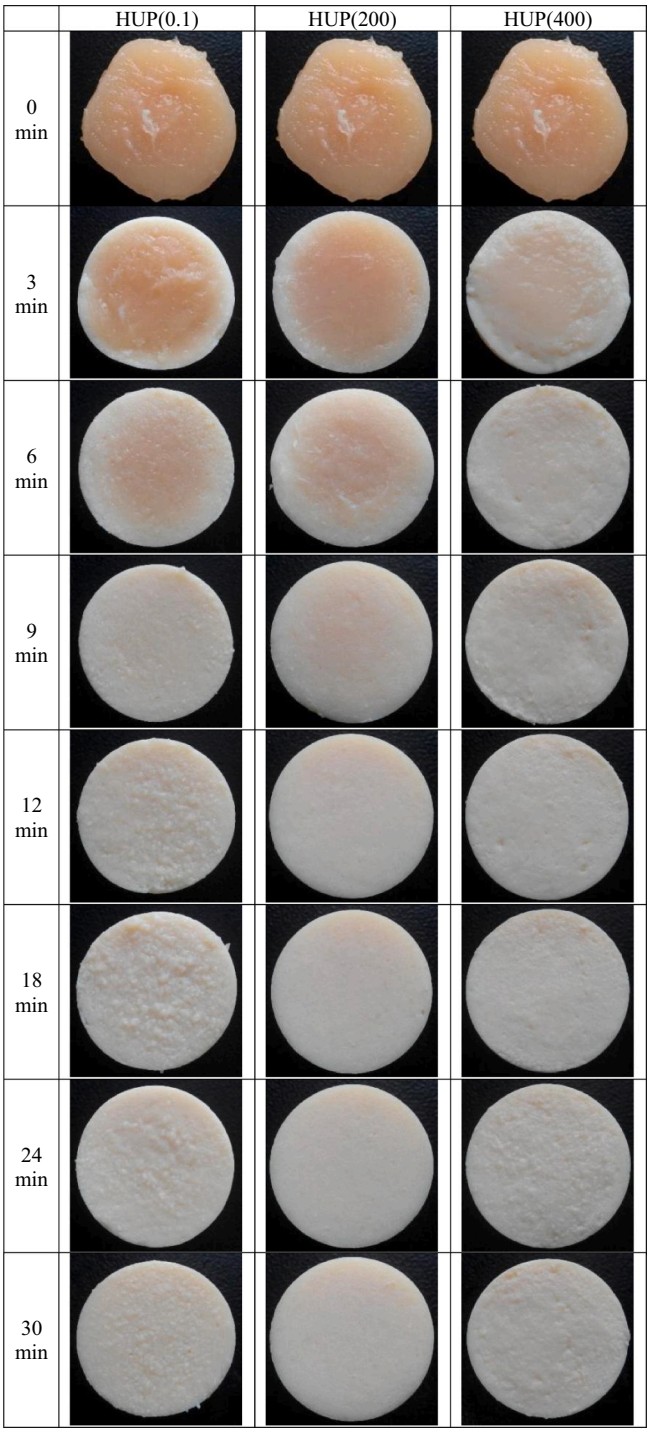

Fig. 2. Cross-sections of chicken sausages heated at 75 °C and 0.1, 200, and 400 MPa for 0, 3, 6, 9, 12, 18, 24, and 30 min.

3.2. Gel hardness

The hardness values of chicken meat batters are shown in Fig. 3. Hardness of batters heated at 0.1 and 200 MPa increased rapidly from 568.7 g (3 min) and 500.3 g (3 min) to 4285.2 g (12 min) and 4179.8 g (12 min), respectively. The hardness of chicken batters heated at 400 MPa, however, only increased a little, from 428.7 g (3 min) to 1378.7 g (12 min). That is consistent with previous reports where heating at 200

