Food Science of Animal Products

Research Article

Superheated steam cooking improved meat quality: evidenced by water status and protein degradation

Jiajia Fang[1,2], Jiapeng Li[2] ✉, Junna Yang[2], Biao Qi[2], Chunjiang Zhang[1] ✉

[1] *Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences (CAAS), Technical Integration Laboratory of Chinese Meat Dishes Processing, Ministry of Agriculture and Rural Affairs, Key Laboratory of Meat Processing and Logistics (IFST-CAAS), China General Chamber of Commerce, Beijing 100193, China*

[2] *China Meat Research Center, Beijing Key Laboratory of Meat Processing Technology, Beijing Academy of Food Sciences, Beijing 100068, China*

✉Address correspondence to Jiapeng Li, ljp7915@126.com; Chunjiang Zhang, chjiang1976@126.com

Received: June 16, 2023; Revised: June 30, 2023; Accepted: July 5, 2023

Abstract: This study aimed to investigate the relationship among physicochemical quality, water status and protein degradation of pork samples during superheated steam (SHS) cooking. Pork samples were cooked with SHS (120, 150 and 180 ℃ and traditional steam (TS) to 40, 60 and 80 ℃. The results showed that SHS cooking at 150 and 180 ℃ significantly reduced the values of lightness (L^*), yellowness (b^*), cooking loss and increased the value of redness (a^*). Moreover, SHS cooked samples had lower shear force, hardness and chewiness value than TS cooked samples, indicating a better mouth feel quality. Low-field nuclear magnetic resonance (LF-NMR) analysis results showed that relaxation time T_{21}, T_{22} and T_{23} increased with SHS temperature, T_2 (TS-cooked) < T_2 (SHS-cooked), SHS had higher P_{22} values but lower P_{23} values than TS. The secondary structure of pork protein cooked by TS tends to be loose than SHS, promoting more immobilized water into free water. Furthermore, SHS led to a low exposure of hydrogen bonds and hydrophobic bonds which reduced protein aggregation. The protein degradation and water status could explain the quality differences between SHS and TS cooked pork.

Keywords: superheated steam cooking; texture; cooking loss; pork; low-field nuclear magnetic resonance; chemical forces

Citation: J. J. Fang, J. P. Li, J. N. Yang, et al., Superheated steam cooking improved meat quality: evidenced by water status and protein degradation, Food Sci. Anim. Prod. 1 (2023) 9240023. https://doi.org/10.26599/FSAP.2023.9240023.

1 Introduction

Pork contains high-quality proteins, vitamins, and mineral compounds and amino acids which is essential for the human body. It is frequently consumed after thermal treatment, as steaks, burgers or roasts[1]. Nowadays, more and more consumers are realizing that reducing the consumption of highly processed meat products such as grilled and fried foods is crucial for a healthy diet. Traditional steaming is a simple, convenient, healthy, smoke-free cooking method which is becoming increasingly popular for preparing pork in restaurants and households[2–3]. It uses the steam generated from boiling water as a heat transfer medium and can better meet people's requirements for healthy eating. However, the common disadvantage of traditional steaming is long processing time and usually requires large quantities of water which is energy consuming.

Heating steam to a temperature above its boiling point is referred to as superheated steam (SHS). SHS is a suitable heating medium in food processing and it is an innovative technology that offers many potential benefits to the food industry[4]. Compared to traditional processing methods, SHS has the advantages of high heat transfer coefficients, low energy consumption, non-oxidative conditions, and low environmental impact[5–6]. Recently, SHS technology has been successfully applied to vegetables and fruit blanching and drying, meat cooking and roasting, and bread baking[7–9]. Previous studies indicated that SHS can improve the nutritional value in terms of lipid in fish or meat products[10]. SHS could also significantly lower hazardous compounds in meat products[11–12]. In addition, meat products processed by SHS were lighter in color and had better textural properties compared with those processed by conventional heating methods[13]. It is well known that meat quality attributes are directly related to heating medium, cooking temperature and final cooking temperature, etc.[14]. Water distribution and proteins degradation are vital in understanding the other physical and chemical properties change[15–17]. Nevertheless, the relationship between water distribution, protein degradation and meat quality attributes after SHS cooking need research and further exploration for improving the uniformity of products quality.

The objective of this study was to investigate the effects of SHS cooking on qualities of pork samples and compare the meat quality of the SHS cooking process with those of a TS cooking process. According to Fang et al.[5] and our pre-experiment, SHS at high temperature can lead to intensive color which is undesirable for steaming. Therefore, the temperature of SHS were determined 120, 150 and 180 ℃. The cooking loss, color, texture profile of meat samples cooked with SHS and TS were characterized. The principal component analysis (PCA) was used to explore the difference in meat quality among the two cooking methods. Water status of pork meats was determined using low-field nuclear magnetic resonance (LF-NMR) technique. Meanwhile, Protein denaturation and

degradation were evaluated by protein secondary structure, hydrogen bonds, ionic bonds and hydrophobic interaction.

2 Materials and methods

2.1 Materials

Longissimus muscles from 9 pigs (Duroc × Landrace × Yorkshire) were obtained from a supermarket in Beijing. Visible connective tissue and fat were removed and *Longissimus* muscles of each pig were sliced along the direction of muscle fibers into twelve meat blocks (about (25 ± 2) g, 5 cm × 5 cm × 1 cm). Meat blocks from the *Longissimus* muscle of same pig were equally assigned to 9 treatment groups, resulting in 9 replicates per treatment ($n = 9$).

2.2 SHS and TS cooking experiments

The SHS cooking is carried out in the SHS oven (Naomoto 350, Japan). A Schematic diagram of the SHS oven was shown in Figure 1. The SHS oven is mainly consists of four parts: a steam generator, a heater, a closed processing chamber and a controller. During the SHS process, saturated steam generated from the steam generator is heated by heaters to produce SHS and enters the processing chamber from upper and lower nozzles. The temperature of SHS is controlled by the controller. In our study, three temperatures in terms of 120, 150 and 180 ℃ was carried out. The meat sample placed on the grid was put into the preheated oven, the thermocouple probe was insert into the center of the pork sample, and the sample was taken quickly when the center temperature of the pork reaches 40, 60, and 80 ℃.

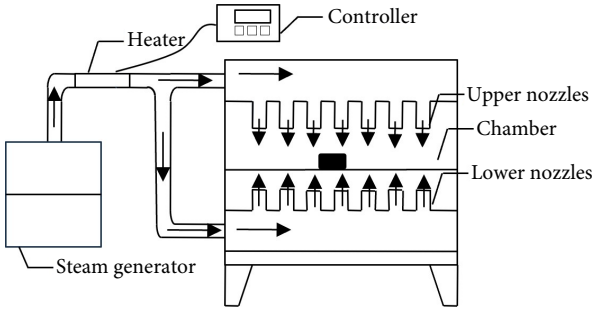

Figure 1 A schematic diagram of the SHS oven.