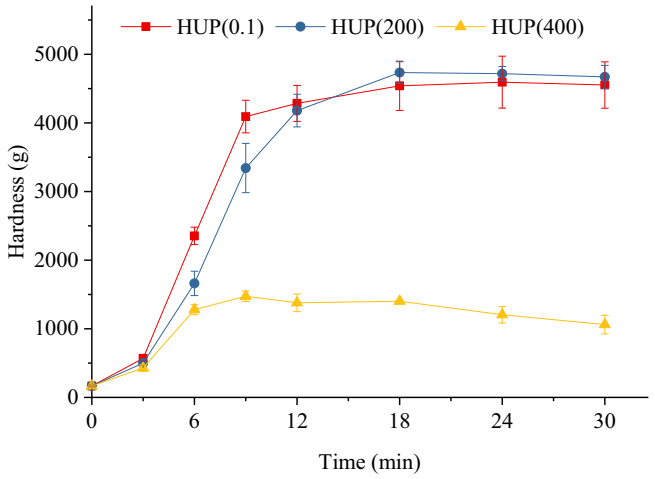

Fig. 3. Hardness of chicken batters heated at 75 °C and 0.1, 200, and 400 MPa for 0, 3, 6, 9, 12, 18, 24, and 30 min. All values are the mean ± SD (n = 4).

MPa improved the texture of chicken meat products, and heating at 400 MPa impaired the texture (Zheng et al., 2017). It should be noted that the hardness growth curve (Fig. 3) and temperature rise curve (Fig. 1) showed the same trend, which reflected the role of heating in forming a hard gel. The early stage of heating is critical for the hardness of the product, longer processing times either heating at atmospheric or high pressure had a limited effect on hardness.

Fig. 1 shows that the center temperature increased to around 70 °C at 18 min for batters heated under 200 MPa , so it was expected that the hardness of meat batter was almost at its maximum, as most of the protein would have been denatured and gelled at 70–75 °C. However, the batter heated at 400 MPa did not show a persistent increase after reaching its maximum hardness at 6 min when the center temperature reached around 50 °C, indicating the heat-induced gelation was impaired by high pressure at 400 MPa. To figure out whether this impairment was exerted by high pressure itself or by the interaction of high pressure and high temperature, the chicken batters were further heated immediately at atmospheric pressure after removing them out from high-pressure chamber, in order to apply a total of 30 min heating at 75 °C in all of the samples. This also guaranteed that a center temperature of 75 °C was reached. The hardness of the these further heated chicken batters is shown in Fig. 4.

Fig. 4 shows that the hardness of the batter treated at 200 MPa was about 5000 g. There was little improvement to the hardness as compared to that of the batter treated for 30 min at 200 MPa. The hardness of the batter treated at 400 MPa decreased steadily from 4351.97 g to 1221.40 g with increasing of pressurization time (see Fig. 4). In other words, the hardness of the HUP(400)-treated batter would increase if the heating time under high pressure was shortened or the heating time under atmospheric pressure was prolonged. This decline was a slow process, indicating that the gelling process of meat batter was gradually damaged when heating under high pressure. According to the principle of Le Chatelier, the transmission of pressure is rapid and uniform, while the decrease of hardness is a much slower process, which is similar to the slow heating process (see Fig. 1). According to the principle of heat conduction, the outer part of the chicken sausage was heated faster than the inner part, so the outer part went into gelling first and then the inner part followed. Therefore, the gelation of meat proteins in chicken sausage under heating was a continuous and lasting procedure. Because of the presence of high pressure (400 MPa), the heated meat batter was soft. This indicated that the impairment at 400 MPa was exerted mainly by the interaction of high pressure and high temperature.

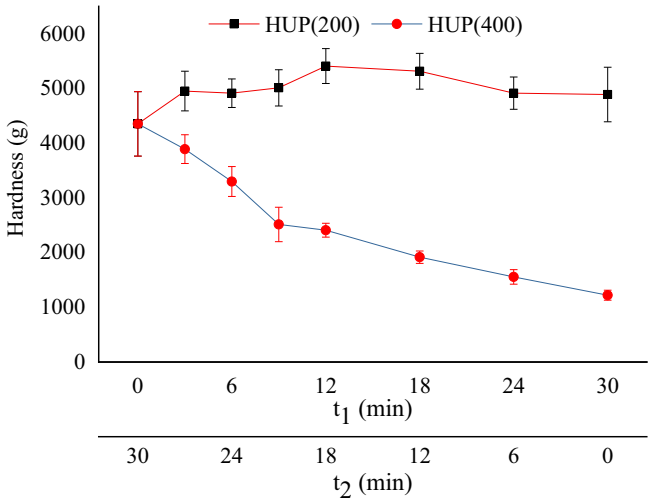

Fig. 4. Hardness of chicken batters treated by heating under pressure (t_1) that were subsequently heated without applying pressure (t_2) to achieve a total of 30 min of cooking time ($t_1 + t_2$). All values are the mean ± SD (n = 4).
t_1: heating time under high pressure.
t_2: heating time under atmospheric pressure.
$t_1 + t_2 = 30$ min.

3.3. DSC

Three endothermic peaks at approximately 54, 64, 70 °C, as shown in Fig. 5, were found and attributed to myosin, sarcoplasmic proteins together with collagen, and actin respectively, according to the descriptions by Speroni, Szerman, and Vaudagna (2014) and Jiménez-Colmenero et al. (1998).

As shown in Fig. 6 (HUP(0.1)), with increasing temperature, the meat proteins were gradually denatured upon reaching of their denaturation temperatures. When heated for 3 min, no proteins were obviously denatured. When heated for 6, 9, and 12 min, myosin, collagen, and sarcoplasmic proteins were denatured successively. After heating for more than 18 min, all of the proteins were denatured.

By heating at 200 MPa, most of the myosin denatured at 3 min and completely denatured at 6 min; actin and collagen were partly denatured at 3 min and 6 min (Fig. 6 (HUP(200)). As compared to Figs. 1 and 6 (HUP(400)), at the time of 6 min, the center temperature was about 40 °C, lower than their denaturation temperatures, indicating their denaturation was caused by high pressure. With time increased to 9 min, collagen was denatured entirely, and only a few actins remained non-

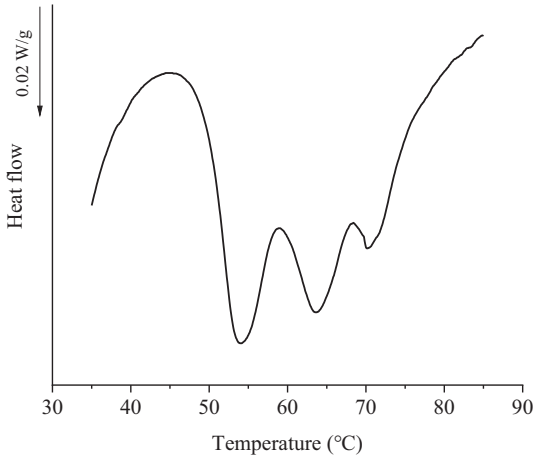

Fig. 5. Thermograms of raw chicken batters.