The steam cooking was conducted with a steaming pot (inner diameter 24 cm, 1 kW) according to the method of Nieva-Echevarría et al.[18]. Add boiling water (3 L, 98 ℃) to the pot, put the meat pieces on the steaming grid, and heat the pork with the steam generated by boiling water. When the center temperature of pork reaches 40, 60 and 80 ℃, take samples quickly.

After cooking, the meat samples were placed in a self-sealing bag and soaked in ice water for 10 min to terminate the reaction. When measuring the indicators, the samples were wiped dry gently with filter paper, and stored the remaining samples at −80 ℃.

2.3 Color measurement

The color of meat samples was measured using Chroma Meter CR-400 colorimeter (Minolta, Osaka, Japan). The colorimeter was calibrated using a standard whiteboard. The cooked samples were cut in the center perpendicular to the longitudinal direction of muscle fibers. The brightness (L^*), redness (a^*) and yellowness (b^*) values of fresh cut surface of samples were measured. For each sample 3 points were selected randomly.

2.4 Cooking loss

The weight of the initial pork sample m_0 and the weight of the pork sample after cooking m_1 were recorded. The cooking loss were calculated using the following formula:

$$\text{cooking loss } (\%) = \frac{m_0(\text{g}) - m_1(\text{g})}{m_0(\text{g})} \times 100$$

2.5 Textural profile analysis (TPA) and shear force

TPA was evaluated using a TA-XT Plus analyzer (Stable Micro System, UK). The pork sample was cut along the direction of muscle fiber into 1 cm × 1 cm × 1 cm. The P35 probe was used to compress the meat piece twice. The speed before the test was 2.0 mm/s, the speed during the test was 1.0 mm/s, the speed after the test was 2.0 mm/s, and the compression distance was 60%.

For measuring shear force, the pork sample was cut along the direction of muscle fiber into 3 cm × 1cm × 1 cm. Then the meat piece was cut by Warner Bratzler probe along the direction perpendicular to the muscle fiber. The cutting position is 0.5 cm away from the edge of the sample. The speed before the test is 5 mm/s, the speed during the test is 10 mm/s, and the speed after the test is 10 mm/s. Shear force is the peak force, and the result is expressed in Newton.

2.6 Acquisition of transverse relaxation signals in LF-NMR

LF-NMR analyzer (PQ-001, Niumag Electric Corporation, Shanghai, China) was used for the measurement of transverse relaxation time (T_2). Each meat sample was individually placed into 25 mm NMR glass tubes. The T_2 measurements were measured with CPMG sequence at 32 ℃ with τ-value for 12 and 24 μs with 90° and 180° pulse from 4 000 echoes.

2.7 Extraction of myofibrillar protein (MP)

Extraction of MP was carried out as described by Hughes et al.[19], with minor modifications. Five gram of the cooked pork sample was mixed with 50 mL 0.03 mol/L phosphate buffered saline (pH 7.0) and homogenized using an Ultra Turrax T25 (I.K.A., Germany) for 2 min. The homogenate was centrifuged for 20 min at 10 000 × g at 4 ℃ and the MPs were extracted from the precipitate by homogenizing with 40 mL extractant solution containing 8 mol/L urea and 1% (m/V) β-mercaptoethanol for 2 min. The homogenate was recentrifuged as above and the supernatant was filtered with Whatman 1# filter paper at 4 ℃, and the filtrate was MP.

2.8 Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) and secondary structure

ATR-FTIR was performed to assess secondary protein structure. ATR-FTIR is an infrared spectroscopy technique used for analyzing solid and liquid samples. It combines the advantages of attenuated total reflection technology and Fourier transform spectroscopy technology, and can obtain high-quality infrared spectra without any sample processing[20]. In this study, ATR-FTIR of the samples were recorded using a Bruker Tensor 27 spectrometer (Bruker Optics, Germany) equipped with an ATR accessory. Pork sample MP was taken and placed on the ATR attachment for scanning. The spectral scanning range was set at 400–4 000 cm^{-1}, and the resolution was 4 cm^{-1}. Peak Fit 4.12 software (SPSS Inc., Chicago, IL, USA) was used to analyze the obtained infrared spectrum.

2.9 Measurement of chemical forces

The chemical forces were determined by the method of Yu et al.[17], with minor modifications. The four following denaturing solutions were selected for cleaving specific bonds: 0.05 mol/L NaCl (SA), 0.6 mol/L NaCl (SB), 0.6 mol/L NaCl + 1.5 mol/L urea (SC), 0.6 mol/L NaCl + 8 mol/L urea (SD). Two grams of pork sample were homogenized in 10 mL of each denaturing solutions with Ultra Turrax T25 (I.K.A., Germany) for 1 min. The homogenates were stirred at 4 ℃ for 2 h, and then centrifuged at 10 000 × *g* for 10 min. Protein concentration in supernatants were determined by the Lowry method. Ionic bonds were determined by difference between SB and SA, hydrogen bonds were determined by difference between SC and in SB, and hydrophobic interactions were determined by difference between SC and in SB.

2.10 Statistical analysis

SPSS Version 22.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Data are presented as the mean ± standard deviation (SD). One-Way analysis of variance (ANOVA) using Duncan adjustment was used to analyze the differences among different internal temperatures for the same cooking method and the differences in different cooking methods at the same internal temperature. The statistical significance was defined as $P < 0.05$.

3 Results and discussion

3.1 Internal color, cooking loss

Table 1 shows the changes in internal color and cooking loss of pork samples cooked to different temperatures by TS and SHS at 120, 150 and 180 ℃. It was found that L^* and b^* of TS and SHS cooked samples gradually increased with the increase of internal temperature, whereas a^* decreased. Chotigavin et al.[2] also observed an increase in the L^* and b^* but a reduction in the a^* during cooking. The L^* value is related to the moisture content of the sample, and during the cooking process, the moisture in the sample continuously migrates to the surface, causing an increase in the L^* value. The value of a^* is related to the state of myoglobin in the sample. There is no significant difference in color between pork cooked by SHS at 120 ℃ and TS. Pork samples cooked by SHS at 150 and 180 ℃ had significant lower value in L^* and b^* but higher value in a^* compared with that cooked by TS, suggesting less myoglobin degradation by use of SHS cooking.

Cooking loss of pork was no significant difference ($P > 0.05$) between TS and SHS at 40 ℃. At an internal temperature of 60 ℃, pork cooked by SHS at 150 and 180 ℃ caused less ($P < 0.05$) cooking loss (7.47% and 7.46%) than those cooked by SHS at 120 ℃ and TS (8.35% and 8.78%). Similarly, at an internal temperature of 80 ℃, SHS cooked samples at 120 ℃ and TS showed higher ($P < 0.05$) cooking loss (21.89% and 22.05%) than those cooked by SHS at 150 and 180 ℃ (15.95% and 14.26%). In addition, the cooking loss significant increased with increasing internal temperature ($P < 0.05$). This phenomenon possible attributed to the denaturation of the pork protein after heating. The structure of the protein was destroyed and the hydrophobic groups was gradually exposed. Therefore, as the central temperature increases, the cooking loss gradually increases. On the other hand, SHS resulted in the less damage of the organizational structure of pork proteins, making it more difficult to convert from immobilized water to free water, resulting in less cooking loss.

3.2 Shear force, texture

The pork samples cooked by TS and SHS showed variation in shear force and texture properties (Table 2). The value of shear force gradually increased with increasing internal temperature. The SHS cooked samples with 60 and 80 ℃ internal temperature showed lower values in shear force compared to TS cooked samples, indicating that SHS cooked meats were tender than TS cooked meats. However, at an internal temperature of 40 ℃, SHS cooked samples showed higher shear force values. This is probably due to TS cooked meats uneven, the steam generated from the boiled water will flow upward and mainly heat the top surface of the meats, while SHS cooked meats uniformly, hard film formed at both top and bottom surface of meat at the beginning of the heating, which need more cutting force, resulting in a higher shear force value.

Table 1 Changes in internal color and cooking loss of pork samples cooked to different internal temperatures by TS and SHS at 120, 150 and 180 ℃.

Group	Internal treatments (℃)	L^*	a^*	b^*	Cooking loss (%)
TS	40	59.27 ± 1.69^{cA}	6.53 ± 0.51^{aB}	4.92 ± 0.54^{cA}	4.48 ± 0.04^{cA}
	60	76.76 ± 0.49^{bA}	5.54 ± 0.79^{bB}	8.48 ± 0.35^{bB}	8.78 ± 0.21^{bA}
	80	81.33 ± 0.79^{aA}	4.93 ± 0.50^{bB}	10.33 ± 0.45^{aB}	22.05 ± 0.08^{aA}
SHS 120 ℃	40	58.21 ± 2.51^{cA}	7.06 ± 0.16^{cB}	5.30 ± 0.59^{bA}	4.47 ± 0.19^{cA}
	60	75.68 ± 1.70^{bAB}	6.62 ± 0.16^{bA}	9.31 ± 0.42^{aA}	8.35 ± 0.20^{bA}
	80	80.51 ± 0.38^{aA}	5.32 ± 0.25^{aB}	10.00 ± 0.38^{aB}	21.89 ± 0.10^{aA}
SHS 150 ℃	40	55.11 ± 0.28^{cB}	7.75 ± 0.21^{aA}	3.66 ± 0.41^{cB}	4.43 ± 0.40^{cA}
	60	74.08 ± 0.55^{bB}	6.85 ± 0.10^{bA}	9.39 ± 0.04^{bA}	7.47 ± 0.65^{bB}
	80	78.82 ± 1.04^{aB}	6.64 ± 0.50^{bA}	10.97 ± 0.15^{aA}	15.95 ± 1.20^{aB}
SHS 180 ℃	40	51.49 ± 1.12^{cC}	8.14 ± 0.14^{aA}	2.97 ± 0.18^{cB}	3.95 ± 0.50^{cA}
	60	71.04 ± 1.63^{bC}	7.18 ± 0.02^{bA}	4.88 ± 0.09^{bA}	7.46 ± 0.40^{bB}
	80	75.08 ± 0.46^{aC}	7.02 ± 0.15^{bA}	8.98 ± 0.30^{aC}	14.26 ± 0.60^{aC}

Note: Different uppercase letters (A–C) indicate significant difference between different cooking methods at same internal temperature ($P < 0.05$); Different lowercase letters (a–c) indicate significant difference between different internal temperature for same cooking method ($P < 0.05$). Values are presented as mean ± SD.

Table 2 Shear force and texture properties of pork samples cooked to different internal temperatures by TS and SHS at 120, 150 and 180 ℃.

Group	Internal temperature (℃)	Shear force (N)	Hardness (g)	Cohesiveness	Springiness (%)	Gumminess	Chewiness
TS	40	3.43 ± 0.32^{bB}	9 067.36 ± 614.71^{cB}	0.43 ± 0.02^{aC}	40.45 ± 0.96^{cC}	4 283.06 ± 492.14^{cC}	1 700.19 ± 65.52^{cC}
	60	5.67 ± 0.10^{aA}	11 341.36 ± 77.92^{bA}	0.57 ± 0.01^{bA}	42.50 ± 1.97^{bA}	5 454.06 ± 1 102.73^{bA}	2 507.68 ± 117.63^{bA}
	80	5.95 ± 0.62^{aA}	15 356.79± 1 332.12^{aA}	0.59 ± 0.01^{bAB}	58.64 ± 2.05^{aA}	9 049.66 ± 602.85^{aA}	5 141.06 ± 135.36^{aA}
SHS 120 ℃	40	4.17 ± 0.13^{bA}	11 213.00 ± 705.68^{bA}	0.52 ± 0.19^{bB}	40.45 ± 1.61^{cC}	5 741.97 ± 240.63^{bA}	2 284.25 ± 146.62^{bA}
	60	5.17 ± 0.51^{aAB}	8 773.88 ± 452.88^{cC}	0.58 ± 0.01^{aA}	45.18 ± 1.40^{bA}	5 303.28 ± 67.30^{cA}	2 175.03 ± 155.12^{bB}
	80	5.66 ± 0.59^{aAB}	12 172.62 ± 726.47^{aB}	0.60 ± 0.01^{aA}	55.73 ± 2.05^{aB}	7 639.84 ± 216.62^{aB}	4 001.66 ± 232.82^{aB}
SHS 150 ℃	40	3.77 ± 0.20^{bAB}	9 859.22 ± 674.64^{bB}	0.48 ± 0.01^{cA}	45.65 ± 0.71^{bA}	5 673.32 ± 813.16^{bAB}	2 050.22 ± 165.22^{bB}
	60	5.17 ± 0.51^{aAB}	9 879.02 ± 812.26^{bB}	0.57 ± 0.11^{bA}	43.30 ± 1.14^{cA}	5 210.95 ± 1 007.47^{bA}	2 195.12 ± 50.53^{bB}
	80	5.66 ± 0.59^{aB}	11 935.52 ± 241.48^{aC}	0.59 ± 0.04^{B}	52.69 ± 1.88^{aD}	7 008.38 ± 505.60^{aBC}	3 515.96 ± 304.19^{aC}
SHS 180 ℃	40	3.29 ± 0.36^{bB}	9 166.49 ± 594.79^{bB}	0.46 ± 0.03^{cA}	43.39 ± 0.81^{bB}	4 855.23 ± 504.84^{bBC}	1 988.98 ± 72.39^{cB}
	60	4.72 ± 0.35^{aB}	9 572.27 ± 178.14^{bBC}	0.57 ± 0.01^{bA}	43.26 ± 1.86^{bA}	5 248.85 ± 445.44^{bA}	2 264.17 ± 198.21^{bAB}
	80	5.16 ± 0.10^{aAB}	10 869.90 ± 789.21^{aD}	0.59 ± 0.01^{aAB}	54.40 ± 2.95^{aC}	6 568.58 ± 286.25^{aC}	3 507.74 ± 136.84^{aC}

Note: Different uppercase letters (A–C) indicate significant difference between different cooking methods at same internal temperature ($P < 0.05$); Different lowercase letters (a–c) indicate significant difference between different internal temperature for same cooking method ($P < 0.05$). Values are presented as mean ± SD.