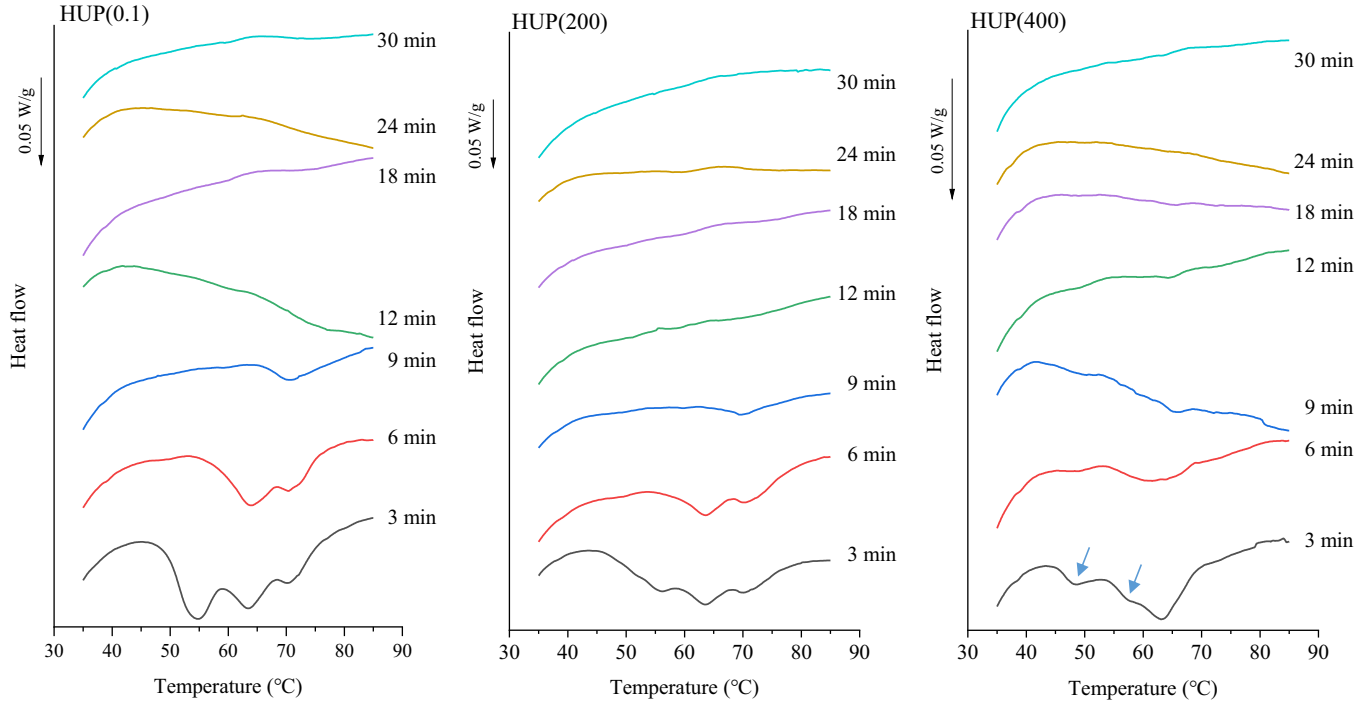

Fig. 6. Thermograms of chicken meat batters after cooking at 75 °C at 0.1, 200, and 400 MPa for 3, 6, 9, 12, 18, 24, 30 min.

denatured. Over 12 min, it seems that all proteins were denatured.

As shown in Fig. 6 (HUP(400)), it was apparent that the myosin peak of batter heated under 400 MPa at 3 min changed from one peak at 54 °C into two peaks at 48 °C and 58 °C, probably because the myosin was transferred into two different derivatives (Chapleau, Mangavel, Compoint, & Lamballerie-Anton, 2004). Actin was almost denatured, but collagen was still mostly non-denatured at 3 min. At treating times of 9, 12, and 18 min, most of the meat proteins were denatured, except a few collagen (64 °C) and myosin derivatives (48 °C). After treating for over 18 min, no peak could be found, indicating all proteins were denatured.

In general, the denaturation occurred at the very beginning of the HUP treatment because of the instant and uniform transmission of high pressure. It have been reported that high pressure (100–500 MPa) induced protein unfolding, exposure of interior hydrophobic and sulfhydryl groups (Zhang, Yang, Zhou, Zhang, & Wang, 2017). HPP treatment above 300 MPa induced severe protein unfolding, thus weakened the gelation properties (Zhang et al., 2017). This was consistent with the findings in this study, as high pressure at 400 MPa exerted a stronger influence than 200 MPa on protein denaturation, and HUP(400) treated batter was weaker than HUP(200) treated batter. The denaturation effect of high pressure on protein in the early stage of treatment may affect gel formation.

Table 1
Corresponding percentage of protein secondary structures of chicken batters after heating at 75 °C under pressure 0.1, 200, and 400 MPa for 0–30 min. All data were expressed as mean ± SD (n = 4).