Texture such as hardness, springiness, chewiness, etc. are important quality characteristics of meat, which have significant impact on consumers' eating experience. In our study, pork texture is highly related to cooking method and internal temperature. Hardness, springiness, cohesiveness, gumminess and chewiness increased significantly ($P < 0.05$) with increasing internal temperature. At an internal temperature of 40 ℃, hardness was no significant difference ($P > 0.05$) between the SHS and TS cooking. Meats cooked by TS at 60 ℃ internal temperature had a higher ($P < 0.05$) hardness ((11 341.36 ± 77.92) g) than those cooked by SHS at 120, 150 and 180 ℃ ((8 773.88 ± 452.88), (9 879.02 ± 812.26) and (9 572.27 ± 178.14) g, respectively). Similarly, TS treatment at 80 ℃ internal temperature caused higher ($P < 0.05$) hardness ((15 356.79 ± 1 332.12) g) than those cooked by SHS at 120, 150 and 180 ℃ ((12 172.62 ± 726.47), (11 935.52 ± 241.48), and (10 869.90 ± 789.21) g, respectively). At 80 ℃ internal temperature, TS cooked meats had significant higher ($P < 0.05$) springiness ((58.64 ± 2.05)%) than those cooked by SHS at 120, 150 and 180 ℃ ((55.73 ± 2.05)%, (52.69 ± 1.88)%, and (54.40 ± 2.95)%, respectively). Furthermore, cohesiveness was no significant difference ($P > 0.05$) between the SHS and TS cooking. At 80 ℃ internal temperature, SHS cooked pork had lower ($P < 0.05$) gumminess and chewiness than TS cooked pork. Rahman et al.[21] found that there was a significant positive relationship between hardness and chewing ability. The lower the chewiness value, the lower the force required for chewing, and the better the taste of the sample, indicating SHS cooked meat had better mouth feel quality. The differences in texture properties between SHS and TS cooked meat is probably related to the changes in internal chemical forces and conformation of pork protein during heating. Therefore, it is necessary to further study the internal chemical forces and conformational changes of proteins.

3.3 PCA result

PCA was used to further characterize the difference in meat quality cooked to different internal temperatures among the TS cooking and SHS cooking as shown in Figures 2A–C, respectively. Parameters of meat quality attributes including color, textual properties, and cooking loss were included in the PCA model. The total contribution rates of the first 2 PC1 and PC2 were 76.7%, 66.1% and 77.4% respectively.

At an internal temperature of 40 ℃, there were clear separation among samples after different cooking methods (Figure 2A), indicating significant difference in meat quality. The SHS cooking at 150 and 180 ℃ gathered in green and blue circle respectively showing with less cooking loss and a more red appearance.

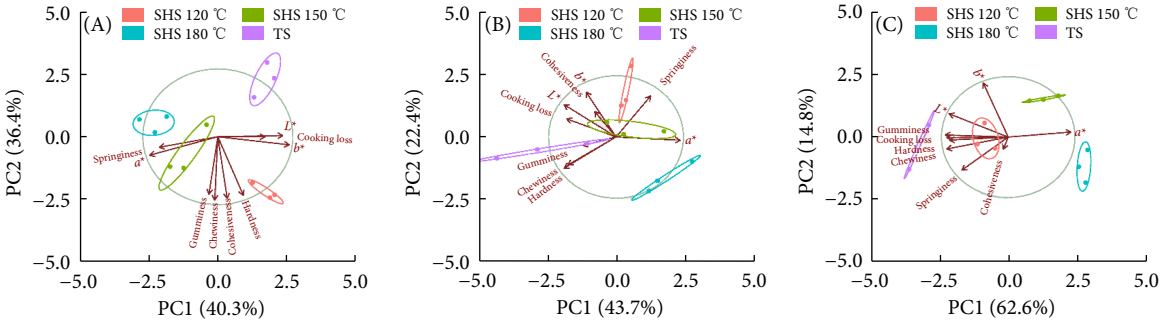

Figure 2 PCA score plots of meat quality cooked by TS and SHS at 120, 150 and 180 ℃. (A) Internal temperature of 40 ℃; (B) Internal temperature of 60 ℃; (C) Internal temperature of 80 ℃.

TS cooking and SHS cooking at 120 ℃ showing higher value of cooking loss and lightness, whereas SHS cooking at 120 ℃ has the highest value of hardness, cohesiveness, gumminess and chewiness. In contrast, PCA of meat quality for 60 ℃ internal temperature (Figure 2B) showed relative short distance from pink, purple and green circle, suggesting similarities in meat quality. The blue area was the SHS cooking at 180 ℃, which was scattered from other groups and it was exhibited with the lowest value of cooking loss, and the highest value of redness. At 80 ℃ internal temperature (Figure 2C), Groups of TS cooking and SHS cooking at 120 ℃ are in the 2nd and 3rd quadrants and had a relative short distance, indicating similar meat quality. SHS cooking at 150 and 180 ℃ are in the 1st and 4th quadrants respectively, suggesting higher SHS temperatures were associated with less cooking loss and shear force, and a more red appearance.

3.4 Water status

NMR transverse relaxation time (T_2) can explain the variation of water state in the sample with relaxation time from a microscopic perspective, and can real-time observe the flow and distribution of hydrogen protons in the sample[22]. If the relaxation time T_2 is long, it indicates that the degree of freedom of hydrogen protons is greater, the binding degree with protein is weaker, and the water is more easily removed; On the contrary, if the T_2 is short, water is difficult to remove. The water in the sample usually has three forms, namely, bound water T_{21} (0.1–10 ms), immobilized water T_{22} (10–100 ms), and free water T_{23} (> 100 ms) which is outside myofibril[23–24].

Figure 3 illustrates the T_2 of pork samples during different cooking methods, which is obtained through inversion and fitting. All samples have three distinguishing component peaks. The bound water (T_{21}) was mainly in the range of 0.52–1.12 ms, the immobilized water (T_{22}) was in the range of 23.82–41.50 ms, and the range of free water (T_{23}) was 178.34–289.94 ms. As expected, most water in pork samples is immobilized water. In addition, with increasing internal temperature, T_2 of both TS and SHS cooked sample gradually shifted to the left and peak amplitude gradually decreased. This was mainly due to protein denaturation caused by heating, which destroyed the hydrogen bond between water and protein, the immobilized water transfer to free water, leading to water evaporation[25]. T_1 and T_2 intensity decreased with increasing internal temperature during pork cooking process was also observed by Song et al.[14].