Secondary structure (%)	Pressure (MPa)	Time (min)							
		0	3	6	9	12	18	24	30
α-helix	0.1	$68.8 \pm 2.1^{a,A}$	$67.5 \pm 3.0^{a,A}$	$59.0 \pm 3.3^{b,A}$	$33.0 \pm 4.9^{c,A}$	$27.1 \pm 1.3^{c,B}$	$25.6 \pm 1.6^{c,B}$	$28.9 \pm 3.9^{c,A}$	$26.6 \pm 3.4^{c,A}$
	200	$68.8 \pm 2.1^{a,A}$	$65.3 \pm 3.4^{a,A}$	$57.8 \pm 3.6^{b,A}$	$41.1 \pm 2.1^{c,A}$	$29.8 \pm 1.8^{d,B}$	$28.1 \pm 2.2^{d,B}$	$29.7 \pm 1.1^{d,A}$	$30.0 \pm 2.3^{d,A}$
	400	$68.8 \pm 2.1^{a,A}$	$61.1 \pm 4.2^{a,A}$	$45.0 \pm 6.1^{b,B}$	$40.0 \pm 6.3^{bc,A}$	$36.6 \pm 1.7^{bc,A}$	$37.2 \pm 4.4^{bc,A}$	$34.5 \pm 5.9^{c,A}$	$31.2 \pm 1.5^{c,A}$
β-sheets	0.1	$9.0 \pm 1.6^{c,A}$	$10.1 \pm 2.3^{c,A}$	$16.6 \pm 2.5^{b,B}$	$36.5 \pm 3.8^{a,A}$	$41.0 \pm 1.0^{a,A}$	$42.1 \pm 1.2^{a,A}$	$39.7 \pm 3.0^{a,A}$	$41.4 \pm 2.6^{a,A}$
	200	$9.0 \pm 1.6^{d,A}$	$11.7 \pm 2.6^{d,A}$	$17.5 \pm 2.8^{c,B}$	$30.3 \pm 1.6^{b,A}$	$38.9 \pm 1.4^{a,A}$	$40.3 \pm 1.7^{a,A}$	$39.0 \pm 0.8^{a,A}$	$38.8 \pm 1.8^{a,A}$
	400	$9.0 \pm 1.6^{c,A}$	$15.0 \pm 3.2^{c,A}$	$27.3 \pm 4.6^{b,A}$	$31.1 \pm 4.8^{ab,A}$	$33.7 \pm 1.3^{ab,A}$	$33.2 \pm 3.4^{ab,B}$	$35.4 \pm 4.5^{a,A}$	$37.9 \pm 1.2^{a,A}$
β-turns	0.1	$13.1 \pm 0.3^{c,A}$	$13.3 \pm 0.5^{c,A}$	$14.6 \pm 0.5^{b,B}$	$18.7 \pm 0.8^{a,A}$	$19.6 \pm 0.2^{a,A}$	$19.8 \pm 0.3^{a,A}$	$19.3 \pm 0.6^{a,A}$	$19.7 \pm 0.5^{a,A}$
	200	$13.1 \pm 0.3^{d,A}$	$13.6 \pm 0.5^{d,A}$	$14.8 \pm 0.6^{c,B}$	$17.4 \pm 0.3^{b,A}$	$19.2 \pm 0.3^{a,A}$	$19.5 \pm 0.3^{a,A}$	$19.2 \pm 0.2^{a,A}$	$19.2 \pm 0.4^{a,A}$
	400	$13.1 \pm 0.3^{c,A}$	$14.3 \pm 0.7^{c,A}$	$16.8 \pm 1.0^{b,A}$	$17.6 \pm 1.0^{ab,A}$	$18.1 \pm 0.3^{ab,B}$	$18.0 \pm 0.7^{ab,B}$	$18.5 \pm 0.9^{ab,A}$	$19.0 \pm 0.2^{a,A}$
Random coil	0.1	$9.4 \pm 0.1^{d,A}$	$9.5 \pm 0.2^{d,A}$	$10.0 \pm 0.2^{c,B}$	$11.6 \pm 0.3^{b,A}$	$12.0 \pm 0.1^{ab,A}$	$12.1 \pm 0.1^{a,A}$	$11.9 \pm 0.2^{ab,A}$	$12.0 \pm 0.2^{ab,A}$
	200	$9.4 \pm 0.1^{d,A}$	$9.7 \pm 0.2^{d,A}$	$10.1 \pm 0.2^{c,B}$	$11.1 \pm 0.1^{b,A}$	$11.8 \pm 0.1^{a,A}$	$11.9 \pm 0.1^{a,A}$	$11.8 \pm 0.1^{a,A}$	$11.8 \pm 0.1^{a,A}$
	400	$9.4 \pm 0.1^{c,A}$	$9.9 \pm 0.3^{c,A}$	$10.9 \pm 0.4^{b,A}$	$11.2 \pm 0.4^{ab,A}$	$11.4 \pm 0.1^{ab,B}$	$11.5 \pm 0.2^{ab,B}$	$11.5 \pm 0.4^{a,A}$	$11.7 \pm 0.1^{a,A}$

Notes: a, b and c: statistically significant differences between average values in rows in reference to the time, $P < 0.05$. A, B: statistically significant differences between average values in columns in reference to the pressure, $P < 0.05$.

3.4. Secondary structure

The spectra profile of the amide I band (centered near 1655 cm^{-1}) is used to quantify the secondary structure of proteins. The quantitative estimation of the secondary structure fractions using Alix's method (Alix et al., 1988) is shown in Table 1.