As shown in Figures 4A–C, At an internal temperature of 60 ℃, T_{21}, T_{22} and T_{23} increased with SHS temperature, indicates decreasing protein-water interactions upon increasing SHS temperature. The T_2 of TS-cooked meats were shorter than that of SHS-cooked samples. Furthermore, SHS cooking at 150 and 180 ℃ had relatively higher P_{22} values but lower P_{23} values, compared to TS and SHS cooking at 120 ℃ (Figures 4E–F). This was mainly due to that the muscle structure of pork cooked by SHS was relatively complete, while the secondary structure of pork protein cooked by TS tends to be loose, promoting more immobilized water into free water.

3.5 ATR-FTIR and secondary structure

Proteins are biological macromolecules with special structures, formed by amino acids connected by peptide bonds. Secondary structure is the foundation of protein spatial structure and is closely

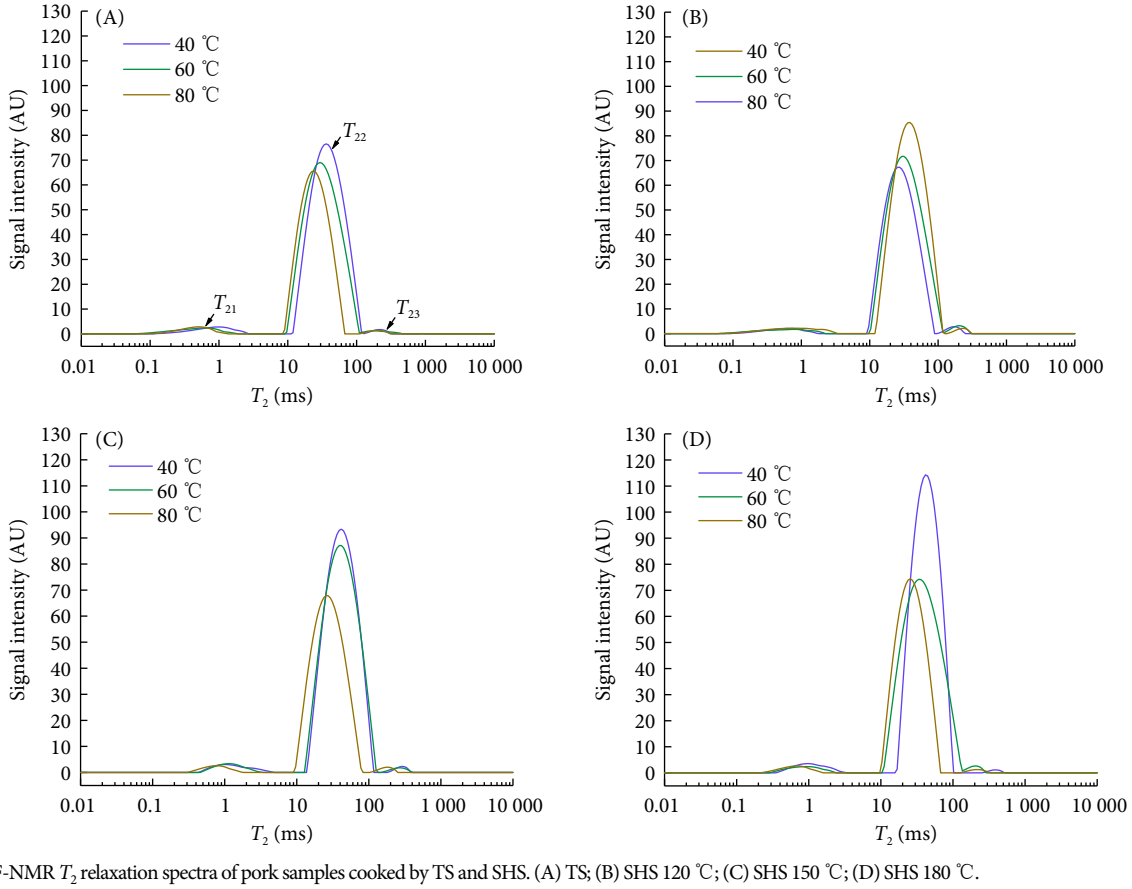

Figure 3 LF-NMR T_2 relaxation spectra of pork samples cooked by TS and SHS. (A) TS; (B) SHS 120 ℃; (C) SHS 150 ℃; (D) SHS 180 ℃.

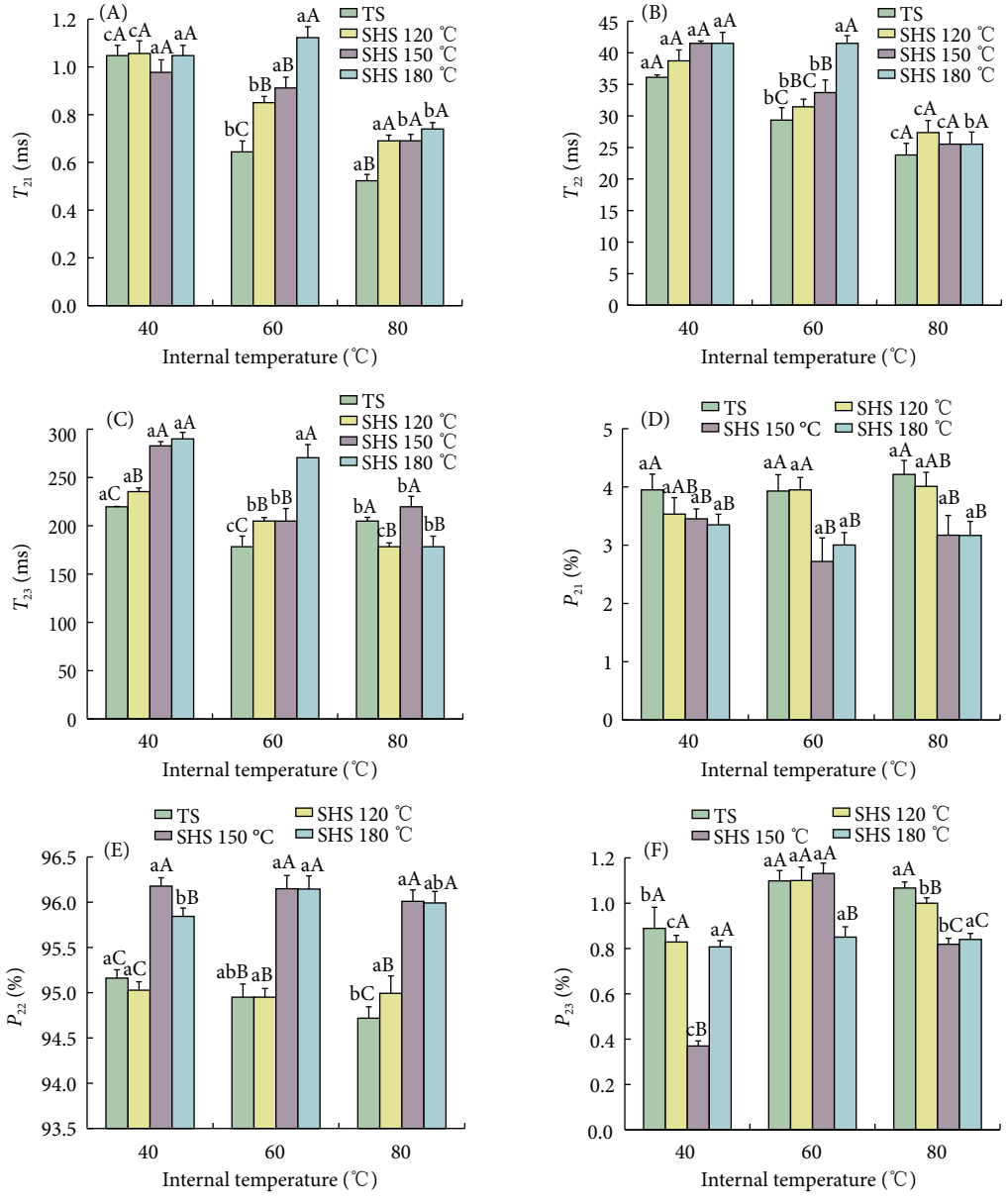

Figure 4 (A–C) T_2 relaxation time and (D–F) relative area of pork samples cooked to different temperatures by TS and SHS at 120, 150 and 180 ℃. Different uppercase letters (A–C) indicate significant difference between different cooking methods at same internal temperature ($P < 0.05$); Different lowercase letters (a–c) indicate significant difference between different internal temperature for same cooking method ($P < 0.05$). Values are presented as mean ± SD.

related to amide band I located between 1 600 and 1 700 cm^{-1} [26]. The α-helix was assigned to 1 646–1 664 cm^{-1}, β-sheet was assigned to 1 615–1 637 and 1 682–1 700 cm^{-1}, random coil was assigned to 1 637–1 645 cm^{-1}, and β-turn was assigned to 1 664–1 681 cm^{-1}. The amide I band of the original protein infrared spectrum can be decompose into several sub peaks by using second derivative and deconvolution techniques[27]. Changes in MP secondary structure content of pork after different cooking methods can be observed through peak height and peak area.

As shown in Figure 5, β-sheet was the main secondary structure in different cooked pork samples MP. The α-helix and β-turn content decreased slightly with increasing internal temperature while the β-sheet and random coil content increased slightly in pork MP for both TS and SHS treatment. The α-helix is mainly maintained by intramolecular hydrogen bonds formed between carbonyl and amino groups on the polypeptide chain. The reduction of α-helix content indicated that heat treatment can destroy hydrogen bonds, causing the unfolding of the helical structure and a transformation of α-helix to β-sheet and random coil. In addition, SHS resulted in increase of α-helixes and β-turns in MP compared to TS, but the β-sheets and random coils showed the opposite trend. According to Mitra et al.[28], the degree of protein denaturation depends on both temperature and time of heat treatment. Compared to TS, SHS has higher heat transfer coefficient and needs less time to reach the desired internal temperature, proteins are denatured with insufficient heat, thus leading to increased α-helixes and β-turns and decreased β-sheets and random coils. Moreover, Cando et al.[29] concluded that tenderness was related to decreased α-helix content and increased β-structure. In our study, we did find that at 80 ℃ internal temperature, samples in SHS 180 ℃ had a higher α-helix content and lowest β-sheet content, which had the lowest shear force ((5.16 ± 0.10) N) among the samples with an internal temperature of 80 ℃.

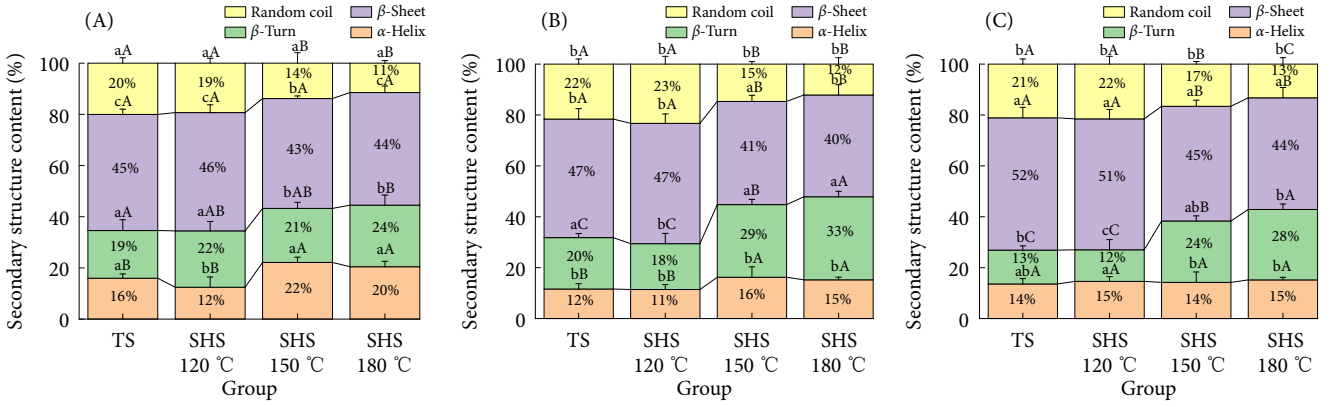

Figure 5 The percentage of secondary protein structure in pork samples cooked by TS and SHS at 120, 150 and 180 ℃. (A) Internal temperature of 40 ℃; (B) Internal temperature of 60 ℃; (C) Internal temperature of 80 ℃. Different uppercase letters (A–C) indicate significant difference between different cooking methods at same internal temperature ($P < 0.05$); Different lowercase letters (a–c) indicate significant difference between different internal temperature for same cooking method ($P < 0.05$). Values are presented as mean ± SD.

3.6 Chemical forces

The changes in chemical forces including hydrogen bonds, ionic bonds and hydrophobic interaction of pork samples during TS and SHS cooking were shown in Figure 6. Ionic and hydrogen bonds significant decreased with internal temperature ($P < 0.05$), and reached a relative low value at 80 ℃, which indicated that ionic and hydrogen bonds were destroyed during cooking process. At 60 ℃ and 80 ℃ internal temperature, greater ionic and hydrogen bonds in pork samples contributed to higher stability for SHS cooked pork protein compared to TS cooked pork protein, and indirectly influenced the gelation properties.