The fresh meat batter presented a typical meat protein secondary structure with a high content of α-helix (68.8%), and low content of β-sheets (9.0%), β-turns (13.1%), and random coil (9.4%). With continuous heating, the content of α-helix in batters heated without applying high pressure (0.1 MPa) decreased with the increase of temperature, and a sharp decrease from 67.5% to 33.0% occurred at 3 mins to 9 mins when the internal temperature increased from around 15 °C to around 55 °C. Similar trends can be seen in batters heated at 200 MPa, but the decline was relatively small, as the α-helix in batter treated at 200 MPa was dropped from 65.3% at 3 min to 41.1% at 9 min, down 24.2%, lower than 34.5% in batter treated at 0.1 MPa. For batters treated at 400 MPa, the sharp drop occurred in the range of 3 min to 6 min, which was from 61.1% decreased to 45.0%. Compared to heating under atmospheric pressure, it was pronounced that high pressure significantly promoted the transformation of protein's secondary structure. Table 1 shows that heating under 200 MPa and 400 MPa decreased α-helical structure by 3.5% and 7.7% in 3 min, respectively. While the α-helical structure in batters treated at 0.1 MPa only decreased 1.4%. The sharp decrease of α-helix in batter treated at 400 MPa was consistent with DSC results (Fig. 6), which showed that a large portion of the protein was denatured at 3 min. When the treatment time exceeds 9 min, the transformation of secondary structure in HUP(400)-treated batter became slow, resulted in higher content of α-helix, and lower content of β-sheets, β-turns, random coil than that of HUP(200) and HUP(0.1) treated batters.

As it can be observed in Table 1, the speed of the transformation of the secondary structure in protein was different. It was supposed that the speed of protein unfolding and aggregation should be balanced to form a fine fibrous network (Zhang et al., 2017). The structural transformation speed of the batter heated at 200 MPa or 400 MPa was very fast at the beginning, but then it was slow. This phenomenon was more obvious at 400 MPa. Table 1 shows that the α-helix in batter heated at 400 MPa was lower at 6 min, but higher at 12 min and 18 min significantly ($P < 0.05$) than that of other batters. There was no significant ($P > 0.05$) difference between HUP(0.1) and HUP(200) treated samples. This is in agreement with the results of appearance and hardness, indicating that the disturbed balance of denaturation may be an important reason for the weak texture of batter heated under 400 MPa.