Compared to TS cooked pork protein, SHS cooked pork protein resulted in less hydrophobic interactions. In addition, with increasing temperature, hydrophobic interactions increased, followed by a decrease (Figure 6). The maximum value was obtained at 60 ℃. At the beginning of heat treatment, proteins denatured and the hydrophobic groups exposed, prompting the hydrophobic interactions to enhance, while when the temperature further increased, protein-protein interactions will enhance by disulfide bonds and covalent cross-links. A small amount of hydrophobic residues will be buried in the molecule again, resulting in the reduction of hydrophobic interaction[30].

Chemical forces such as ion bonds, hydrogen bonds, hydrophobic bonds, and disulfide bonds all play an important role in the hardness, elasticity, cohesiveness, chewiness, and resilience changes of pork. During heating, pork muscle protein forms gel, and each chemical force is the main force to maintain the appearance of the gel[31]. At a lower temperature range (40–60 ℃), hydrogen bond, ionic bond and hydrophobic bond play the main role. With the increase of temperature, disulfide bond becomes the main force to maintain the stability of gel. The results of this study showed that at a lower temperature range (40–60 ℃), the content of hydrogen bond and ionic bond was higher, the hydrophobic group exposed, and the pork MP formed a softer gel with less hardness and elasticity. With the temperature further increased, the chemical forces between protein molecules were destroyed during heating, and hydrophobic residues exposed and were oxidized into disulfide bonds, leading to complete denaturation and aggregation of proteins, forming a new ordered three-dimensional network structure. At this time, the hardness, elasticity, cohesiveness and resilience of gel increased. The differences in hardness and springiness in pork samples cooked by different cooking methods were mainly due to the degree of protein denaturation. Compared to TS cooking, SHS cooking resulted in low degree of denaturation of protein. This led to a low exposure of hydrogen bonds and hydrophobic bonds, and low protein aggregation, resulted in low hardness and springiness.

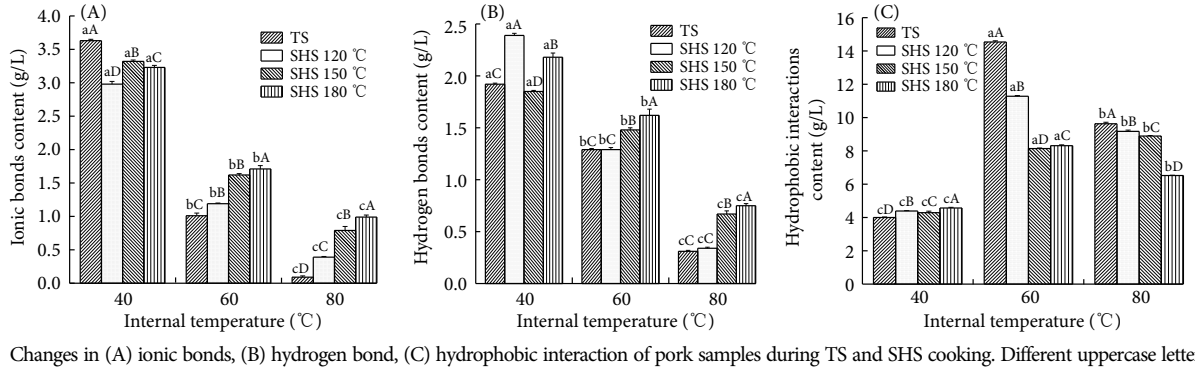

Figure 6 Changes in (A) ionic bonds, (B) hydrogen bond, (C) hydrophobic interaction of pork samples during TS and SHS cooking. Different uppercase letters (A–C) indicate significant difference between different cooking methods at same internal temperature ($P < 0.05$); Different lowercase letters (a–c) indicate significant difference between different internal temperature for same cooking method ($P < 0.05$). Values are presented as mean ± SD.

4 Conclusion

This study aimed to evaluate the physicochemical quality, water status and protein degradation of pork samples cooked by SHS. The treatments varied in temperature (120, 150, and 180 ℃) and pork samples were cooked to 40, 60 and 80 ℃, respectively; Samples cooked by TS were included as controls. Compared to TS, SHS resulted in lower value of L^*, b^*, cooking loss, shear force, hardness and chewiness and higher value of a^* in pork. Protein structure tended to unfold and more loosen in TS cooked pork during heating, leading to a high exposure of hydrophobic bonds, and high protein aggregation, which promoted more immobilized water transferred into free water, while promoting the gel formation, which in turn affected the hardness and springiness of pork samples. These results provide information for the use of SHS as an alternative cooking method for pork and other meats. Furthermore, the quality of pork during SHS cooking can be monitored through protein conformation and water migration to improve product uniformity.

Author statement

Jiajia Fang: Conceptualization, data curation, methodology, formal analysis, writing-original draft; Jiapeng Li: supervision, funding acquisition, formal writing-review and editing; Junna Yang: Supervision; Biao Qi: Supervision; Chunjiang Zhang: Supervision.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

This research was funded by the National Key R & D Program of China (2022YFD2100500).

References

[1] B. M. Bohrer, Review: nutrient density and nutritional value of meat products and non-meat foods high in protein, Trends Food Sci. Tech. 65 (2017) 103–112. https://doi.org/10.1016/j.tifs.2017.04.016.

[2] N. Chotigavin, W. L. Kerr, W. Klaypradit, et al., Novel sous-vide pressure technique affecting properties of local beef muscle, LWT-Food Sci. Technol. 175 (2023) 114439. https://doi.org/10.1016/j.lwt.2023.114439.

[3] M. Modzelewska-Kapituła, R. Pietrzak-Fiećko, K. Tkacz, et al., Influence of sous vide and steam cooking on mineral contents, fatty acid composition and tenderness of *Semimembranosus* muscle from Holstein-Friesian bulls, Meat Sci. 157 (2019) 107877. https://doi.org/10.1016/j.meatsci.2019.107877.

[4] A. Alfy, B. V. Kiran, G. C. Jeevitha, et al., Recent developments in superheated steam processing of foods: a review, Crit. Rev. Food Sci. Nutr. 56 (2016) 2191–2208. https://doi.org/10.1080/10408398.2012.740641.

[5] J. Fang, C. Liu, C. L. Law, et al., Superheated steam processing: an emerging technology to improve food quality and safety, Crit. Rev. Food Sci. Nutr. (2022) 1–17. https://doi.org/10.1080/10408398.2022.2059440.

[6] R. Sehrawat, P. K. Nema, B. P. Kaur, Quality evaluation and drying characteristics of mango cubes dried using low-pressure superheated steam, vacuum and hot air drying methods, LWT-Food Sci. Technol. 92 (2018) 548–555. https://doi.org/10.1016/j.lwt.2018.03.012.

[7] P. Pongmalai, S. Devahastin, Profiles of prebiotic fructooligosaccharides, inulin and sugars as well as physicochemical properties of banana and its snacks as affected by ripening stage and applied drying methods, Dry. Technol. 38 (2020) 724–734. https://doi.org/10.1080/07373937.2019.1700517.

[8] H. Takemitsu, M. Amako, Y. Sako, et al., Reducing the undesirable odor of barley by cooking with superheated steam, J. Food Sci. Tech. 56 (2019) 4732–4741. https://doi.org/10.1007/s13197-019-03907-2.

[9] R. Liu, S. M. Zhao, B. J. Xie, et al., Contribution of protein conformation and intermolecular bonds to fish and pork gelation properties, Food Hydrocoll. 25 (2011) 898–906. https://doi.org/10.1016/j.foodhyd.2010.08.016.

[10] R. M. Bórquez, E. R. Canales, H. R. Quezada, Drying of fish press-cake with superheated steam in a pilot plant impingement system, Dry. Technol. 26 (2008) 290–298. https://doi.org/10.1080/07373930801897986.

[11] K. Y. Hsu, B. H. Chen, Analysis and reduction of heterocyclic amines and cholesterol oxidation products in chicken by controlling flavorings and roasting condition, Food Res. Int. 131 (2020) 109004. https://doi.org/10.1016/j.foodres.2020.109004.

[12] R. Suleman, T. Hui, Z. Wang, et al., Comparative analysis of charcoal grilling, infrared grilling and superheated steam roasting on the colour, textural quality and heterocyclic aromatic amines of lamb patties, Int. J. Food Sci. Tech. 55 (2020) 1057–1068. https://doi.org/10.1111/ijfs.14388.

[13] R. Wang, F. Huang, L. Zhang, et al., Changes in the texture, microstructures, colour and volatile compounds of pork meat loins during superheated steam cooking, Int. J. Food Sci. Tech. 54 (2019) 2821–2830. https://doi.org/10.1111/ijfs.14198.

[14] Y. Song, F. Huang, X. Li, et al., Water status evolution of pork blocks at different cooking procedures: a two-dimensional LF-NMR T_1-T_2 relaxation study, Food Res. Int. 148 (2021) 110614. https://doi.org/10.1016/j.foodres.2021.110614.

[15] S. Wang, R. Lin, S. S Cheng, et al., Water dynamics changes and protein denaturation in surf clam evaluated by two-dimensional LF-NMR T_1-T_2 relaxation technique during heating process, Food Chem. 320 (2020) 126622. https://doi.org/10.1016/j.foodchem.2020.126622.

[16] L. Chen, D. Jiao, H. Liu, et al., Effects of water distribution and protein degradation on the texture of high pressure-treated shrimp (*Penaeus monodon*) during chilled storage, Food Control 132 (2022) 108555. https://doi.org/10.1016/j.foodcont.2021.108555.

[17] Y. Yu, M. Tang, H. Dai, et al., Dominating roles of protein conformation and water migration in fish muscle quality: the effect of freshness and heating process, Food Chem. 388 (2022) 132881. https://doi.org/10.1016/j.foodchem.2022.132881.

[18] B. Nieva-Echevarría, M. J. Manzanos, E. Goicoechea, et al., Changes provoked by boiling, steaming and sous-vide cooking in the lipid and volatile profile of European sea bass, Food Res. Int. 99 (2017) 630–640. https://doi.org/10.1016/j.foodres.2017.06.043.

[19] M. C. Hughes, J. P. Kerry, E. K. Arendt, et al., Characterization of proteolysis during the ripening of semi-dry fermented sausages, Meat Sci. 62 (2002) 205–216. https://doi.org/10.1016/S0309-1740(01)00248-0.

[20] M. Lopes, V. Mouillet, L. Bernucci, et al., The potential of attenuated total reflection imaging in the mid-infrared for the study of recycled asphalt mixtures, Constr. Build. Mater. 124 (2016) 1120–1131. https://doi.org/10.1016/j.conbuildmat.2016.08.108.

[21] M. S. Rahman, S. A. Al-Farsi, Instrumental texture profile analysis (TPA) of date flesh as a function of moisture content, J. Food Eng. 66 (2005) 505–511. https://doi.org/10.1016/j.jfoodeng.2004.04.022.

[22] H. C, Bertram, P. P. Purslow, H. J. Andersen, Relationship between meat structure, water mobility, and distribution: a low-field nuclear magnetic resonance study, J. Agric. Food Chem. 50 (2002) 824–829. https://doi.org/10.1021/jf010738f.

[23] X. Zang, Y. Zu, T. Zhang, et al., Dynamic water mobility in sea cucumber (*Stichopus japonicas*) during drying process assessed by LF-NMR and MRI *in situ*, Int. J. Food Eng. 13 (2017). https://doi.org/10.1515/ijfe-2016-0360.

[24] Y. Song, F. Huang, X. Li, et al., Effects of different wet heating methods on the water distribution, microstructure and protein denaturation of pork steaks, Int. J. Food Sci. Tech. 56 (2021) 4627–4638. https://doi.org/10.1111/ijfs.15248.

[25] J. Wang, A. S. Mujumdar, L. Z. Deng, et al., High-humidity hot air impingement blanching alters texture, cell-wall polysaccharides, water status and distribution of seedless grape, Carbohyd. Polym. 194 (2018) 9–17. https://doi.org/10.1016/j.carbpol.2018.04.023.

[26] A. Zając, L. Dymińska, J. Lorenc, et al., Fourier transform infrared and Raman spectroscopy studies of the time-dependent changes in chicken meat as a tool for recording spoilage processes, Food Anal. Method 10 (2017) 640–648. https://doi.org/10.1007/s12161-016-0636-x.

[27] H. Yang, S. Yang, J. Kong, et al., Obtaining information about protein secondary structures in aqueous solution using Fourier transform IR spectroscopy, Nat. Protoc. 10 (2015) 382–396. https://doi.org/10.1038/nprot.2015.024.

[28] B. Mitra, Å. Rinnan, J. Ruiz-Carrascal, Tracking hydrophobicity state, aggregation behaviour and structural modifications of pork proteins under the influence of assorted heat treatments, Food Res. Int. 101 (2017) 266–273. https://doi.org/10.1016/j.foodres.2017.09.027.

[29] D. Cando, B. Herranz, A. J. Borderías, et al., Different additives to enhance the gelation of surimi gel with reduced sodium content, Food Chem. 196 (2016) 791–799. https://doi.org/10.1016/j.foodchem.2015.10.022.

[30] Y. Sun, L. Ma, M. Ma, et al., Texture characteristics of chilled prepared Mandarin fish (*Siniperca chuatsi*) during storage, Int. J. Food Prop. 21 (2018) 242–254. https://doi.org/10.1080/10942912.2018.1451343.

[31] J. Liu, J. Xue, Q. Xu, et al., Drying kinetics and quality attributes of white radish in low pressure superheated steam, Int. J. Food Eng. (2017) 20160365. https://doi.org/10.1515/ijfe-2016-0365.