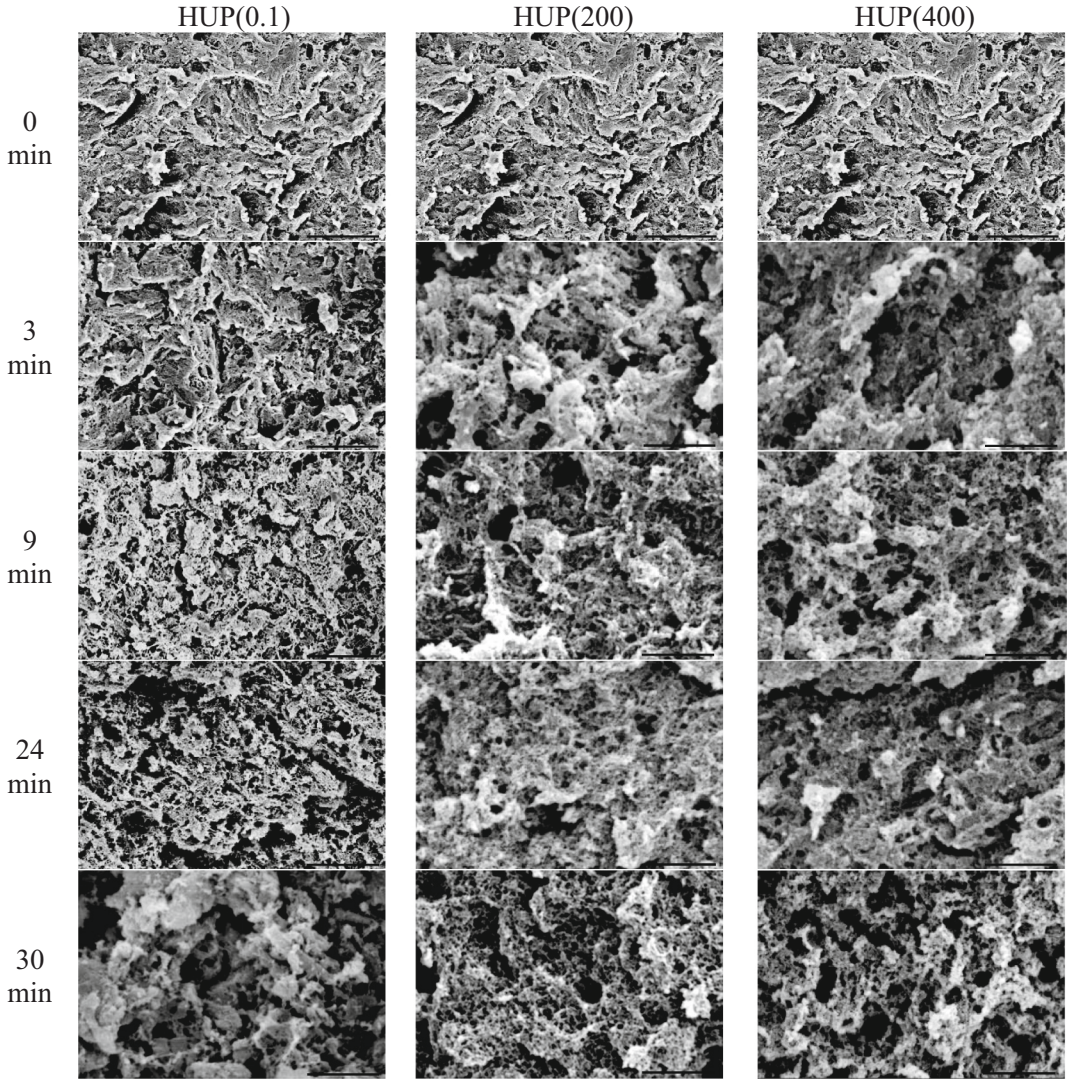

Fig. 7. Scanning electron micrographs of chicken sausages after heating at 75 °C under pressure 0.1, 200, and 400 MPa for 0, 3, 9, 24 min. The black line in the lower right corner represents 5 μm.

3.5. Microstructure

Fig. 7 shows that myofibrils were disrupted in batters heated at 200 and 400 MPa. At a treating time of 3 min, no obvious myofibrils could be found in 200 and 400 MPa heated samples, indicating that the myofibrils were disrupted very quickly before the temperature increased to the denaturing temperature. It can also be seen that gel networks were formed in batter treated at 200 MPa at the same time, while no significant network could be found in batter treated at 400 MPa and 0.1 MPa. With increasing time (or internal temperature), the gel network of batter treated at 200 MPa became dense and porous, a similar tendency had also been observed in batter treated at 400 MPa, but less interconnected and porous. After heating for 9 min, the porous structure could also be found in batter treated at 0.1 MPa, but very different from the structure found in high pressure treated sample as the muscle fibers were not disrupted as those found in high pressure treated samples.

The disruption of the myofibrillar structure by high pressure was the most obvious observable change in the early stage of HUP treatment. High pressure promoted the formation of gel network, which was helpful to improve the water holding capacity (Zheng, Han, Yang, Xu, & Zhou, 2018). The microstructure did not show much change after reaching the myosin denaturation temperature of around 55 °C at 9 min. It should be noted that high pressure was not able to disrupt the myofibrillar structure of heat-denatured batter (Zheng et al., 2015), so the compression speed should be fast enough to avoid denaturation before pressurization when applying HUP treatment in meat product processing.

4. Discussion

The high pressure-induced disruption of myofibrillar structure has been accepted as a critical factor that improves gel texture (Iwasaki et al., 2006; Zheng et al., 2017). High pressure lead to the disappearance of M-line, disorder of *Z*-line, and depolymerization of thick and thin filaments (Bolumar et al., 2021; Iwasaki et al., 2006). The higher the pressure (100–300 MPa) the more intense the destruction (Iwasaki et al., 2006). Many researches have showed that heating at 200 MPa contributed to form a porous gel network and a firm texture (Iwasaki et al., 2006; Zheng et al., 2015; Zheng et al., 2017). This research was consistent with these researches in terms of microstructure and texture. However, heating at 75 °C in a chamber at 400 MPa showed a negative impact on texture, although myofibrils were also disrupted as those heated under 200 MPa. The deterioration in microstructure was also observed in HUP(400)-treated batter, which showed that the under 400 MPa heated batter had a thick fibrous gel network with obviously bigger cavities. Many researches ascribed this impairment to the preservation of protein denaturation, as high pressure was found to be able to hinder protein from being heat-denatured (Fernández Martín et al., 1997; Jiménez-Colmenero et al., 1998). Myosin and actin were vulnerable to high pressure and denatured before being heated. The main protein stabilized by high pressure was collagen (Fig. 6), because it has a large amount of hydrogen-bonded structure, which is favored under pressure (Buckow et al., 2013). This stabilizing effect is relative, as the protein conformation could be destabilized at higher pressures or higher temperatures (Potekhin, Senin, Abdurakhmanov, & Tiktopulo, 2009). Although collagen, which accounts for about 2% of the total proteins (Rigdon et al., 2021), can be preserved from being denatured, the amount of non-denatured proteins was much fewer than the total proteins. What is more, as the temperature was high enough that all proteins went denaturation at last, as shown by DSC (Fig. 6). In general, high pressure promoted the denaturation of meat proteins, as myosin and actin were pressure-sensitive and only collagen was relatively stable. Therefore, it was not proper to ascribe the soft texture of HUP(400)-treated batter to the preservation effect of high pressure on heat-induced protein denaturation.

The effect of high pressure and heating on the gelation of meat batter was not simultaneous. Fernández Martín et al. (1997) divided the whole process into two phases according to the temperature is higher or lower than the protein denaturation temperature. When processing at non-denaturing temperatures, lower than 40 °C, the protein denaturation was caused by high pressure dependently and related to pressure level; When processing at denaturation temperatures, higher than 50 °C, the net effect was interdependent with both pressure and temperature (Fernández Martín et al., 1997). It is generally accepted that in the first phase, high pressure causes myosin to disintegrate, unfold and then aggregate to form large aggregates (Orlien, 2021), along with disruption of myofibrillar structure. In the second stage, the protein structure was further modified and the weak gel that formed in the first stage was turned into rigid gel. The main driving force in the second stage was heating, but this process was disturbed by high pressure. The results showed that the structural transformation of proteins was slowed down at the denaturation temperature, after a rapid transformation below the denaturation temperature.

With heating for 3 min, the inner temperature of the meat batter was still lower than 40 °C. Myosin, the main functional protein in gel-type muscle foods, was greatly affected by high pressure at 400 MPa and the α-helix content in meat batter dropped sharply from 68.8% (0 min) to 61.1% (3 min), but decreased slightly to 65.3% (3 min) at 200 MPa. Dong et al. (2021) found out that the reduction of α-helix contents and the increase of β-turn, β-sheet, and random coil were consistent with the change of pressure. This pressure-induced protein's secondary structure transformation before heating was an unignorable reason for the gel weakness (Zhang et al., 2017). However, it seems that it was not the main reason, as the gelation ability could be recovered to a certain extent, if the pressure is removed (see Fig. 4). It was considered that the change of protein tertiary structures induced by high pressure (< 300 MPa) is reversible (Sun & Holley, 2010). Fig. 4 shows that those samples that later were heated at atmospheric pressure had a more rigid texture than those only heated under high pressure. Therefore, it indicated the interaction between heating and high pressure impaired the normal gelling ability of meat protein when heated under pressure. The protein was compressed under high pressure. Thus, its inner cavity was minimized, resulting in the exposure of hydrophobic regions and aggregation of the protein. The hydrogen bonding was strengthened under high pressure, as it contributed to minimize system volume (Sun, et al., 2010). Any reactions that increase the system volume were hindered by high pressure. Heating, on the contrary, tends to increase system volume by breaking hydrogen and covalent bonds (Royer & Winter, 2011). Finally, due to the contrary effects of heating and high pressure, the structural transformation and aggregation balance of proteins were disturbed or modified. The amplitude of modification depended on the pressure level. Excessive high pressure at 400 MPa resulted in the soft texture of the HUP(400)-treated sample. It should be emphasized that this kind of impairment occurred only when the high pressure persisted during heating.

5. Conclusions

Heating and high pressure both affected the gel-structure forming process, but the main driving force for gelling was the heating. The disruption of the myofibrillar structure was an important and positive influence that high pressure produced in the initial stage of the HUP treatment. High pressure has both promoting and hindering effects on protein denaturation, which are related to the type of protein. Myosin and actin were prone to be denatured by high pressure, but collagen denaturation was retarded by high pressure. With the temperature increased to the denaturing temperature, the heat-induced protein structural transformation was hindered by high pressure. High pressure at 200 MPa was able to promote the formation of a highly uniform and desirable gel-like meat product structure without excessive interference in the thermal denaturation process of proteins. While heating at 400 MPa not only disrupted the myofibrils but also seriously interfered the

structural transformation of proteins. The imbalance of protein conformational modification and denaturation caused by the presence of excessive high pressure during heating could be the main reason that resulted in the weak texture.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

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